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Macrophage CD31 Signaling in Dissecting Aortic Aneurysm

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ABSTRACT

BACKGROUND The authors recently found that a CD31 agonist peptide reaches macrophages in injured aortas and exerts beneficial effects on apolipoprotein E-knockout (Apo $E^{-/-}$) mice subjected to angiotensin (Ang) II infusion, a model of experimental acute aortic dissection and intramural hematoma (ADIM).

OBJECTIVES The purpose of this study was to evaluate the therapeutic potential of a drug-suitable agonist peptide in experimental ADIM.

METHODS P8RI, a retro-inverso sequence of the best candidate identified by functional in vitro screening of a peptide library, passed an absorption, distribution, metabolism, excretion and toxicology analysis. Apo $E^{-/-}$ mice (male, 28-week-old) implanted with Ang II-releasing pumps received P8RI (2.5 mg/kg/day) or vehicle from day 14 ($n = 10$/group). Leukocytes were analyzed by flow cytometry. Healing features of human and mouse dissected aortic segments were assessed by histology and immunofluorescence. The effect of CD31 on macrophages was evaluated using cells from $CD31^{-/-}$ mice and P8RI, in vitro.

RESULTS Human and experimental ADIM were characterized by the infiltration of proinflammatory macrophages. The absence of CD31 enhanced the proinflammatory polarization of macrophages, whereas the CD31 agonist P8RI favored reparative macrophages both in vitro and in vivo. The administration of P8RI after the occurrence of ADIM prevented aneurysmal transformation by promoting the resolution of intramural hematoma and the production of collagen in dissected aortas in vivo, associated with enrichment of M2 macrophages at the site of injury.

CONCLUSIONS CD31 signaling promotes the switching of proinflammatory macrophages to the reparative phenotype and favors the healing of experimental dissected aortas. Treatment with a drug-suitable CD31 agonist may facilitate the clinical management of ADIM. (J Am Coll Cardiol 2018;72:45–57) © 2018 by the American College of Cardiology Foundation.

Acute aortic dissection and intramural hematoma (ADIM) (dissecting aneurysm) is a life-threatening disease with a mortality of 50% within the first 48 h. In the absence of involvement of the ascending aorta, the clinical management is essentially palliative and is directed at reducing the systemic blood pressure and heart rate as much as possible, which limits the propagation of the false

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ABBREVIATIONS AND ACRONYMS

ADIM = acute aortic dissection and intramural hematoma

Ang II = angiotensin II

Apo E$^{-/-}$ = apolipoprotein E knockout

BMDM = bone marrow–derived macrophage

IL = interleukin

iNOS = inducible nitric oxide synthase

US = ultrasound

lumen and prevents end-organ damage and risk of rupture (1).

Due to the lack of specific therapeutic agents aimed at favoring rapid tissue healing, 25% to 30% of ADIM patients require subsequent intervention because of aneurysmal expansion, progressive dissection, and other complications from the unresolved dissection process (2). Interestingly, the rate of recurrent events is independent of the treatment strategy (open surgery, endovascular repair, or aggressive antihypertensive treatment) and occurs more frequently in patients affected by connective tissue disorders (3), highlighting the importance of an appropriate extracellular matrix response for the healing process and long-term prognosis.

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The outcomes of tissue healing after an acute injury essentially depend on the resolution of the initial inflammatory phase, and macrophages play a crucial role in this process. Immediately after entering the wound site, circulating monocytes contribute to the demolition phase of wound healing by acquiring a proinflammatory phenotype; however, for appropriate tissue healing, the monocytes must exert fundamental functions including the acquisition of a reparative phenotype (4). Importantly, the wound healing process may be consistently delayed and can even remain unachieved in the presence of blood-derived elements, as in the case of dissected aortas, which prevent the macrophages from switching from the proinflammatory to the reparative phase (5).

In this work, we sought to assess the role of CD31, an immunoregulatory receptor, in macrophage polarization and ADIM outcome in apolipoprotein E-knockout (Apo E$^{-/-}$) mice subjected to chronic angiotensin (Ang) II infusion, an experimental model of ADIM (6).

METHODS

IDENTIFICATION OF A DRUG-SUITABLE CD31 AGONIST PEPTIDE. The drug-suitable CD31 agonist used in this study was selected from 2 peptide libraries, as detailed in Online Table 1.

The absorption, distribution, metabolism, excretion, and toxicology analysis was performed on the retro-inverso sequence of the best candidate, termed P8RI, and included the measurement of potassium currents mediated by the human ether-a-go-go-related gene channel; bacterial reverse mutation test; blood half-life (pharmacokinetic studies) after intravenous, oral, and subcutaneous administration in C57BL/6 mice; and in vivo toxicity evaluated during a 14-day subcutaneous dose-range study in C57BL/6 mice, as detailed in Online Appendix 1.

ANALYSIS OF MOUSE MACROPHAGE POLARIZATION IN VITRO. Bone marrow–derived macrophages (BMDMs) were prepared and analyzed as described in Online Appendix 1 from the femurs of 10-week-old male CD31$^{+/+}$ and CD31$^{-/-}$ (7) mice derived in our animal facility by breeding CD31$^{+/-}$ mice (C57BL/6 background), which were generously provided by Dr. Debra K. Newman (Blood Center of Wisconsin, Milwaukee, Wisconsin).

ANG II INFUSION IN APO E$^{-/-}$ MICE. Twenty-eight-week-old male Apo E$^{-/-}$ mice (B6.129P2-ApoEtm1Unc/Crl, Charles River Laboratories, Saint Germain Nuelles, France) were maintained on a regular chow diet under standard conditions. Ang II (#A9525, 1 mg/kg/day, Sigma-Aldrich, St. Louis, Missouri) was continuously infused into the experimental mice via osmotic pumps (Model 2004, Alzet, Charles River Laboratories). All investigations conformed to the Directive 2010/63/EU of the European Parliament, and the local animal ethics committee (Comité d'éthique Bichat–Debré) granted formal approval.

ULTRASOUND IMAGING, HISTOLOGY, IMMUNOFLUORESCENCE, AND FLOW CYTOMETRY. Ultrasound imaging (US) was used to monitor aortic diameter changes over time and heart function in experimental mice. Microscopy and flow cytometry analyses were performed as detailed in Online Appendix 1.

STATISTICAL METHODS. The results are expressed as mean ± SEM. The differences among groups were evaluated by 1-way analysis of variance with Fisher post hoc tests or by Mann-Whitney nonparametric tests, as appropriate. Any differences between groups were considered to be significant when p values were <0.05. All analyses were performed with JMP 6.0 Software (SAS Institute Inc., Cary, North Carolina).

RESULTS

PROINFLAMMATORY MACROPHAGES LACKING CD31 EXPRESSION ACCUMULATE AT THE SITE OF INJURY IN ADIM. The sites of arterial wall dissection (detected as sites of elastin rupture) were consistently associated with the presence of proinflammatory M1 macrophages in both human (**Figure 1**) and experimental ADIM (Online Figure 1), further supporting the key role played by macrophages in the pathophysiology of aortic wall injury. Of note, CD31