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Inflammation Research

1. Histamine in allergy, inflammation, tissue gowth and repair

Evaluation of the effects of a novel carbon monoxide releasing molecule (CORM-3) in an in vitro model of cardiovascular inflammation.

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Introduction

Heme oxygenase 1 (HO-1) is a stress-responsive enzyme that is active during inflammatory reactions. Expression of HO-1 regulates inflammatory and immune responses, such as those involved in cardiac anaphylaxis [1], in allergic reactions [2] and in the rejection of transplanted organs [3]. Three products of HO-1 catalyzed heme breakdown can mediate these effects: CO, biliverdin and free iron (Fe^{2+}). Among them, a relevant role could be played by CO, a gaseous mediator that has been shown to be highly protective in several disease models, mimicking the action of HO-1 [4].

Previous reports from our group showed that exogenous CO or water-insoluble CO-releasing molecules (CORMs) were able to mimic the anti-allergic and anti-anaphylactic effects of heme oxygenase in isolated guinea pig hearts [1], in guinea pig mast cells and in human basophils [2, 5], mainly through the activation of the soluble guanylyl cyclase. Because the available CORMs were not water soluble, Motterlini's group synthesized new CO carriers, chemically modified to allow solubility in water [6]. Among them, tricarbonylchloro(glyc inate)ruthenium(II) (CORM-3) was chosen for the present study. The inactivated form of CORM-3 (iCORM), unable to release CO, was used as a negative control. The aim of the present study was to evaluate the effects of CORM-3 in a coincubation model of rat coronary endothelial cells with human neutrophils, activated with a chemotactic peptide.

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Materials and methods

Rat coronary endothelial cells (EC) were cultured in a Petri dish at the density of 8,000–10,000 cell/cm^2. About 50,000 human polimorphonucleated neutrophilic granulocytes (PMN), isolated from healthy donors, were suspended in the supernatant of the EC cultures. PMN were then activated by means of the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (fMLP) 10 nM, in the presence or in the absence of CORM-3 (10 nM–100 μM). In some experiments, superoxide dismutase (SOD, 300 IU/ml) was also used in the place of CORM-3 to assess the involvement of superoxide anion generation in the PMN-induced activation of EC. The activation of PMN was assessed through flow cytometric evaluation of CD11b expression on the cell surface, while EC activation was measured assessing the membrane expression of the activation marker CD54.

Results and discussion

The expression of CD54 on EC membranes was increased after incubation with PMN stimulated by fMLP (Fig. 1A). CORM-3 reduced the activation of EC, while the inactivate form, (iCORM), unable to release CO, was ineffective (Fig. 1B). PMN significantly increased the production of ROS upon activation with fMLP (data not shown) and, consistent with the hypothesis that superoxide anions play a role in endothelium activation, treatment of the cells with SOD mimicked the effects of CORM-3 (Fig. 1B). Finally, CORM-3 also reduced the activation of human PMN, assessed as the membrane expression of CD11b (Fig. 2A). The inactivated form of CORM-3 (iCORM) and SOD were ineffective (Fig. 2B).

In summary, CORM-3 was highly effective at reducing PMN-induced CD54 expression on EC. The effect was

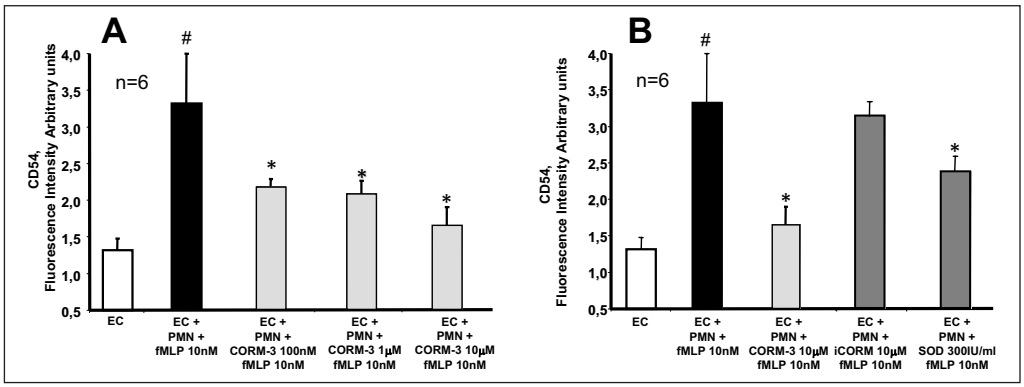

Fig. 1. The expression of CD54 on endothelial cell (EC) membranes was increased after incubation with neutrophils (PMN) stimulated by fMLP (*A*). CORM-3 reduced the activation of EC (A), while the inactivated form of the drug, iCORM, unable to release CO, was ineffective (*B*). Treatment of the cells with SOD mimicked the effects of CORM-3 (B). #p < 0.01 vs. basal; *p < 0.05 vs. EC + PMN + fMLP.

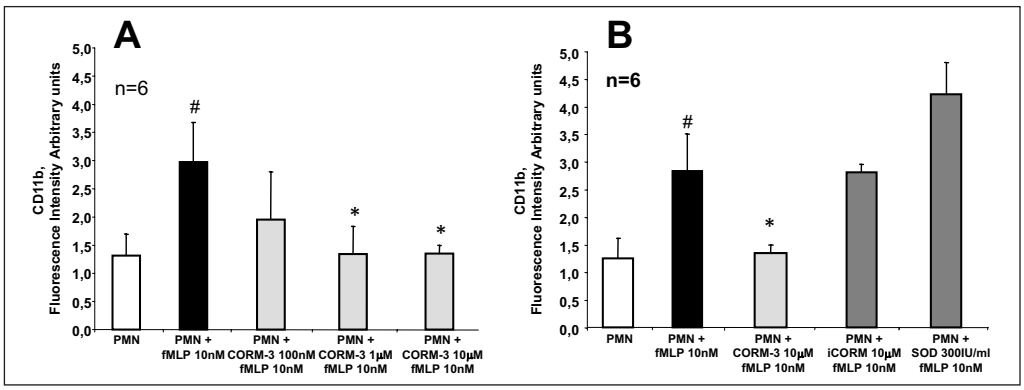

Fig. 2. CORM-3 reduced the activation of human PMN, assessed as membrane expression of CD11b (*A*). The inactivated form of CORM-3 (iCORM) and SOD were ineffective (*B*). #p < 0.01 vs. basal; *p < 0.05 vs. PMN + fMLP.

mediated by the release of CO, since the inactivated form of CORM-3 (iCORM) was completely ineffective. We can also suggest an involvement of superoxide anions, since the activation of EC was completely reversed by incubating the cells with SOD. CORM-3 also reduced the activation of PMN, as evidenced by decreased expression of CD11b on the cell membrane, while SOD was ineffective. The production of reactive oxygen species and the degranulation of PMN appear to be two parallel aspects of cell activation, and the scavenging role of SOD is apparent after CD11b expression has occurred.

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