COMMENTARY

A novel definition and treatment of hyperinflammation in COVID-19 based on purinergic signalling

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Abstract

Hyperinflammation plays an important role in severe and critical COVID-19. Using inconsistent criteria, many researchers define hyperinflammation as a form of very severe inflammation with cytokine storm. Therefore, COVID-19 patients are treated with anti-inflammatory drugs. These drugs appear to be less efficacious than expected and are sometimes accompanied by serious adverse effects. SARS-CoV-2 promotes cellular ATP release. Increased levels of extracellular ATP activate the purinergic receptors of the immune cells initiating the physiologic pro-inflammatory immune response. Persisting viral infection drives the ATP release even further leading to the activation of the P2X7 purinergic receptors (P2X7Rs) and a severe yet physiologic inflammation. Disease progression promotes prolonged vigorous activation of the P2X7R causing cell death and uncontrolled ATP release leading to cytokine storm and desensitisation of all other purinergic receptors of the immune cells. This results in immune paralysis with co-infections or secondary infections. We refer to this pathologic condition as hyperinflammation. The readily available and affordable P2X7R antagonist lidocaine can abrogate hyperinflammation and restore the normal immune function. The issue is that the half-maximal effective concentration for P2X7R inhibition of lidocaine is much higher than the maximal tolerable plasma concentration where adverse effects start to develop. To overcome this, we selectively inhibit the P2X7Rs of the immune cells of the lymphatic system inducing clonal expansion of Tregs in local lymph nodes. Subsequently, these Tregs migrate throughout the body exerting anti-inflammatory activities suppressing systemic and (distant) local hyperinflammation. We illustrate this with six critically ill COVID-19 patients treated with lidocaine.

Keywords COVID-19 · P2X7 receptor antagonist · Lidocaine base · Hyperinflammation · Cytokine storm · Immune paralysis

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Introduction

Hyperinflammation and acute respiratory distress syndrome (ARDS) caused by coronavirus disease 2019 (COVID-19) have become the world's number 1 challenge. The exponential pattern in the number of severe cases in the second and third waves of the SARS-CoV-2 pandemic has shown to reach nations' maximum ICU capacities in weeks rather than months after outbreak of the disease irrespective of rigorous population-based preventive measures. In a recently published systematic review, the case fatality rates in patients in the ICU across 7 countries vary between 14.9 and 66.7%, while the case fatality rates among those who required mechanical ventilation vary between 16.7 and 97.0% [1]. In addition, the case fatality rate in a cohort of 1035 critically ill COVID-19 patients requiring extracorporeal membrane oxygenation (ECMO, artificial lungs) is alarmingly high (37.4%) [2].

The clinical manifestations of severe COVID-19 consist of pneumonia with dyspnoea and hyperinflammation. Hyperinflammation is thought to be the basis of the development of severe and critical COVID-19 [3–5]. Currently, a clear-cut definition of hyperinflammation is lacking. Some authors describe the condition of hyperinflammation as a form of very severe inflammation with cytokine storm [6]. The criteria of hyperinflammation are not consistent and include clinical data and/or different combinations of the parameters of the activation of the pro-inflammatory response of the immune system (i.e. fever, rapid respiratory deterioration, cytokine, ferritin and/or CRP concentrations, changes in blood levels of several types of immune cells, etc., examples are presented in Table 1) [3, 4, 6–8, 9, 10–14]. In addition, the current definitions of hyperinflammatory syndrome do not provide an

Table 1 Examples of the criteria of hyperinflammation. These criteria are not consistent and include different combinations of symptoms and laboratory parameters of the activation of the pro-inflammatory response of the immune system

Author	Year of publication	Criteria of hyperinflammation		Reference number
		Clinical	Laboratory or pathogenesis	
Webb BJ et al.	2020	Fever (temperature of more than 38.0°C)	Macrophage activation (ferritin concentration of 700 μg/l or more) Haematological dysfunction (neutrophil to lymphocyte ratio of 10 or more or both haemoglobin concentration of 9.2 g/dl or less and platelet count of 110 × 10^{9}cells/L or less) Haematological dysfunction (neutrophil to lymphocyte ratio of 10 or more or both haemoglobin concentration of 9.2 g/dl or less and platelet count of 110 × 10^{9}cells/L or less) Coagulopathy (D-dimer concentration of 1.5 μg/ml or more) Hepatic injury (lactate dehydrogenase concentration of 400 U/L or more, or an aspartate aminotransferase concentration of 100 U/L or more) Cytokinaemia (defined as an IL-6 concentration of 15 pg/ml or more, or a triglyceride concentration of 150 mg/ml or more, or a CRP concentration of 15 mg/dl or more)	[7]
Fajgenbaum DC and June CH	2020		Very severe inflammation with cytokine storm	[6]
Manson JJ et al.	2020		C-reactive protein (CRP) concentration greater than 150 mg/L Doubling of CRP concentration within 24 h from a concentration of greater than 50 mg/L Ferritin concentration of greater than 1500 μg/L	[3]
Gustine JN and Jones D	2021		Cytokine storm, dysregulated macrophage activation, impaired natural killer cell response, lymphopenia, elevated absolute neutrophil count and neutrophil/lymphocyte ratio and increased levels of neutrophil extracellular traps (NETs)	[4]
Anka AU et al.	2021		Excessive secretion of pro-inflammatory cytokines and the recruitment of pro-inflammatory cells such as granulocytes and macrophages caused by tissue injury result in a snowballing of cytokine secretion leading to a systemic inflammatory response such as macrophage activation syndrome (MAS), secondary haemophagocytic lymphohistiocytosis (sHLH—cytokine storm)	[11]
Cardone MC et al.	2020		Increased plasma levels of pro- and anti-inflammatory cytokines (IL-1β, IL-6, IL-7, IL-8, IL-9, IL-10, IFN-γ, TNF), chemokines (MCP1, MIP1A, MIP1B) and growth factors (G-CSF, GM-CSF)	[8]
Mehta P et al.	2020		Trends in laboratory results such as increasing ferritin, decreasing platelet counts or high erythrocyte sedimentation rate	12
Freeman TL et al. (2020)	2020		Vigorous stimulation of the innate immune response activating the Nod-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome pathway. This causes the release of the pro-inflammatory cytokines IL-6 and IL-1β	[13]
De Luca G et al.	2020		Elevation of CRP to ≥100 mg/L or ferritin to ≥900 μg/L in the presence of any increase in lactate dehydrogenase (LDH)	[14]
Bozzi G et al.	2021		Ferritin plasma levels of ≥1000 ng/mL and/or CRP of >10 mg/dl	[9]
Landewé RBM et al.	2021	Rapid respiratory deterioration on or during admission	Plus fulfilment of at least two out of three biomarker criteria: CRP of >100mg/L, serum ferritin of >900 μg/L, D-dimer of >1500 μg/L	[10]

explanation for the frequently observed co-infections or secondary infections in COVID-19 [15, 16].

The results of non-randomised cohort studies with controls and of retrospective observational studies suggest that IL-1 receptor blockade (anakinra) [9, 17], monoclonal antibodies against IL-6 receptors [18–21] and the combination of both drugs [22, 23] may improve survival rate in at least a subgroup of patients with COVID-19. However, in prospective randomised controlled trials with the exception of one trial with tocilizumab and sarilumab in critically patients [24], anti-inflammatory therapy with anakinra [25] or tocilizumab [26–32] did not improve the outcome in moderate, severe and critically ill COVID-19. On December 10, 2020, an editorial commented that it is disappointing that nearly 10 months into the COVID-19 pandemic, a breakthrough treatment has not been identified [33]. Researchers of the US National Institute of Allergy and Infectious Diseases stated that although Remdesivir is effective to reduce time to recovery in hospitalised COVID-19 patients [34] and dexamethasone reduces mortality in critically ill COVID-19 patients [35], there is no treatment for early or mild infection [36]. Moreover, dexamethasone raises concerns because it increased the 28-day mortality in patients who did not receive respiratory support [35] and it dampens the "alarm phase" of the inflammation process including the capacity of detecting pathogens in mammals by the immune system [37]. In addition, administration of methylprednisolone (1 mg/kg/day intravenously) in COVID-19 reduced the blood levels of NK cells, CD4$^+$ and CD8$^+$T-cells and increases the duration of throat viral RNA detectability indicating immune cell dysfunction [38]. Furthermore, targeted anti-viral medication has failed to treat COVID-19 effectively [39]. According to the World Health Organisation, after a record-breaking development, vaccine deployment is slow and has many challenges to overcome [40]. Vaccine hesitancy is relatively high [41] even among health care workers [42, 43]. It could take more than a year to vaccinate enough people required to make an impact on SARS-CoV-2 spreading, while therapeutic measures that can immediately attenuate the course of SARS-CoV-2-related lung damage are promptly needed on a global scale. To make the matters worse, many scientists expect that SARS-CoV-2 may become endemic and is here to stay [44].

In this report, we developed a novel definition of hyperinflammation based on purinergic signalling. Subsequently, we describe our discovery of an old drug capable of attenuating hyperinflammation and illustrate this with six critically ill patients suffering from COVID-19. Finally, we present the future development of a new and more accessible administration route for this drug as shown in Fig. 1.

Purinergic signalling

In 1929 adenylic acid (identical to adenosine) was identified [45], and in the same year, the adenosine triphosphate (ATP) molecule was discovered and isolated [46]. Ten years later (1939), researchers contributed to the understanding of intracellular ATP as an intracellular energy transport molecule [47–50]. In 1948 and in 1959, it was reported that extracellular ATP has a different function than ATP within the cytoplasm [51, 52]. The authors showed that extracellular ATP molecules have an intercellular signalling function. The intercellular signalling by nucleotides (ATP, ADP, UTP and UDP) and nucleoside (adenosine) is referred to as purinergic signalling. The purinergic co-transmission in neurons was discovered by Geoffrey Burnstock in 1972 [53]. It took over 20 years for the importance of purinergic signalling to be accepted [54, 55]. Finally, researchers of the University of Ferrara first reported that the P2Z receptor (the former name of the P2X7R) plays an intriguing role in immunity, inflammation and cell death [56].

The intracellular levels of ATP are high at millimolar concentrations (2–8 mM) [57], and the ATP concentrations in synaptic vesicles are even higher in the range of 5 to 100 mM [58]. In contrast, under normal resting conditions, the extracellular levels of ATP are quite low at nanomolar concentrations (<3 nM) [57, 59]. Under specific conditions, ATP release can rise by more than 1000-fold [53, 57, 60, 61] and leads to a significant increase in the extracellular levels of ATP. The resulting significant increase in extracellular nucleotides and adenosine concentrations activates their purinergic receptors inducing certain cellular functions. Examples of such conditions are membrane depolarisation (i.e. sympathetic neuron endings) [53], mechanical stress (i.e. high mechanical power ventilation) [59–63], hypoxia [64], hyperosmosis, hypotonic and isotonic stress of endothelial cells [65–68], inflammation [69, 70], surfactant release by alveolar epithelial type II cells [59–61], mucine release by airway smooth muscle cells [71], insulin release by pancreatic islet beta-cells [72, 73], etc. There is an exception to this concept: Although a spontaneous ATP-induced inward Ca^{2+} current through the P2X7R could not be detected below extracellular ATP levels of 200 μmol/ml [74], low tonic basal activation of P2X7R at nanomolar extracellular ATP concentrations promotes serum independent cellular proliferation [75], promotes closure of the wound area in scratch wound assay [76], protects from apoptosis [77], initiates anaerobic glycolysis independent of the oxygen contents [78], etc. (Table 2, rows 80–85). However, low tonic basal activation of the P2X7Rs by extracellular ATP does not cause a pro-inflammatory response of the immune system. Therefore, this topic is beyond the scope of this paper and will not be discussed here.

Clearance of the ATP molecule in order to avoid accumulation in the extracellular space is performed by enzymes attached to the outside of the cell membranes (ecto-enzymes)
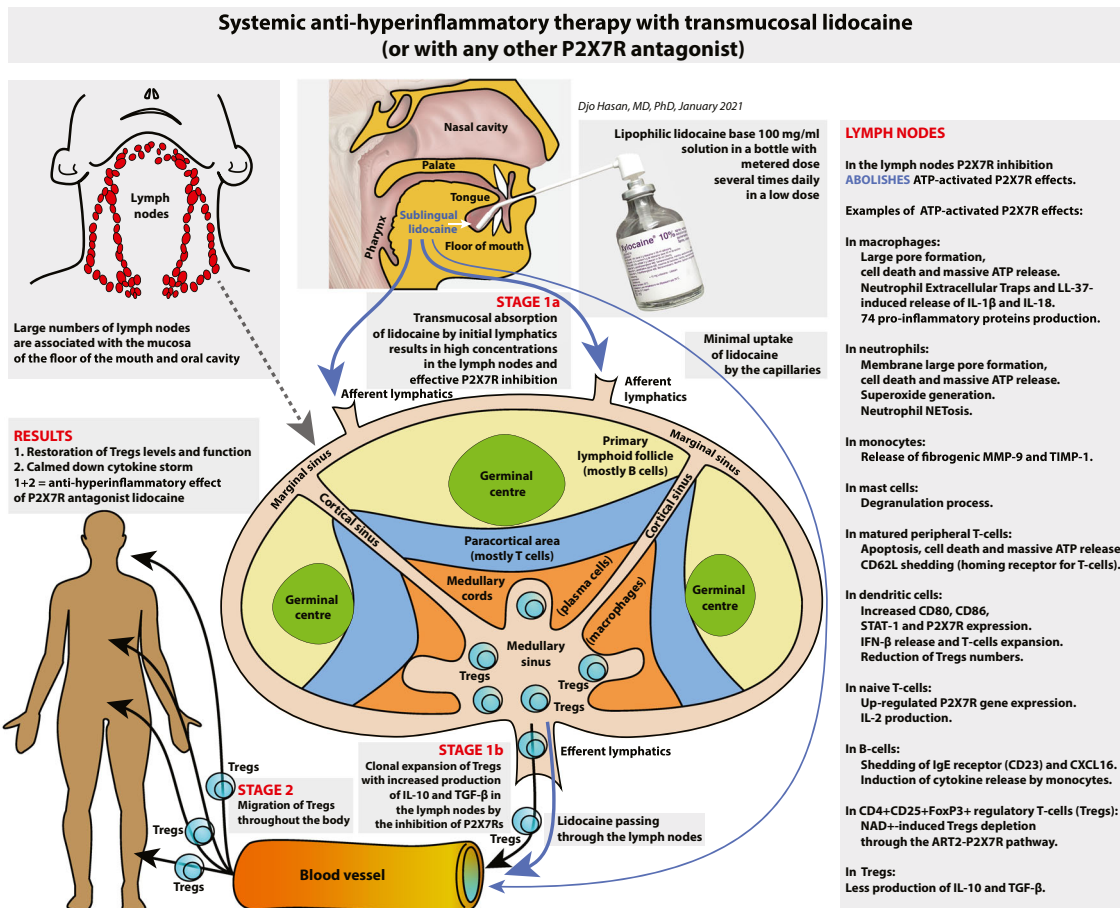
Fig. 1 A graphical summary of the future development of the administration of lipophilic lidocaine base in the sublingual region or elsewhere in the oral cavity. We postulate that selective inhibition of the P2X7Rs of the immune cells of the lymphatic system by lidocaine suppresses hyperinflammation in two stages. Stage 1: The selective inhibition of the P2X7Rs of the immune cells residing in the lymph nodes (stage 1a) induces clonal expansion of Tregs with improved function in these lymph nodes (stage 1b); Stage 2: Subsequently, these Tregs migrate throughout the body exerting anti-inflammatory activities reducing systemic and (distant) local hyperinflammation. See text under the heading "Future development" for explanation

and by soluble enzymes excreted to the extracellular space (Fig. 2) [57, 272–275]. A proportion of the enzymatic-breakdown product of ATP adenosine enters the cells via the equilibrative nucleoside transporters (ENT1 and ENT2) and concentrative nucleoside transporters (CNT1 and CNT2) (Fig. 2) [57, 60, 61]. The release and subsequently clearance of the extracellular nucleotides and adenosine cause fluctuation in the extracellular levels of ATP, other nucleotides and adenosine. These fluctuations in extracellular concentrations are indispensable for the receptor resensitisation after desensitisation following receptor activation as discussed below.

Purinergic signalling in inflammation and hyperinflammation

The purinergic control of cellular processes including the pro-inflammatory and anti-inflammatory responses of the immune system is depending on the activation and the desensitisation

phenomenon of the nucleotides and adenosine receptors of the immune cells [74, 276–282]. Except for the P2X7R, all other purinergic receptors, i.e. P2XRs, P2YRs and P1 receptors (adenosine receptors—AdoRs), are subject to desensitisation [279–283]. In addition, a certain extent of desensitisation occurs after every activation, and this desensitisation requires time to return to the state of complete resensitisation [279, 280]. The higher and the longer the stimulus of the activation, the higher the extent of desensitisation and the longer the recovery time to the state of complete resensitisation [278]. One of the P2 receptors, the P2X7 receptor, is not prone to desensitisation, and apart from the low tonic basal activation of this receptor at low nanomolar concentrations as mentioned above, the extracellular concentration of ATP required to activate this receptor is much higher. Activation of the P2X7R starts at 100 µM with an EC_{50} of >1 mM [74, 279, 284].

Summary of the effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors is presented in Table 2, rows 1–

Table 2 Summary of the effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors. *AdoR* adenosine receptor; *TNF-α* tumour necrosis factor alpha; *FcγR* receptors belonging to the immunoglobulin superfamily; *IFN-γ* interferon gamma; *IFN-β* interferon beta; *MAC-1* macrophage-1 antigen comprised CD11b (integrin αM) and CD18 (integrin β2); *CpG-A* oligodeoxynucleotides; *PARP* Poly ADP ribose polymerase; *FMLP* N-Formylmethionyl-leucyl-phenylalanine, a chemotactic factor; *COX-2* cytochrome C oxidase polypeptide II; *PGE2* prostaglandin E2; *MIP-1α* macrophage inflammatory protein 1 alpha (MIP-1α = CCL3 chemokine ligand 3), MIP-1β (CCL4), MIP-2α (CXCL2 chemokine CXC motif ligand 2) and MIP-3α(CCL20);*RANTES* (Regulated on Activation, Normal T cell Expressed and Secreted, CCL5); *LTB4* Leukotriene B4; *LTA4* Leukotriene A4; *VCAM-1* vascular cell adhesion molecule 1 (CD106);*ICAM-1* intercellular adhesion molecule 1 (CD54);*HMGB-1* high-mobility group box 1 (belongs to danger-associated molecular patterns); *MCP-1* monocyte chemoattractant protein 1 (CCL2);*FoxP3* Forkhead box P3; *CTL* cytotoxic T lymphocyte; *Th* T helper cell; *CTLA-4 cytotoxic T-lymphocyte-associated protein 4 (CD152); CD39* nucleoside triphosphate diphosphohydrolase 1 (NTPD1);*CD735'*-nucleotidase (5'-NT); *VEGF* vascular endothelial growth factor; *IDO*Indoleamine-pyrrole 2,3-dioxygenase; *α-SMA* alpha smooth muscle actin; *CTGF* connective tissue growth factor (CCN2);*bFGF* basic fibroblast growth factor; *TCR*T-cell receptor; *NFAT* nuclear factor of activated T cells; *NLRP3*Nod-like receptor family pyrin domain containing 3 gene; *ART2-P2X7 pathway* extracellular NAD+-induced ATP-independent p2X7R activation involving ADP-ribosyltransferase 2; *MMP-9* matrix metalloproteinase-9;*TIMP-1* tissue inhibitor of metalloproteinase 1; *LC-MS/MS* liquid chromatography and tandem mass spectrometry; *STAT-1* signal transducer and activator of transcription 1. Updated table, source: Hasan D, et al. (2017) [60] with permission

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
1	AdoRA1	Adenosine	Neutrophils	Promotes chemotaxis	[79, 80]
2			Neutrophils	Increases adherence to endothelial cells	[81]
3			Neutrophils	Inhibits TNF-α release	[82]
4			Neutrophils	At low concentrations adenosine enhances FcγR phagocytosis and actin dynamics	[83–85]
5			Neutrophils	Restores LPS-inhibited chemotaxis	[86]
6			Resting DCs (rDCs)	Inhibits vesicular MHC class I cross-presentation	[87]
7			Plasmacytoid DCs (pDCs)	Potent chemoattractants, reduces IL-6, IL-12 and IFN-γ release	[88]
8	AdoRA1 and AdoR-A2A		CD39^high B-cells (Bregs)	Promotes expansion and function of CD39^high B-cells	[89, 90]
9	AdorA2A	Adenosine	Monocytes	Inhibits IL-12 and TNF-α release	[91, 92]
10			Neutrophils	Promotes chemotaxis	[80]
11			Neutrophils	Inhibits oxygen radical generation	[79]
12			Neutrophils	Inhibits upregulation of beta2 integrins or MAC-1 (CD11/CD18) and shedding of L-selectin by FMLP	[93, 94]
13			Neutrophils	Promotes Cox-2 and PGE2 release	[95]
14			Neutrophils	Decreases adherence to endothelial cells	[81]
15			Neutrophils	Decreases adherence to fibrinogen coated surfaces	[96]
16			Neutrophils	Inhibits TNF-α release and chemokines MIP-1α (CCL3), MIP-1β (CCL4), MIP-2α(CXCL2) and MIP-3α (CCL20)	[82, 97]
17			Neutrophils	At high concentrations adenosine inhibits FcγR functions and actin dynamics	[83–85]
18			Neutrophils	Inhibits leukotriene (LTB4, LTA4) synthesis	[98–102]
19			Neutrophils	Inhibits degranulation and superoxide release or oxidative burst	[96, 103–106]
20			Neutrophils	Delays neutrophil apoptosis	[107]
21			Neutrophils	Inhibits autophagy suppressed apoptosis of neutrophils by blocking caspase-8, caspase-3 and PARP signalling	[108]
22			Mast cells	Increases IL-1β, IL-3 and IL-8 release	[109]
23			Macrophages	Inhibits LPS-induced TNF-α release	[110]
24			Endothelial cells		[111]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
				Reduces thrombin-induced permeability. Inhibits thrombin-mediated expression of VCAM-1, ICAM-1 an E-selectin. Inhibits thrombin induced increase of IL-6, HMGB-1; chemokines, MCP-1 (CCL-2), CXCL-1 and CXCL-3	
25			Naïve T-cells	Promotes the differentiation towards CD4$^+$FoxP3$^+$Lag3$^+$ Tregs, inhibits Th1 and Th17 differentiation, inhibits IL-6 secretion and increases TGF-β secretion	[112]
26			Th1, Th2 and Th17cells	Reduces release of IL-2, IL-4, TNF-α and IFN-γ	[113–115]
27			CD8$^+$CTLs, Th1, Th2	Reduces release of IL-2, TNF-α, IFN-γ. Inhibits CD8$^+$CTL and Th1 expansion to alloantigens	[116]
28			CD4$^+$ T-cells	Inhibits TCR-mediated IFN-γ release	[117]
29			CD4$^+$CD25$^+$FoxP3$^+$ Tregs	Increases number of Tregs and increases the expression of CTLA-4 receptor	[118]
30			CD4$^+$CD25$^+$FoxP3$^+$ Tregs	Upregulates ecto-enzymes CD39 and CD73 expression accelerating adenosine generation from extracellular ATP	[118]
31			AdoRA2A-knockout mice	Bleomycin-induced fibrosis is more severe and elevated TGF-β is higher than in wild-type mice	[119]
32			Human leukaemia monocytic cell line THP-1 cells	TNF-α upregulates the expression of AdoRA2A followed by the increase of the expression of CD163 and TGF-β1	[120]
33			Human CD4+ CD25+ CD127low/− Tregs and CD8+ T-cells	Tregs from gastric cancer patients hydrolyse ATP into adenosine. Adenosine synthesised by Tregs promotes apoptosis and suppresses proliferation of CD8+ T-cells. Tregs reduces CD8+ T-cell activity by promoting cAMP synthesis. Tregs Inhibit the immune function of CD8+ T-cells through A2aR pathway	[121]
34	AdoRA2A and AdorA2-B	Adenosine	Macrophages	Differentiation of monocytes towards M2 macrophages with VEGF and IL-10 release	[122–126]
35			Macrophages	Inhibits LPS-induced IL-6, MIP-2 and TNF-α release	[127, 128]
36	AdoRA2B	Adenosine	Neutrophils	Inhibits neutrophil recruitment and transmigration, release of TNF-α, IL-6, MIF-1α and IL-8	[129, 130]
37			Neutrophils	Inhibits superoxide generation	[131]
38			Neutrophils	Inhibits TNF-α release	[82]
39			Mast cells	Stimulates degranulation (mice), IL-13, IL-4 (Th2 cytokines)	[109]
40			Macrophages	Stimulates IL-10 release	[132]
41			DCs	Differentiation and maturation towards regulatory DCs: High level expression of angiogenic (VEGF), wound healing (IL-6), chemokine (IL-8), immune suppressing (IL-10) and tolerogenic (IDO) factors	[133]
42			DCs	Promotes Th17 differentiation via stimulation of IL-6 release	[134]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
43			Bone marrow cells	Promotes differentiation towards CD11c$^+$Gr-1$^+$ DCs that promotes Th17 response	[135]
44			Myeloid cells in systemic bleomycin-induced pulmonary fibrosis	Myeloid cells AdorA2B knock out mice show a reduction in CD206 and arginase-1 (markers for M2 macrophages). 10-fold reduction in IL-6 and 5-fold reduction in hyaluronan (both linked to pulmonary fibrosis)	[136]
45			Mast cells	Upregulates the IL-4 and IL-13 release	[109]
46			B-cells	Induces Ig-E release through IL-4 and IL-13 release by the adenosine-activated mast cells	[109]
47			Endothelial cells	Reduces endothelial permeability, ICAM-1, P-selectin and E-selectin (adhesion molecules)	[137]
48			Endothelial cells	Stimulates basic fibroblast growth factor (bFGF) and insulin-like factor-1 release	[138]
49			Bronchial epithelial cells	Increases IL-19 release	[139]
50			Human leukaemia monocytic cell line THP-1 cells	Increases TNF-α release through mast cell-released IL-19	[139]
51			Renal fibroblasts	Increases the expression of α-SMA, IL-6, TGF-β, CTGF and fibronectin (pro-fibrotic mediators)	[140]
52			AdoR2B knock-out mice	Negligible effect on bleomycin-induced acute lung injury. Enhanced loss of barrier function	[141]
53			AdorR2B knock-out mice exposed to systemic bleomycin	Substantial reduction of fibrosis and IL-6 production	[141]
54			Specific pathogen-free male Sprague-Dawley rats	Inhibition of AdoRA2B: Attenuates necrotizing enterocolitis in newborn rats and protects against body weight loss, decreases myeloperoxidase activity, decreases TNF-α, IFN-γ and IL-6 intestinal levels and increases IL-10 intestinal levels	[142]
55			RAW 264.7 murine macrophage cells with and without transfection with AdoRA2B siRNA cultured with B. abortus 544 biovar 1 strain (ATCC 23448)	Blocking of Adora2b using siRNA induces productions of IL-6, MCP-1 and TNF-α in cells without infection. Adora2b siRNA macrophages have reduced uptake of *B. abortus*. Inhibition of AdoRA2B results in higher total weight of the spleens and less Brucella colonisation in this organ, decreases IL-10, elevates the levels of IFN-γ and IL-12 at three days p.i. and elevates the levels of IL-6, TNF-α and IL-12 at 14 days p.i.	[143]
56	AdoRA2B and AdoR-A3	Adenosine	Mast cells	Stimulates IL-8(chemokine) and VEGF (angiogenic) release	[144]
57			Peritoneal macrophages from wild type, AdoRA2A knockout and AdoRA3 knockout FVB or C57BL/6 male mice	Simultaneous adenosine AdoRA2B and AdoR3 signalling is required to promote chemotactic migration of macrophages towards the apoptotic cells	[145]
58	AdoRA3	Adenosine	Neutrophils	Synergistic AdorA3 and P2Y2R neutrophil chemotaxis through autocrine ATP release by pannexin-1, extracellular	[146–152]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
				conversion of ATP to adenosine by the ecto-enzymes (CD39 and CD73), strategic translocation of the FPR, AdorA3, P2Y2, pannexin-1 receptors and CD39, Cd73 to the leading edge of the neutrophils. This results in the amplification of the chemoattractant gradient sensing and the self-generated gradients	
59			Microglial cells and colonic epithelial cells	Suppresses LPS-induced TNF-α production	[153, 154]
60			Anti-CD3-activated CD8+ CTLs	Reduces the expression of mRNAs coding for granzyme B, perforin, Fas ligand and TNF-related apoptosis-inducing ligand (TRAIL). Diminishes Nalpha-CBZ-L-lysine thiobenzylester esterase activity (enzyme with cytotoxic activity). Reduces IL-2 sand IFN-γ release.	[155]
61			Microglia BV-2 cell line	Reduces elevated hydrostatic pressure-induced inducible nitric oxide synthase (iNOS) expression, microglia migration and phagocytosis in BV-2 cells	[156]
62			AdoRA3 knock-out mice exposed to intratracheal bleomycin	Increase in eosinophil numbers and selective upregulation of eosinophil-related chemokines and cytokines. But decreased eosinophil peroxidase activity in the BALF	[157]
63			Human colonic mucosa biopsies	Significantly decreases TNF-α and IL-1β production and attenuates the NF-κBp65 activation	[158]
64	P2X1R	ATP	Neutrophils and platelets	Promotes thrombosis and fibrinogenesis: Keeps circulating neutrophils in quiescent state, recruit neutrophil to the injury site, activate adhered neutrophils and platelets	[159]
65			Bovine polymorphonuclear leukocytes (PMNs)	Oleic acid (OA) and linoleic acid (LA) induce Neutrophil Extracellular Traps (NETs) formation and ATP release via PANX1 and activation of P2X1	[160]
66	P2X1R, P2X4R and P2X7	ATP	Naïve T-cells	TCR stimulation results in the translocation of pannexin-1 hemichannels, P2X1Rs and P2X4Rs to the immune synapse. While the P2X7Rs remain uniformly distributed. This process is required to induce calcium entry, NFAT and release of IL-2	[161]
67	P2X3R	ATP	Mast cells	Increases the expression of IL-4, IL-6, IFN-γ, TNF-α, RANTES and M IP-2. Increases the release of IL-6 and IL-13	Article retracted due to figure irregularities [162]
68	P2X4R	ATP	γδ T-cells	Activates and upregulates TNF-α and IFN-γ release	[163]
69			Microglial cells	Promotes survival after LPS-activation	[164]
70			CD4+T-cells from Human peripheral blood mononuclear cells (PMBCs)	Chemokine stromal-derived factor-1α (SDF-1α) triggered mitochondrial ATP	[165]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
				production, rapid bursts of ATP release and increased migration of primary human CD4+ T cells. This process depended on pannexin-1 ATP release channels and autocrine stimulation of P2X4Rs. SDF-1α stimulation caused localised accumulation of mitochondria with P2X4Rs near the front of cells, resulting in a feed-forward signalling mechanism that promotes cellular Ca2+ influx and sustains mitochondrial ATP synthesis at levels needed for pseudopod protrusion, T cell polarisation and cell migration	
71			Chinese hamster ovary (CHO) cells transfected with human Kv1.3 cDNA and rat P2X4 construct	The voltage-gated potassium channel Kv1.3 is required for microglia activation . Inhibition of Kv1.3 channels completely nullified the ability of Kv1.3 to normalise membrane potential changes, resulting in excessive depolarisation and reduced calcium transients through P2X4 receptors	[166]
72	P2X4R and P2Y11R	ATP	CD4+T-cells from Human peripheral blood mononuclear cells (PMBCs), Jurkat T cells (clone E6-1) and U-937 cells	P2Y11Rs retract from the immune synapse (IS) towards the back of cells where their stimulation by extracellular ATP induces cAMP/PKA signalling that redirects mitochondrial trafficking to the IS. P2Y11Rs thus reinforce IS signalling by promoting the aggregation of mitochondria with panx1 ATP release channels and P2X4 receptors at the IS. This dual purinergic signalling mechanism involving P2X4Rs and P2Y11Rs focuses mitochondrial metabolism to the IS where localised ATP production sustains synaptic activity in order to allow successful completion of T cell activation responses	[167]
73			CD4+ T-cells from Human peripheral blood mononuclear cells (PMBCs)	Autocrine P2X4R and simultaneous P2Y11R activation regulate mitochondrial metabolism, T-cell polarisation, pseudopod formation and redistribution of P2Y11Rs to the back of polarised T-cells resulting in T-cell trafficking. Exogenous activation of P2Y11R blocks T-cell trafficking	[168]
74	P2X4R and/or P2X7R	ATP	Neutrophils, monocytes, macrophages, DCs, CD4+ T-cells, CD8+ T-cells, iNKTs, adenoviral infected macrophages and alveolar epithelial cells	Mediates NLRP3 inflammasome-dependent IL-1β and IL-18 secretion (signal 2, non-classical pathway), increases IL-6 production	[169–176]
75	P2X5R		C57BL/6J mice: wild type, P2X5R knockout, P2X7R knockout and P2X5R/P2X7R knockout and their bone marrow-derived macrophages (BMMs)	P2X5R-deficient BMMs exhibit defective cytosolic killing of L. monocytogenes P2X5R is required for L. monocytogenes-induced inflammasome activation and IL-1β production and that defective L. monocytogenes killing in P2X5R-deficient BMMs is substantially rescued by exogenous IL-1β or IL-18. The P2X5-dependent anti-L.	[177]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
				monocytogenes immunity is independent of the ATP-P2X7 inflammasome activation pathway	
76	P2X7R	Unactivated state in the absence of ATP	Macrophages and P2X7R-transfected HEK-293 cells	P2X7 is a scavenger receptor for apoptotic cells in the absence of i ts ligand ATP	[178, 179]
77		ATP release channel	Alveolar epithelial type I cells (AT I cells), mice osteoclast cells, murine neuroblastoma cells, astrocytic cell line, mice astrocytes, B16 melanoma cells	Release ATP after mechanical deformation (AT I cells), spontaneously (osteoblast cells), after activation (neuroblastoma cells, astrocytic cell line), after γ irradiation (melanoma cells)	[180–185]
78		P2X7R-mediated ATP release	Mouse 3T3fibroblasts	P2X7R-mediated ATP secretion is accompanied by depletion of cytosolic ATP	[186]
79			Bone marrow–derived dendritic cells from WT mice and Panx1−/− C57BL/6 mice	Upon stimulation of the P2X7 receptor by ATP, Panx1 contributed to fast DC motility by increasing the permeability of the plasma membrane, which resulted in supplementary ATP release	[187]
80		ATP, low tonic basal activation	HET293 and HELA cells	Elevates mitochondrial calcium and potential, cellular ATP levels and promotes serum-independent growth. This process requires a full pore-forming function	[75]
81			In-vitro scratch wound assay with HaCat cells (human skin keratinocytes)	Medium hyaluronan fragment (MMW-HA, between 100 and 300 kD) increases tight junction ZO-1 protein expression and induces a low activation of P2X7 receptor resulting in improved closure of the wound area. This is accompanied by pore formation as shown by Yo-Pro-1 cellular uptake. The P2X7R antagonist brilliant blue G (BBG) completely inhibits this process	[76]
82			HEK293 and NIH3T3 cells	Increases the Ca2+ content of the endoplasmic reticulum, activates NFATc1 and protects from apoptosis	[77]
83			PC3 cells LNCaP, Kelly, RPMI-8226, DU145 and SK-MEL-5 cells	Drives the expression of nfP2X7, a key mediator of cell survival	[188]
84			Osteoclast-like cells	Promotes the increase in the extracellular adenosine concentrations	[189]
85			HEK293 cells	The initiation of anaerobic glycolysis independent of the oxygen content: Upregulates glucose transporter Glut1 (thus enhances intracellular glycogen stores); Upregulates glycolytic enzymes (PFK, G3PDH, PKM2), phosphorylated Akt/PKB and hypoxia-inducible factor 1a (HIF-1a) expression. Impedes the Krebs cycle independent of the oxygen concentrations: Promotes pyruvate dehydrogenase kinase 1 (PDHK1) and inhibits of pyruvate dehydrogenase (PDH, conversion of pyruvate to acetyl-coA)	[78]
86		ATP >1 mM, vigorous activation	C57BL/6 mice	P2X7 activation inhibits the suppressive potential and stability of Tregs. In contrast, P2X7R inhibition promotes the conversion of the cell-autonomous	[190]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
				conversion CD4+ T cells into Tregs after stimulation of their T-cell receptors (TCRs)	
87			C57BL/6 wild type and P2X7 knockout mice	P2X7 knock-out mice show an increase of CD90/CD45RB^{low} FoxP3$^+$ Tregs in colon lamina propria, prevents Tregs death in mesenteric lymph nodes and these Tregs produce more IL-10. Colitis is prevented or reduced and P2X7 knock-out mice. Treg cells lacking the P2X7 receptor have higher levels of integrin CD103	[191]
88			C57BL/6 mice	P2X7R activation reduces the frequency of Tregs and P2X7R inhibition increases the expansion of Tregs	[192]
89			C57BL/6 wild type, P2X7 knockout mice and foetal thymus organ culture	Selectively increases immature γδ+CD25+ cells which are much more competent to release ATP than pre-TCR expressing cells following TCR stimulation and Ca2+ influx. Genetic ablation as well as pharmacological antagonism of P2X7 results in impaired ERK phosphorylation, reduction of early growth response (Egr) transcripts induction, diversion of γδTCR-expressing thymocytes towards the αβ lineage fate and increased representation of the Id3-independent NK1.1-expressing γδ T-cell subset in the periphery	[193]
90			C57BL/6J mice implanted with melanoma B16F10 cells	P2X7 activation in tumour infiltrating CD8+ lymphocytes (TILs) promotes cell cycle arrest and p38 MAPK mediated cellular senescence in the tumour microenvironment	[194]
91			BAC1.2F5 macrophage cell line	P2X7 receptor-dependent blebbing and the activation of Rho-effector kinases, caspases and IL-1μβ release	[195]
92			Dendritic cells cultured from mice bone marrow precursor cells	Autocrine-mediated (pannexin-1 channels) fast migration of dendritic cells through the reorganisation of the actin cytoskeleton	[187]
93			RAW 264.7 murine macrophages	mediates actin reorganisation and membrane blebbing via p38 MAP kinase and Rho	[196]
94			Monocytes	Induces MMP-9 and TIMP-1 release, fibrosis markers	[197]
95			M1 macrophages	Induces release of 74 pro-inflammatory proteins detected by antibody protein array and 33 inflammatory proteins detected by LC-MS/MS	[198]
96			M2 macrophages	Induces release of 21 anti-inflammatory proteins detected by LC-MS/MS	[198]
97			Macrophages	Enhances intracellular bacterial killing	[199]
98			Macrophages and P2X7R-transfected HEK-293 cells	Mediates rapid uptake of beads and bacteria in the absence of serum after ATP activation	
99			Mast cells	Induces degranulation	[200]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
100			Naïve NKTs	Facilitates NAD$^+$-induced inhibitory signal through the ART2-P2X7 pathway resulting in non-functional NKTs	[201]
101			Activated NKTs	Facilitates NAD$^+$-induced stimulatory signal through the ART2-P2X7 pathway resulting in functional NKTs with increased IFN-γ and IL-4 release	[201]
102			B cells	Induces shedding of IgE receptor (CD23) and CXCL16. Soluble CD23 sustains growth of B-cell precursors, promotes B and T cell differentiation and drives cytokine release from monocytes. CXCL16 is a chemoattractant for lymphocytes	[202, 203]
103			CD11c$^+$CD103$^+$ DCs	Mediates infection-induced rapid recruitment of CD11c$^+$CD103$^+$ DC subsets into the epithelial layer of the gut	[204]
104			Naïve T-cells	TCR stimulation triggers rapid release of ATP and upregulates P2X7 gene expression. Autocrine ATP stimulation through the P2X7R is required to for the TCR-mediated calcium influx, NFAT activation and IL-2 production	[205]
105			T follicular B helper cells (Tfh cells)	Reduces and thus controls the number of Tfh cells in Peyer's patches in the gut with high-affinity IgA responses to promote host-microbiota mutualism	[206]
106			CD4$^+$CD25$^+$FoxP3$^+$ regulatory T-cells (Tregs)	Facilitates NAD$^+$-induced Tregs depletion through the ART2-P2X7 pathway	[207]
107			DCs	Increases CD80, CD 86, STAT-1 and P2X7R expression, IFN-β release and T-cells expansion. Reduces Tregs numbers	[208]
108			AT I cells	Induces VCAM-1 shedding and neutrophil transmigration in acute lung injury	[209]
109			Human endometrial mesenchymal stem cells, murine luteal cells	Causes cell cycle arrest in G0/G1 phase and suppresses cell replication	[210, 211]
110			Brain-derived type-2 astrocyte cell, mesangial cells	Stimulates TGF-β mRNA expression	[212, 213]
111					
112			Sprague-Dawley rats with and without spinal cord injury	After spinal cord injury P2X7R of microglia was upregulated by BzATP and down-regulated by P2X7R antagonist A-438079. Upregulation of P2X7R on microglia coincides with increase of neuroinflammation after spinal cord injury. P2X7R of microglia participates in spinal cord-mediated neuroinflammation via regulating NLRP3 inflammasome-dependent inflammation	[214]
113			Abdominal cells of male Kunming mice of clean grade	Transfection of the long non-coding siRNA uc.48+ decreases the upregulated mRNA and protein levels of the P2X7 receptor in diabetes mellitus type 2 mice model	[215]
114			Human embryonic kidney cells (HEK293T)	Promotes paxillin and NLRP3 migration from the cytosol to the plasma membrane and facilitates P2X7R-paxillin interaction and	[216]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
				PaxillinNLRP3 association, resulting in the formation of the P2X7R-Paxillin-NLRP3 complex. Paxillin is essential for ATP-induced NLRP3 inflammasome activation in mouse bone marrow-derived macrophages and bone marrow-derived dendritic cells (PMDCs) as well as in human PBMCs and THP-1-differentiated macrophages	
115			P2Y1R knockout, P2Y12R knockout, P2Y13R knockout, P2X7R knockout, NLRP3 knockout and wild type C57BL/6 mice	Aggravates inflammatory bowel disease through ERK5-mediated tyrosine phosphorylation of the adaptor protein ASC essential for NLRP3 inflammasome activation and the secretion of IL-1β	[217]
116			C57BL/6 mice: Wild-type, P2X7 knockout, NLRP3 knockout and caspase-1/11 knockout	Induces the release of extracellular vesicles containing CD14. Extracellular CD14 induced during sepsis controls bacterial dissemination and cytokine secretion	[218]
117			C57BL/6 J mice and their peritoneal macrophages, immortalised human liver stellate cell line LX-2 and immortalised human leukaemia monocytic cell line THP-1 cells	Blockade of P2X7R reverses TAA-induced liver fibrosis thioacetamide and attenuates thioacetamide-induced inflammatory response by inhibiting NLRP3 and NF-κB activation in mice liver. P2X7R overexpression significantly enhances TGF-β-increasedα-SMA and collagen I protein and mRNA level in LX-2 cells. Macrophages increase fibrogenesis in LX-2 HSCs through the release of IL-1β by P2x7R stimulation	[219]
118			Macrophages derived from human leukaemia monocytic cell line THP-1 cells cultured with T. pallidum with and without P2X7R gene siRNA-transfection	T. pallidum increases both the mRNA and protein levels of P2X7R, increases levels of NLRP3 mRNA expression and IL-1β. SiRNA transfection of the macrophages reduces the percentage of spirochete-positive macrophages and spirochete internalisation	[220]
119			Human and mice macrophages	Enhances the Neutrophil Extracellular Traps (NETs) and LL-37 formation (an antibacterial protein externalised on NETs) activated caspase-1, the central enzyme of the inflammasome, in both human and murine macrophages, resulting in release of active IL-1β and IL-18. LL-37 activation of the NLRP3 Inflammasome utilises P2X7R-mediated potassium efflux. IL-18 can stimulate NETosis (NET activation and release) in human neutrophils	[221]
120		ATP >1 mM, prolonged vigorous activation	Macrophage, HeLa cells, 1321-N1 astrocytes and HEK293 cells	Induces pannexin-1 mediates large pore formation and IL-1β release	[222]
121			Human neutrophils and HL-60 cells	Mediates membrane large pore formation and superoxide generation	[223]
122			Matured peripheral T-cells	High dose ATP promotes apoptosis, cell death and CD62L shedding (homing	[224–226]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
				receptor for central T-cells) independent from the NAD$^+$-induced ART2-P2X7 pathway	
123			J774 cells and HEK cells expressing the P2X7 receptor	Promotes the formation of pores permeable to very large ions leading to cytolysis	[227]
124	P2X7R and P2Y13R		Human mast cell line HMC-1 and rat basophilic leukaemia cell line (RBL-2H3) with and without transfection of P2Y13-siRNAs and P2X7-siRNAs	P2Y13R mediates nanomolar mechanical-induced ATP release. P2X7R mediates micromolar mechanical-induced ATP release	[228]
125	P2Y1R and P2Y12R	ADP>ATP	Platelets	P2Y1R and P2Y12R synergistic action in thrombin-induced platelet activation	[229]
126			Platelets	C-activation of P2Y1R and P2Y12R by ADP upregulates the expression of P-selectin (CD62P)	[230]
127	P2Y2R	UTP≥ATP	Neutrophils	Synergistic AdorA3 and P2Y2R neutrophil chemotaxis (see under AdoRA3 above)	[146, 147]
128			Neutrophils and fibroblasts	Mediates recruitment of neutrophils into the lungs, proliferation and migration of lung fibroblasts and IL-6 production	[231]
129			Monocyte-derived DCs (moDCs), eosinophils	Promotes chemotaxis	[232]
130			Eosinophils	Induces VCAM-1 expression	[233]
131			Peritoneal macrophages (RPMs) isolated from resting C57/BL6 mice	P2Y2R-Induced c-Jun N-terminal kinase (JNK) activation is responsible for Increased in IL-1β production	[234]
132			Murine model of cutaneous leishmaniasis	Induces CASP-1 activation and IL-1β secretion during *L. amazonensis* infection. IL-1β/IL-1R signalling is crucial for P2Y2R-mediated protective immune response in an experimental model of cutaneous leishmaniasis	[235]
133			ChAT^{BAC}-eGFP mice	Elicits tracheal brush cells generation of cysteinyl leukotrienes. Aeroallergens elicit P2Y2-dependent tracheal brush cells cysteinyl leukotrienes generation and tracheal brush cells -dependent airway eosinophilia	[236]
134	P2Y4R and P2Y12	UTP≥ATP, ADP>ATP, respectively	Microglial cells	P2Y4R and P2Y12R synergistic action increases microglial chemotaxis	[237, 238]
135	P2Y6R	UDP>UTP>>ATP	Neutrophils	Induces neutrophil activation and extracellular trap formation	[239]
136			Human leukaemia monocytic cell line THP-1 cells	Induces IL-1β release	[240]
137			Macrophages	Induces MCP-3(CCL7) expression in response to necrotic tissue cells	[241]
138			Microglial cells	Facilitates phagocytosis	[242]
139			Microglial cells	Induces the expression of MCP-1 (CCL-2)	[243]
140			Microglial cells	Promotes phagocytosis	[244, 245]
141			Basophils	UDP promotes IgE-dependent degranulation	[246]
142			Plasmacytoid DCs	UDP and UTP strongly inhibit IFN-alpha secretion induced by influenza virus or CpG-A	[247]
143			Tissue cells	Induces IL-1α, IL-8/CXCL8 and IL-6 release	[240, 248, 249]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
144			Tissue cells	Induces IFN-β release	[250]
145			Wild-type C57BL/6 mice and their DCs	Inhibits the maturation and activation of DCs via suppressing the activation of the transcription factor NF-κB. In-vitro studies show that P2Y6 signalling inhibits the production of IL-12 and IL-23 and the polarisation of Th1 and Th17 subsets mediated by DCs. Mice lacking P2Y6 develop more severe experimental autoimmune encephalomyelitis compared with wild-type mice	[251]
146			Institute of Cancer Research (ICR) mice, primary microglial cells and neurons from Sprague Dawley rat	Transient middle cerebral artery occlusion (tMCAO) increases P2Y6R expression. P2Y6 receptor-specific inhibitor blocked the phagocytosis of primary microglia under LPS and UDP stimulation. P2Y6 receptor-specific inhibitor down-regulates myosin light-chain kinase (MLCK) required for the cytoskeletal remodelling for the formation of the phagocytic cup. Inhibition of P2Y6R does not reduce the tMCAO-induced upregulation of mRNA levels of IL-1α, IL-1β, IL-6, IL-10, TNF-α and TGF-β	[252]
147	P2Y11R	ATP	Neutrophils	Inhibits neutrophil apoptosis	[253]
148			Neutrophils	Enhances chemotactic response	[254]
149			Neutrophils and moDCs	Induces maturation of the granulocytic progenitors and monocyte differentiation	[255, 256]
150			moDCs	Inhibits migratory capacity	[257]
151			moDCs	Induces IL-8 release	[258]
152			Monocytes	Autocrine differentiation towards M1 macrophages, induces IL-1β, IL-6, IL-12 and TNF-α release	[259]
153	P2Y12R	ADP>ATP	Monocytes	Increases monocyte adhesion	[260]
154			Vascular smooth muscle cells	Upregulates MCP-1 (CCL-2)	[260]
155			DCs	Increases antigen endocytosis with subsequent enhancement of specific T-cell activation	[261]
156			Microglial cells	Induces movement of juxta-vascular microglial processes to close the injured blood-brain barrier (BBB) and microglial activation	[262, 263]
157			Microglial cells	Promotes migratory, inflammatory (TNF-α and IL-6 release) responses	[264]
158			Microglial cells	ADP treated microglial cells induces CCL3 expression in activated T-cells	[265]
159			Murine model of sepsis, caecal ligation and puncture (CLP). Co-cultures of human platelets and T-cells with or without anti-CD3/CD28	Blockade of the P2Y12 signalling pathway restrains Treg proliferation in vivo and in vitro	[266]
160			Male C57BL/6 mice microglial cells	Mediates microglial activation via Ras homolog family member A/Rho-associated protein kinase (RhoA/ROCK) pathways	[267]
161	P2Y13R	ADP>ATP	Red blood cells	Inhibits ATP release	[268]
162	P2Y14R	UDP>UDP-glucose	Neutrophils		[269, 270]

Table 2 (continued)

Effects of extracellular nucleotides and nucleoside on the innate and adaptive immune system through different purinergic receptors

Row number	Receptor	Ligand [52]	Immune cell expression or experimental model	Results of receptor signalling	Reference number
163			Sprague-Dawley Rats and human leukaemia monocytic cell line THP-1 cells	Enhances neutrophil chemotactic response through IL-8 dependent manner P2Y14R knockout reduces in-vivo and in-vitro monosodiu m urate-indu ced NLRP3 inflammasome activation, increased expressions of NLRP3, ASC, active Caspase-1 and downstream active IL-1β. Therefore, increases resistance to monosodium urate-induced acute gouty arthritis. Decreased AMP reverses the in-vivo and in-vitro protective effect of P2Y14R knockout	[271]

163. Coronaviruses can induce inflammation by the activation of the intracellular sensing molecules IRIG1/MDA5 [285, 286]. Reportedly, acute inflammation [69, 70] and infection with SARS-CoV-2 virus induce ATP release [287]. The vesicular exocytosis-mediated release of ATP, connexin-43 (Cx43)-mediated ATP release and pannexin-1 (Panx-1)-mediated ATP release can be triggered by the activation of Toll-like receptor 4 (TLR4) and TLR2 by pathogen-associated molecular patterns (PAMPs) and by the activation of P2X7Rs [180–182, 187]. In turn, activation of the P2X7Rs upregulates the protein expression of TLR 2, TLR3, TLR4 and TLR 5 [288]. Additionally, increased levels of TNF-α during inflammation induce ATP release via Panx-1 [289]. Pro-inflammatory immune response is initiated by the increase in the extracellular ATP, ADP and adenosine levels in the microenvironment of immune cells activating the P2XRs, P2YRs and AdoRs (Fig. 3) [57, 60, 169, 290]. In this case, ATP acts as a danger-associated molecular pattern (DAMP) [291, 292]. Increased ADP levels promote platelet activation and intravascular thrombosis (Table 2, rows 125 and 126). Reportedly, the pathological changes in the lung in patients with COVID-19 pneumonia showed marked microvascular thrombosis [293]. The EC_{50} for AdoRs is in the range of 26 nM to 1.4 μM [281] and for ATP, UTP or ADP receptors (P2XRs and P2YRs with the exception of the P2X7R) in the range of 0.01 nM to 10 μM [284, 294]. Obviously, the extent of the cellular ATP release is proportional to the severity of the infection. A severe infection with SARS-CoV-2 causes massive extracellular ATP release by the infected cells. This may be confined to the airway mucosa and the lung or may be extensive in multiple organs. Although increased extracellular ATP concentrations upregulate the expression of ecto-nucleotidases [295] these high ATP concentrations

exceed the capacity of these ecto-enzymes (CD39, CD73, etc.) to clear the extracellular space from ATP molecules [60] ending in ATP concentrations of >1 mM. This is demonstrated in a report where the authors show that TLR-mediated CD39 internalisation (causing the deactivation of the ecto-enzyme CD39) in mice bone marrow-derived dendritic cells (BMDCs) leads to the accumulation of extracellular ATP to 1.4 mM [296]. The activation of P2X7Rs in ongoing inflammation is the hallmark of severe pro-inflammatory immune response (Table 2, rows 74, 86–119 and Fig. 3) [297] including COVID-19 [298]. If these levels of extracellular ATP are accompanied by the absence of the required fluctuations for other purinergic receptor to recover from desensitisation, all P1 and P2 (other than P2X7) purinergic receptors will become fully desensitised demarcating the initiation of hyperinflammation (Fig.3 and Table 2, rows 120–123) [279–283].

Hyperinflammation is characterised by the activation of P2X7Rs and desensitisation of other P2 receptors and AdoRs

As mentioned above, hyperinflammation starts when fluctuation of the extracellular nucleotides and adenosine no longer occurs and leads to prolonged activation of the P2X7Rs of the immune cells. Prolonged vigorous activation of the P2X7Rs leads to macropore formation and cytolysis with uncontrolled ATP release [222, 223, 227, 299] (Table 2, rows 120–123) causing hyperinflammation with massive pro-inflammatory immune response, massive pro-inflammatory and anti-inflammatory cytokine release: the cytokine storm (Fig. 3). In the early phase of COVID-19, hyperinflammation may be confined to the site of viral entry (i.e. airway mucosa and conjunctivae) but as viral replication and viral spreading progress, systemic hyperinflammation devel ops.
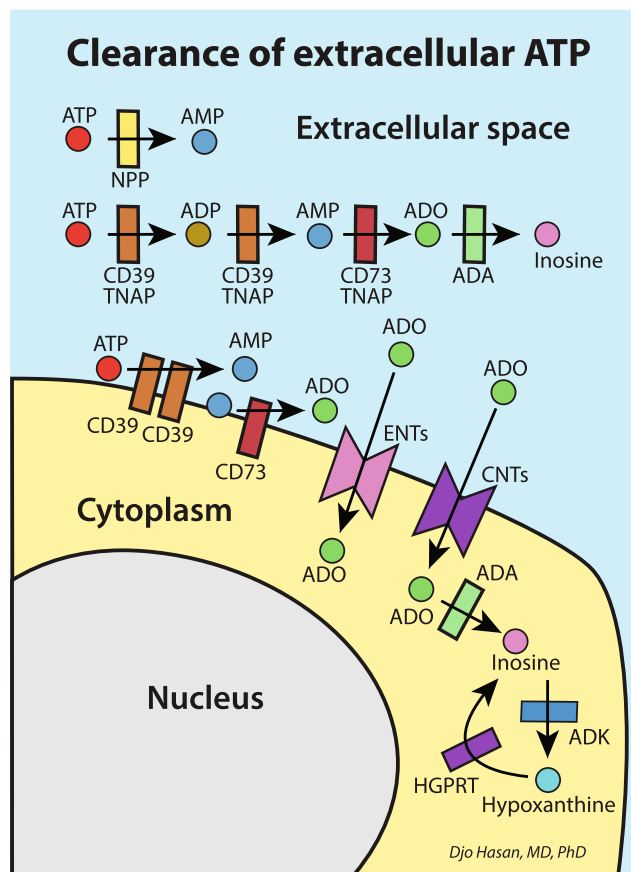
Fig. 2 Clearance of *extracellular* ATP and adenosine by ectonucleotidases and soluble extracellular nucleotidases [272–274275]. This process is indispensable to enable receptors to recover from desensitisation following receptor activation (resensitisation, see text under the heading "Purinergic signalling in inflammation and hyperinflammation" for explanation). CD39,Ecto-nucleoside triphosphate diphosphohydrolase 1-3 (ENTPD 1-3); CD73, Ecto-5′-nucleotidase (5′-NT); NPP, *n*ucleotide pyrophosphatase/phosphodiesterase; TNAP, *t*issue *n*onspecific *a*lkaline *p*hosphatase; ADA, *a*denosine deaminase; ADK, adenosine kinase; HGPRT, *h*ypoxanthine-*g*uanine phosphoribosyltransferase; ATP, *a*denosine triphosphate; ADP, *a*denosine diphosphate; AMP, adenosine monophosphate; ADO, adenosine; ENTs, *e*quilibrative nucleoside transporters; CNTs, *c*oncentrative nucleoside transporters

The upregulation of the expression of ectonucleotidases also leads to an increase in the concentrations of other nucleotides (i.e. ADP) and adenosine. These high extracellular concentrations of nucleotides and adenosine do not show concentration fluctuations required for the recovery (resensitisation) time from desensitisation causing a state of persistent desensitisation of all P2XRs, P2YRs [279, 280, 283, 300, 301] and AdoRs [282] with the exception of P2X7Rs. Consequently, the physiological function in the affected organs and inflamma tory response of the immune system are deactivated. This leads to the failure of organ function (i.e. ARDS in the lungs as we reported earlier [61]) and the immune system (immune paralysis) rendering the host susceptible to secondary co-infections(Fig. 3). Sepsis-induced

immunosuppression [302, 303] or compensatory anti-inflammatory response syndrome (CARS) in critically ill patients [304] was already raised by researchers in 1996 [305] and is a well-known phenomenon in critically ill patients [302]. Secondary bacterial infections occurred in 34.4% of 274 surviving elderly patients (age over 60 years) with COVID-19 and in 81.7% of 65 deceased patients [15]. In addition, it was found that 76 co-infections with other respiratory pathogens occurred in another cohort of 354 COVID-19 patients (16 of 115 mild cases (13.9%), 33 of 155 severe cases (21.3%) and 27 in 84 critical cases (32%)) [16]. In a meta-analysis involving 118 scientific reports on patients with COVID-19, co-infection with other pathogens at admission was observed in 19% and superinfection with other pathogens during admission in the hospital in 24% [306].

Control of hyperinflammation is annihilated by the downregulation of Tregs through the activation of P2X7R and the desensitisation of adenosine receptors

Tregs are key elements in the control of hyperinflammation [307]. Activation of AdoRA2As promotes the differentiation of naïve T-cells towards regulatory T-cells(Tregs) [112], increases the frequency of Tregs and the expression of CTLA-4 receptor and upregulates ecto-enzymes CD39 and CD73 expression accelerating adenosine generation from extracellular ATP [118] (Table 2, rows 25, 29, 30 and 33). This process is upset in case of desensitisation of AdoRs. In addition, activation of P2X7Rs inhibits the suppressive potential and stability of Tregs, inhibits the clonal expansion of Tregs, promotes Treg death, induces Treg depletion and reduces Treg IL-10 production (Table 2, rows 86–88, 106 and 107). In COVID-19 patients, significant lower Treg frequencies [308–310], lower expression of forkhead box protein P3 (FoxP3), lower expression of transforming growth factor-β(TGF-β) and lower cytokine TGF-β secretion [309] are observed compared to healthy control. Additionally, a reduced proportion of specific SARS-CoV-2-reactive Tregs was reported [311]. The desensitisation of AdoRs and the activation of P2X7Rs may well be the underlying mechanism of the low Tregs frequency in severe and critically ill COVID-19.

P2X7R antagonist restores the reduced Tregs population and Tregs function in hyperinflammation

As stated above, infected cells release ATP into the extracellular space. Obviously, the P2X7R antagonist blocks the activation of the P2X7Rs. Because a significant proportion of the ATP release to the extracellular space is mediated by the P2X7R (Table 2, rows 77–79), P2X7R a ntagonism combined with the upregulated ATP hydrolysing activity of the ecto-enzymes results in the decrease of the extracellular ATP concentrations. This can potentially abrogate hyperinflammation
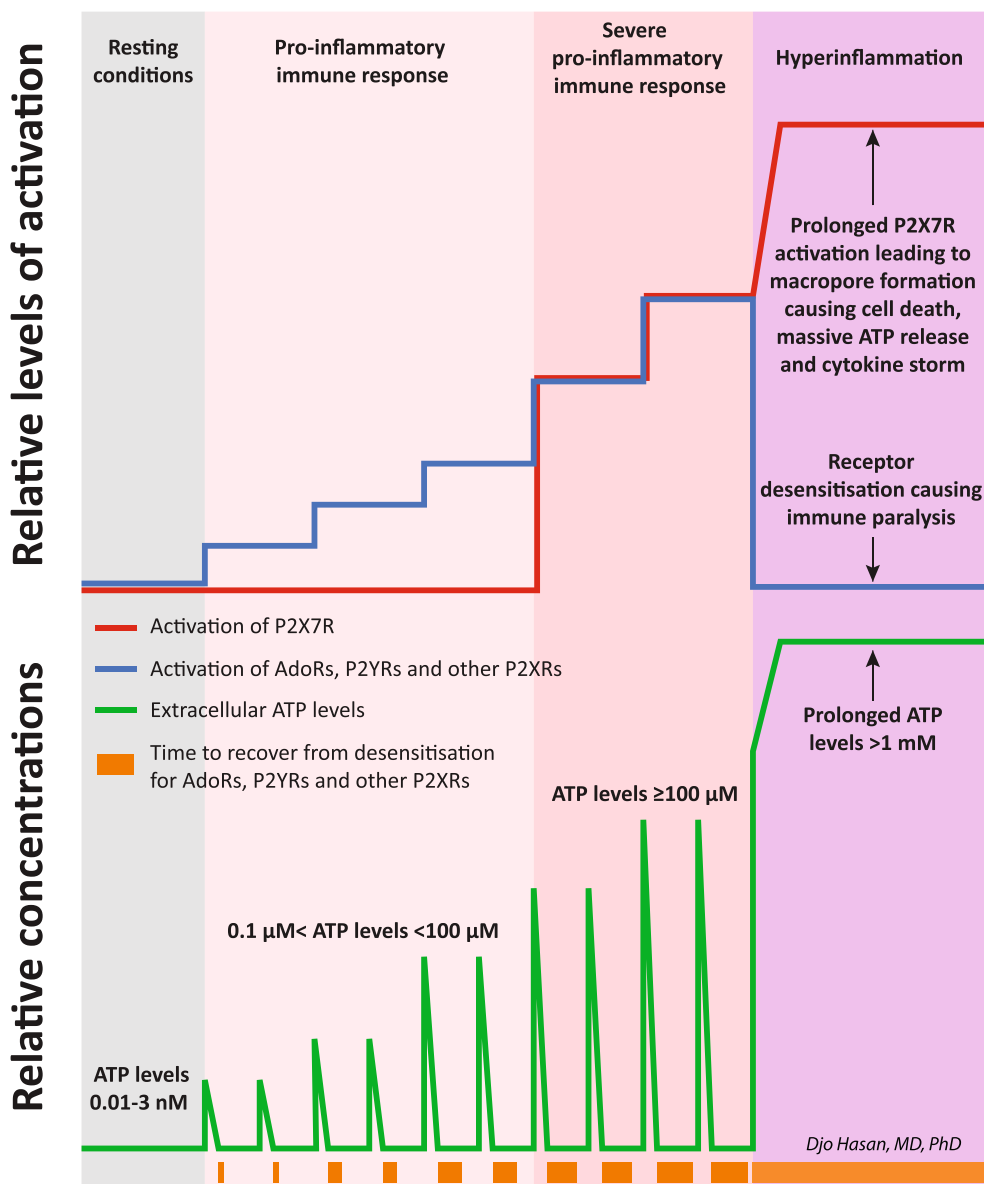
Fig. 3 A schematic presentation of the activation of the purinergic receptors of the immune cells causing a pro-inflammatory response leading to hyperinflammation. Viral infection drives the controlled cellular release of ATP molecules. Increased extracellular nucleotides levels activate P2XRs and P2YRs. Upregulation of the extracellular ATP hydrolysing enzymes as depicted in Fig. 2 results in the increase of extracellular adenosine levels followed by the activation of the adenosine receptors (AdoRs). These processes initiate the physiologic pro-inflammatory response of the immune system. The green line at the bottom of the graph represents the extracellular ATP levels. The ascending part is caused by the ATP release, and the descending part results from the clearance of ATP by the extracellular or membrane-bound ATP hydrolysing enzymes. As the disease progresses and extracellular ATP levels increase above 1 mM, the P2X7R is additionally and effectively activated leading to a severe immune response. Except for P2X7Rs, all these receptors are known to be subject to desensitisation. Desensitisation of a receptor is defined as being unresponsive to activation by the ligand, resulting in (near) zero transmembrane signal transduction. A certain extent of desensitisation occurs after every activation, and this desensitisation requires time to return to the state of complete resensitisation. Increasing intensity and duration of the activation stimuli leads to increasing extent of desensitisation and duration of the recovery time to the state of complete resensitisation (represented by brown boxes with increasing size at the bottom of the graph). Severe viral infection can increase the controlled ATP release beyond the capacity of the extracellular enzymes to clear ATP and adenosine molecules. This causes a sustained high extracellular ATP and adenosine levels preventing the purinergic receptors from recovering from the state of desensitisation. The capacity to clear invading microorganisms diminishes leading to immune paralysis. In addition, prolonged high extracellular levels of ATP and activation of the P2X7R lead to macropore formation and cell death with uncontrolled release of ATP. In turn, this leads to vigorous activation of the P2X7R of the immune cells promoting massive production of cytokines ending in a cytokine storm and hyperinflammation

and the concomitant immune paralysis. Moreover, P2X7R inhibition promotes the cell-autonomous conversion of CD4+ T cells into Tregs after stimulation of their T-cell receptors (TCRs) [190]. In addition, P2X7R knock-out mice, mimicking the state of complete P2X7R inhibition, show an increase in tissue Tregs, prevent Tregs death and the Tregs produce more IL-10 and TGF-β [191]. Experimental inhibition of P2X7Rs restores the Tregs levels and function (Table 2, rows 86–88, 106 and 107) [190–192]. Inhibition of the P2X7R or P2X7R knock-out can attenuate severe inflammation in abdominal sepsis [312] and in acute lung injury [313, 314]. Apparently, amelioration of hyperinflammation by P2X7R inhibition is based on the increased activation and clonal expansion of the anti-inflammatory Tregs population (Table 2, rows 86–88, 106 and 107).

Some authors proposed that the P2X7R is an ideal candidate to target in COVID-19-associated severe pneumonia [298, 315], and others suggested that hyperactivation of the P2X7R plays a key role in the neuropathology of COVID-19 and that P2X7R antagonism may prevent or treat neurological manifestations of COVID-19 [316].

Lidocaine is a P2X7R antagonist

In 2015 it was discovered that lidocaine is a P2X7R antagonist [74], and therefore, lidocaine can potentially reduce the clinical symptoms of hyperinflammation significantly. In experimental sepsis, lidocaine improves organ failure [317–319] and survival [317]. In septic patients, lidocaine reduces neutrophil recruitment by the mitigation of chemokine-induced arrest and transepithelial neutrophil migration [320]. Neutrophil recruitment is an important facilitating process in the pathogenesis of multiple organ failure [320] and hyperinflammation in COVID-19 [321–324]. In patients with skin lesions from atopic dermatitis, lidocaine increases the proportion of Tregs and upregulates the FoxP3 expression [325]. In addition, lidocaine increases the IL-10 levels in mechanically ventilated mice [326] and decreases the TNF-α in BAL, plasma and lung samples in pigs undergoing surgery for lung resection [327].

The P2X7R antagonist dose-response relationship of lidocaine is presented in Fig. 4. The IC_{50} for the inhibition of the P2X7R by lidocaine is about 66.07 μg/ml (0.28 mM) [74] where IC_{50} is defined as the required extracellular concentrations of the receptor antagonist to reach an inhibitory effect of halfway between maximal activation and maximal inhibition (half-maximal inhibitory concentration). The main issue is that the IC_{50} for P2X7R inhibition is much higher than the maximal tolerable plasma concentration for mammals. The maximal tolerable plasma concentration in humans is about 4.7 μg/ml (0.02 mM); this corresponds with an IC_{10} or lower (<10% inhibitory concentration, Fig. 4). Above this lidocaine plasma concentration, adverse effects in increasing severity occur as presented in Table 3 [328, 329]. Thus, systemic lidocaine plasma concentrations of >4.7 μg/ml must be avoided [328, 329]. Caveat: The inhibitory concentrations of lidocaine for P2X7R as presented in Fig. 4 are not corrected for the series resistance (in the range of 1–3 MΩ) of the used whole-cell voltage clamp method with two puller microelectrodes [74]. One should bear in mind that after correction for series resistance, the reported inhibitory concentration values including IC_{50} are expected to be higher [330].

In addition to the P2X7R antagonist properties, lidocaine is also known to have several other inhibitory pharmacological targets: the voltage-gated sodium channels (VGSC: Nav1.2 [331], Nav1.3 [332], Nav1.4[333], Nav1.5 [334], Nav1.7 [335], 1.8 [336] and Nav 1.9 [337]), the Toll-like receptor 2 (TLR 2) [338], TLR4 [318] and the N-methyl-D-aspartate receptor (NMDAR) [339].

VGSCs conduct sodium ions inward and are essential for the transduction of sensory stimuli, the generation of the action potential and the release of neurotransmitters from sensory neuron terminals. Lidocaine inhibition of VGSCs can effectively reduce pain signalling [340]. In addition, VGSCs are present on dendritic cells (maintain chemokine-induced migration) [341], macrophages (regulate phagocytosis and endosomal pH during LPS-mediated endosomal acidification) [342], microglia (regulate phagocytosis cytokine release ad migration) [343], neutrophils (regulate attachment,

Table 3 Adverse effects in relation to the plasma concentrations of lidocaine. The maximal tolerable plasma levels for human are about 4.7 μg/ml (0.02 mmol/L). Serious adverse effects start at 9.84 μg/ml (0.042 mmol/L). Source: Hermanns H et al. [328] and Weinberg L et al. [329]

| Symptoms of toxic plasma levels of lidocaine	Lidocaine concentration		
	mmol/L	μmol/L	μg/ml
No noticeable symptoms	<0.020	<20	<4.69
Anxiety, dizziness	0.020	20	4.69
Decreased spinal reflexes	0.042	42	9.84
Central nervous system (confusion, diplopia, nausea and vomiting, twitching and tremors, seizures with reduced consciousness, respiratory depression, coma, etc.)	0.080	80	18.74
Cardiac toxicity (bradycardia, hypotension, cardiovascular depression, cardiac arrest, etc.)	0.130	130	30.46
Cytotoxicity	3.0	3000	702.9
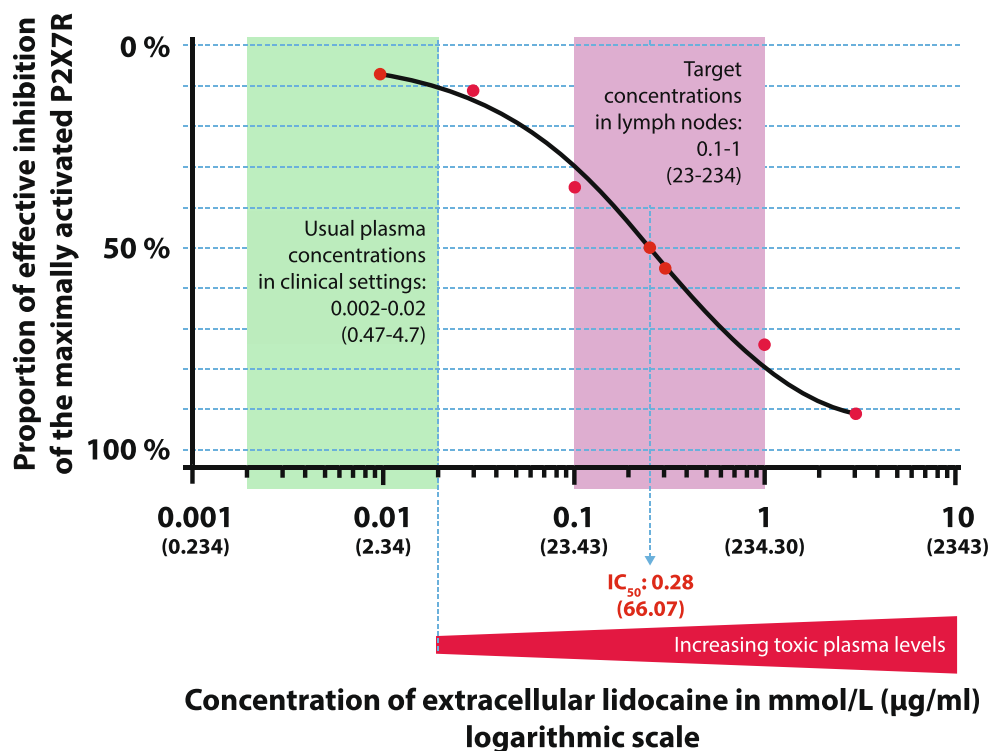
Fig. 4 Dose-response relationship of lidocaine suppressing the ATP-induced currents in oocytes expressing P2X7R. We reconstructed the fitted curve from the inhibitory concentrations data of lidocaine for P2X7R from the original article: 7% inhibition: 0.01 mM (2.34 μg/ml); 11% inhibition: 0.03 mM (7.03 μg/ml); 35% inhibition: 0.10 mM (23.43 μg/ml); 50% inhibition (IC_{50}): 0.28 mM (66.07 μg/ml); 55% inhibition: 0.30 mM (70.29 μg/ml); 74% inhibition: 1.00 mM (234.30 μg/ml); 91% inhibition: 3.00 mM (702.90 μg/ml); and 98% inhibition: 10.00 mM (2343.00 μg/ml), respectively. The usual plasma concentrations in clinical settings are indicated by the green box, and the targeted concentrations in the lymph nodes are indicated by the magenta box. Note that the maximal tolerable plasma levels for human (about 4.7 μg/ml–0.02 mmol/L) are much lower than the required extracellular concentrations of lidocaine to effectively inhibit the P2X7R. Source: Okura D, et al. [74]

transmigration and chemotaxis) [344] and T-cells (regulate positive selection of $CD4^+$ T cells) [345]. However, until date no relevant data have been published suggesting that other VGSC antagonists (such as HYP-17 [346], A-803467 [347, 348], PF-05089771 [349], phenytoin [350] or tetrodotoxin [351, 352]) may substitute non-steroidal anti-inflammatory drugs let alone may suppress COVID-19-related hyperinflammation [353]. A plausible reason is that during hyperinflammation—including hyperinflammation in COVID-19—the cytokine levels (i.e. IL-1β [354], IL-6, IL-10 [355, 356] and IL-12 [357]) are high. Reportedly, IL-1β [358] and IL-6 [359] inhibit sodium currents of VGSCs, and IL-10 downregulates the expression of VCSCs [360]. Moreover, activation of the P2X7R reduced the density and currents of VGSCs [361]. Therefore, we do not consider the inhibitory properties of lidocaine on VGSCs to be relevant for the treatment of hyperinflammation in COVID-19.

At first glance, the downregulation of the expression of TLR 2 [338] and TLR 4 [318] is an important anti-inflammatory mechanism directly induced by lidocaine. But at a closer look, it appeared that activation of P2X7R by the agonist cathelicidin (LL-37) leads to the upregulation of the protein expression of TLR2, TLR3, TLR4 and TLR 5 [288].

This is in line with the MyD88 (myeloid differentiation primary-response protein 88)-dependent activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) following the activation of the P2X7R by BzATP [362, 363]. The MyD88-dependent activation of NF-κB is part of the TLR4/NF-κB pathway. Therefore, it is unsurprising that the inhibition of P2X7R by its antagonists (Brilliant Blue G, A-438079 and A-740003) neutralises the above-mentioned P2X7R-induced upregulation of TLRs [362]. Consequently, we argue that lidocaine inhibits inflammation directly by blocking P2X7Rs independent from the neutralisation of the P2X7R-induced upregulated TLR2 and TLR4.

The subpopulation of NMDA receptors present on the peripheral neurons are involved in nociception, and their number increases during inflammation contributing to the sensitisation of peripheral nerves to nociceptive stimuli. NMDA receptor antagonists have anaesthetic-like effects [364]. In addition, NMDA receptor antagonist can prevent hypoxic neuronal death, IL-1β and TNFα release [365], reduce the activation of inflammatory experimental colitis [366] and suppress glial pro-inflammatory cytokine expression [367]. Moreover, the NMDA receptor antagonist memantine can increase IL-10

production in BCR/CD40-activatedB-cells [368]. Lidocaine inhibits NMDA receptors [339, 369, 370], and thus the anti-inflammatory properties of lidocaine could be attributed to the inhibition of NMDA receptors. However, it has been reported that the anti-inflammatory effect in T-cell functions (inhibition of antigen-specific T-cell proliferation, T-cell cytotoxicity, T-cell migration towards chemokines and decrease in IL-2 and IFN-γ production by Th1 effector cells in favour of IL-10 and IL-13 production by Th2 cells) of the NMDA receptor antagonist ifenprodil is effective both in wild-type and in NMDA receptor (GluN1) knockout mice [371]. Moreover, it was found that KN-62, an inhibitor of Ca2+/calmodulin-dependent kinase type II and a potent P2X7R antagonist, provides neuroprotection against NMDA-induced cell death [372]. Therefore, we argue that the anti-inflammatory properties of NMDA receptor antagonists (including lidocaine) should be attributed to the inhibition of P2X7Rs rather than to the inhibition of NMDA receptors.

Selective inhibition of the P2X7Rs of the immune cells in the lymphatics avoids exceeding the maximal tolerable plasma concentration of lidocaine and inhibits hyperinflammation in two stages

As mentioned above, the main issue is that the IC_{50} for P2X7R inhibition is much higher than the maximal tolerable plasma concentration for mammals because P2X7Rs are indispensable for normal physiological functions (i.e. in the central nervous system [373], the peripheral nervous system [374] and in the lungs [60, 61]). Therefore, intravenous or oral administration aimed at achieving an effective concentration of lidocaine to inhibit P2X7Rs in serum and in target organs will hamper organ functions and is potentially dangerous.

The lymphatic system is populated exclusively by trafficking immune cells, i.e. naïve T cells, activated T cells, B cells [375], dendritic cells [376], monocytes [377], macrophages [378], neutrophils [379], mast cells [380], eosinophils [381] and basophils [382]. We postulate that selective inhibition of the P2X7Rs of the immune cells of the lymphatic system by lidocaine suppresses hyperinflammation in two stages: stage 1, the selective inhibition of the P2X7Rs of the immune cells residing in the lymph nodes induces clonal expansion of Tregs in these lymph nodes; stage 2, subsequently, these Tregs migrate throughout the body exerting anti-inflammatory activities reducing systemic and (distant) local hyperinflammation (Fig. 1).

The endothelium of the dermal capillaries of the skin belongs to the structural type "continuous endothelium" [383]. Although capillary walls can transport substances from blood to tissue, the absorption of substances from tissue to blood is, if any, extremely low [384]. Apparently, specialised initial lymphatics harbouring one-way valve leaflets capable of absorbing fluid and molecules from the interstitium are localised in the dermis. The absorbed lymph fluid is then propelled forward in the lymphatic network by collecting lymphatic vessels harbouring a rhythmic contracting muscle layer [385]. This system brings fluids and particles into the lymph nodes where numerous immune processes take place. The administration route to target the lymphatic system in a domestic swine model is illustrated by the subcutaneous or intradermal injection of compounds (isosulfan blue, fluorescein and radioactive technetium-99 isotope—Tc^{99}) and by tracing the extent and the transit time of the distribution of these compounds using whole body scintigraphy in pigs [386]. The absorption of intradermal application of radioactive Tc^{99} into the lymph nodes is 10 times faster than after deep subcutaneous application and leads to higher concentrations in the lymph nodes related to these lymphatic vessels [386]. Radionuclide lymphoscintigraphy with molecules of different sizes after intradermal and subcutaneous injections showed that smaller particles (i.e. 99mTc-dextran and 99mTc-human serum albumin) migrate more rapidly towards the lymphatic vessels and lymphatic nodes than larger particles (i.e. radiocolloids of larger molecular size) [387]. The rate of clearance of ^{99m}Tc-pertechnetate and ^{99m}TcDTPA after subcutaneous and intradermal administration in the back of the hand in humans is 1 %/min and 8 to 10 %/min, respectively [387].

The additional advantage is that the plasma concentrations of subcutaneously administered lidocaine are much lower than intravenously administered lidocaine. Intravenous administration of 2 mg/kg lidocaine in cats is almost immediately followed by a peak plasma concentration of 3.6 μg/mL [388]. In contrast, the achieved mean peak plasma concentrations after the subcutaneous administration of 30 mg/kg, 20 mg/kg and 10 mg/kg lidocaine are much lower: 1.69, 1.07 and 0.77 μg/mL, respectively [389]. Note that the applied subcutaneous dose [389] is 15, 10 and 5 times higher than the intravenous dose, respectively [388, 389]. Reportedly, the difference in the plasma concentrations after intravenous and subcutaneous administration of lidocaine is caused by the fact that, in contrast to the intravenous administration, a large proportion of the subcutaneously administered lidocaine is drained into the lymphatic system [390–392]. Obviously, this slows down the release of lidocaine to the venous blood. This is confirmed for bevacizumab in mice [390], for trastuzumab in rats [391] and for docetaxel in rats by [392].

As stated above, lymphatic absorption after intradermal administration is much higher than after deep subcutaneous administration [386, 387]. Practically, the intradermal infusion with lidocaine is not an accepted administration route for lidocaine. Therefore, we argue that a subdermal administration of lidocaine using a catheter inserted just beneath the dermis (subdermal infusion, Fig. 5) will result in higher

concentrations of lidocaine in the draining local lymph nodes than a deep subcutaneous or intravenous infusion as depicted in the schematic presentation of the putative distribution of lidocaine in Fig. 6.

In summary, by means of the subdermal administration of lidocaine, we can ensure high concentrations of lidocaine in the local lymph nodes enabling an effective inhibition of the P2X7R of the immune cells while keeping the lidocaine plasma concentrations <4.7 µg/ml(stage 1a and 1b in Fig. 1). The induced Tregs clonal expansion in these local lymph nodes produces Tregs which migrate throughout the body controlling the ongoing hyperinflammation (stage 2 in Fig. 1). Obviously, the subdermal administration route may also apply to other P2X7R antagonists.

Three other P2X7R antagonists have been tested in human: CE-224,535 500 (Pfizer), AZD-9056(Astra-Zeneca) and JNJ-54175446 (Johnson and Johnson). A phase IIa study with CE-224,535 in patients with rheumatoid arthritis not responding adequately to methotrexate was recently reported [393]. Patients in the treatment arm received oral CE-224,535 500 mg twice/day for 12 weeks. Although the safety and tolerability for the compound were acceptable, CE-224,535 was not effective in this group of patients. The results of a phase II study with AZD-9056 in patients with active rheumatoid arthritis despite treatment with methotrexate or sulphasalazine was published. The treatment arm consists of oral AZD-9056 100 or 400 mg/day for 6 months [394]. The AZD-9056 used in this trial is non-lipophilic as indicated by the fact that this compound cannot penetrate the blood-brain barrier [395]. The authors conceded that "AZD-9056 does not have significant efficacy in the treatment of RA, and the P2X7 receptor does not appear to be a therapeutically useful target in RA" [394]. Recently, a randomised, placebo controlled, sequential-group, single-centre ascending dose phase I study was reported. The

patients in the 5 treatment arms received 0.5, 2.5, 10, 50, 150 and 300 mg JNJ-54175446, respectively. The authors reported dose-dependent plasma levels, no serious adverse events, ex vivo attenuation of lipopolysaccharide-induced IL-1β release in peripheral blood and confirmation of passive brain penetration of JNJ-54175446 [396]. The approach of the P2X7R antagonist therapy of the above-mentioned authors is quite different from ours: While these authors directly targeted the diseased organs via the gut absorption of the drug, we target the immune cells in local lymph nodes inducing an anti-inflammatory immune response which in turn targets the diseased organs (Fig. 1). This is illustrated by the following study concerning a placebo-controlled, multicentre, double-blind phase IIa study in patients with moderately to severely active Crohn's disease. The patients in the treatment arm received oral AZD-9056 200 mg/day for 28 days. The authors found a significant improvement in the Crohn's Disease Activity Index (CDAI) at day 28 [397]. In contrast to the skin, the endothelium of the mucosal capillaries of the mouth and the gastrointestinal tract are fenestrated allowing molecules to pass from the submucosal tissue into the capillaries [383]. Unlike the failure of the treatment of rheumatoid arthritis described above, the successful treatment of gut inflammation here can be attributed to the absorption of non-lipophilic oral AZD-9056 by the mucosa-associated lymphoid tissue (MALT). This is the inductive site of the mucosal immune system consisting of mesenteric lymph nodes, Peyer's patches and isolated lymph follicles [398, 399]. Although lymphatic transport to the lymph nodes of the non-lipophilic oral AZD-9056 is limited [400, 401], AZD-9056 inhibits P2X7Rs of the local T-cells via absorption by the inductive sites of MALT. This induces a local anti-inflammatory immune response executed by the effector sites of MALT consisting of lamina propria lymphocytes and intraepithelial lymphocytes [398, 399].

Fig. 5 *The cannula for subdermal infusion of lidocaine is superficially positioned just below the dermis* to promote the uptake of lidocaine by the initial lymphatics of the dermis and to avoid accumulation of lidocaine in the subcutaneous fat tissue
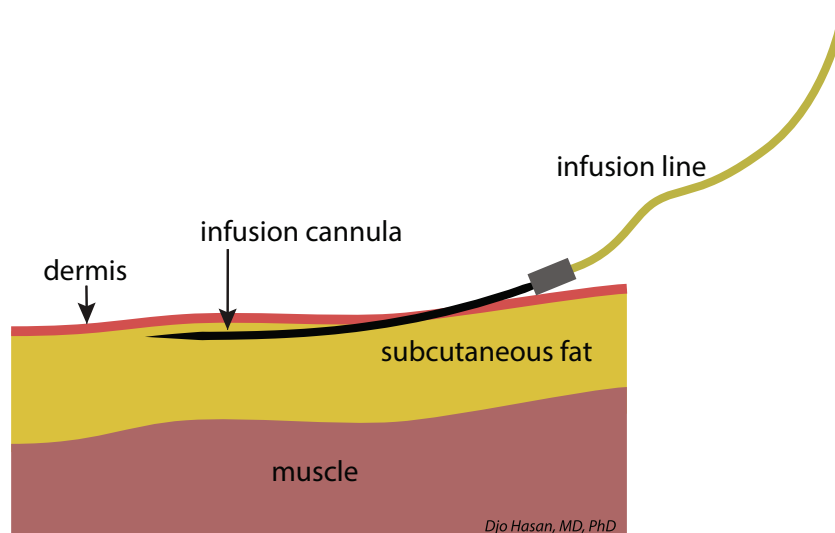
Schematic presentation of the putative distribution of lidocaine (or of any other P2X7R antagonist) shortly after administration
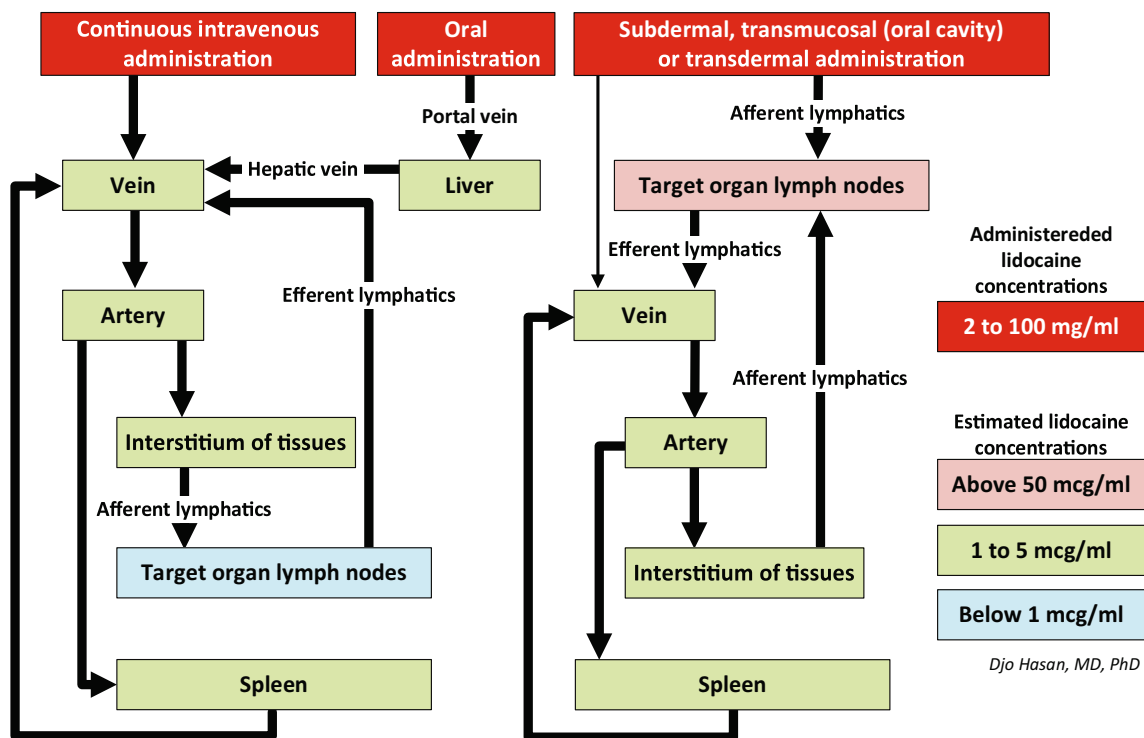

Fig. 6 *Schematic presentation of the putative distribution of intravenous*, oral, transmucosal (i.e. in the oral cavity) and subdermal administered lidocaine. Administration of hydrophilic lidocaine (lidocaine HCL) through a (central) venous catheter or by oral intake results in concentration gradients with the highest value in the venous blood and the lowest value in the lymph nodes. The reason is that by the time lidocaine reaches the lymph nodes, the drug is massively diluted and may never reach the effective concentration required to adequately inhibit the P2X7Rs of the immune system. In contrast, after subdermal injection of hydrophilic lidocaine, apart from a minimal absorption by the dermal capillaries, almost all the lidocaine is absorbed by the lymphatic system via the initial lymphatics. Because the fluid in the afferent collecting lymphatics originates from the interstitial fluid of the tissues, dilution of the concentration of lidocaine occurs. This fluid is then drained into the local lymph nodes. The extent of the dilution of lidocaine in the targeted lymph nodes is far less drastic compared to the (central) venous administration of the drug. We postulate that with continuous subdermal infusion, we can achieve concentrations of lidocaine in the lymph nodes sufficient to effectively inhibit the P2X7Rs of the immune cells. Theoretically, similar results may be expected from transmucosal and transdermal administration of lipophilic lidocaine base with a high concentration. Obviously, the subdermal, transmucosal and transdermal administration routes may also apply to other P2X7R antagonists

Real-world subdermal administration of lidocaine in critically ill COVID-19 patients

Six COVID-19-induced ARDS patients

From April 2020 until end of July 2020, two of the authors of this report (AS and TK) have successfully treated six critically ill patients with COVID-19 admitted to the ICU of the Showa University in Tokyo, Japan, with lidocaine. The lidocaine treatment was based on off-label use. The Medical Ethical Committee of the Showa University, School of Medicine, Tokyo, approved the collection, analysis and publication of patients on mechanical ventilation admitted to the ICU (protocol number 3313). The administration was initially intravenously in the two first patients, followed by subdermally (a superficially inserted subcutaneous catheter as illustrated in Fig. 5). In the other four patients, only the subdermal administration was further applied. The concentration of the intravenous lidocaine infusion solution is 20 mg/ml (2%), the route for continuous administration of lidocaine commonly used in daily practice. The dose for intravenous administration is 0.6 mg/kg/h as recommended earlier [402]. Due to the limited efficacy of intravenous lidocaine and based on the hypothesis of selectively targeting the inhibition of the P2X7Rs of the immune cells, the infusion in both patients was converted to subdermal infusion of 1.0 mg/kg/h (dosage as reported by Japanese researchers [403]) after 7 and 6 days, respectively. The time course of clinical parameters of these six patients

is presented in Figures 7, 8, 9, 10. In about 20% of the inserted subdermal cannulae, local subdermal indurations were observed. Whenever this occurred, the infusion cannula was removed and replaced with a new cannula at a different location.

The first patient (Fig.7), a 63-year-old male (75 kg, 168 cm), developed fever and nausea on March 27, 2020, and 3 days later, he started to cough and became dyspnoeic. After 5 days, the PCR SARS-Cov-2 test was positive, and he was admitted to the hospital with SARS-Cov-2-induced ARDS. Co-morbidities include COPD and smoking 60 cigarettes per day for more than 40 years. About 40 years earlier, the patient suffered from pneumothorax. On day 3, the patient deteriorated and was intubated and mechanically ventilated due to poor blood gases. No haemodynamic instability was

observed. The CT scan showed bilateral ground glass opacities compatible with ARDS. On day 5, the patient was transferred to the ICU of the university hospital because of further respiratory deterioration. The patient received favipiravir for 14 days after admission; the patient did not receive dexamethasone. Prone position mechanical ventilation was initiated due to the progression of the respiratory disease with an extremely low PaO_2/FiO_2 ratio of 63.3 mm Hg (severe ARDS according to the Berlin definition. The Berlin definition of ARDS includes severe PaO_2/FiO_2 ratio $\leq$100 mm Hg, moderate PaO_2/FiO_2 >100 to 200 mm Hg, mild PaO2/FiO2 >200 to 300 mm Hg, no ARDS PaO2/FiO2 >300 mm Hg [404]). The initial ventilator settings include APRV, P_{high}27 cm H_2O, T_{high} 7.0 s, P_{low} 0 cm H_2O and T_{low} 0.32 s. The
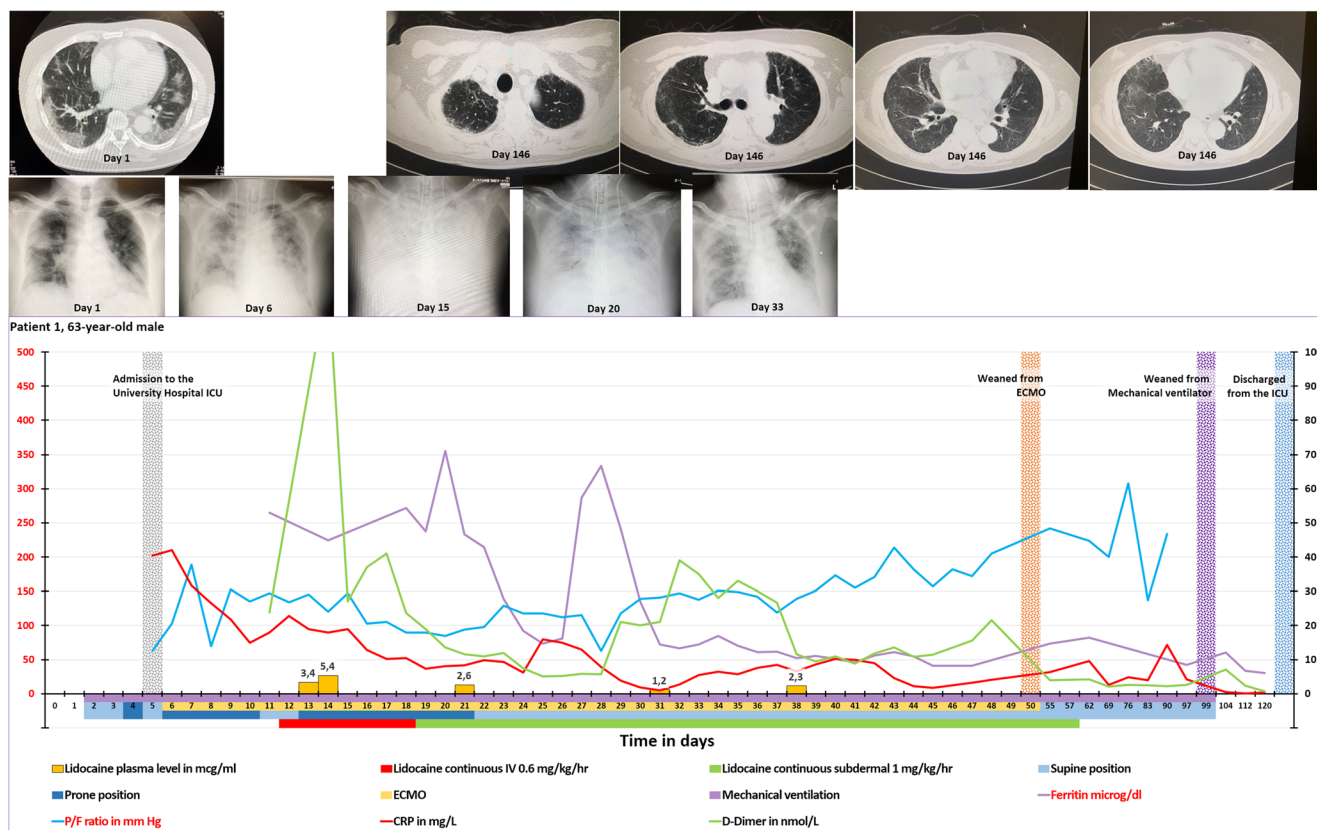

Fig. 7 Patient 1, the first of the six cases with severe COVID-19 treated with subdermal lidocaine in the ICU of the Showa University, Tokyo, Japan. A 63-year-old male with COVID-19-induced ARDS, was admitted to the hospital. The CT scan showed bilateral ground glass opacities. Co-morbidities: COPD, smoking 60 cigarettes per day for more than 40 years. About 40 years before admission, *the patient suffer*ed from pneumothorax. After admission the clinical condition deteriorated requiring an ICU admission and mechanical ventilation on day 4. On day 11, *continuous intravenous lidocaine of 0.6 mg/kg/h was initiated, but the patient's* condition kept worsening with high pulmonary artery pressures and reduced aeration of the lung. On day 19, *the continuous intravenous lidocaine of 0.6 mg/kg/h was changed to continuous subdermal lidocaine of 1 mg/kg/h. This was followed by improvement of the clinical condition, and on day 20, the aeration of the lung was improved, but the pulmonary artery pressures remain*ed high. Despite this the P/F ratio was gradually improving, *and ECMO weaning was done on day 50. No new ECG*

changes were observed during treatment with lidocaine. *Bloo*d metHb were within the normal range (0.3–0.8%). On day 99, *he was weaned from the mechanical ventilator and was discharged from the ICU on day 121.* CT scan on day 146 showed reduced ground glass opacities in both lungs and some interstitial change in upper and middle fields of the lung and improvement of the pneumothorax. The patient was discharged from the hospital on day 187, he went home, and he could walk but needed extra oxygen supply of 2L/min. Nine months after admission, the patient is doing well and has returned to work. The patient visited the hospital 3 months after discharge: He only uses oxygen 1 L/min to go shopping and during physical training (out-patient rehabilitation). He talked to the treating intensivist without requiring oxygen and had no shortness of breath or tachypnoea. The red-coloured labels of the legends refer to graph plots using the (left) primary Y-axis, and the black-coloured labels of the legends refer to graph plots using the (right) secondary Y-axis
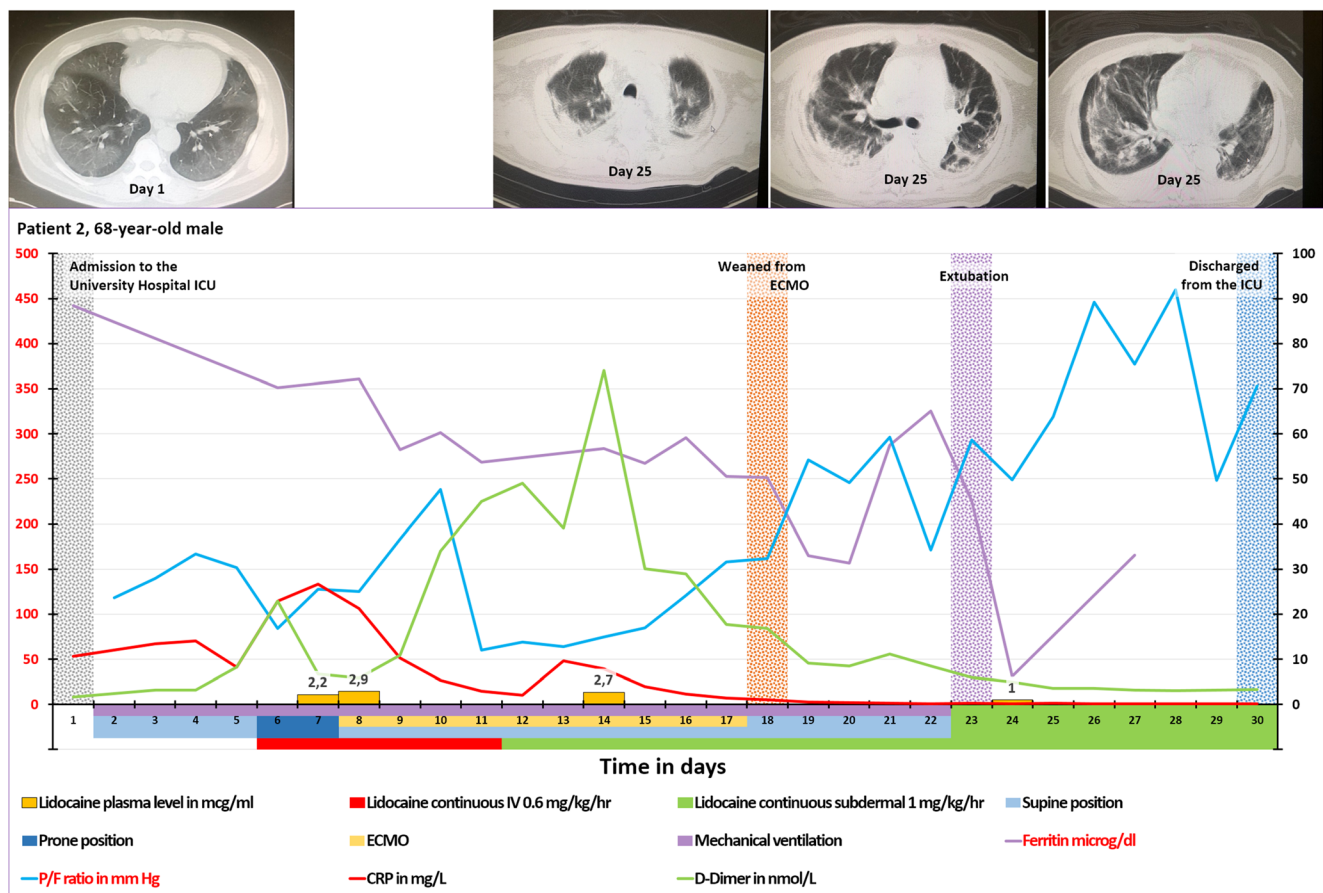
Fig. 8 Patient 2. A 68-year-old male with COVID-19-induced ARDS admitted to the ICU and required mechanical ventilation. The CT scan showed bilateral ground glass opacities. Co-morbidity: Asthma. After admission the patient's condition was deteriorating. On day 5, *continuous intravenous lidocaine of 0.6 mg/kg/h was initiated, but the clinical condition and the P/F ratio kept worsening. On day 11, the intravenous lidocaine of 0.6 mg/kg/h was changed to continuous subdermal lidocaine of 1 mg/kg/h. A few days later, this was followed by improvement of the* clinical condition and the P/F ratio. *No new ECG changes were observed during treatment with lidocaine. Blood metHb were within the normal range (0.1–0.6%). The patient was discharged from the ICU on day 30 home on day 37. At 3 months after admission, the patient is doing* well. The red coloured labels of the legends refer to graph plots using the (left) primary Y-axis, and the black-coloured labels of the legends refer to graph plots using the (right) secondary Y-axis

$PaCO_2$ was normal. The echocardiographic estimated pulmonary arterial systolic pressure (PASP) was 80 mm Hg. The Krebs von Lungen 6 (KL-6, a marker for lung fibrosis [405]) plasma level was highly elevated (1299 U/mL; normal value <425 U/mL), CRP was also high (40.4 mg/L; normal value <10 mg/L), and albumin was 2.2 g/dl. The white blood cell count, platelet count and urine production were normal. On day 4, the chest X-ray was not improved. On day 6, the PaO_2/FiO_2 ratio was slightly increased but remained low at 103 mm Hg, and the chest X-ray showed progression of the ARDS. ECMO was initiated due to exhausted ventilatory strategy. On day 9, the PaO_2/FiO_2 ratio improved but remained low at around 153 mm Hg, but the CRP declined to around 21.8 mg/L. The patient was put on muscle relaxants. The patient's ARDS status had improved from severe to moderate ARDS. From day 10 until day 30, the ferritin levels were well >1000 ng/ml (>100 µg/dl, normal values <300 ng/ml). From day 11 until day 62, D-Dimer was very high reaching

121.9 nM/L day 14. On day 11, no improvement of the blood gases was observed, and it was decided to treat the patient with continuous intravenous lidocaine 0.6 mg/kg/h. The CRP showed a progressive decline from 19 (on day 12) to 12.8 (on day 16) and 7.4 (on day 19), but the PaO_2/FiO_2 ratio remained poor at around 90 mm Hg (severe ARDS according to the Berlin criteria) and the chest X-ray image on day 15, 3 days after the initiation of the intravenous lidocaine infusion, deteriorated dramatically. The lidocaine plasma concentrations were 3.4 µg/ml on day 13 and 5.4 µg/ml on day 14. On day 19, the continuous intravenous lidocaine infusion was replaced by continuous subdermal lidocaine infusion of 1 mg/kg/h. Although the PaO_2/FiO_2 ratio remained unchanged on day 20 (1 day after the switch to the continuous subdermal lidocaine), the chest X-ray improved clearly. On day 21, the lidocaine plasma concentration was 2.6 µg/ml, and albumin was 2.5 g/dl. From day 22, the PaO_2/FiO_2 ratio was gradually improving reaching 151 mm Hg on day 34
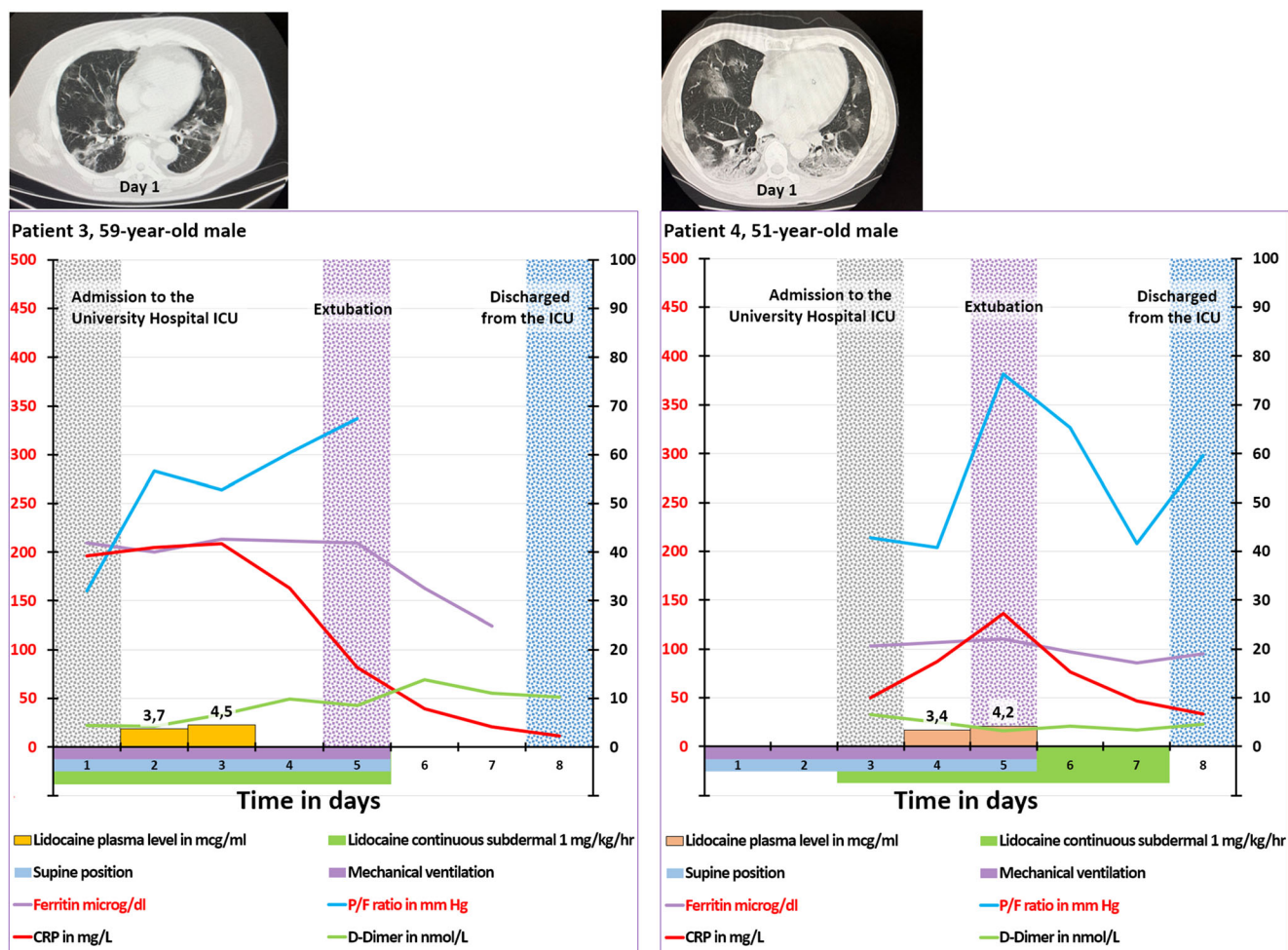
Fig. 9 Left graph: Patient 3. A 59-year-old male with respiratory distress and bilateral ground glass opacities on the CT scan. Co-morbidity: Obesity, diabetes mellitus and gout. No new ECG changes were observed during treatment with lidocaine. Blood metHb were within the normal range (0.1–0.4%). The patient was discharged from the ICU on day 8 and was discharged home on day 20. After 3 months, he is doing well. Right graph: Patient 4. A 51-year-old male with fever, dyspnoea and cough due to COVID-19. The CT scan showed bilateral ground glass opacities. Co-

morbidity: none. No new ECG changes were observed during treatment with lidocaine. Blood metHb were within the normal range (0.1–0.3%). The patient was discharged from the ICU on day 8 and was disc harged home on day 28. At *3 months, he is doing well and has returned to work.* The red-coloured labels of the legends refer to graph plots using the (left) primary Y-axis, and the black-coloured labels of the legends refer to graph plots using the (right) secondary Y-axis

(moderate ARDS). The KL-6 on day 22 dropped to 458 U/L (this is only slightly above the normal value of <450 U/l). On day 31, the CRP was low at 1 mg/L, and the lidocaine plasma concentration was 1.2 µg/ml. The muscle relaxants were discontinued. Albumin was 2.3 g/dl. On day 33, the chest X-ray was further improved, and the CRP remained low at 5.5 mg/L. The patient was awake and could communicate with the nurses. On day 38, the lidocaine plasma level was 2.3. On day 43, the PaO2/FiO2 ratio was increased to 214 mm Hg. According to the Berlin definition of ARDS [404], the patient's ARDS status had changed from moderate to mild. Albumin was 2.8 g/dl. On day 50, the patient was weaned from ECMO. On day 51, the patient underwent tracheotomy. Because the clinical condition of the patient was stabilised with a low CRP of 6.3 mg/L on day 55, the continuous

subdermal lidocaine was discontinued on day 57. On day 69, he developed pneumothorax requiring pleural drainage. On day 99, he was weaned from the mechanical ventilator and was discharged from the ICU on day 121. No new ECG changes were observed during treatment with lidocaine. Blood methaemoglobin (metHb) were within the normal range (0.3–0.8%). CT scan on day 146 showed reduced ground glass opacities in both lungs, some interstitial change in upper and middle fields of the lung and improvement of the pneumothorax. The patient left the hospital on day 187, he went home, and he could walk but needed extra oxygen supply of 2L/min. Nine months after admission, the patient is doing well and has returned to work. The patient visited the hospital 3 months after discharge: He only uses oxygen 1 L/min to go shopping and during physical training (out-patient
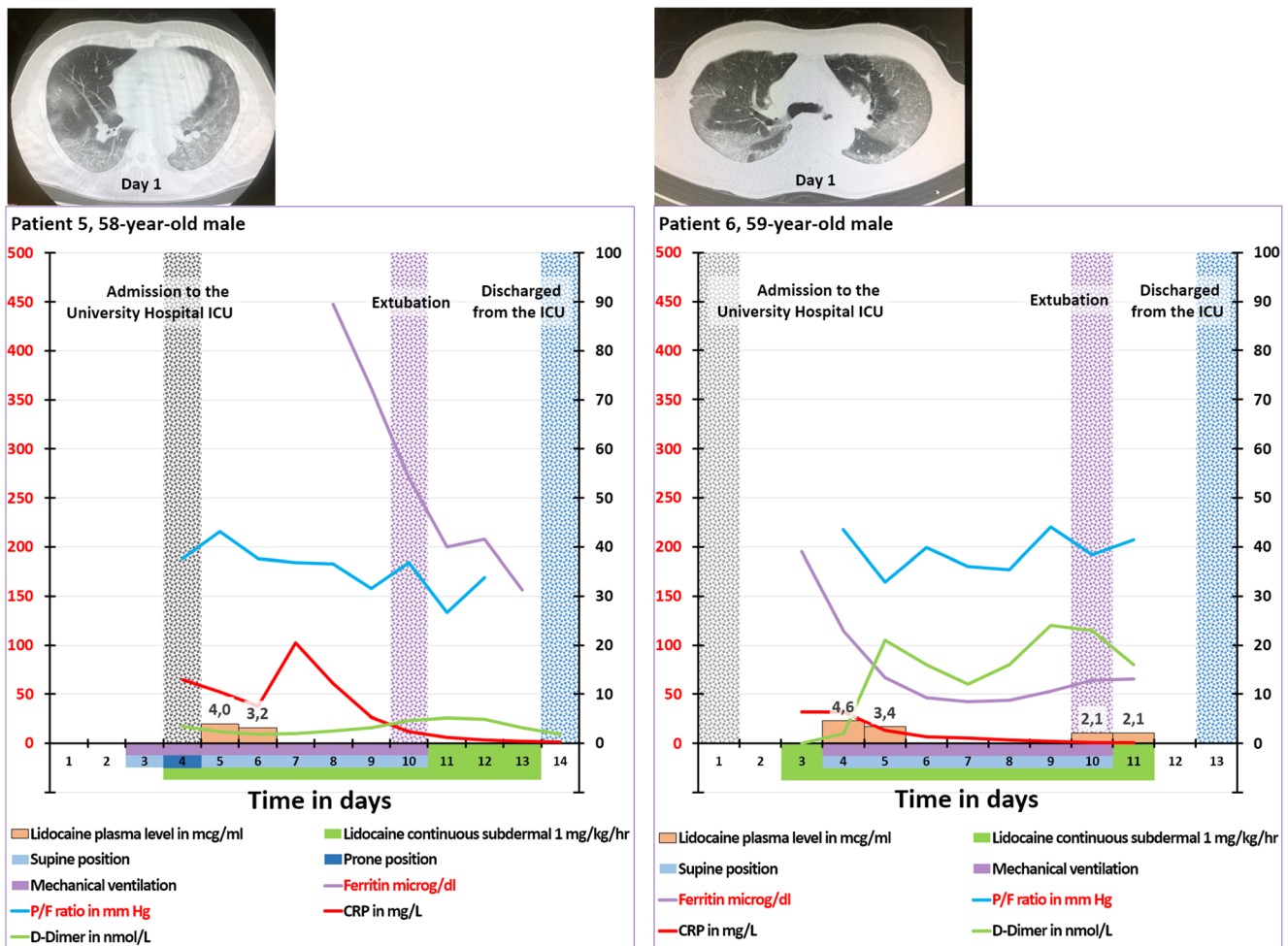
Fig. 10 Left gr aph: P atient 5. A 5 8 -year-o ld male with fever, dyspnoea and cough due to COVID-19. The CT scan showed bilateral ground glass opacities. Co-morbidity: Fatty liver. No new ECG changes were observed during treatment with lidocaine. Blood metHb were within the normal range (0.1–0.3%). On day 14, *the patient was discharged from the ICU. On day 20, the patient was* discharged home and is doing well at *3 months after admission*. Right graph: Patien ts 6. A 59-year-old male with fever, dyspnoea and cough due to COVID-19. CT scan showed bilateral ground glass opacities. Co-morbidity: Hypertension on medication. No new ECG changes were observed during treatment with lidocaine. MetHb were within the normal range (0.1–0.3%). On day 13, *the patient was discharged from the ICU. He was discharged from the hospital on day 20, and a t 3 months after admission, he is doing well, played golf* regularly and has returned to work. The red-coloured labels of the legends refer to graph plots using the (left) primary Y-axis, and the black-coloured labels of the legends refer to graph plots using the (right) secondary Y-axis

rehabilitation). He talked to the treating intensivist without requiring oxygen and had no shortness of breath or tachypnoea.

The second patient (Fig. 8) is a 68-year-old male (75 kg, 164 cm) with SARS-Cov-2-induced ARDS and positive SARS-Cov-2 PCR test admitted to the university hospital. Co-morbidity is asthma. The CT scan showed bilateral ground glass opacities. Haemodynamically the patient was stable. The patient received tocilizumab on day 8 and favipiravir for 14 days; he did not receive dexamethasone. On day 2, the respiratory conditions deteriorated, and the PaO_2/FiO_2 ratio is 118 mm Hg (moderate ARDS according to the Berlin ARDS definition [404]). The patient was intubated and required mechanical ventilation. The initial ventilator settings include pressure control, peak inspiratory pressure 28 cm

H_2O, PEEP 13 cm H_2O and respiratory rate 30/min. CRP was 10.6 mg/L, and KL-6 was 486 U/ml. White blood cell count, platelet count and urine production were normal. The ferritin levels remained >1000 ng/ml (100 µg/dl) during the entire ICU stay. Albumin was 2.9 g/dl. In the following 3 days, the PaO_2/FiO_2 ratio improved to around 150 mm Hg. The PaO_2/FiO_2 ratio dropped from 152 on day 5 to 84 mm Hg on day 6. CRP was increased to 22.9, and the KL-6 was increased to 762 U/ml. The patient was put in prone position and given muscle relaxants. Continuous intravenous lidocaine of 0.6 ml/kg/h was started. Albumin was 1.8 g/dl. On day 7, the PaO_2/FiO_2 ratio increased to 128 mm Hg, CRP dropped to 10.3 mg/mL and the lidocaine plasma concentration was 2.2 µg/ml. From day 3 until discharge from the ICU, D-dimer values were elevated reaching 75 nM/L on day 14. On day

8, although the PaO2/FiO2 ratio improved from 84 to 125 mm Hg, the mechanical ventilatory strategies were exhausted, and the patient was put on ECMO. The KL-6 was increased to 845 U/L, and lidocaine plasma level was 2.9 µg/ml. The PaO_2/FiO_2 ratio improved to 238 mm Hg on day 9, but on day 10, a sharp drop of the PaO_2/FiO_2 ratio to 60 mm Hg was observed, and CRP was 2.0 mg/ml. The patient's ARDS status had changed from moderate to severe according to the Berlin ARDS criteria [404]. Lidocaine treatment was switched from continuous intravenous to continuous subdermal (dosage: 1 mg/kg/h). On day 14, the lidocaine plasma level was 2.7 µg/ml. KL-6 dropped to 549 U/l. On day 17, the clinical condition of the patient was improving, and the PaO_2/FiO_2 ratio reached 158 mm Hg. The patient was weaned from ECMO. The PaO_2/FiO_2 ratio improved further reaching 291 mm Hg on day 21, and the patient's ARDS status has changed from moderate to mild ARDS [404]. On day 22, mechanical ventilation was discontinued, and the patient was extubated. The patient was orientated, and no signs of confusion were detected. CT scan on day 25 showed persistent ground glass opacities in both lungs, some pulmonary effusion (right >left), and no signs of vascular thrombosis. In addition, no signs of deep venous thrombosis were found in the lower extremities. Lidocaine treatment was continued until discharge from the ICU on day 30. No new ECG changes were observed during treatment with lidocaine. Blood metHb were within the normal range (0.1–0.6%). The patient was discharged home on day 37. At 3 months after admission, the patient is doing well.

The third patient (Fig. 9*left*), a 59-year-old male (109 kg, 170 cm), was admitted to the university hospital with respiratory distress and bilateral ground glass opacities on the CT scan with a positive SARS-CoV-2 test. Co-morbidities include obesity (BMI 37.7 kg/m^2), diabetes mellitus and gout. The patient required immediate intubation and mechanical ventilation. The patient received tocilizumab on day 3 and favipiravir for 15 days and did not receive dexamethasone. The initial ventilator settings are pressure control, peak inspiratory pressure 30 cm H_2O, PEEP 15 cm H_2O and respiratory rate 25/min. The PaO_2/FiO_2 ratio on admission was 160 mm Hg (moderate ARDS according to the Berlin definition [404]), CRP was 39.3 mg/L and KL-6 was 294 U/ml. White blood cell count was increased (13.10^{-9}/L) and platelet count and urine production were normal. Albumin was 2.1 g/dl. Haemodynamic parameters were stable. On the admission day, continuous subdermal lidocaine was started at 1 mg/kg/h. On day 2, the PaO_2/FiO_2 ratio improved to 283 mm Hg, and the patient's ARDS status had changed from moderate to mild ARDS. CRP was 41 mg/L, KL-6 was 268 U/L and the lidocaine plasma level was 3.7 µg/ml. Albumin was 1.7 g/dl. On day 4, the PaO_2/FiO_2 ratio was 302 mm Hg, and the patient's ARDS status had

changed from mild ARDS to no ARDS according to the Berlin ARDS criteria. On day 5, the PaO_2/FiO_2 ratio was improved further to 328 mm Hg, and CRP dropped to 16.4, and the patient was extubated. The patient was orientated, no signs of confusion were detected. The patient was discharged from the ICU on day 8; CRP was 2.3 mg/ml. Albumin was 2.5 g/dl. No new ECG changes were observed during treatment with lidocaine. Blood metHb were within the normal range (0.1–0.4%). The patient was discharged home on day 20. After 3 months, he is doing well.

The fourth patient (Fig. 9*right*) is a 51-year-old male (68 kg, 175 cm). Ten days before admission, he developed fever and 2 days before admission dyspnoea and coughing. On the day of admission, the PCR SARS-CoV-2 test was positive. The CT scan showed bilateral ground glass opacities. Co-morbidity is none. The patient was intubated and put on mechanical ventilation on admission. The patient received favipiravir for 14 days; he did not receive dexamethasone. On day 3, he was transferred to the university hospital because of deterioration of pulmonary condition. The initial ventilator settings include pressure control, peak inspiratory pressure 24 cm H_2O, PEEP 12 cm H_2O and respiratory rate 15/min. The haemodynamic conditions were stable. White blood cell count and platelet count were normal. Albumin was 2.6 g/dl. Continuous subdermal lidocaine was started immediately. On day 3, the PaO_2/FiO_2 ratio was 214 (moderate ARDS according to the Berlin definition [404]). KL-6 was 177 U/L, and CRP was 17.4 mg/L. On day 5, the PaO_2/FiO_2 ratio was increased to 382 (the patient's ARDS status had changed from mild ARDS to no ARDS), and lidocaine plasma concentration was 5.2 µg/ml. CRP was 27.3mg/L. Lidocaine plasma levels on day 3 and 4 were 3.4 and 4.2 µg/ml, respectively. KL-6 was 163 U/L. The patient was extubated. The patient was orientated, and no signs of confusion were detected. The patient was discharged from the ICU on day 8, and the CRP was 9.3 mg/L. No new ECG changes were observed during treatment with lidocaine. Blood metHb were within the normal range (0.1–0.3%). He was discharged home on day 28. At 3 months, he is doing well and has returned to work.

The fifth patient (Fig. 10*left*) is a 58-year-old male (80 kg, 175 cm). Nine days before admission, he developed a sore throat. A day later, he developed fever. Two days before admission, he started coughing and was dyspnoeic. On the day of admission, the PCR SARS-Cov-2 test was positive. Co-morbidity includes fatty liver. The CT scan showed bilateral ground glass opacities. The patient was initially admitted to the hospital ward. The patient received tocilizumab on day 7 and favipiravir for 10 days; dexamethasone was not prescribed. On day 3, the patient deteriorated and had to be intubated and put on mechanical ventilation. On day 4, the patient was transferred to the university hospital due to deterioration of the pulmonary condition. The initial ventilator

settings include pressure control, peak inspiratory pressure 27 cm H2O, PEEP 12 cm H2O and respiratory rate 25/min. PaO$_2$/FiO$_2$ ratio was 188 (moderate ARDS according to the Berlin definition). Haemodynamic parameters were stable, and CRP was 12.9 mg/ml. White blood cell count was increased (14.4.10^9/L), but platelet count was normal. KL-6 was 330 U/L. Continuous subdermal lidocaine was started at 1 mg/kg/h at arrival at the ICU of the university hospital. Albumin was 2.8 g/dl. On day 5, the PaO$_2$/FiO$_2$ ratio was unchanged, CRP was 10.4 mg/L and the lidocaine plasma level was 4 μg/ml. On day 6, the lidocaine plasma level was 3.2 μg/ml. KL-6 remained stable at 400 U/L. Albumin was 2.3 g/dl. On day 10, the respiratory insufficiency had cleared; although the PaO2/FiO2 ratio remained 184, the CRP dropped to 2.4 mg/L, and KL-6 was 322 U/L. The patient was extubated, and he was orientated; no signs of confusion were detected. On day 14, the patient was discharged from the ICU. No new ECG changes were observed during treatment with lidocaine. Blood metHb were within the normal range (0.1–0.3%). On day 20, the patient was discharged home and is doing well at 3 months after admission.

The sixth patient (Fig. 10*right*) is a 59-year-old male (65 kg, 175 cm) with fever, dyspnoea and cough due to COVID-19. CT scan showed bilateral ground glass opacities. Co-morbidity includes hypertension on medication. The patient was admitted to the general ward. KL-6 233 U/L, white blood cell count and platelet count were normal. Albumin was 3.6 g/dl. On day 3, there is a deterioration of the respiratory function necessitating a transfer to the ICU and mechanical ventilation. Tocilizumab was given on day 4. The patient received favipiravir for 11 days, and the patient did not receive dexamethasone. The initial ventilator settings include pressure control, peak inspiratory pressure 22 cm H$_2$O, PEEP 10 cm H$_2$O and respiratory rate 20/min. Continuous subdermal lidocaine of 1 mg/kg/h was initiated after admission to the ICU. Haemodynamic parameters were stable. CRP was 6.3 mg/L, and KL-6 was 263 U/L. On day 4, a progressive respiratory failure occurred requiring intubation and mechanical ventilation. PaO$_2$/FiO$_2$ ratio was 218 mm Hg; the haemodynamic parameters remained stable. CRP was 6.3 mg/L, and the white blood count and platelet count were normal. Lidocaine plasma level was 4.6 μg/ml. On day 5, the PaO$_2$/FiO$_2$ ratio dropped further to 164 mm Hg. Lidocaine plasma level was 3.4 μg/ml. Albumin was 3.2 g/dl. On day 9, the clinical condition of the patient improved. The ventilator settings could be decreased, the PaO$_2$/FiO$_2$ ratio remained 207 mm Hg during the weaning period, and CRP was 0.7 mg/L. On day 10, the patient was extubated, he was orientated, and no signs of confusion were detected. On day 13, the patient was discharged from the ICU. No new ECG changes were observed during treatment with lidocaine.

Blood metHb were within the normal range (0.1–0.3%). He was discharged from the hospital on day 20, and at 3 months after admission, he is doing well, played golf regularly and has returned to work.

Additional 14 patients with COVID-19-induced ARDS

From July 2020 until beginning of December 2020, 14 additional critically ill patients with COVID-19-induced ARDS requiring mechanical ventilation were treated in the ICU of the Showa University with continuous subdermal lidocaine infusion (1 mg/kg/h) plus intravenous or oral dexamethasone (6 mg/day) as reported earlier [35]. Of these 20 patients, 19 survived, but an 87-year-old female patient died of invasive aspergillosis. No other patient developed secondary co-infections (unpublished data, personal communication by AS and TK).

Discussion of the clinical cases

After completing the novel definition of hyperinflammation, we developed a new approach to target the lymphatic system with continuous subdermal administration of lidocaine. This is meant to increase the anti-hyperinflammatory effect of lidocaine while avoiding toxic plasma levels. We described the treatment of six critically ill patients with COVID-19 with lidocaine. Two patients required mechanical ventilation and ECMO, and four patients were treated with mechanical ventilation. As mentioned under the heading "Introduction", the case fatality rates of patients requiring mechanical ventilation and/or ECMO are alarmingly high [1, 2]. Patient 1 and patient 2 were older than 60 years. Additionally, patient 1 had COPD and had smoked 60 cigarettes per day for more than 40 years. Patient 3 suffered from obesity and diabetes mellitus. These are serious prognostic factors for bad outcome COVID-19 [406, 407]. Patient 1 and patient 2 were initially treated with continuous intravenous lidocaine through a central venous line. In both patients, the pulmonary conditions deteriorated after the initiation of intravenous lidocaine: Patient 1 who was already on ECMO showed progressive pulmonary deterioration on the chest X-rays, and patient 2 deteriorated further necessitating the initiation of ECMO therapy. Remarkably, the pulmonary conditions of both patients improved within 48 h after the switch from intravenous to subdermal continuous lidocaine. The lidocaine plasma levels remained around 5 μg/ml. To our knowledge, these six cases represent the first observations of the promising treatment of critically ill COVID-19 patients with lidocaine targeting P2X7Rs of the immune cells in the lymphatics. All patients recovered completely from their illness. None of the patients showed the feared side effect of cardiac arrhythmia and methaemoglobinaemia during lidocaine therapy. Our findings suggest that continuous subdermal lidocaine infusion at the

rate of 1 mg/kg/h has the potential to mitigate hyperinflammation and ARDS in critically ill COVID-19-patients. Obviously, although all six patients appeared to respond positively to the treatment and no severe adverse effects were observed, no final conclusions can be made on the efficacy of lidocaine in critically ill COVID-19 patients.

Researchers from Lima, Peru, reported the treatment of 28 (three mild, 21 moderate and four severe) COVID-19 patients with 0.5% lidocaine HCL solution with an intravenous dose of 1 mg/kg once a day for 2 days and 2% lidocaine HCL solution with a subcutaneous dose of 1 mg/kg once a day for 2 days [408]. The authors aimed at the improvement of pain, cough, respiratory rate and oxygen saturation. They found improvement in most patients. In severe cases, this treatment did not improve the oxygen saturation. As expected, treatment with a low daily dose of lidocaine once per day for a total treatment duration of 2 days could not adequately inhibit the P2X7R-induced hyperinflammation in COVID-19.

Recently, a group of researchers from Strasbourg, France, announced a study entitled: "Impact of intravenous lidocaine on clinical outcomes of patients with ARDS during COVID-19 pandemia (LidoCovid): A structured summary of a study protocol for a randomised controlled trial" (ClinicalTrials.gov Identifier: NCT04609865) [409].

Lately, an extraordinary treatment of COVID-19 ARDS was reported [410]. The authors performed lung transplantations in three critically ill COVID-19 ARDS patients: a 28-year-old female, a 62-year-old male and a 43-year-old male. The first patient underwent lung transplantation after weeks on veno-venous ECMO support with elevated pulmonary arterial pressures and severe secondary Serratia marcescens pneumonia. The second patient underwent lung transplantation after 100 days on veno-venous ECMO support complicated by Pseudomonas aeruginosa pneumonia, haemothorax and empyema, while the third patient after 90 days on the mechanical ventilator. This patient suffered from many complications: asystolic cardiac arrest, heparin-induced thrombocytopenia, a left frontal lobe infarct of the cerebral cortex, Serratia marcescens-mediated pneumonia with bacteraemia, acute kidney injury, a left haemothorax requiring thoracotomy and lung decortication, a right pneumothorax requiring tube thoracostomy, hypernatremia associated with seizures and malnutrition. Before lung transplantation, the patient developed increasing clinical signs of pulmonary fibrosis and severe pulmonary hypertension. The first two patients are reported to have achieved independence in daily life activities several months after lung transplantation. Three months after lung transplantation, the third patient made improvements in the neurocognitive status and muscular strength at an inpatient rehabilitation centre.

Far less drastic is our proposed treatment of hyperinflammation in COVID-19-induced ARDS with lidocaine, an old drug that is readily available to hospitals all over the world at a low cost. In November 1948, Xylocaine was approved by the Food and Drug Administration (FDA) in the USA [411]. Lidocaine is used as a local anaesthetic [411], treatment of chronic neuropathic pain [412] but also for the prophylaxis or treatment of ventricular arrhythmia [328, 329]. Recently, intravenous lidocaine has been administered as general anaesthetic replacing opioids in the perioperative settings [413]. Potentially, lidocaine, as a P2X7R antagonist, can abrogate hyperinflammation, can restore the capacity of the immune system to combat secondary co-infections and can improve the clinical condition in critically ill COVID-19 patients. Despite several in vitro [326, 327, 414, 415], animal studies [319, 416–420] and patient cohorts [408, 421] on the anti-inflammatory properties of lidocaine, completed clinical trials which deliver a proof of concept (i.e. a randomised controlled trial) have not yet been performed. We postulate that because the maximal tolerable plasma concentration of lidocaine is much lower than the required extracellular concentration to effectively inhibit P2X7Rs, intravenous systemic administration of lidocaine simply cannot not be used to effectively treat hyperinflammation. This is a plausible reason why 5 years after the discovery of lidocaine as a P2X7R inhibitor (published in 2015) [74] the drug is still not used as an anti-hyperinflammatory treatment in clinical practice.

Concluding remarks

As stated in the introduction, therapeutic measures that can immediately attenuate the course of SARS-CoV-2-related lung damage are promptly needed on a global scale. In contrast to the investigational P2X7R antagonists described above, continuous subdermal infusion of 2% lidocaine solution to primarily deposit lidocaine into the lymphatics is readily available and can be used in the daily practice immediately and, in principle, even outside the ICU and is very well affordable. Therefore, this therapy deserves to be investigated in larger placebo controlled randomised clinical studies with COVID-19 patients.

Future development

However, our experience with subdermal administration of lidocaine in the ICU made clear that this method may not be routinely suitable outside hospital settings. Needless to say that high complexity and high-cost treatments (requiring highly skilled nurses and infusion pump equipment) are inaccessible to low-income COVID-19 patients in developing

countries. Also, as the severity and case fatality rate of COVID-19 increase with age [406], the case fatality rate in elderly patients in nursing homes is strikingly high, and many residents have poor access to medical care [422]. This encouraged us to explore alternative uncomplicated methods of lidocaine administration accessible to everyone, particularly elderly COVID-19 patients and COVID-19 patients in developing countries.

Recently, researchers stated in their article on targeting the P2X7R in COVID-19 that the P2X7R antagonists for human use are available only in oral form and that this might be an inefficient route of drug delivery [298]. We found a solution to this problem. Permeability of the skin and mucous membrane to water, drugs, etc. is said to be dependent on the site of the administration [423, 424]. For example, the permeability constant of the floor of the mouth (sublingual mucosa), lateral border of the tongue and buccal mucosa for tritium-labelled water is 22, 17 and 13 times as high as human skin, respectively [423]. We argue that this also applies to lidocaine. As mentioned above, the endothelium of the mucosal capillaries of the mouth and the gastrointestinal tract belong to the structural type "fenestrated endothelium" allowing molecules to pass from the submucosal tissue into the capillaries [383]. Lidocaine hydrochloride is highly soluble in water (solubility of 680 mg/ml in water) [425] and therefore will mainly be absorbed by the submucosal capillary [426] and the inductive sites of MALT [398, 399]. In contrast, the highly lipophilic lidocaine base (solubility of 4 mg/ml in water, 760 mg/ml in 95% ethanol and 790 mg/ml in chloroform) [425] is preferably absorbed by the local initial lymphatics in the submucosal tissue [426, 427]. In addition, the lymphatic drainage of the floor of the mouth is extensive, involving many lymph nodes [428–431].

We estimate that with a sublingual administration of lipophilic lidocaine base (Fig. 1), we may reach the IC_{50} of the P2X7Rs in the draining lymph nodes to control systemic hyperinflammation and avoid toxic lidocaine plasma levels (Figs. 4 and 6). Obviously, such solution may also apply to other P2X7R antagonists. We stress that sublingual and buccal administration of lipophilic lidocaine is different from oral administration of lidocaine. Oral administration of lidocaine is aimed at the resorption of the drug in the gastrointestinal tract (Fig.6).

There are other methods of targeting the immune cells in the lymphatics, i.e. transdermal administration of lipophilic P2X7R antagonist with skin penetration enhancers (i.e. alpha-terpineol [432], ethanol [433] and lipid based nanoformulations [434]), intravenous administration of a P2X7R antagonist using nano-sized drug delivery systems [435], liposomes or polymer micelles [436] and oral administration of a P2X7R antagonist using delivery systems for intestinal lymphatic drug transport such as chylomicrons [437].

Data Availability The datasets generated during and/or analysed during the current study are not publicly available due to privacy reasons but are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval, consent to participate and consent for publication The Medical Ethical Committee of the Showa University, School of Medicine, Tokyo, approved the collection, analysis and publication of patients on mechanical ventilation admitted to the ICU (protocol number 3313).

Conflicts of interest DH: The author declares to have filed patent applications that are based on the content of the paper.

AS: none.
CvK: none.
PJvdS: none.
EPK: none.
TK: none.

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