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Inflammation Research

N-desulfated heparin improves concanavalin A-induced liver injury partly through inhibiting T lymphocyte adhesion

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Abstract. *Objective and Design*: To examine the effect of *N*-desulfated heparin on Concanavalin A (Con A)-induced liver injury and its mechanisms of action.
Materials and methods: Liver injury was induced in mice by Con A. The in vitro assays for examining the adhesions of spleen T lymphocytes and Jurkat cells to extracellular matrix (ECM) were also performed.
Results: *N*-desulfated heparin significantly inhibited the elevation in alanine transaminase, aspartate transaminase and lactic dehydrogenase activities in serum, and recovered the superoxide dismutase activity in the liver tissue of mice with liver injury. In liver histological examination, the inflammatory infiltration, hepatocyte degeneration and Kupffer cell hyperplasia were remarkably improved by *N*-desulfated heparin. Multiple administrations of the heparin derivative for one day showed a more potent prevention of the liver injury than did single dosing for three days. *N*-desulfated heparin significantly inhibited the adhesion of spleen cells and purified T lymphocytes isolated from the liver injured mice to either type I collagen or fibronectin but not to laminin. The heparin derivative also showed a similar inhibition of the adhesion of spleen cells from normal mice, stimulated in vitro with Con A, whereas it did not affect their proliferation. Moreover, the adhesion of human leukemia Jurkat cells to collagen I was inhibited by *N*-desulfated heparin.
Conclusion: *N*-desulfated heparin may improve immunological liver injury partly via reducing the functions, such as the adhesion to ECM, of T lymphocytes.

Key words: *N*-desulfated heparin – Con A – Liver injury – Lymphocytes – Adhesion

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Introduction

Hepatitis is an inflammatory liver disease induced by various causes such as virus infection, alcohol, chemicals, etc. Although there are many unsolved problems related to the mechanisms, it is generally accepted that activated T lymphocytes play crucial roles in both acute and chronic hepatitis, and the development of cirrhosis. Therefore, animal models of T cell-mediated hepatitis are thought to reflect the situations of human clinical hepatitis or hepatocellular damage, and several models have been developed for the pathological and pharmacological studies [1, 2]. For example, concanavalin A (Con A), a plant lectin that activates T lymphocytes in vitro and in vivo, could lead to a liver-selective necrotic injury due to polyclonal T cell activation [2, 3].

Glycosaminoglycans are a family of structurally distinct polyanionic, complex carbohydrates, which include heparin, heparan sulphate, chondroitin sulphate, and hyaluronic acid. Since 1937 heparin has been used as a clinical anti-coagulant agent, and many other physiological effects have been documented, such as inhibition of complement activation [4], prevention of leukocyte adhesion and activation [5, 6], protection of vascular endothelial cells from various damages [7, 8], and modulation of the activities of a number of inflammatory cells including T cells and neutrophils [9, 10]. It is also capable of inactivation of NF-κB, a ubiquitous transcriptional factor and a pleiotropic regulator of many genes involved in inflammatory responses [11].

However, the clinical use of heparin for the treatment of inflammation is hampered by its inherent strong anti-coagulant activity. For this reason, various low molecular weight heparin derivatives as well as chemically and enzymatically modified heparin derivatives have been developed. Although these derivatives have relatively low anti-coagulant activity and preserve anti-inflammatory activities, as reported for *O*-desulfated heparin derivatives [12, 13] and *N*-acetyl heparin [14, 15], they still retained anti-coagulant activities, to

some extent, as compared to heparin [12–15]. Since *N*-sulfation is essential for the anti-coagulant activity of heparin [16], *N*-desulfated heparin may be considered as a non-anticoagulant heparin retaining anti-thrombotic activity and anti-inflammatory activities [17–19]. Previously, Wang et al. have found that *N*-desulfated heparin preserved a potent anti-inflammatory activity in the in vitro assays for human promyeloid HL-60 cell adhesion to the stimulated human umbilical vein endothelial cells (HUVECs) and for human neutrophils transmigration through the monolayers of HUVECs following TNF-α stimulation, as well as in the in vivo assays for acute peritonitis and for ischemia and reperfusion injury [20].

In the present study, *N*-desulfated heparin was applied to Con A-induced liver damage model for examining its efficacy against T cell-mediated immunological liver inflammation. Then, its mechanism of action was investigated by focusing on the T lymphocyte adhesion.

Materials and methods

Animals

Male and female Kunming and ICR mice at 5–6 weeks old were obtained from Experimental Animal Center of China Pharmaceutical University (Nanjing, China), and maintained in plastic cages at 21 ± 2ºC with free access to pellet food and water, and kept on a 12-h light/dark cycle. All animal experiments were performed according to the regulations of Chinese Academy of Sciences.

Cell line

Human leukemia Jurkat cell line was obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China, and maintained in RPMI 1640 (GIBCO BRL) medium supplemented with 100 U/ml penicillin (Shandong Lukang Pharmaceutical Co., Ltd., Jining, China), 100 U/ml streptomycin (Dalian Merro Pharmaceutical Factory, Dalian, China) and 10% fetal calf serum (FCS, Hangzhou Sijiqing Biological Engineering Material Co., Ltd., Hangzhou, China) under a humidified 5% (v/v) CO_2 atmosphere at 37 °C.

Drugs and reagents

N-desulfated heparin was prepared by mixing the pyridine salt of heparin (Shanghai Bio Life Science & Technology Co. Ltd., Shanghai, China) (100 mg in double distilled and deionized water) with 4.75 ml of dimethylsulfoxide (Sigma St. Louis, MO) at 50 °C for 1 h. Then, the sample was diluted with equal volume of water and adjusted to pH 9.0 with 0.1 mol/L NaOH to terminate the reaction. The *N*-sulfate amount in the obtained product was only 11.7% of unfractionated heparin. The *N*-desulfated heparin showed 188-fold reduction of the anticoagulant activity but retained a strong anti-inflammatory effect, which has been reported previously [20]. Reagents employed in this study were as follows: concanavalin A (Con A, Sigma, St. Louis, MO); kits for determining serum alanine transaminase (ALT), aspartate transaminase (AST), lactic dehydrogenase (LDH) and superoxide dismutase (SOD) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China); type I collagen (Collaborative Biomedical Products, MA); fibronectin, laminin and bovine serum albumin (BSA) (Sigma, St. Louis, MO); mouse T cell Enrichment columns and column wash buffer (R & D systems, USA); phorbol 12, 13-dibutyrate (PDBu, Wako Pure Chemical Industry Ltd., Japan); crystal violet (Shanghai Yuanhang Reagent Factory, Shanghai, China).

Con A-induced hepatitis in mice

Acute liver injury was induced by injecting mice with Con A in phosphate buffered saline (PBS) at 25 mg/kg via the tail vein. Eight hours after the injection, the mice were bled. The serum was collected for assaying the ALT, AST and LDH activities, and the liver homogenate was used to determine the SOD activity by commercial kits as the protocols indicated. Portion of livers were fixed in 4% paraformaldehyde, and embedded in paraffin. The tissue sections were stained with haematoxylin-eosin and read on a 0 to 3 scale (0, no change; 1, mild; 2, moderate; and 3, severe) by a pathologist who had no prior knowledge of the induction of liver injury or other experimental data.

Preparation of spleen cell suspensions and purification of T cells

Spleen was aseptically taken from mice, crushed gently and separated into single cells by squeezing in Hank's solution. The cells obtained were passed through a gauze of eight-layers and centrifuged at 1000 rpm for 5 min at 4 °C. Pellet was added into 10 ml sterile Tris-NH_4Cl (pH 7.5), followed by centrifugation to remove erythrocytes. After washing twice with RPMI 1640 (GIBCO BRL) medium supplied with 100 U/ml of penicillin, 100 U/ml of streptomycin and 10% FCS, they were resuspended in the medium and used for culture. In some cases, the prepared mouse spleen cell suspensions were loaded onto T cell enrichment columns to purify T cells as the protocol indicated. Briefly, the column was equilibrated with column wash buffer. After the wash buffer had drained down to the level of the white filter, 2 ml cell suspension was applied to the top of the column and incubated at room temperature for 10 min. Then cells were eluted from the column with 4 aliquots of 2 ml of column wash buffer. The collected cells were centrifuged at 250 *g* for 5 min and re-suspended in the appropriate culture medium. The purity ($CD3^+$ cells) of recovered cells reached about 88%.

Adhesion assay

Flat-bottom 96-well microplate was coated with 50 µl of type I collagen (50 µg/ml), fibronectin (10 µg/ml) or laminin (10 µg/ml) in PBS at 4 °C overnight, and nonspecific binding sites were blocked with 0.2% BSA for 2 h at room temperature. Then, the plate was washed 3 times with PBS. Spleen cells or spleen T cells (5×10^5/well) were allowed to adhere in the microplate at 37 °C for 3 h in the presence or absence of *N*-desulfated heparin. Jurkat cells (1×10^5/well) were treated or non-treated with *N*-desulfated heparin for 2 h followed by washing, and were then added to the microplate for 45 min adhesion under the stimulation of PDBu (100 ng/ml). After adhesion, nonadherent cells were removed by washing three times with RPMI 1640. Then, the cells were fixed with methanol/acetone (1:1), and stained with 0.5% crystal violet in 20% methanol:water. The unbound dye was removed in tap water and the plate was air-dried. Bound dye was extracted with 1% SDS. The absorbance of the samples was measured at 592 nm with an ELISA reader. All assays were run in triplicate, and results were expressed as percentage of bound cells. The absorbance of 5×10^5 spleen cells or spleen T cells or 1×10^5 Jurkat cells, which were fixed and stained without previous washing, was considered as 100% cell adhesion. Specificity of the cell adhesion assay was corroborated using BSA as substratum. The inhibitory rate of drug was calculated as follows: % inhibition = (% bound $cells_{control}$ – % bound $cells_{drug}$)/% bound $cells_{control}$.

Statistical analysis

One-way analysis of variance (ANOVA) for multiple comparisons was used to detect whether there were any significant differences among the different treatments. Once significant differences were detected ($P < 0.05$), Student two-tailed *t*-test was used to evaluate the difference between two

groups, and Dunnett's *t*-test between control group and multiple dose groups. All experimental results were shown as the mean ± SEM.

Results

Effect of N-desulfated heparin on Con A-induced liver injury in mice

Mice were administered i.p. for 3 times at an interval of 8 h with *N*-desulfated heparin in different doses. One h after the final administration, 25 mg/kg of Con A were injected i.v. to induce the liver damage. As shown in Fig. 1A and 1B, the serum levels of ALT, AST, and LDH were greatly increased 8 h after Con A injection. Compared with the control, all three doses from 0.5 to 2 mg/kg of *N*-desulfated heparin significantly inhibited the elevation in serum ALT, AST and LDH. At the same time, SOD activity in liver homogenate was greatly decreased due to the injection with Con A. The pre-treatment with 1 and 2 mg/kg of *N*-desulfated heparin increased the SOD almost to the normal level (Fig. 1C).

In addition, we administered mice *N*-desulfated heparin at the dose of 1 mg/kg at different schedules, once a day for 3 days and twice a day or three times a day. As shown in Fig. 2, all three sets of dosing with *N*-desulfated heparin significantly inhibited the elevation in serum ALT levels. Among them, the multiple administrations in a day showed a more effective prevention from the Con A-induced ALT elevation than did its single dosing for three days.

In histological examination, marked inflammatory infiltration, severe hepatocyte degeneration and Kupffer cell hyperplasia were observed in the control group without medication. Compared with this, a significant improvement was made by *N*-desulfated heparin at the dose of 1 mg/kg for three times a day (Table 1).

Effect of N-desulfated heparin on Con A-induced spleen cell proliferation

Spleen cells (5×10^5) from normal mice, which co-cultured with or without *N*-desulfated heparin, were stimulated with Con A (5 μg/ml) for 96 h. *N*-desulfated heparin did not show any influence on the spleen cell proliferation induced by Con A (data not shown).

Effect of N-desulfated heparin on the adhesion of spleen cells and purified spleen T lymphocytes from Con A-injected mice to type I collagen, fibronectin and laminin

Spleen cells isolated from mice at different times after Con A administration were applied to the adhesion assay. The result showed that the adhesion of spleen cells to type I collagen was in a very low level at 0 h (before Con A injection), and it increased remarkably 2 h after administrating Con A, remained plateau until 6 h, and began to decrease at 8 h (Fig. 3). Therefore, the spleen cells isolated at 2 h of Con A injection was used to examine the effect of *N*-desulfated heparin.

The isolated spleen cells were allowed to adhere to type I collagen, fibronectin and laminin for 3 h in the presence or absence of *N*-desulfated heparin. As shown in Fig. 4A, the bound cells from the mice with Con A hepatitis were remarkably increased as compared with those from normal mice. Against this, *N*-desulfated heparin decreased the adhesion of spleen cells to type I collagen and fibronectin in a concentration-dependent manner. However, the heparin derivative showed no effect on the adhesion of spleen cells to laminin. Furthermore, the isolated spleen cells were used for purify-

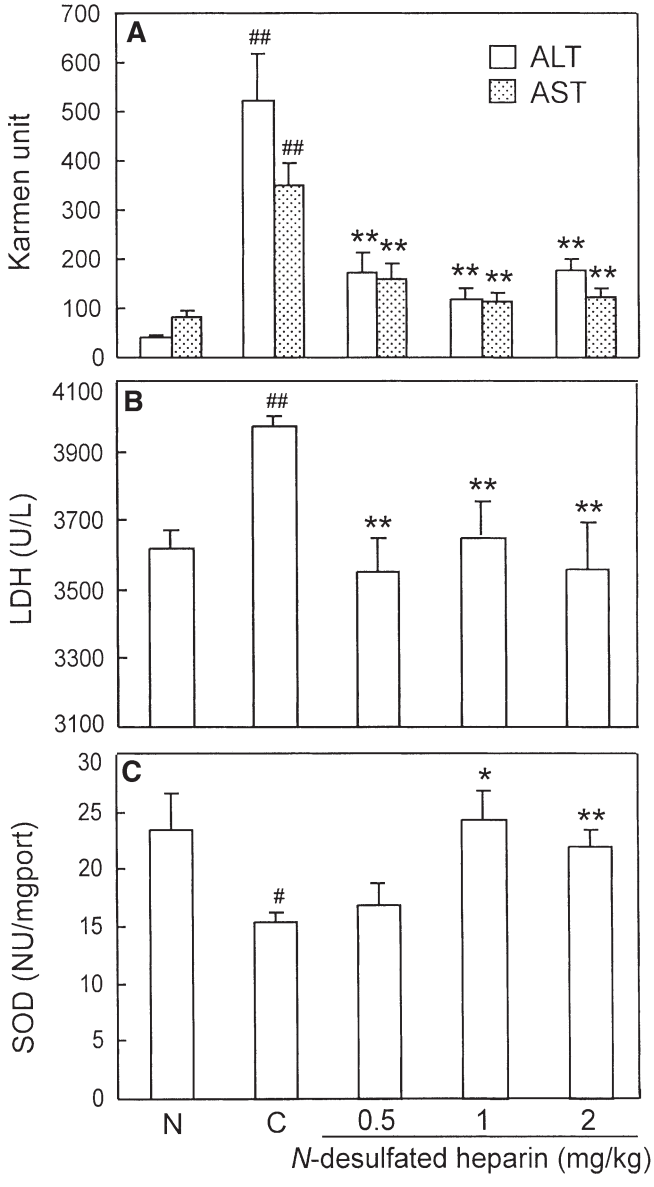

Fig. 1. Effect of *N*-desulfated heparin on Con A-induced liver injury in mice. Mice were administered i.p. with *N*-desulfated heparin at doses of 0.5, 1 and 2 mg/kg for three times a day at the interval of 8 h. One h after the final administration, mice were injected i.v. with 25 mg/kg of Con A, followed 8 h later by bleeding. The serum was used for the measurement of alanine transaminase (ALT), aspartate transaminase (AST) and lactic dehydrogenase (LDH) activities and the liver tissue was homogenized and used for the measurement of superoxide dismutase (SOD) activity. Each column indicates the mean ± SEM of ten animals. NU/mgprot: nitrite unit per mg protein; N: Normal mice; C: Control mice without medication. $^{*}P < 0.05$, $^{**}P < 0.01$ vs Control (Dunnett's *t*-test); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Normal (Student's *t*-test).

Table 1. Histopathological changes of livers in mice with Con A-induced liver injury.

Group	Dose (mg/kg)	No. of animals	Serum ALT (Karmen unit)	Hepatocyte necrosis	Hepatocyte degeneration	Inflammatory infiltration	Kupffer Cell hyperplasia
Control	–	8	728 ± 49	1.00 ± 0.19	2.88 ± 0.23	1.75 ± 0.16	2.50 ± 0.38
N-desulfated heparin	1.0	8	144 ± 38**	0.75 ± 0.16	0.88 ± 0.23**	0.75 ± 0.16**	0.75 ± 0.16**

Mice were administered i.p. with *N*-desulfated heparin at doses of 1 mg/kg for three times a day at the interval of 8 h. One h after the final administration, mice were injected i.v. with 25 mg/kg of Con A, followed 8 h later by bleeding. The serum was used for the measurement of alanine transaminase (ALT) activity. The liver tissue sections were stained with haematoxylin-eosin. The histological changes were read on a scale of 0–3 (0, no change; 1, mild; 2, moderate; and 3, severe) and expressed as an average score. Each figure indicates the mean ± SEM of 8 animals. ** P < 0.01 vs control (Student's *t*-test).

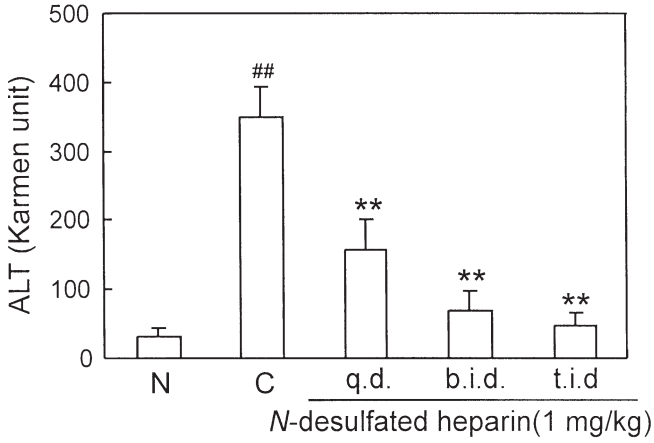

Fig. 2. Effect of different administration schedules of *N*-desulfated heparin on Con A-induced liver injury in mice. Mice were administered i.p. with *N*-desulfated heparin at the dose of 1 mg/kg once a day for three days (q.d.), twice a day (b.i.d.) and three times a day (t.i.d.) for one day respectively before the injection of Con A (25 mg/kg). Eight hours after Con A injection, mice were sacrificed and the serum was used for the measurement of alanine transaminase (ALT). Each column indicates the mean ± SEM of twelve animals. N: Normal mice; C: Control mice without medication. ** $P < 0.01$ vs Control (Dunnett's *t*-test); ## $P < 0.01$ vs Normal (Student's *t*-test).

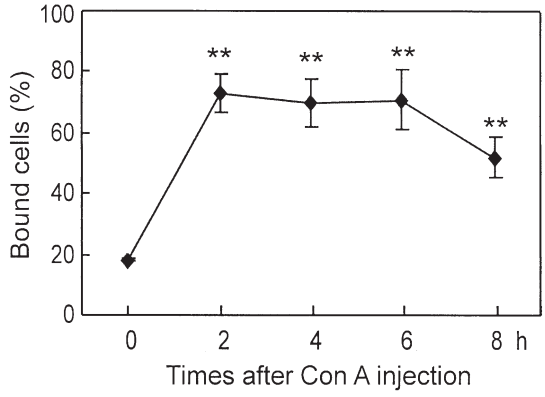

Fig. 3. Kinetics of the adhesion to type I collagen of spleen cells isolated from mice with Con A-induced liver injury. Spleen cells were isolated from naive mice (0 h) and the mice 2, 4, 6 and 8 h after Con A injection (i.v. 25 mg/kg). Then the cells (5×10^5) were used for adhesion assay. Data were expressed as mean ± SEM of three separated experiments and each experiment was performed in triplicate sets. ** $P < 0.01$ vs 0 h (Dunnett's *t*-test).

ing T lymphocytes. The purified T cells showed more strong adhesion to all three components of extracellular matrix (ECM) and *N*-desulfated heparin also significantly inhibited the adhesion to type I collagen and fibronectin but not to laminin (Fig. 4B). Its inhibitory rates at 10^{-5} g/ml were 33.3% for spleen cells and 38.1% for T lymphocytes to collagen, 37.8% for spleen cells and 36.0% for T lymphocytes to fibronectin, respectively.

Fig. 5 shows the in vivo effect of *N*-desulfated heparin on the adhesion potential of spleen cells and purified T lymphocytes. The i.p. administration of *N*-desulfated heparin at 0.5, 1 and 2 mg/kg showed a dose-dependent inhibition on the cell adhesion ability to type I collagen and fibronectin, but not to laminin, when tested in vitro. Its inhibitory rates at 2 mg/kg were 32.9% for spleen cells and 26.4% for T lymphocytes to collagen, 38.5% for spleen cells and 38.0% for T lymphocytes to fibronectin, respectively.

Effect of N-desulfated heparin on the adhesion of spleen cells, activated by Con A in vitro, to type I collagen, fibronectin and laminin

Spleen cells from normal mice were stimulated with Con A (5 µg/ml) for 24 h. After washing, they were applied to the adhesion assay and co-cultured with or without *N*-desulfated heparin. As shown in Fig. 6, the Con A stimulation resulted in a remarkable increase in bound cell numbers as compared with Con A-non-treated cells. *N*-desulfated heparin decreased the adhesion of spleen cells to type I collagen and fibronectin in a concentration-dependent manner. However, the heparin derivative did not affect the adhesion of spleen cells to laminin.

Effect of N-desulfated heparin on the adhesion of Jurkat cells to type I collagen

Jurkat cells were pretreated for 2 h with or without *N*-desulfated heparin. After washing three times, they were applied for 45 min adhesion under the stimulation of PDBu (100 ng/ml). As shown in Fig. 7, the stimulation with PDBu resulted in a remarkable increase in bound cell numbers as compared with PDBu-non-treated cells. The pretreatment with *N*-desulfated heparin at 10^{-6} and 10^{-8} g/ml caused a significant decrease in the cell adhesion to type I collagen.

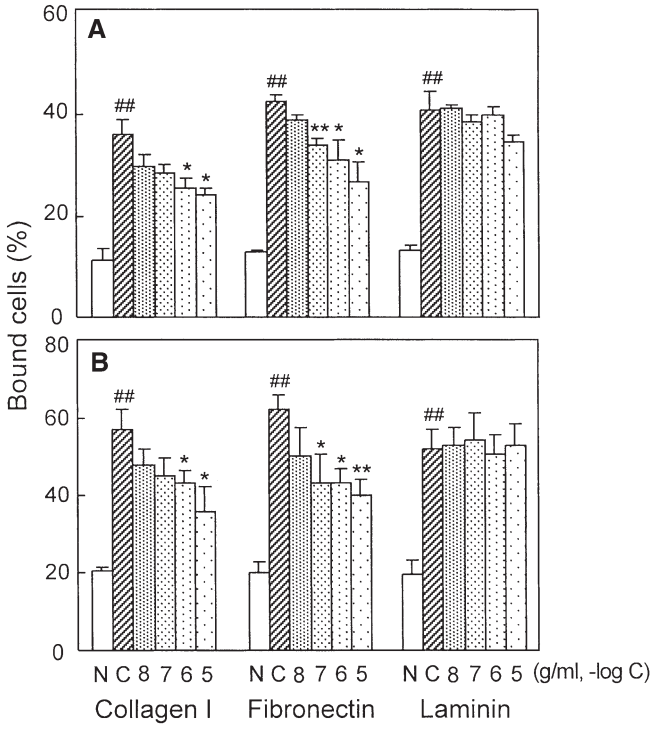

Fig. 4. In vitro effect of *N*-desulfated heparin on the increased adhesion activity to collagen I, fibronectin and laminin of spleen cells and purified T lymphocytes isolated from mice with Con A liver injury. Spleen cells were isolated from naive mice or mice 2 h after Con A injection (i.v., 25 mg/kg). Spleen cells (5×10^5) (A) and spleen T lymphocytes (5×10^5) (B) that were isolated from the spleen cells were applied to the adhesion assay in the presence or absence of *N*-desulfated heparin for 3 h. Data were expressed as mean ± SEM of three animals and each assay was performed in triplicate. N: cells from normal mice; C: Control (cells from Con A-treated mice without *N*-desulfated heparin). $^*P < 0.05$, $^{**}P < 0.01$ vs Control (Dunnett's *t*-test); $^{\#\#}P < 0.01$ vs Normal (Student's *t*-test).

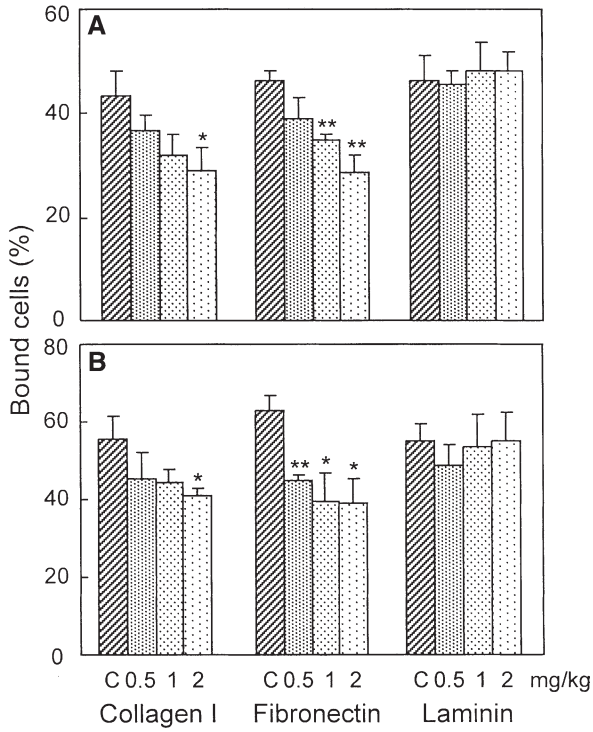

Fig. 5. Inhibition of increased adhesion activities of spleen cells and of isolated T lymphocytes in vitro to collagen I, fibronectin and laminin from mice treated by Con A and *N*-desulfated heparin. Mice were administered i.p. with 0.5, 1 and 2 mg/kg of *N*-desulfated heparin for three times a day at the interval of 8 h prior to Con A injection. Spleen cells were isolated from mice 2 h after Con A injection (i.v. 25 mg/kg). Spleen cells (5×10^5) (A) and spleen T lymphocytes (5×10^5) (B) that were isolated from the spleen cells were applied to the adhesion assay for 3 h. Data were expressed as mean ± SEM of three animals and each assay was performed in triplicate. C: Control without medication. $^*P < 0.05$, $^{**}P < 0.01$ vs Control (Dunnett's *t*-test).

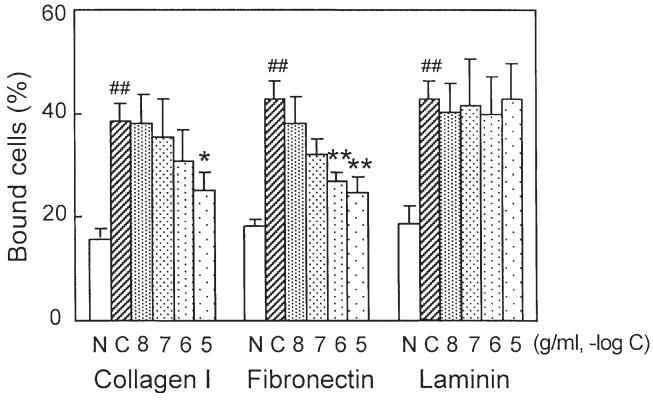

Fig. 6. In vitro effect of *N*-desulfated heparin on the increased adhesion activity to collagen I, fibronectin and laminin of spleen cells from normal mice. Spleen cells isolated from normal mice were stimulated with Con A (5 μg/ml) for 24 h. After washing, they were applied to the adhesion assay and co-cultured with or without *N*-desulfated heparin for 3 h. Data were expressed as mean ± SEM of three animals and each assay was performed in triplicate. N: normal cells without *N*-desulfated heparin or Con A; C: control cells stimulated with Con A but without *N*-desulfated heparin. $^*P < 0.05$, $^{**}P < 0.01$ vs Control (Dunnett's *t*-test); $^{\#\#}P < 0.01$ vs Normal (Student's *t*-test).

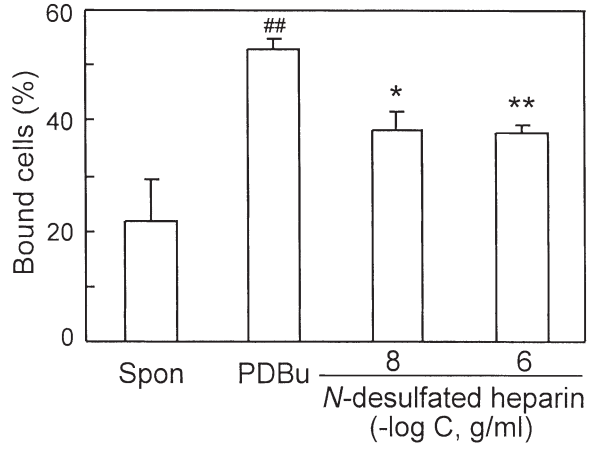

Fig. 7. Effect of *N*-desulfated heparin on the adhesion activity of Jurkat cells to type I collagen. Jurkat cells (1×10^5) were pre-treated with or without *N*-desulfated heparin for 2 h. After washing, the cells were added to type I collagen (50 μg/ml) pre-coated plate under the stimulation of phorbol 12, 13-dibutyrate (PDBu) (100 ng/ml) and incubated for 45 min at 37°C. Cell adhesion was determined as described in Materials and methods. Data were expressed as the mean ± SEM of three separated experiments and each assay was performed in triplicate. Spon: spontaneous adhesion without PDBu stimulation. $^*P < 0.05$, $^{**}P < 0.01$ vs Control (Dunnett's *t*-test); $^{\#\#}P < 0.01$ vs Normal (Student's *t*-test).

Discussion

In the present study, we first demonstrated the anti-inflammatory effects of *N*-desulfated heparin on the immunological liver injury at very low doses (0.5–2 mg/kg), as assessed by the serum ALT, AST, LDH levels and liver SOD activity. Namely, *N*-desulfated heparin significantly reduced the serum ALT, AST and LDH activities and raised liver SOD activity to almost normal levels (Fig.1). Considering that the *N*-desulfation greatly shortened the elimination of the half-life of heparin [21], we designed several administration schedules and found that the repeated dosing, twice and three times, of *N*-desulfated heparin within a day showed a stronger anti-inflammatory effect on the liver damage than did the dosing once a day for three days. This result suggested an importance to maintain an enough concentrations in blood for the anti-hepatitis effect of *N*-desulfated heparin. The pathological observation also showed that the liver injury induced by Con A was improved by *N*-desulfated heparin including the inhibition of inflammatory infiltration (Table 1).

In Con A-induced hepatitis, the activated T cells are obligatory to start the immunological response and finally result in liver injury [2, 3]. To explore the anti-inflammatory mechanisms of *N*-desulfated heparin that led to improvement of the liver damage, we examined the effects of *N*-desulfated heparin on the T cell proliferation in vitro. *N*-desulfated heparin did not show inhibition on the Con A-induced spleen cell transformation, suggesting that the improvement of Con A hepatitis by the heparin derivative was not due to the inhibition of the T cell proliferation.

The localization of lymphocytes to the inflammation site is a complicated coordinated multi-step processes, which is mediated by the sequential actions of adhesion molecules and chemokines. In these processes, the activated T lymphocytes not only express cell surface proteins that bind to ECM [22], but also produce an endoglycosidase that degrades the heparin sulfate moiety of the vascular endothelial cells or subendothelial ECM [23, 24]. Since the ECM components have heparin and heparin-like molecular binding domains [25–27] and the cell surface proteins bound to ECM proteins would be partly blocked by the glycosaminoglycan heparin sulfate [22, 28], we checked the adhesion ability of spleen cells from mice with Con A-induced hepatitis. The injection of Con A to mice remarkably increased the adhesion ability of spleen cells to type I collagen, fibronectin and laminin, which are known as the important components of ECM and localized in normal liver [29]. Figure 3 showed that the adhesion capacity of the spleen cells from naive mice to type I collagen was minimum. Two h after Con A injection, the adhesion potential of isolated spleen cells increased greatly, which remained at a high level until 6 h. By using the spleen cells isolated at 2 h, we found a concentration-dependent inhibition by *N*-desulfated heparin of the increased adhesion potential of spleen cells and purified T lymphocytes to type I collagen and fibronectin in vitro (Fig. 4). The inhibitory effect on the adhesion was further confirmed by in vivo administration of the *N*-desulfated heparin at 0.5–2 mg/kg (Fig. 5). These results suggest that *N*-desulfated heparin could inhibit the adhesion ability of spleen cells and purified T lymphocytes activated by Con A to type I collagen and fibronectin both in vitro and in vivo, though it did not influence Con A-induced cell proliferation. It should be noted that *N*-desulfated heparin showed almost same inhibitory rate on the adhesion of pure T lymphocytes to that of spleen cells in spite of the remarkable increase in their adhesion percentage after purification (Figs 4 and 5). This finding suggests that T cells are main populations to perform the adhesion in the spleen cells. However, it did not affect the adhesion ability to laminin either in vitro or in vivo. Furthermore, we also examined the adhesion of spleen cells from normal mice when stimulated by Con A in vitro and *N*-desulfated heparin showed the same inhibition pattern (Fig. 6) as the case when Con A was used in vivo.

To confirm the effect of *N*-desulfated heparin to the adhesion ability of lymphocytes, we further examined adhesion activity of human T lymphoma Jurkat cells in vitro. As shown in Fig. 7, PDBu significantly enhanced the adhesion of Jurkat cells to type I collagen, and *N*-desulfated heparin significantly inhibited the enhancement at the concentration of 10^{-8} and 10^{-6} g/ml.

Since *N*-desulfated heparin inhibited the adhesion when pretreated for T cells (Fig. 7) or co-cultured with spleen cells (Figs. 4 and 6), its effective mechanisms may possibly include the blockade of both expression of ligands for ECM components and interaction between ECM and their ligands. It is well known that various adhesion molecules are involved in the inflammatory process of hepatitis. In Con A hepatitis model, a massive induction of ICAM-1, VCAM-1 and E-selectin in the liver has also been reported after Con A injection [30]. Wang et al. reported that various heparinoids including *N*-desulfated heparin showed an anti-inflammatory activity primarily through blocking the interaction between P- and L-selectin and their ligand SLe^x [31]. In addition, considering that heparin could directly bind with integrin [32, 33], type I collagen [34, 35] and fibronectin [25], it is possible to suggest that *N*-desulfated heparin inhibited the cell adhesion through directly interfering the binding between type I collagen, fibronectin and their receptors. These results indicated the relation of the anti-adhesion activity of heparinoids to the blockade of adhesion process. A further investigation to examine the detailed mechanisms of *N*-desulfated heparin is now in progress.

Overall, *N*-desulfated heparin could improve Con A-induced hepatitis at low doses. Its mechanism of action may be related to the inhibition of the increased adhesion capacity of T lymphocytes to ECM after Con A activation. Our findings suggest that *N*-desulfated heparin may be useful for the treatment of various inflammatory diseases including hepatitis.

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