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Involvement of high mobility group box 1 in the development and maintenance of chemotherapy-induced peripheral neuropathy in rats

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ABSTRACT

Given that high mobility group box 1 (HMGB1), a nuclear protein, once released to the extracellular space, promotes nociception, we asked if inactivation of HMGB1 prevents or reverses chemotherapy-induced painful neuropathy in rats and also examined possible involvement of Toll-like receptor 4 (TLR4) and the receptor for advanced glycation endproduct (RAGE), known as targets for HMGB1. Painful neuropathy was produced by repeated i.p. administration of paclitaxel or vincristine in rats. Nociceptive threshold was determined by the paw pressure method and/or von Frey test in the hindpaw. Tissue protein levels were determined by immunoblotting. Repeated i.p. administration of the anti-HMGB1-neutralizing antibody or recombinant human soluble thrombomodulin (rhsTM), known to inactivate HMGB1, prevented the development of hyperalgesia and/or allodynia induced by paclitaxel or vincristine in rats. A single i.p. or intraplantar (i.pl.) administration of the antibody or rhsTM reversed the chemotherapy-induced neuropathy. A single i.pl. administration of a TLR4 antagonist or low molecular weight heparin, known to inhibit RAGE, attenuated the hyperalgesia caused by i.pl. HMGB1 and also the chemotherapy-induced painful neuropathy. Paclitaxel or vincristine treatment significantly decreased protein levels of HMGB1 in the dorsal root ganglia, but not sciatic nerves. HMGB1 thus participates in both development and maintenance of chemotherapy-induced painful neuropathy, in part through RAGE and TLR4. HMGB1 inactivation is considered useful to prevent and treat the chemotherapy-induced painful neuropathy.

1. Introduction

High mobility group Box 1 (HMGB1), a nuclear DNA-binding protein, regulates transcriptional activities as a structural co-factor in mammalian cells. HMGB1, once released passively from dying cells, plays pro-inflammatory roles as one of damage-associated molecular patterns (DAMPs), while it is also actively secreted by certain cells including macrophages (Malarkey and Churchill 2012; Yanai et al., 2012). The nuclear HMGB1 can be acetylated by histone acetyltransferases (HATs) such as, cyclic-AMP regulatory element binding protein (CREB)-binding protein (CBP) and p300/CBP-associated factor (PCAF) (Ong et al., 2012; Wu et al., 2012), and deacetylated by histone deacetylases (HDACs), particularly HDAC1 and HDAC4 (Evankovich et al., 2010). The acetylated HMGB1 is translocated to the cytoplasm, and then released to the extracellular space following its packaging into secretory lysosomes that is regulated by calcium/calmodulin-dependent protein kinase (Zhang et al., 2011). Accumulating evidence strongly suggests that HMGB1 plays a pronociceptive role in processing somatic and visceral pain (Agalave and Svensson 2014; Tanaka et al., 2013, 2014). We have demonstrated the pronociceptive role of peripheral HMGB1 in rodents with inflammatory hyperalgesia induced by intraplantar administration of lipopolysaccharide (Tanaka et al.,

Abbreviations: APC, activated protein C; CBP, cyclic-AMP regulatory element binding protein (CREB)-binding protein; DIC, disseminated intravascular coagulation; DRG, dorsal root ganglia; GAPDH, glyceraldehyde-3-phosphoate dehydrogenase; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMGB1, high mobility group box 1; i.pl., intraplantar; LPS-RS, lipopolysaccharide from *Rhodobacter sphaeroides*; LMWH, low molecular weight heparin; PCAF, p300/CBP-associated factor; RAGE, receptor for advanced glycation endproducts; rhsTM, recombinant human soluble thrombomodulin; TLR4, Toll-like receptor 4.

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2013) and with bladder pain accompanying cyclophosphamide-induced cystitis (Tanaka et al., 2014). The pronociceptive roles of HMGB1 in the spinal cord and/or dorsal root ganglia (DRG) have been well described in the inflammatory pain mouse model with the collagen antibody-induced arthritis (Agalave et al., 2014) and in the rodents with the neuropathy induced by surgical nerve injury (Allette et al., 2014; Feldman et al., 2012; He et al., 2013; Kuang et al., 2012; Nakamura et al., 2013; Otoshi et al., 2011; Shibasaki et al., 2010) or by genetic type 2 diabetes (Ren et al., 2012). Nonetheless, it remains unclear whether HMGB1 participates in chemotherapy-induced neuropathic pain which could limit the life-saving cancer therapy.

Thrombomodulin, expressed on vascular endothelial cells, is a membrane protein which is composed of five domains: the N-terminal lectin-like domain [TM domain (TM-D) 1], EGF like domain (TM-D2), O-glycosylation-rich domain (TM-D3), *trans*-membrane domain (TM-D4) and C-terminal cytoplasmic domain (TM-D5) (Conway, 2012). TM-D2 directly or indirectly inhibits thrombin-dependent coagulation by binding to thrombin or promoting the formation of activated protein C (APC). On the other hand, TM-D1 binds to HMGB1 and facilitates its degradation by thrombin. Recombinant human soluble thrombomdulin (rhsTM) that lacks TM-D4 and TM-D5 is used for the clinical treatment of disseminated intravascular coagulation (DIC) in Japan. Our previous study has shown that rhsTM prevents inflammatory pain and bladder pain and is available as a novel analgesic for treatment of HMGB1-mediated pain (Tanaka et al., 2013, 2014).

In the present study, to inactivate endogenous extracellular HMGB1, we used the rat anti-HMGB1-neutralizing monoclonal antibody (Liu et al., 2007) and rhsTM that blocks HMGB1-dependent pain by sequestering HMGB1 and promoting it degradation by thrombin (Ito et al., 2008; Tanaka et al., 2013, 2014), and evaluated their preventive and/or therapeutic potentials in rats with the neuropathy induced by paclitaxel or vincristine, anti-cancer agents.

2. Materials and methods

2.1. Animals

Male Wistar rats (7–10 weeks old) were obtained from Kiwa laboratory Animals Co., Ltd. (Wakayama, Japan). Three to five rats were group-housed in each cage, maintained under a 12-h light/dark cycle and allowed access to water and food *ad libitum*. All animals were used with approval by Kindai (formerly Kinki) University's Committee for the Care and Use of Laboratory Animals, and all procedures were in accordance with the Guiding Principles approved by The Japanese Pharmacological Society and with the guidelines for animal experimentation of the International Association for the Study of Pain.

2.2. Major chemicals

The rat anti-HMGB1-neutralizing monoclonal antibody and control rat IgG were made in house, the specificity of the antibody being described elsewhere (Liu et al., 2007). The chicken anti-HMGB1-neutralizing polyclonal antibody, the control chicken IgY and HMGB1 from bovine thymus were purchased from SHINO-TEST Corporation (Kanagawa, Japan). Recombinant human soluble thrombomodulin (rhsTM) was provided by Asahi Kasei Pharma (Tokyo, Japan). Lipopolysaccharide from *Rhodobacter sphaeroides* (LPS-RS) was obtained from InvivoGen (San Diego, CA, USA). Low molecular weight heparin (LMWH; molecular weight, 4500–6500; 79.5 U/mg), known to inhibit receptor for advanced glycation endproducts (RAGE) (Liu et al., 2009; Myint et al., 2006; Takeuchi et al., 2013), was a gift from Fuso Pharmaceutical Industries, Ltd. (Osaka, Japan). The rat anti-HMGB1 monoclonal antibody and rat IgG were dissolved in 0.01 M PBS. The chicken anti-HMGB1 polyclonal antibody and chicken IgY were dissolved in 0.2 M PBS. The rhsTM was dissolved in 0.002% Tween 80-containing saline. HMGB1, LPS-RS or LMWH was dissolved in saline.

2.3. Assessment of nociceptive threshold and paw swelling in rats

Nociceptive threshold was measured by the paw pressure test using an analgesia meter (MK-300, Muromachi Kikai Co., Tokyo, Japan) or von Frey test according to the up-down method. In the paw pressure test, pressure was applied to the hindpaw of rats at a linearly increasing rate of 30 g/s. The paw withdrawal threshold was determined and expressed as the percentage of the baseline value, and a cut-off value of 500 g was used to avoid damage to the paw (Kawabata et al., 2007). In the von Frey test, rats were placed in a plastic cage (34.2 × 29.4 × 17.8 cm) with a wire mesh bottom. After 1-h acclimatization in the cage, the hindpaw was stimulated with one of 8 distinct von Frey filaments with strengths of 2, 4, 6, 8, 10, 15, 26 and 60 g for 6 s and the 50% nociceptive threshold was determined as reported elsewhere (Chaplan et al., 1994). After the baseline threshold was determined 2–3 times daily for 3-5 days, the animals were randomly allocated to test groups without considering the responses to the chemotherapeutic drugs. The paw swelling was assessed by measuring the paw thickness using a tissue caliper with 0.05 mm accuracy, as described previously (Tanaka et al., 2013).

2.4. Creation of chemotherapy-induced neuropathic pain models in rats

A chemotherapy-induced neuropathic pain model was created as described previously (Polomano et al., 2001). After measurements of baseline nociceptive thresholds, rats were administered i.p. paclitaxel or vincristine according to the following schedules. Paclitaxel at 2 mg/kg [prepared with saline from 6 mg/mL solution in 50% Cremophor EL® (polyethoxylated castor oil) and 50% ethanol, Taxol®, Bristol-Myers Squibb, Co. Ltd., Tokyo, Japan] was repeatedly administered i.p. every two days (day 0, 2, 4 and 6), 4 times in all. Vincristine at 0.1 mg/kg (prepared in saline, Wako Pure Chem., Osaka, Japan) was administered i.p. daily in two 5-day cycles with 2-day break between cycles (day 0–4 and 7–11), 10 times in total (Flatters and Bennett 2004; Weng et al., 2003).

2.5. Drug administration schedules

For the assessment of their preventive effects on the chemotherapy-induced neuropathy, the rat anti-HMGB1 antibody at 1 mg/kg or rhsTM at 1–10 mg/kg was repeatedly administered i.p. to rats once a day for 7 days during paclitaxel treatment or for two 5-day cycles of vincristine treatment. Some rats received daily intraplantar (i.pl.) administration of the rat anti-HMGB1 antibody at 50 μg/paw in a volume of 100 μl, according to the same schedules during paclitaxel treatment. To assess their therapeutic effects on the chemotherapy-induced neuropathy, the rat anti-HMGB1 antibody at 1 mg/kg or rhsTM at 10 mg/kg was administered i.p. 14 days or more after the onset of paclitaxel or vincristine treatment. In some experiments, rats received i.pl. administration of the rat or chicken anti-HMGB1-neutralizing antibody at 50 μg/paw, LPS-RS at 50 μg/paw or LMWH at 10 μg/paw 14 days or more after the onset of paclitaxel or vincristine treatment. In separate experiments, the rats received i.pl. administration of LPS-RS at 50 μg/paw or LMWH at 10 μg/paw in a volume of 20 μl, 30 min before i.pl. administration of HMGB1 in a volume of 100 μl.