Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
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Hyaluronic acid as a rescue therapy for trinitrobenzene sulfonic acid-induced colitis through Cox-2 and PGE_2 in a Toll-like receptor 4-dependent way*

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Received Oct. 17, 2010; Revision accepted Mar. 10, 2011; Crosschecked Aug. 16, 2011

Abstract: We hypothesized whether systemic administration of high-molecular-weight hyaluronic acid (HMW HA) could rescue trinitrobenzene sulfonic acid (TNBS)-induced colitis through Toll-like receptor 4 (TLR4) signal. C3H/HeN mice and C3H/HeJ mice were used. Mice were divided into four groups: control, 50% ethanol treatment group, TNBS treatment group, and TNBS plus HA treatment group. The weight changes, clinical scores, macroscopic scores, and histological scores were recorded. Cyclooxygenase 2 (Cox-2) and prostaglandin E_2 (PGE_2) expressions were measured both in colons and peritoneal macrophages from these mice. HA was a rescue therapy for the colitis induced by TNBS only in C3H/HeN mice. The clinical score, macroscopic score, and histological score were much lower in C3H/HeN mice receiving TNBS plus HA treatment. Cox-2 and PGE_2 expressions only increased in C3H/HeN mice. These Cox-2 expressing cells were macrophages. HA can also promote the production of Cox-2 and PGE_2 in peritoneal macrophages from C3H/HeN mice. Our data demonstrated that HMW HA can rescue TNBS-induced colitis through inducing Cox-2 and PGE_2 expressions in a TLR4-dependent way. Macrophages may be the effector cells of HMW HA.

Key words: Trinitrobenzene sulfonic acid (TNBS) colitis, Therapy, Hyaluronic acid, Toll-like receptor
doi:10.1631/jzus.B1000362 **Document code:** A **CLC number:** R574.6

1 Introduction

Hyaluronic acid (HA), an important component of the extracellular matrix, is composed of repeating disaccharide units containing alternating D-glucuronic acid and *N*-acetyl glucosamine. Two kinds of HA have been found: high-molecular-weight (HMW HA, >500 kDa) and low-molecular-weight (LMW HA, <500 kDa) (Spicer and McDonald, 1998; Itano *et al*., 1999). These two kinds of HA have different functions. LMW HA exists mainly in the inflammatory conditions (Horton *et al*., 1998). It can act as an intracellular signaling molecule and enhance productions of many cytokines, such as interleukin-8 (IL-8) (Bai *et al*., 2005; Mascarenhas *et al*., 2004; Boodoo *et al*., 2006). HMW HA exists predominantly in the physiological conditions and maintains the structural integrity of the extracellular matrix. It has been shown to be protective in many inflammation models including acute liver injury, bleomycin-induced lung injury, and sepsis-induced lung injury, etc. (Jiang *et al*., 2005; Liu *et al*., 2008).

Studies showed that HA may play an important role in inflammatory bowel disease. HA expression increased in inflamed tissue sections from patients with ulcerative colitis or Crohn's disease compared to non-inflamed and less inflamed areas (de la Motte *et al*., 1999). Majors *et al*. (2003) incubated human colonic smooth muscle cells with dextran sulfate sodium (DSS) in vitro, and the results showed that the production of HA in smooth muscle cells increased after DSS stimulation. In a genetic model of ileitis

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* Project (No. C140404) supported by National Natural Science Foundation of China

in which tumor necrosis factor (TNF)-α is over-expressed, the production of HA also significantly increased (Collins *et al*., 2008).

Toll like receptors (TLRs) are widely distributed in the gastrointestinal tract. For example, TLR4 and myeloid differential protein-2 (MD-2) are highly expressed in the normal colonic epithelium (Janeway and Medzhitov, 1999). A deficiency in TLR4 and Myd88 leads to more severe symptoms in DSS colitis model, which suggests that TLR4 is important in colonic defence response. HA is one of the endogenous receptors of TLR4. HA binding to TLR4 can promote the protective component of the host response in sterile skin and lung injury models (Jiang *et al*., 2005; Taylor *et al*., 2007). Whether HA can work through TLR4 to participate in the host response to gastrointestinal injury is not widely explored. In this paper, we used C3H/HeN and C3H/HeJ mice to confirm this hypothesis. These two substrains are similar genetically, but there is a spontaneous mutation in C3H/HeJ mice at lipopolysaccharide response locus (mutation in TLR4 gene, *Tlr4lps*).

2 Materials and methods

2.1 Animals

Adult (6 weeks old) female C3H/HeN and C3H/HeJ mice were bought from Charles River Laboratories (Wilmington, MA, USA) and Jackson Laboratories (Bar Harbor, ME, USA), separately. All mice were fed standard laboratory food and tap water ad libitum. All the experimental procedures affecting the mice were approved by the Animal Studies Committee of West China Hospital of Sichuan University, China.

2.2 Experimental protocol

The colitis was induced by trinitrobenzene sulfonic acid (TNBS) as previously described (Fukata *et al*., 2006). Briefly, 25 mg/ml TNBS solution (100 μl) in 50% (v/v) ethanol was administrated into the colon via a thin round-tip needle equipped with a 1-ml syringe. The needle was inserted so that the tip was 4 cm proximal to the anal verge. Mice were held in a vertical position for 30 s after the injection.

All the mice were weighed and randomized into four groups: control, 50% ethanol treatment, TNBS plus HA treatment, and TNBS plus phosphate-buffered saline (PBS) treatment. Control mice received 0.1 ml of saline using the same technique. For mice treated with TNBS plus HA (30 mg/kg), HA was given by intraperitoneal injection at 2 h, 3 d, and 5 d after beginning the TNBS treatment. For mice receiving TNBS plus PBS treatment, PBS was given by intraperitoneal injection at the same time as HA injection. The weight changes were recorded at 1, 3, 5, and 7 d after TNBS treatment. Mice were sacrificed at 7 d after TNBS administration. At least eight mice were used for each group. The clinical-grade HMW HA (M_w 2×10^3 kDa; Healon) was from Physician Sales and Service (St. Louis, MO, USA).

Clinical severity was scored based on the following standards: (1) appearance of diarrhea, (2) signs of fecal blood (macroscopic signs of fecal blood), and (3) perfuse bleeding from anus.

Colons were cut longitudinally and the macroscopic damage score was assessed following Majors *et al.* (2003): 0, no damage; 1, localized hyperemia without ulceration; 2, linear ulceration without significant inflammation; 3, linear ulceration with inflammation in one site; 4, two or more major sites of inflammation and ulceration extending 1 cm; 5–8, one point added for each centimeter of ulceration beyond an initial 2 cm.

2.3 Histological assessment of colitis

After opening the colons, a 2-cm segment of the distal colon was fixed in 40 g/L formalin and embedded in paraffin. Hematoxylin and eosin (H & E)-stained sections were evaluated by an expert gastrointestinal tract pathologist who was blinded to the treatment. Each sample was scored according to Lahat *et al*. (2007) with slight modification: percentage of area involved, crypt loss, erosions, number of follicle aggregates, edema, and infiltration of mononuclear and polymorphonuclear cells (Table 1).

2.4 Isolation of peritoneal macrophages

Ice-cold PBS was injected into the peritoneal cavity of each mouse. This fluid was carefully collected and centrifuged at 1000 r/min for 10 min. The supernatant was then withdrawn, and the cell pellet was resuspended in RPMI 1640 medium to obtain 10^5 cells/ml. The cells were allowed to adhere for 3 h, and then washed three times with pre-warmed

Table 1 Histological scoring system*

Parameter	Scoring (0–20)
Percentage of area involved, crypt loss	0: Normal 1: <10% 2: 10% 3: 10%–15% 4: 15%–50%
Erosions	0: Intact epithelium 1: Involvement of the lamina propria 2: Involvement of the submucosa 3: Transmural ulceration
No. of follicle aggregates, edema, infiltration of mononuclear and polymorphonuclear cells	0: Absent 1: Weak 2: Moderate 3: Severe

* Lahat *et al*. (2007) with slight modification

PBS to remove nonadherent cells. The medium was then replaced with fresh RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum. A total of 2×10^6 to 3×10^6 cells were obtained from each mouse.

2.5 Immunofluorescent analysis for double staining

Horseradish peroxidase (HRP)-catalyzing immunohistochemical staining method was used as described as our previous study (Zhang *et al*., 2010). Non-specific bindings were blocked with 3% bovine serum albumin (BSA) and avidin-biotin blocking kit (Vector). Antigen retrieval was performed by immersing the slides in 10 mmol/L citrate buffer for 15 min in microwave. Primary antibodies included mouse monoclonal anti-cyclooxygenase 2 (anti-Cox-2) (BD Pharmingen; 1:50), rat anti-mouse F4/80 (for macrophage staining, AbD Serotec, Raleigh, NC; 1:50), rabbit monoclonal anti-TLR4 (Santa Cruz; 1:100), and goat anti-mouse macrophage inflammatory protein (MIP)-2 (R & D Systems, Minneapolis, MN; 1:100). Secondary antibodies included Cy3-labeled donkey anti-rat IgG (Jackson ImmunoResearch; 1:500), fluorescein isothiocyanate (FITC)-labeled goat anti-mouse (Jackson ImmunoResearch; 1:200), and FITC-labeled donkey anti-S-rabbit/goat IgG (Jackson ImmunoResearch; 1:250).

For hyaluronan staining, distal colon sections were stained with biotinylated hyaluronan-binding proteins (North Star; 1:100) and then detected with Alexa Fluor® 488 conjugate streptavidin (Invitrogen; 1:500).

2.6 Western blot analysis

Proteins were extracted from the colon via homogenization in ice-cold lysis buffer. The concentrations were measured using the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). Protein samples were separated on 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. Then the membranes were incubated with the primary antibody (mouse Cox-2, Cayman Chemical; 1:600) overnight at 4 °C, followed by the secondary antibodies (HRP-conjugated donkey anti-mouse/rabbit immunoglobulin; Amersham, GE Healthcare; 1:3000) at 24 °C for 1 h. The blots were detected by an enhanced chemiluminescence (ECL) system (Amersham, GE Healthcare, UK). Bands were scanned and semi-quantified by densitometry.

2.7 Prostaglandin E_2 assay

Prostaglandin E_2 (PGE_2) concentration was measured by enzyme-linked immunosorbent assay (ELISA) using a PGE_2 kit (Cayman Chemical) according to the manufacturer's directions. Triplicate aliquots of supernatant were measured for each sample.

2.8 Statistic analysis

All the data were expressed as mean±standard deviation (SD). The difference between groups was analyzed by Student's *t*-test. All the calculations were performed using Microsoft Excel 2003.

3 Results

3.1 HA expression only increased in C3H/HeN mice

HA expression was located in the lamina propria. After TNBS administration for 7 d, HA expression increased only in C3H/HeN mice, while no change was observed in C3H/HeJ mice (Fig. 1).

3.2 HA rescued the colitis induced by TNBS only in C3H/HeN mice

TNBS-treated mice developed a severe illness characterized by diarrhea, hematochezia, and weight loss. The mortality in mice only receiving TNBS was as high as 40%, while the mortality was 0% in the other three groups. TNBS administration in

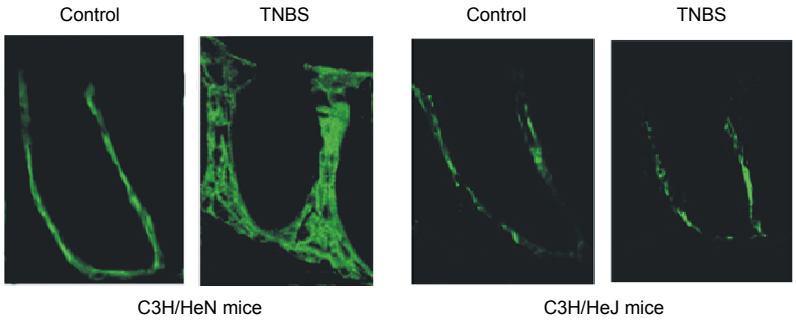

Fig. 1 HA expression in C3H/HeN and C3H/HeJ mice

After TNBS administration for 7 d, HA expression increased in C3H/HeN mice, but did not change in C3H/HeJ mice

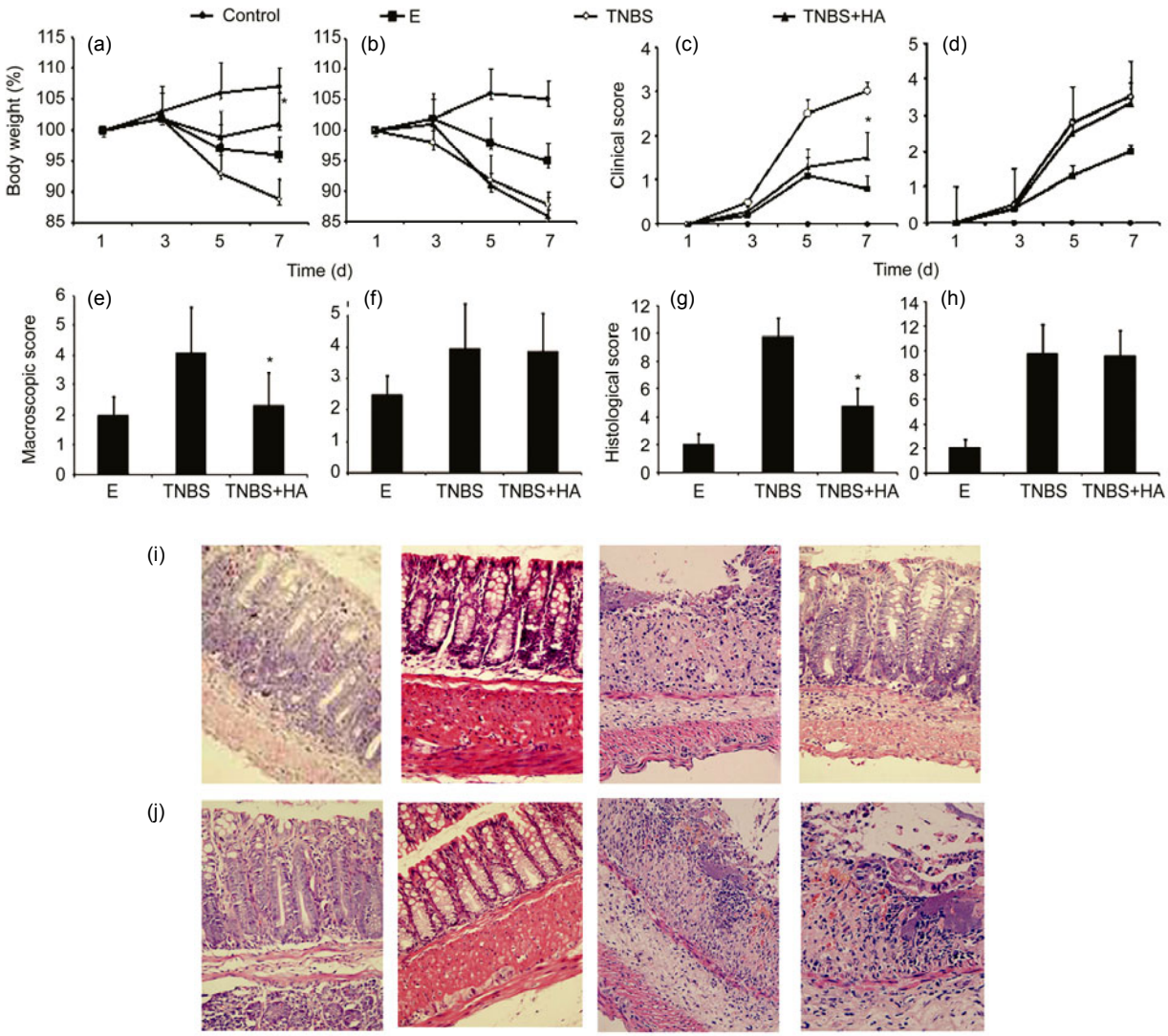

Fig. 2 Effect of HA on TNBS colitis in C3H/HeN and C3H/HeJ mice

(a, b) Body weight. HA maintained the body weight in C3H/HeN mice (a), but not in C3H/HeJ mice (b). (c, d) Clinical score. The clinical score was decreased by HA in C3H/HeN mice (c), but not in C3H/HeJ mice (d). (e, f) Macroscopic damage score. The macroscopic damage score was decreased by HA in C3H/HeN mice (e), but not in C3H/HeJ mice (f). (g, h) Histological score. The histological score was much lower in C3H/HeN mice (g) receiving HA administration, but remained unchanged in C3H/HeJ mice receiving HA (h). (i, j) Microscopic view of the colon. HA can alleviate the damage caused by TNBS only in C3H/HeN mice (i), not in C3H/HeJ mice (j). E: 50% ethanol; TNBS+HA: TNBS plus HA; * $P<0.05$, TNBS plus HA group compared to TNBS group

C3H/HeN mice resulted in a 12% loss of body weight over 7 d, whereas C3H/HeN mice receiving TNBS plus HA maintained their weight. There was no difference in body weight between TNBS and TNBS plus HA groups in C3H/HeJ mice (Figs. 2a and 2b).

The severity of colitis was decreased by HA in C3H/HeN mice. Seven days after TNBS or TNBS plus HA administration, the clinical score and the macroscopic damage score were much lower in TNBS plus HA treatment group (2.8±0.4 vs. 1.3±0.5; 4.2±1.2 vs. 2.2±1.3; P<0.05; Figs. 2c and 2e). Microscopic examination showed hyperemia, inflammation, and ulcer in mice receiving TNBS administration. The histological score was much lower in TNBS plus HA treatment group (9.5±1.5 vs. 4.5±1.1, P<0.05; Figs. 2i and 2g).

There were no differences in the clinical score, macroscopic damage score, or histological score between TNBS and TNBS plus HA groups in C3H/HeJ mice 7 d after administration (3.6±0.8 vs. 3.4±0.6; 4.2±1.2 vs. 4.1±1.3; 11.2±2.1 vs. 10.6±1.9; P>0.05; Figs. 2d, 2f, 2h, and 2j).

3.3 Cox-2 and PGE_2 expressions only increased in C3H/HeN mice

In C3H/HeN mice receiving TNBS plus HA treatment, the expression of PGE_2 significantly increased compared to mice receiving TNBS administration ((423±35) pg/ml vs. (250±26) pg/ml, P<0.05, Fig. 3a). On the contrary, the expression of PGE_2 was not affected by HA administration in C3H/HeJ mice (Fig. 3b). Cox-2 expression increased approximately three-fold in the distal colon in C3H/HeN mice, but remained unchanged in C3H/HeJ mice after TNBS plus HA treatment (Fig. 3c). These Cox-2 expressing cells were located in the lamina propria. Most of them were double stained with macrophages (Fig. 3d). The latter also expressed TLR4 and MIP-2 (Figs. 3e and 3f).

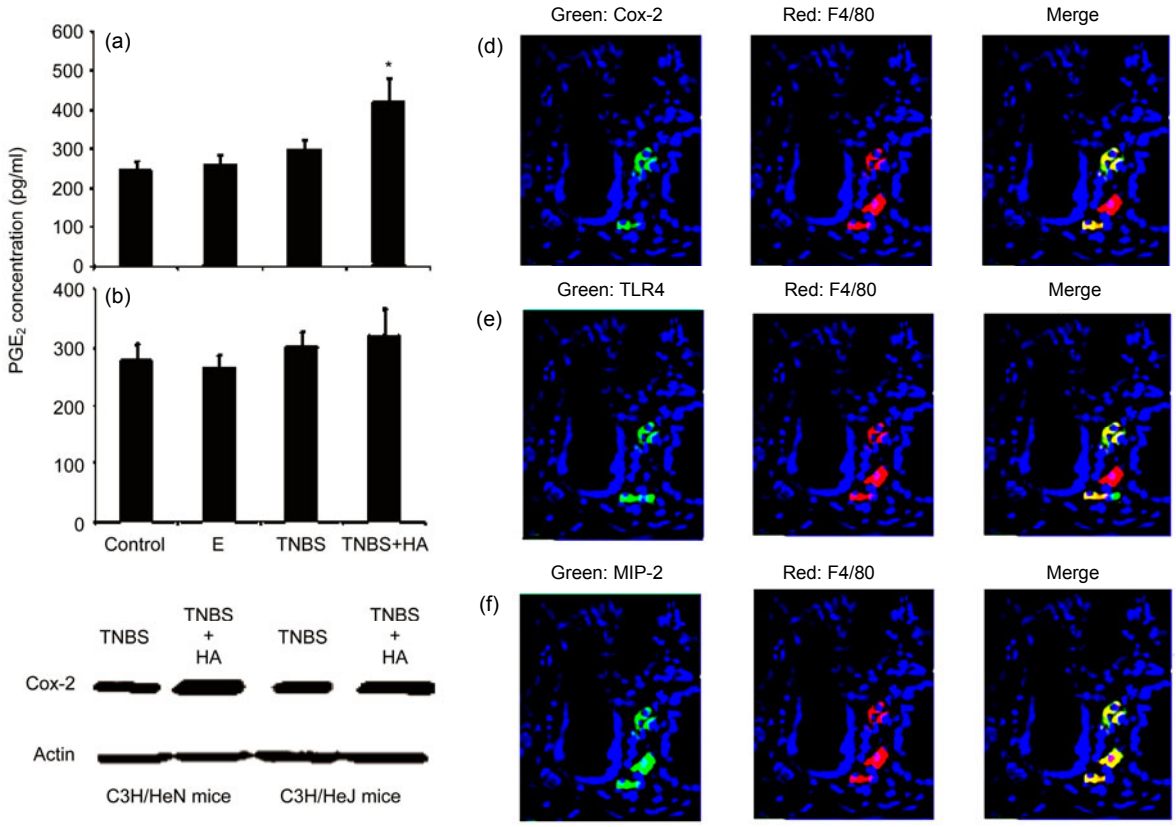

Fig. 3 Cox-2 and PGE_2 expressions in C3H/HeN and C3H/HeJ mice

(a, b) PGE_2 expression significantly increased in C3H/HeN mice (a), but not in C3H/HeJ mice (b); (c) Cox-2 expression increased three times in C3H/HeN mice, but not in C3H/HeJ mice; (d) Most of the Cox-2 expressing cells were macrophages (F4/80); (e) Most of the macrophages expressed TLR4; (f) All of the macrophages expressed MIP-2. E: 50% ethanol; TNBS+HA: TNBS plus HA. * P<0.05, TNBS plus HA group compared to TNBS group

3.4 HA increased Cox-2 and PGE_2 expressions in peritoneal macrophages

Incubation of C3H/HeN mice peritoneal macrophages with HA (100 g/ml) induced two- to three-fold increases in Cox-2 and PGE_2 expressions (Fig. 4). These increases were not seen in peritoneal macrophages from C3H/HeJ mice.

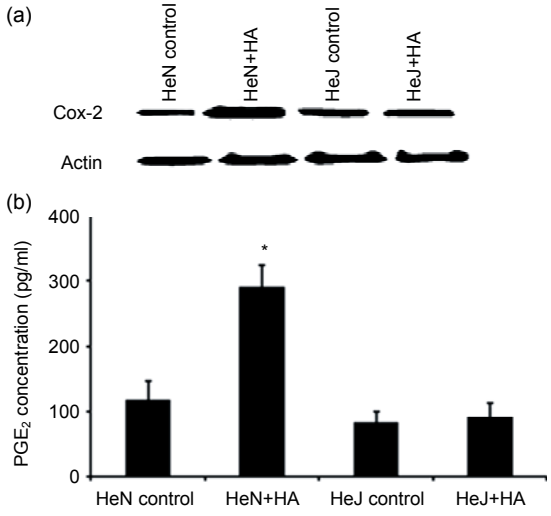

Fig. 4 Effect of HA on Cox-2 and PGE_2 expressions in peritoneal macrophages of C3H/HeN and C3H/HeJ mice
(a) Cox-2 expression increased about three times in C3H/HeN mice peritoneal macrophages, but not in C3H/HeJ mice; (b) PGE_2 expression increased about two times in C3H/HeN mice peritoneal macrophages. HeN: C3H/HeN mice; HeJ: C3H/HeN mice. HeN+HA: C3H/HeN mice treated with HA. HeJ+HA: C3H/HeJ mice treated with HA. * $P<0.05$, HA group compared to control group

4 Discussion

Our results showed that HMW HA can rescue TNBS-induced colitis, and this protective effect occurred only in C3H/HeN mice. This result is consistent with the study of Zheng *et al*. (2009). Their study showed HA can rescue DSS colitis in wild type mice, but not in $TLR4^{-/-}$ mice. These data suggested HA may work through TLR4 signal to participate in the host response to gastrointestinal injury.

Except highly conserved pathogen-associated molecular patterns (PAMPs), TLRs also act to some endogenous molecules, such as heat shock proteins, which are capable of signaling the presence of danger to surrounding cells and tissues (Deng *et al*., 2007; Földes *et al*., 2008; Zhang *et al*., 2005). Studies have showed that HA is one of the endogenous receptors of TLR4. HMW HA can reduce bleomycin-induced apoptosis in alveolar epithelial cells from wild type mice and the protective effect was not seen in $TLR4^{-/-}$ mice (Jiang *et al*., 2005). Using TLR4-specific small interfering RNA (siRNA), the expressions of matrix metalloprotease 2 (MMP2) and IL-8 induced by HA were blocked (Wallet *et al*., 2010). Taylor *et al*. (2007) also found that recognition of hyaluronan released in sterile injury involves a unique receptor complex dependent on TLR4, CD44, and MD-2. These results showed that the interaction between TLR4 and HA may be crucial in host defense against noninfectious injury.

The expression of HA increased not only in patients with DSS colitis but also in patients with inflammatory bowel disease (Majors *et al*., 2003). Our results showed HA expression also increased in TNBS colitis and this occurred only in C3H/HeN mice, which suggested that the expression of endogenous HA also relied on TLR4 signaling. There may be a positive feedback between endogenous HA expression and TLR4 signaling. The consequence is most likely related to the interaction of the repair process and the mobilization of antimicrobial defense at the site of tissue disruption.

The protective effect of HWM HA has been confirmed in many inflammatory diseases, such as emphysema, acute liver injury, and bleomycin-induced lung injury (Połubinska *et al*., 2000; Breborowicz *et al*., 2001; Jiang *et al*., 2005). The anti-inflammatory activity of HA is not well understood. It depends not only on direct interaction with inflammatory cells, but also on the physical properties of the molecule itself. It can decrease many cytokines production, such as TNF-α and Interferon-γ (IFN-γ) (Wang C.T. *et al*., 2006). Our study showed that after HMW HA injection, the expressions of Cox-2 and PGE_2 in colon mucosa increased only in C3H/HeN mice. This may suggest that HMW HA may work through Cox-2 and PGE_2 production to protect the colon from injury. Cox-2 and PGE_2 are very important for mucosa protection. Deficiency in Cox-2 results in the colitis induced by DSS becoming more severe. PGE_2 can increase the blood flow and activate the epidermal growth factor receptor. Supplementation

of PGE_2 in $TLR4^{-/-}$ mice resulted in improvement in clinical signs of colitis (Fukata *et al*., 2006).

Our results showed that Cox-2 expressing cells were double stained with macrophages and most of macrophages were TLR4 expressing cells. We also isolated peritoneal macrophages and found that HA can promote the production of Cox-2 and PGE_2 in C3H/HeN mice macrophages. These data suggested that macrophages may be the effector cells of HMW HA. The interaction between HA and macrophage has been confirmed in many studies. For example, HA fragments can induce the expression of many chemokines in macrophages, such as the T helper 1 (TH1) cytokine IL-12p70 (Wang M.J. *et al*., 2006; Wallet *et al*., 2010). In a study using macrophages from bleomycin-injured rat lungs, HA fragments can induce metalloelastase and inducible nitric oxide synthase (iNOS) production in macrophages (McKee *et al*., 1997; Horton *et al*., 1999). Noble *et al*. (1996) also demonstrated that HA fragments can induce the nuclear factor-kappa B (NF-κB) activation in murine macrophages. Our results together with these data suggested that HMW HA could activate macrophages to improve Cox-2 and PGE_2 production.

In summary, our data demonstrated that HMW HA can rescue the TNBS colitis through inducing Cox-2 and PGE_2 production in a TLR4-dependent way. The interactions between HA and macrophages may have an important role in determining how inflammatory responses are resolved. This may help to find a new therapy for human inflammatory bowel disease.

References

Bai, K.J., Spicer, A.P., Mascarenhas, M.M., Yu, L., Ochoa, C.D., Garg, H.G., Quinn, D.A., 2005. The role of hyaluronan synthase 3 in ventilator induced lung injury. *Am. J. Respir. Crit. Care Med.*, **172**(1):92-98. [doi:10.1164/rccm.200405-652OC]

Boodoo, S., Spannhake, E.W., Powell, J.D., Horton, M.R., 2006. Differential regulation of hyaluronan-induced IL-8 and IP-10 in airway epithelial cells. *AJP Lung Cell. Mol. Physiol.*, **291**(3):L479-L486. [doi:10.1152/ajplung.00518.2005]

Breborowicz, A., Połubinska, A., Moberly, J., Ogle, K., Martis, L., Oreopoulos, D., 2001. Hyaluronan modifies inflammatory response and peritoneal permeability during peritonitis in rats. *Am. J. Kidney Dis.*, **37**(3):594-600. [doi:10.1016/S0272-6386(01)80018-4]

Collins, C.B., Ho, J., Wilson, T.E., Wermers, J.D., Tlaxca, J.L., Lawrence, M.B., Solga, M., Lannigan, J., Rivera-Nieves, J., 2008. CD44 deficiency attenuates chronic murine ileitis. *Gastroenterology*, **135**(6):1993-2002. [doi:10.1053/j.gastro.2008.08.053]

de la Motte, C.A., Hascall, V.C., Calabro, A., Yen-Lieberman, B., Strong, S.A., 1999. Mononuclear leukocytes preferentially bind via CD44 to hyaluronan on human intestinal mucosal smooth muscle cells after virus infection or treatment with poly(I.C). *J. Biol. Chem.*, **274**(43):30747-30755. [doi:10.1074/jbc.274.43.30747]

Deng, J.F., Geng, L., Qian, Y.G., Li, H., Wang, Y., Xie, H.Y., Feng, X.W., Zheng, S.S., 2007. The role of Toll-like receptors 2 and 4 in acute allograft rejection after liver transplantation. *Transplant. Proc.*, **39**(10):3222-3224. [doi:10.1016/j.transproceed.2007.02.102]

Földes, G., von Haehling, S., Okonko, D.O., Jankowska, E.A., Poole-Wilson, P.A., Anker, S.D., 2008. Fluvastatin reduces increased blood monocyte Toll-like receptor 4 expression in whole blood from patients with chronic heart failure. *Int. J. Cardiol.*, **124**(1):80-85. [doi:10.1016/j.ijcard.2006.12.024]

Fukata, M., Chen, A., Klepper, A., Krishnareddy, S., Vamadevan, A.S., Thomas, L.S., Xu, R., Inoue, H., Arditi, M., Dannenberg, A.J., *et al*., 2006. Cox-2 is regulated by Toll-like receptor-4 (TLR4) signaling: role in proliferation and apoptosis in the intestine. *Gastroenterology*, **131**(3):862-877. [doi:10.1053/j.gastro.2006.06.017]

Horton, M.R., McKee, C.M., Bao, C., Liao, F., Farber, J.M., Hodge-DuFour, J., Puré, E., Oliver, B.L., Wright, T.M., Noble, P.W., 1998. Hyaluronan fragments synergize with interferon-γ to induce the C–X–C chemokines Mig and interferon-inducible protein-10 in mouse macrophages. *J. Biol. Chem.*, **273**(52):35088-35094. [doi:10.1074/jbc.273.52.35088]

Horton, M.R., Shapiro, S., Bao, C., Lowenstein, C.J., Noble, P.W., 1999. Induction and regulation of macrophage metalloelastase by hyaluronan fragments in mouse macrophages. *J. Immunol.*, **162**(7):4171-4176.

Itano, N., Sawani, T., Yoshida, M., Lenas, P., Yamada, Y., Imagawa, M., Shinomura, T., Hamaguchi, M., Yoshida, Y., Ohnuki, Y., *et al*., 1999. Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties. *J. Biol. Chem.*, **274**(35):25085-25092. [doi:10.1074/jbc.274.35.25085]

Janeway, C.A., Medzhitov, R., 1999. Lipoproteins take their toll on the host. *Curr. Biol.*, **9**(23):R879-R882. [doi:10.1016/S0960-9822(00)80073-1]

Jiang, D., Liang, J., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G.D., Mascarenhas, M.M., Garg, H.G., Quinn, D.A., *et al*., 2005. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat. Med.*, **11**(11):1173-1179. [doi:10.1038/nm1315]

Lahat, G., Halperin, D., Barazovsky, E.L., Shalit, I., Rabau, M., Klausner, J., Fabian, I., 2007. Immunomodulatory effects of ciprofloxacin in TNBS-induced colitis in mice. *Inflamm. Bowel Dis.*, **13**(5):557-565. [doi:10.1002/ibd.20077]

Liu, Y.Y., Lee, C.H., Dedaj, R., Zhao, H., Mrabat, H., Sheidlin,

A., Syrkina, O., Huang, P.M., Garg, H.G., Hales, C.A., *et al*., 2008. High-molecular-weight hyaluronan—a possible new treatment for sepsis-induced lung injury: a preclinical study in mechanically ventilated rats. *Crit. Care*, **12**(4):R102. [doi:10.1186/cc6982]

Majors, A.K., Austin, R.C., de la Motte, C.A., Pyeritz, R.E., Hascall, V.C., Kessler, S.P., Sen, G., Strong, S.A., 2003. Endoplasmic reticulum stress induces hyaluronan deposition and leukocyte adhesion. *J. Biol. Chem.*, **278**(47): 47223-47231. [doi:10.1074/jbc.M304871200]

Mascarenhas, M.M., Day, R.M., Ochoa, C.D., Choi, W.I., Yu, L., Ouyang, B., Garg, H.G., Hales, C.A., Quinn, D.A., 2004. Low molecular weight hyaluronan from stretched lung enhances IL-8 expression. *Am. J. Respir. Cell Mol. Biol.*, **30**(1):51-60. [doi:10.1165/rcmb.2002-0167OC]

McKee, C.M., Lowenstein, C.J., Horton, M.R., Wu, J., Bao, C., Chin, B.Y., Choi, A.M., Noble, P.W., 1997. Hyaluronan fragments induce nitric-oxide synthase in murine macrophages through a nuclear factor κB-dependent mechanism. *J. Biol. Chem.*, **272**(12):8013-8018. [doi:10.1074/jbc.272.12.8013]

Noble, P.W., McKee, C.M., Cowman, M., Shin, H.S., 1996. Hyaluronan fragments activate an NF-κB/I-κBα autoregulatory loop in murine macrophages. *J. Exp. Med.*, **183**(5):2373-2378. [doi:10.1084/jem.183.5.2373]

Połubinska, A., Pawlaczyk, K., Kużlan-Pawlaczyk, M., Wieczorowska-Tobis, K., Chen, C., Moberly, J.B., Martis, L., Breborowicz, A., Oreopoulos, D.G., 2000. Dialysis solution containing hyaluronan: effect on peritoneal permeability and inflammation in rats. *Kidney Int.*, **57**(3): 1182-1189. [doi:10.1046/j.1523-1755.2000.00946.x]

Spicer, A.P., McDonald, J.A., 1998. Characterization and molecular evolution of a vertebrate hyaluronan synthase gene family. *J. Biol. Chem.*, **273**(4):1923-1932. [doi:10.1074/jbc.273.4.1923]

Taylor, K.R., Yamasaki, K., Radek, K.A., di Nardo, A., Goodarzi, H., Golenbock, D., Beutler, B., Gallo, R.L., 2007. Recognition of hayluronan released in sterile injury involves a unique receptor complex dependent on Toll-like receptor 4, CD44, and MD-2. *J. Biol. Chem.*, **282**(25):18265-18275. [doi:10.1074/jbc.M606352200]

Wallet, M.A., Wallet, S.M., Guiulfo, G., Sleasman, J.W., Goodenow, M.M., 2010. IFN-γ primes macrophages for inflammatory activation by high molecular weight hyaluronan. *Cell. Immunol.*, **262**(2):84-88. [doi:10.1016/j.cellimm.2010.02.013]

Wang, C.T., Lin, Y.T., Chiang, B.L., Lin, Y.H., Hou, S.M., 2006. Weight hyaluronic acid down-regulates the gene expression of osteoarthritis-associated cytokines and enzymes in fibroblast-like synoviocytes from patients with early osteoarthritis. *Osteoarthritis and Cartilage*, **14**(12):1237-1247. [doi:10.1016/j.joca.2006.05.009]

Wang, M.J., Kuo, J.S., Lee, W.W., Huang, H.Y., Chen, W.F., Lin, S.Z., 2006. Translational event mediates differential production of tumor necrosis factor-α in hyaluronan-stimulated microglia and macrophages. *J. Neurochem.*, **97**(3):857-871. [doi:10.1111/j.1471-4159.2006.03776.x]

Zhang, X., Shan, P., Qureshi, S., Homer, R., Medzhitov, R., Noble, P.W., Lee, P.J., 2005. Cutting edge: TLR4 deficiency confers susceptibility to lethal oxidant lung injury. *J. Immunol.*, **175**(8):4834-4838.

Zhang, Y., Chen, H., Yang, L., 2010. Toll-like receptor 4 participates in gastric mucosal protection through Cox-2 and PGE_2. *Dig. Liver Dis*., **42**(7):472-476. [doi:10.1016/j.dld.2009.10.007]

Zheng, L., Riehl, T.E., Stenson, W.F., 2009. Regulation of colonic epithelial repair in mice by Toll-like receptors and hyaluronic acid. *Gastroenterology*, **137**(6):2041-2051. [doi:10.1053/j.gastro.2009.08.055]

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