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Methylene blue in covid-19

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ABSTRACT

SARS-CoV-2 infection generally begins in the respiratory tract where it can cause bilateral pneumonia. The disease can evolve into acute respiratory distress syndrome and multi-organ failure, due to viral spread in the blood and an excessive inflammatory reaction including cytokine storm. Antiviral and anti-cytokine drugs have proven to be poorly or in-effective in stopping disease progression, and mortality or serious chronic damage is common in severely ill cases. The low efficacy of antiviral drugs is probably due to late administration, when the virus has triggered the inflammatory reaction and is no longer the main protagonist. The relatively poor efficacy of anti-cytokine drugs is explained by the fact that they act on one or a few of the dozens of cytokines involved, and because other mediators of inflammation – reactive oxygen and nitrogen species – are not targeted. When produced in excess, reactive species cause extensive cell and tissue damage. The only drug known to inhibit the excessive production of reactive species and cytokines is methylene blue, a low-cost dye with antiseptic properties used effectively to treat malaria, urinary tract infections, septic shock, and methaemoglobinaemia. We propose testing methylene blue to contrast Covid-related acute respiratory distress syndrome, but particularly suggest testing it early in Covid infections to prevent the hyper-inflammatory reaction responsible for the serious complications of the disease.

Background

SARS-CoV-2 enters the human host mainly through the respiratory tract, although faecal transmission could also occur [1]. In some cases the infection evolves to acute respiratory distress syndrome (ARDS) [2], a form of respiratory failure that develops rapidly in critically ill patients [3]. ARDS is often associated with multi-organ damage (mainly lung, heart, brain, kidney, liver and small intestine) and septic or vasoplegic shock [4]. The lungs are affected early as they are the first structures encountered by the virus after entry through the respiratory tract, and because alveolar epithelial cells abundantly express ACE2 receptors that the virus uses to enter cells [5]. Pervasive alveolar injury is accompanied by severe damage to the vascular endothelium [6] allowing the virus to enter the bloodstream. Viraemia and high viral RNA titres in liver, kidney, and heart, have been found of patients who died of Covid-19 [7].

It is unclear whether the blood-brain barrier can prevent viral access to the central nervous system. However capillary endothelial cells-fundamental constituent of the blood-brain barrier-express abundant ACE2 receptors and could well be a CNS entry route. Direct viral invasion of the brain through the olfactory pathways is also a possibility [8].

ACE2 receptors are present on the vascular endothelial cells of all organs [9], which would account for the endothelial inflammation across vascular beds of different organs reported in a series of autoptic Covid-19 cases [10]. After entering cells, the virus triggers an inflammatory reaction by activating signal transduction pathways, including transcription factor NF-kB, that stimulate the production of cytokines to counteract viral replication and spread [11].

What Didn't work

Evolution to ARDS and multi-organ failure occurs, on average, seven or so days after symptoms onset, when the viral load has decreased significantly [12,13], and correlates with a violent systemic inflammatory reaction-accompanied by a cytokine storm – in excess of what is necessary to eliminate the virus. Antiviral drugs, assuming they work against Covid-19, are only likely to be effective against ARDS/multi-organ failure if administered early, at the onset of symptoms, before

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the inflammatory reaction has started. Thus they should be administered to all infected patients, at a cost of several thousand dollars per patient.

Anti-cytokine drugs have been tried, but the results have been disappointing, probably for two main reasons: first, these drugs inhibit just one or a few of the fifty or more cytokines involved; second, there are other protagonists in the complicated inflammatory scenario, including free radicals (reactive oxygen and nitrogen species, ROS and RNS), that have not been taken into account [14,15]. The role of kinins in ARDS from Covid-19 has also been underestimated. There is strong evidence supporting the role of bradikinin, histamine, and serotonin, as key mediators of acute lung inflammation and respiratory distress [16].

Free radicals

Free radicals are produced during intracellular redox reactions in mitochondria, peroxisomes, and endoplasmic reticulum, and are involved in many physiological functions including signal transduction, gene expression and apoptosis. Bronchial, alveolar and endothelial cells also produce abundant free radicals (ROS and RNS) to kill pathogens but which may also damage endogenous proteins, lipids and nucleic acids. Activated phagocytes (neutrophils, monocytes and macrophages) are recruited to sites of inflammation where they adhere to endothelium and also release a range of ROS to contribute to pathogen destruction and organ damage. The inflammatory response, initially local and subsequently systemic, is driven by ROS [17] whose production increases following viral infection to activate NF-kB and other transcription factors that control inflammation-related genes. NF-kB moves from the cytoplasm to the nucleus and binds DNA to trigger cytokine synthesis [17,18]. The role of ROS in inducing cytokine synthesis has been highlighted in experimental models of systemic inflammation [19,20]. In physiological conditions, the superoxide anion (progenitor of ROS) is rapidly inactivated by superoxide dismutase. However, following specific inflammatory or septic stimuli, superoxide production exceeds the scavenging capacity of superoxide dismutase and it undergoes reactions leading to more reactive ROS, such as the hydroxyl radical [21]. Nitric oxide (progenitor of RNS) is also formed in excess in response to inflammatory/septic stimuli [22] and reacts with the superoxide anion to form peroxynitrite, which is highly toxic. All these reactive species damage cell membranes and DNA, and can lead to cell death [23].

Lowest common denominator: endothelial damage

Lung and vascular endothelium are most affected in severe Covid-19, whose cells are rich in the ACE2 receptors used by the virus to effect cell entry. When the production of ROS, RNS and cytokines exceeds the ability of tissues to neutralize them, they invade the interstitial space and induce oxidative stress, causing necrosis or apoptosis, and damaging lung alveoli and vascular endothelium [10,24]. Cytokines, ROS and RNS can also spill over into the systemic circulation to cause endothelial injury in distant organs [25,26]. The endothelium of distant organs is therefore the target of both circulating virus and mediators of systemic inflammation, and, at the same time, is the source of inflammatory mediators.

Several lines of evidence suggests that vascular endothelial damage and subsequent thrombophilia [27] are the central factors in Covid-related multi-organ failure. For example, endothelialitis and thrombosis have been identified as the cause of skin lesions in the form of chilblains in pediatric Covid-19 patients [28]. Furthermore, histologic involvement of the digestive system has been observed in a limited number of autoptic Covid cases; and ischemic necrosis from recent thrombosis and severe inflammatory infiltration of the endothelium of mesenteric vessel were observed in an autoptic case [29]. In addition, a high incidence of thromboembolic events in deep veins of the lower extremities was found in another series of autoptic Covid cases [7]. Cardiovascular complications such as myocarditis, pericarditis, vasculitis, and heart failure are frequently observed in Covid-19 patients. However, SARS-CoV-2 has not always been isolated from the myocardium of patients who have died of Covid-19, suggesting that myocardial damage is due to altered microcirculation caused by endothelial dysfunction and hyper-inflammatory reaction [30].

It is also noteworthy that Covid cases with neurological disorders caused by ischemic stroke, hemorrhage and diffuse microbleeds, suggesting secondary microangiopathy, have been reported [31]. Endothelial rupture of cerebral microvessels with bleeding into brain parenchyma can have serious consequences. Red blood cells spilled into brain release iron, which reacts with endogenous hydrogen peroxide to afford the highly reactive hydroxyl radical [32]. The persistence of iron perpetuates hydroxyl radical production,

What could work

Thus, radicals and cytokines are intimately involved in the genesis of the endothelial damage, which is the common denominator of multi-organ failure in Covid-19. When ARDS occurs, ROS, RNS and cytokine production is out of control, and attempting to contrast all with a single cytokine inhibitor is doomed to fail. To our knowledge, only one drug is capable of inhibiting the production of all three of these classes of substance: methylene blue. Methylene blue inhibits the formation of superoxide anion (ROS precursor) by blocking the xanthine oxidase pathway [33]; it counteracts the synthesis of nitric oxide (RNS precursor) by direct inhibition of NO-synthase [34], and inhibits cytokine expression via attenuation of NF-kB signaling [35,36].

Methylene blue is a tricyclic phenothiazine, approved by the FDA and EMA for the treatment of methaemoglobinaemia and malaria. It is also used to inactivate viruses in blood products for transfusion, in the presence of UV light. Recent (non peer-reviewed) in vitro studies indicate that it has antiviral activity in the absence of UV light [37,38], strengthening the rationale for its use in Covid-19. However methylene blue use in patients with glucose-6-phosphate dehydrogenase deficiency is contraindicated due to increased risk of haemolytic anemia [39]. Its concomitant use with serotonin reuptake inhibitors is also contraindicated. Methylene blue is a potent reversible inhibitor of monoamine oxidase A-the enzyme that catalyses serotonin breakdown- and concomitant use with serotonin reuptake inhibitors can inhibit the degradation of serotonin and increase its concentration to toxic levels (serotonin syndrome) [40].

Administered intravenously at doses not exceeding 2–3 mg/kg, methylene blue has been successfully and safety used against septic shock [41,42], also in pediatric patients [43]. Methylene blue can also be administered intravenously in severely affected Covid-19 patients. Studies conducted in small groups of critically ill patients appear to demonstrate the efficacy of methylene blue combined with other antioxidants [44]. “However, since it is impossible to reverse tissue destruction that has already developed, it is important to start treatment early. We suggest starting oral treatment with methylene blue at first Covid-19 symptoms with the aim of preventing the excessive inflammatory reaction. The usual daily oral dose is 200 mg [45], although 300 to 1000 mg/day in divided oral doses for 7–23 days used to be given to malaria cases [46].

We suggest trying oral methylene blue at 2–3 mg/kg body weight/day divided into three daily doses for seven to ten days in patients newly infected with SARS-CoV-2. However, optimal dosage should also be object of a clinical study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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