High mobility group box 1 suppresses smooth muscle tension in rat aorta via Toll-like receptor 4-dependent upregulation of iNOS

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<Background> Activation of Toll-like receptor 4 (TLR4) by lipopolysaccharide (LPS) causes upregulation of iNOS in vascular endothelial cells followed by reduction of vascular smooth muscle tension, which plays a crucial role in development of septic shock. High mobility group box 1 (HMGB1), a nucleus protein containing three cysteine residues, is passively and actively released from necrotic cells and activated macrophages, respectively, thereby playing a pro-inflammatory role. HMGB1 exists in redox-dependent distinct active forms, i.e. all-thiol-HMGB1 (at-HMGB1) and disulfide-HMGB1 (ds-HMGB1) which activate the receptor for advanced glycation end-products (RAGE) and TLR4, respectively. Functional RAGE and TLR4 are expressed in endothelial cells, whereas the effects of HMGB1 on expression of iNOS or vascular tension have yet to be evaluated. In the present study, we thus examined if HMGB1 alters muscular tonus and iNOS expression in rat aorta.

<Methods> Isometric tension was recorded in endothelium-intact ring preparations prepared from rat thoracic aorta. Before the recording, the preparations were preincubated with LPS, at-HMGB1, ds-HMGB1 or vehicle for 20 h at 37ºC in a standard cell culture medium. Inhibitors were added 10 min before the onset of preincubation. Phenylephrine (Phe)-induced vasoconstriction was observed in the absence and presence of L-NAME, an NOS inhibitor, in order to estimate the L-NAME-induced tension augmentation. Levels of iNOS protein expression were determined by Western blotting.

<Results> Preincubation with LPS at 100 ng/ml markedly suppressed Phe-induced vasoconstriction of the aortic ring. L-NAME augmented the Phe-induced vasoconstriction, and the magnitude of L-NAME-induced acceleration of vasoconstriction was increased by preincubation with LPS. Preincubation with ds-HMGB1, but not at-HMGB1, at 0.01 mg/ml slightly but significantly increased the NAME-induced augmentation of vasoconstriction, an effect accelerated by LPS at 0.1 ng/ml, a subeffective concentration. Treatment with ds-HMGB1 tended to increase iNOS expression, an effect enhanced by LPS at the subeffective concentration. The synergistic effects of ds-HMGB1 and LPS on the L-NAME-induced augmentation of vasoconstriction was prevented by TAK-242, a TLR antagonist, but not by TH1020, a TLR5 antagonist.

<Conclusions> Our data suggest that ds-HMGB1, but not at-HMGB1, reduces vascular tone by accelerating TLR4-dependent upregulation of iNOS in rat aorta.