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Repurposing traditional immunomodulators to target the inflammatory burden of atherosclerosis ☆

1. Introduction

The concept of atherosclerosis as an inflammatory disorder dates back to the seminal observations of Karl von Rokitansky and Rudolph Virchow, who in the mid-19th century noted inflammatory changes in the arterial wall. Many basic and clinical studies have since then implicated immune cells and inflammatory pathways in atherosclerosis development and progression, but these relationships were associative only. The CANTOS (Canankinumab Anti-Inflammatory Thrombosis Outcome) trial [1] provided the first definite causal link and validated the strategy of targeting inflammatory mediators for secondary cardiovascular prevention [2]. The anti-atherogenic mechanism(s) of canakinumab, a monoclonal antibogy against interleukin (IL)-1β, was largely attributed to blunted production of the pro-atherogenic cytokine IL-6 downstream of IL-1 receptor activation. CANTOS clearly constitutes a milestone in cardiovascular medicine, yet several factors still prohibit wide-scale application of biologicals in routine clinical practice. First, the lack of long-term experience with these novel compounds with no chronic safety data. Additionally, the basic rules of pharmacology, particularly pharmacokinetic principles, cannot be directly applied to biologicals. Finally, there are the as yet exorbitant costs. CANTOS reported increased numbers of fatal infections including sepsis in patients receiving canakinumab, and many other biologicals have also been linked with severe infections, malignancies, heart failure, and lipoprotein abnormalities. Lipoproteins critically determine fibroatheromatous cap vulnerability [3], and recent experimental evidence links IL-1β blockade with adverse plaque composition and stability [4], implying an unexpectedly beneficial role of IL-1β in late stage atherosclerosis.

There is a clear unmet need for improved approaches to limit atherosclerosis: biologicals targeting specific cytokine mediators, or broad-spectrum anti-inflammatory strategies. Accordingly, there has been a revived interest in traditional immune modulators such as colchicine, allopurinol, rapamycin, methotrexate and mycophenolate, and, given the current situation with COVID-19, also chloroquine. These generics are relatively inexpensive, and most practitioners are familiar with their pharmacology and safety profile, while biologicals, by contrast, are more susceptible to structural variability due to manufacturing processes, and are limited by pharmacokinetics that cannot be predicted with established models. The recent COLCOT (Colchicine Cardiovascular Outcomes Trial) showed that low-dose colchicine (0.5 mg once-daily) significantly lowers ischemic events in patients with recent myocardial infarction [5], providing independent support for the inflammation-hypothesis of atherosclerosis. This anti-mitotic agent suppresses inflammatory cell proliferation by inhibiting tubulin polymerization, a large part of its anti-inflammatory action is suspected to be attributed to its ability to block the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome [6], an activating platform for caspase-1 and hence maturation of IL-1β. Mycophenolate mofetil, by contrast, may actually trigger NLRP3-dependent caspase-1 activation and cytokine production in monocytic cells [7] and methotrexate, a folate antagonist showing promising anti-inflammatory actions in experimental atherosclerosis, did not meet the expectations in the Cardiovascular Inflammation Reduction Trial (CIRT) [8].

In this issue of the *International Journal of Cardiology Heart and Vasculature*, Sato-Okabayashi and colleagues report that a classic anti-rheumatic drug, cyclophosphamide, may delay atherosclerosis in mice [9]. Cyclophosphamide is a precursor of DNA-alkylating cytotoxic metabolites and is used to treat cancer, certain autoimmune diseases, and nephrotic syndrome in children. After absorption, cyclophosphamide is converted by hepatic cytochrome P450 enzymes to 4-hydroxyphosphamide, a soluble intermediate that is transported in the circulation to target cells. Upon uptake by tumor or immune cells, the compound spontaneously forms aldophosphamide, which degrades to the principal DNA- and protein-adduct forming metabolites phosphoramide mustard and acrolein. Aldehyde dehydrogenase can also convert aldophosphamide to the more weakly alkylating metabolite nitrogen mustard. In the study presented by Sato-Okabayashi et al. [9], a relatively low dose of cyclophosphamide (20 mg/kg/day) applied orally over 12 weeks, slowed atherosclerotic plaque formation in Western diet-fed ApoE $^{-/-}$ mice. This anti-atherogenic action was attributed to suppression of circulating and splenic inflammatory monocytes, CD3-positive Th1 cells and CD19-positive B-cells, lowered macrophage accumulation in the plaque and a modest trend towards a net anti-inflammatory cytokine profile in the aorta. The intra-plaque abundance of IL-6 and NFκB - both key

☆ These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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contributors to coronary inflammation and dysfunction [10,11,12] – was significantly reduced by cyclophosphamide.

The clinical value of these anti-atherogenic effects of cyclophosphamide is unclear. Premature atherosclerosis is commonly seen in patients with systemic lupus erythematosus and this correlates negatively with cyclophosphamide therapy [13]. However, cyclophosphamide use has also been identified as an independent contributor to arterial stiffness and calcification [14,15] Thus, the long-term efficacy and safety of low-dose cyclophosphamide therapy need prospective clinical validation. The typical adverse side-effects of anti-neoplastic doses of cyclophosphamide are neutropenia, fever, alopecia, nausea, and vomiting, diarrhoea and infertility. Cyclophosphamide can also promote malignancies, and - even at the relatively low dose used in the study by Sato-Okabayashi et al. [9] – severe hepatotoxicity [16] Cyclophosphamide-treated mice showed increased neutrophil infiltration, myeloperoxidase activity and IL-6 expression in the liver, and higher serum levels of danger-associated molecular patterns (DAMPs) such as high mobility group box 1 (HMGB1), heat shock protein 60 (HSP60) and glucose-regulated protein 94 (Grp94) [16]. The authors postulated a DAMP-activated inflammatory injury mediated through an augmented toll-like receptor (TLR4)/NFκB pathway. Since this pathway also mediates the major priming mechanism of the NLRP3 inflammasome, cyclophosphamide would be expected to increase IL-1β maturation and downstream IL-6 production, as it did in the liver. How the same dose *blunts* the net inflammatory burden in the murine aorta, as shown in the current study by Sato-Okabayashi et al, [9] warrants further systematic investigation.

The pro-atherosclerotic effects of cyclophosphamide are likely due to its reactive metabolite acrolein, [17] an aldehyde intermediate that readily conjugates with thiol-containing residues of proteins including low-density lipoprotein, glutathione, serum albumin, fibrinogen and thioredoxin. Acrolein-adducts have been identified in human aortic atherosclerotic lesions and in plasma and aorta of rats receiving short-term low-dose cyclophosphamide (30 mg/kg/day for 10 days) [18]. The immediate consequence of oxidative protein-modification by acrolein is depletion of front-line antioxidant defenses, while longer-lasting DAMP-like actions will arise from engagement of CD36 scavenger receptors. One such action is the induction of endoplasmic reticulum (ER) stress, which is functionally upstream of NLRP3 inflammasome activation, and as such, can be seen as a significant individual driver of atherosclerosis. The potential atherosclerosis protective benefits of cyclophosphamide may therefore be self-limited through the generation of pro-atherogenic acrolein. One means of negating acrolein-mediated toxicity may be the adjunct use of organ protectants with acrolein-scavenging capacity, such as mesna. Sodium 2-mercaptoethanesulphonate (Uromitexan®) is used to prevent the uremic toxicity of acrolein-generating chemotherapies, but also shows direct anti-inflammatory, anti-oxidant and anti-apoptotic actions in various tissues. The substance itself is well-tolerated, and merits evaluation as an adjuvant to boost the atheroprotective actions of cyclophosphamide.

The renaissance and repurposing of traditional immunomodulators for cardiovascular protection is an emerging concept that requires careful assessment of risks and benefits. Exploration of co-therapies such as mesna to limit adverse actions is highly warranted, with potential relevance also for newer cardiotoxic agents such as the immune check-point inhibitors [19].

Disclosures

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References

[1] P.M. Ridker, B.M. Everett, T. Thuren, J.G. MacFadyen, W.H. Chang, C. Ballantyne, F. Fonseca, J. Nicolau, W. Koenig, S.D. Anker, J.J.P. Kastelein, J.H. Cornel, P. Pais, D. Pella, J. Genest, R. Cifkova, A. Lorenzatti, T. Forster, Z. Kobalava, L. Vida-Simiti, M. Flather, H. Shimokawa, H. Ogawa, M. Dellborg, P.R.F. Rossi, R.P.T. Troquay, P. Libby, Glynn R.J. Antiinflammatory, Therapy with Canakinumab for Atherosclerotic Disease, N. Engl. J. Med. 377 (1119–1131) (2017), https://doi.org/10.1056/NEJMoa1707914.

[2] M. Vaccarezza, C. Balla, P. Rizzo, Atherosclerosis as an inflammatory disease: Doubts? No more, Int. J. Cardiol. Heart Vasc. 19 (1–2) (2018), https://doi.org/10.1016/j.ijcha.2018.03.003.

[3] Y. Muramatsu, Y. Minami, A. Kato, A. Katsura, T. Sato, R. Kakizaki, T. Nemoto, T. Hashimoto, K. Fujiyoshi, K. Meguro, T. Shimohama, J. Ako, Lipoprotein (a) level is associated with plaque vulnerability in patients with coronary artery disease: An optical coherence tomography study, Int. J. Cardiol. Heart Vasc. 24 (100382) (2019), https://doi.org/10.1016/j.ijcha.2019.100382.

[4] D. Gomez et al, H and Owens GK. Interleukin-1beta has atheroprotective effects in advanced atherosclerotic lesions of mice, Nat. Med. 24 (1418–1429) (2018), https://doi.org/10.1038/s41591-018-0124-5.

[5] J.C. Tardif, S. Kouz, D.D. Waters, O.F. Bertrand, R. Diaz, A.P. Maggioni, F.J. Pinto, R. Ibrahim, H. Gamra, G.S. Kiwan, C. Berry, J. Lopez-Sendon, P. Ostadal, W. Koenig, D. Angoulvant, J.C. Gregoire, M.A. Lavoie, M.P. Dube, D. Rhainds, M. Provencher, L. Blondeau, A. Orfanos, P.L. L'Allier, M.C. Guertin, F. Roubille, Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction, N. Engl. J. Med. 381 (2497–2505) (2019), https://doi.org/10.1056/NEJMoa1912388.

[6] S.M. Nidorf, A.T.L. Fiolet, J.W. Eikelboom, A. Schut, T.S.J. Opstal, W.A. Bax, C.A. Budgeon, J.G.P. Tijssen, A. Mosterd, J.H. Cornel, P.L. Thompson, The effect of low-dose colchicine in patients with stable coronary artery disease: The LoDoCo2 trial rationale, design, and baseline characteristics, Am. Heart J. 218 (46–56) (2019), https://doi.org/10.1016/j.ahj.2019.09.011.

[7] X. Huang, Q. Huang, Y. He, S. Chen, T. Li, Mycophenolic acid enhanced lipopolysaccharide-induced interleukin-18 release in THP-1 cells via activation of the NLRP3 inflammasome, Immunopharmacol. Immunotoxicol. 41 (521–526) (2019), https://doi.org/10.1080/08923973.2019.1652913.

[8] A. Le Bras, No benefit of methotrexate on the risk of cardiovascular events, Nat. Rev. Cardiol. 16 (2–3) (2019), https://doi.org/10.1038/s41569-018-0133-6.

[9] Y. Sato-Okabayashi, K. Isoda, B. Heissig, T. Kadoguchi, K. Akita, K. Kitamura, K. Shimada, K. Hattori, H. Daida, Low-dose Oral Cyclophosphamide Therapy Reduces Atherosclerosis Progression Due to Decreasing of Inflammatory Cells in a Murine Model of Atherosclerosis. Int. J. Cardiol. Heart Vasc. 28 (100529) (2020). https:/doi.org//10.1016/j.ijcha.2020.100529.

[10] K. Kitamura, K. Isoda, K. Akita, K. Miyosawa, T. Kadoguchi, K. Shimada, H. Daida, Lack of IkappaBNS promotes cholate-containing high-fat diet-induced inflammation and atherogenesis in low-density lipoprotein (LDL) receptor-deficient mice, Int. J. Cardiol. Heart Vasc. 23 (100344) (2019), https://doi.org/10.1016/j.ijcha.2019.03.004.

[11] J. Schroder, N.D. Mygind, D. Frestad, M. Michelsen, H.E. Suhrs, K.B. Bove, I. Gustafsson, J. Kastrup, E. Prescott, Pro-inflammatory biomarkers in women with non-obstructive angina pectoris and coronary microvascular dysfunction, Int. J. Cardiol. Heart Vasc. 24 (100370) (2019), https://doi.org/10.1016/j.ijcha.2019.100370.

[12] A.O. Jackson, M.A. Regine, C. Subrata, S. Long, Molecular mechanisms and genetic regulation in atherosclerosis, Int. J. Cardiol. Heart Vasc. 21 (36–44) (2018), https://doi.org/10.1016/j.ijcha.2018.09.006.

[13] C.A. Roldan et al, Premature aortic atherosclerosis in systemic lupus erythematosus: a controlled transesophageal echocardiographic study, J. Rheumatol. 37 (71–78) (2010), https://doi.org/10.3899/jrheum.090665.

[14] L. Hu, Z. Chen, Y. Jin, B. Jiang, X. Wang, H. Yu, X. Yang, Incidence and predictors of aorta calcification in patients with systemic lupus erythematosus, Lupus 28 (275–282) (2019), https://doi.org/10.1177/0961203319826689.

[15] I. Mozos, G. Borzak, Caraba A and Mihaescu R. Arterial stiffness in hematologic malignancies, Onco. Targets Ther. 10 (1381–1388) (2017), https://doi.org/10.2147/ott.S126852.

[16] M. Chen, C. Zhang, J. Zhang, G. Kai, B. Lu, Z. Huang, L. Ji, The involvement of DAMPs-mediated inflammation in cyclophosphamide-induced liver injury and the protection of liquiritigenin and liquiritin, Eur. J. Pharmacol. 856 (172421) (2019), https://doi.org/10.1016/j.ejphar.2019.172421.
[17] A. Iqubal, M.K. Iqubal, S. Sharma, M.A. Ansari, A.K. Najmi, S.M. Ali, J. Ali, S.E. Haque, Molecular mechanism involved in cyclophosphamide-induced cardiotoxicity: Old drug with a new vision, Life Sci. 218 (112–131) (2019), https://doi.org/10.1016/j.lfs.2018.12.018.
[18] D. Arikketh, S. Niranjali, H. Devaraj, Detection of acrolein-lysine adducts in plasma low-density lipoprotein and in aorta of cyclophosphamide-administered rats, Arch. Toxicol. 78 (397–401) (2004), https://doi.org/10.1007/s00204-004-0556-1.
[19] L. Michel, T. Rassaf, M. Totzeck, Cardiotoxicity from immune checkpoint inhibitors, Int. J. Cardiol. Heart. Vasc. 25 (100420) (2019), https://doi.org/10.1016/j.ijcha.2019.100420.

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