Peripheral HMGB1-induced hyperalgesia in mice: Redox state-dependent distinct roles of RAGE and TLR4

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Nuclear HMGB1 that contains 3 cysteine residues is acetylated and secreted to the extracellular space, promoting inflammation via multiple molecules such as RAGE and TLR4. We thus evaluated and characterized the redox state-dependent effects of peripheral HMGB1 on nociception. Intraplantar (i.pl.) administration of bovine thymus-derived HMGB1 (bt-HMGB1), all-thiol HMGB1 (at-HMGB1) or disulfide HMGB1 (ds-HMGB1) caused long-lasting mechanical hyperalgesia in mice. The hyperalgesia following i.pl. bt-HMGB1 or at-HMGB1 was attenuated by RAGE inhibitors, while the ds-HMGB1-induced hyperalgesia was abolished by a TLR4 antagonist. Thus, nociceptive processing by peripheral HMGB1 is considered dependent on its redox states.

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High mobility group box 1 (HMGB1), a DNA binding protein, is passively released from necrotic cells and actively secreted by certain cells such as macrophages, playing a pro-inflammatory role as damage-associated molecular patterns (DAMPs) (1). In macrophages, nuclear HMGB1 is acetylated by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs), and the acetylated HMGB1 is translocated to the cytoplasm and then packaged to secretory lysosomes, followed by its active secretion to the extracellular space (2). The extracellular HMGB1 targets multiple molecules including Toll-like receptor 4 (TLR4), TLR2 and the receptor for advanced glycation endproducts (RAGE), and also preferentially interacts with TLR4 (3). Nuclear HMGB1 that contains 3 cysteine residues is acetylated and secreted to the extracellular space, promoting inflammation via multiple molecules such as RAGE and TLR4. We thus evaluated and characterized the redox state-dependent effects of peripheral HMGB1 on nociception. Intraplantar (i.pl.) administration of bovine thymus-derived HMGB1 (bt-HMGB1), all-thiol HMGB1 (at-HMGB1) or disulfide HMGB1 (ds-HMGB1) caused long-lasting mechanical hyperalgesia in mice. The hyperalgesia following i.pl. bt-HMGB1 or at-HMGB1 was attenuated by RAGE inhibitors, while the ds-HMGB1-induced hyperalgesia was abolished by a TLR4 antagonist. Thus, nociceptive processing by peripheral HMGB1 is considered dependent on its redox states.
kept at 22–24 °C under a 12-h day/night cycle with free access to food and water at least for 1 week before experiments. All animals were used with approval by the Committee for the Care and Use of Laboratory Animals at Kindai University, and all procedures employed in the present study were in accordance with the guidelines of the Committee for Research and Ethical Issues of IASP [www.iasp-pain.org/Education/Content.aspx?ItemNumber=1217]. Bovine thymus-derived HMGB1 (bt-HMGB1) was purchased from Shino-Test Corp. (Tokyo, Japan), and recombinant all-thiol-HMGB1 (at-HMGB1) and disulfide-HMGB1 (ds-HMGB1) were from HMGBiotech (Milan, Italy). Trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA) and AMD3100 were purchased from Sigma–Aldrich (St. Louis, MO, USA), and lipopolysaccharide from Rhodobacter sphaeroides (LPS-RS) was from InvivoGen (San Diego, CA, USA). Low molecular weight heparin (LMWH) was a gift from Fuso Pharmaceutical Industries, Ltd. (Osaka, Japan). TSA and SAHA were dissolved in DMSO and then diluted with saline (the final DMSO concentration was 0.3%), and all other chemicals were dissolved in saline. An anti-human HMGB1 neutralizing rat monoclonal antibody and the control IgG were produced by Dr. Nishibori (Okayama University Graduate School of Medicine, Okayama, Japan), and dissolved in 0.01 M phosphate-buffered

Fig. 1. The mechanical hyperalgesia following intraplantar administration of bovine thymus-derived HMGB1 or HDAC inhibitors in mice. Mice received intraplantar (i.pl.) administration of bovine thymus-derived HMGB1 (bt-HMGB1) at 1–1000 ng/paw (A, D–I) or of TSA and SAHA, HDAC inhibitors, 3 or 30 pmol/paw (B, C) in a volume of 10 μl. The anti-HMGB1 neutralizing antibody or the control IgG at 1 mg/kg (D, E), LMWH, known to inhibit RAGE, at 2.5 mg/kg (F), LPS-RS, a TLR4 antagonist, at 0.5 mg/kg (H) or AMD3100 at 5 mg/kg (I) was administered i.p. 30 min before i.pl. bt-HMGB1 at 100 ng/paw and before i.pl. TSA or SAHA at 30 pmol/paw. The anti-RAGE neutralizing antibody or the control IgG at 100 ng/paw was administered i.pl. 1 h before i.pl. bt-HMGB1 at 100 ng/paw (G). Data show the mean ± SEM of the nociceptive threshold or of the AUC of the time–threshold curve between 1.5 and 3 h after challenge with bt-HMGB1 or HDAC inhibitors. The number of mice: 4–6 (A), 5–10 (B), 6–12 (C), 5 (D), 7–9 (E), 5–9 (F), 6–8 (G), 6–7 (H) and 4 (I). *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle (V) (A–C) or V + V (D–I); |P < 0.05, |P < 0.01 vs. IgG + bt-HMGB1 (D, G), V + TSA or SAHA (E), and V + bt-HMGB1 (F, H, I).
saline (PBS). The anti-rat RAGE rabbit polyclonal antibody and the control rabbit IgG were obtained from Sigma–Aldrich and Cell Signaling Technology (Danvers, MA, USA), respectively, and dissolved in 0.1 M PBS. In the von Frey test, the mice were placed and acclimatized on a risen wire mesh floor, covered with a clear plastic box (10 × 10 × 10 cm), and the mid-plantar surface of the right hindpaw was stimulated with von Frey filaments of distinct strength (0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 0.6 and 1.0 g). The nociceptive threshold was determined according to the up-down method (10). In some experiments, the area under the curve (AUC) was calculated from the time–threshold curve between 1.5 and 3 h after intraplantar (i.pl.) injection of test compounds. Data

Fig. 2. The mechanical hyperalgesia caused by intraplantar administration of all-thiol-HMGB1 or disulfide-HMGB1 in mice. Mice received intraplantar (i.pl.) all-thiol-HMGB1 (at-HMGB1) (A, C, D) or disulfide-HMGB1 (ds-HMGB1) (B, E, F) at 1–100 ng/paw in a volume of 10 μl. LMWH, known to inhibit RAGE, at 2.5 mg/kg (C, E) or LPS-RS, a TLR4 antagonist, at 0.5 mg/kg (D, F) was administered i.p. 30 min before i.pl. HMGB1. Data show the mean ± SEM of the nociceptive threshold or of the AUC of the time–threshold curve between 1.5 and 3 h after HMGB1 challenge. The number of mice: 5–8 (A), 5–7 (B), 5–9 (C), 5–8 (D), 4–6 (E), and 4–6 (F). *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle (V) (A, B) or V + V (C–F); |P < 0.05, ||P < 0.01 vs. V + at-HMGB1 (C) or V + ds-HMGB1 (F).
are shown as mean ± S.E.M. Non-parametric analyses were performed by Mann–Whitney's U-test for comparison of two-group data and by Kruskal–Wallis H-test followed by a least significant difference-type test for comparison of three or more group data. Intraplantar injection of bt-HMG1 caused long-lasting hyperalgesia in a dose rage of 100–1000 ng/paw (Fig. 1A), as seen in our previous study using rats (8). Given evidence that decreased HDAC activity promotes the activation, cytotoxic translocation and extracellular release of HMG1 (11), we asked if HDAC inhibitors produce pro-nociceptive activity. TSA and SAHA, distinct HDAC inhibitors, when administered i.p. at 3–30 pmol/paw, mimicked the long-lasting hyperalgesia (Fig. 1B, C), as caused by i.p. bt-HMG1 (Fig. 1A). Systemic (i.p.) administration of the anti-HMG1 neutralizing antibody at 1 mg/kg abolished the hyperalgesia induced by i.p. bt-HMG1 at 100 ng/paw (Fig. 1D), and partially prevented the hyperalgesia following i.p. TSA or SAHA at 30 pmol/paw (Fig. 1E). The hyperalgesia caused by i.p. bt-HMG1 was partially but significantly inhibited by i.p. administration of LMWH at 2.5 mg/kg, known to directly block RAGE (12), and by i.p. administration of the anti-RAGE neutralizing antibody at 100 ng/paw (Fig. 1E, G). In contrast, the bt-HMG1-induced hyperalgesia was only slightly attenuated by i.p. LPS-RS, a TLR4 antagonist, at 0.5 mg/kg and unaffected by i.p. AMD3100, a CXCR4 antagonist, at 5 mg/kg (Fig. 1H, I). As did bt-HMG1, i.p. administration of recombinant at-HMG1 or ds-HMG1 produced long-lasting hyperalgesia (Fig. 2A, B). The effective dose range of at-HMG1 was 100 ng/paw or more (Fig. 2A), equivalent to bt-HMG1 (see Fig. 1A), while that of ds-HMG1 was 10–100 ng/paw (Fig. 2B), much more potent than at-HMG1 (see Fig. 2A) and bt-HMG1 (see Fig. 1A). The hyperalgesia caused by i.p. at-HMG1 was partially inhibited by i.p. LMWH (Fig. 2C), but not by i.p. LPS-RS (Fig. 2D), being similar to the characteristics of the bt-HMG1-induced hyperalgesia (see Fig. 1E, H). In contrast, the i.p. ds-HMG1-induced hyperalgesia was abolished by i.p. LPS-RS (Fig. 2F), but resistant to i.p. LMWH (Fig. 2E).

Our data demonstrate that peripheral at-HMG1 promotes mechanical nociception at least in part through activation of RAGE, but not TLR4, while peripheral ds-HMG1 produces more potent pro-nociceptive activity exclusively via activation of TLR4, but not RAGE. Considering that the anti-HMG1 neutralizing antibody partially blocked the hyperalgesia induced by i.p. HDAC inhibitors (see Fig. 1E), it is hypothesized that the accelerated acetylation and secretion of endogenous HMG1 following HDAC inhibition might contribute, at least in part, to promotion of nociceptive processing. In general, HDAC inhibition is considered analgesic, whereas there is evidence that the epigenetic modulation by HDAC inhibition restores nerve injury-induced down-regulation of Na\(_\text{v1.8}\) channels and subsensitivity of C-fiber neurons (13). Thus, the possibility cannot be ruled out that the epigenetic modulation of unknown pro-nociceptive molecules by HDAC inhibition is involved in the HMG1-independent portion of the hyperalgesic effect of HDAC inhibitors. The bt-HMG1 is considered almost in the all-thiol form, since the potency and characteristics of the pro-nociceptive activity of bt-HMG1 were similar to the recombinant at-HMG1. The finding that LPS-RS slightly reduced the hyperalgesia induced by bt-HMG1 or at-HMG1 (see Figs. 1H and 2D), suggests that a small amount of bt-HMG1/at-HMG1, administered i.p., might be oxidized to ds-HMG1 in the tissue. It is necessary to test whether the hyperalgesia induced by i.p. ds-HMG1 or at-HMG1 involves the direct or indirect activation of TLR2, another target for ds-HMG1, since TLR2 may also play a pro-nociceptive role (14). The pro-nociceptive role of CXCR4 has been reported elsewhere (15), whereas it does not appear to contribute to the bt-HMG1-induced hyperalgesia (see Fig. 1I). This finding may be in agreement with the report that at-HMG1 is incapable of activating CXCR4 unless forming a heterocomplex with CXCL12 (3).

In conclusion, our study demonstrates that both at-HMG1 and ds-HMG1 in the peripheral tissue are capable of causing mechanical hyperalgesia via activation of RAGE and TLR4, respectively, in mice, and that the latter is more potent than the former as a nociceptive promoter. Thus, the pro-nociceptive property for peripheral HMG1 are considered dependent on its redox-state.

Conflict of interest

The authors declare that there are no conflicts of interest.

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