J Toxicol Pathol 2014; 27: 231–234

Technical Report

Effects of Repeated Intravenous Administration of Dextrans, Water-soluble Macromolecules, in Rats

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Abstract: We investigated the influence of repeated intravenous administration of dextrans (DEXs) to rats. Seven-week-old Sprague Dawley rats (6 males/group) were given intravenously 10% saline solutions of dextrans (DEXs), 40 kDa or 200–300 kDa, at a dose level of 5 mL/kg/day for 28 days and they were examined histopathologically. Another group (3 males/group) was administered saline in a similar manner and served as the control. Histopathological changes indicating accumulation of DEXs in the mononuclear phagocyte system (MPS) and the liver were noted in the treated groups. The incidence and severity of the findings were molecular weight-dependent, except for the lungs. These results are considered useful in interpreting data from preclinical studies, in which DEXs or their derivatives are administered as test or control substances. (DOI: 10.1293/tox.2013–0067; J Toxicol Pathol 2014; 27: 231–234)

Key words: macromolecular substances, intravenous administration, mononuclear phagocyte system

Dextrans (DEXs) are glucose polymers, that is, kinds of polysaccharides produced by bacteria from sucrose or by chemical synthesis. They have been used clinically as plasma volume expanders for more than five decades[1] because of their high biocompatibility and lack of bioactivity (like heparins). Recently, DEXs and their derivatives have been investigated as potential macromolecular carriers for delivery of drugs and proteins to increase the intensity and prolong the pharmacologic action, and to decrease toxicity[1–4]. There are many reports regarding the tissue distribution and pharmacokinetics of DEXs of various molecular weights (MWs) after a single administration[1, 4–13]; however, there are very few reports that describe the influence of repeated administration of DEXs. In this study, we investigated the influence of repeated intravenous administration of DEXs to rats for 28 days and examined the rats histopathologically. To review the difference of MW, the test substances were as follows: DEX 40 kDa, the MW as with a plasma volume expander (Dextran 40, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and DEX 200–300 kDa, the largest MW commercially available (Dextran Clinical Grade, MW 200,000–300,000, MP Biomedicals LLC., Santa Ana, CA, USA). Each type of DEX was dissolved in physiological saline at a concentration of 100 mg/mL (10%), stirred overnight, and then sterilized using a syringe filter (Millex-GV Syringe Filter Unit SLGVO33RS, Millipore Corporation, Billerica, MA, USA) just before use. Male Sprague Dawley rats were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan), and acclimatized to laboratory conditions. The animals were 7 weeks old with a body weight range of 236.5–263.3 g at initiation of treatment. Two groups of rats (6 males/group) were treated with 10% saline solution of DEX 40 kDa or 200–300 kDa via tail vein injection at a dose level of 5 mL/kg/day for 28 days (days 1 to 28). A separate group (3 males/group) was treated with saline and served as the control. On day 29, the day after the final dosing, all animals were sacrificed by exsanguination under deep anesthesia and necropsied. The lungs, spleen, liver, and kidneys were fixed with 10% neutral buffered formalin, trimmed, dehydrated, and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (HE). For detection of polysaccharides, periodic acid schiff (PAS) staining and colloidal iron staining were also performed. Colloidal iron staining is able to detect the negatively charged hydroxyl (OH) groups of DEXs. Additionally, the sections were stained immunohistochemically using the polymeric method (Nichirei-Histofine Simple Stain MAX PO (R), Nichirei Biosciences, Tokyo, Japan) for ED1 (CD68), a marker for rat macrophages (1:500, AbD Serotec, Oxford, UK), and ED2 (CD163), a marker for rat splenic macrophages (1:500, AbD Serotec). The experimental protocol was approved by the Institutional Animal Care and Use Committee of Seikagaku Corporation Central Research Laboratories.

At necropsy, discolored red foci were seen over the partial or entire surface of the lungs. After formalin fixation,

Received: 23 December 2013, Accepted: 25 April 2014
Published online in J-STAGE: 9 June 2014
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discolored white punctate foci over the entire surface of the lungs were noted in all animals in the treatment groups (Fig. 1, A–C). In the kidneys, discolored red punctate foci on the kidney surface were noted in both treatment groups. Microscopically, in the lungs of the treatment animals, diffuse intra-alveolar foam cell aggregation were observed. PAS-positive granules were noted within the foam cells (Fig. 1, D). The severity of the lung lesions was similar between the DEX 40 kDa and DEX 200–300 kDa groups. In the liver, cytoplasmic vacuolation of hepatocytes was noted in the treatment groups. These vacuoles were positive for the colloidal iron staining, which indicates accumulation of DEXs (Fig. 2, A–C). In the DEX 200–300 kDa group, Kupffer cells were increased in number and slightly to moderately hypertrophied, and they contained granules staining positively with PAS (Fig. 2, F). The higher MW DEX resulted in more severe findings and an increase in incidence, i.e., MW-dependency was observed in the liver. In the spleen, foam cell aggregation in the marginal zone and its adjacent area in the red pulp were noted in a MW-dependent manner. PAS-positive granules were noted within the foam cells (Fig. 2, G-I). In the kidneys of the DEX 200–300 kDa group, sparse foamy deposits were seen in the glomerular mesangial area. Sporadic foam cell infiltration was also seen in the cortical interstitial tissue. PAS-positive granules were stained in the foamy deposits as in the case of the foam cells (Fig. 3, A). No histopathological changes were noted that were considered to be related to discolored red punctate foci on the kidney surface found at necropsy. In the immunohistochemistry, the foam cells were ED1-positive in the kidneys (Fig. 3, B), liver, lungs and spleen, and ED2-positive in the liver, lungs, and spleen (data not shown).

Water-soluble macromolecules have generally low clearance and a relatively long plasma half-life, resulting in accumulation in MPS[6], or vacuolization of the renal proximal tubular cells (so-called osmotic nephrosis)[10, 14–16]. In this study, histopathological changes indicating accumulation of DEXs in the MPS and the liver were noted in the treated groups. These results were similar but milder to those in a 1-month repeated intravenous study of a DEX plasma volume expander, 10% saline solution of DEX 40 kDa, at dose levels of 20, 40, and 80 mL/kg/day in rabbits[17]. In the present study, the incidence and severity of the findings were MW-dependent, except for the lungs. Mehvar *et al*. reported the MW-dependency of the pharmacokinetics and tissue distribution of DEXs. After intravenous administration of a single 5 mg/kg dose of DEXs with MWs of 4, 20, 70 and 150 kDa in rats, MW-dependent tissue accumulation of DEXs was found in the liver and spleen but not in the heart, lungs and brain. The tissue:serum AUC ratios (an indication of tissue targetability) for the spleen increased from 0.095 for DEX 4kDa to 9.56 for DEX 150 kDa, while for the lungs, the ratios were 0.367, 0.537, 0.347, and 0.118 for DEX 4, 20, 70 and 150 kDa, respectively[1, 5, 6]. These data are consistent with our histopathological results; however, Mehvar *et al*. also showed that after a single 5 mg/kg dose of DEXs

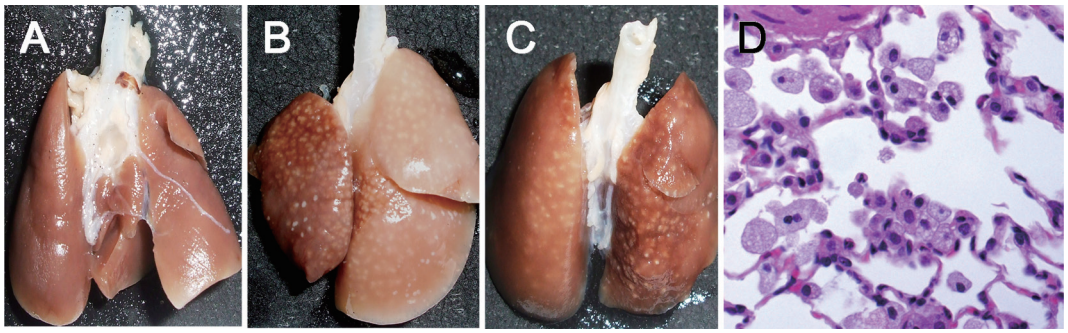

Fig. 1. Gross and histopathological findings in the lungs of SD rats intravenously administered DEXs for 28 days. Discolored white punctate foci were found on the lungs after formalin fixation. The severity of the lesions on the lungs in the DEX 40 kDa and 200–300 kDa groups was similar. (A, saline; B, DEX 40 kDa; C, DEX 200–300 kDa). Intra-alveolar aggregation of foam cells was seen in the lungs of all treated animals (D, a lung of a DEX 40 kDa treated rat; PAS, ×400).

Fig. 2. Histopathological findings of the liver and spleen from SD rats intravenously administered DEXs for 28 days. Cytoplasmic vacuoles were stained blue with colloidal iron staining in the hepatocytes in a MW-dependent manner (A, saline; B, DEX 40 kDa; C, DEX 200–300 kDa; colloidal iron, ×400). In the DEX 200–300 kDa group, Kupffer cells were slightly to moderately hypertrophied (F, arrowheads) and increased in number, and they contained granules stained positively with PAS (D, saline; E, DEX 40 kDa; F, DEX 200–300 kDa; PAS, ×400). Foam cell infiltration was noted in some interstitial tissue (F, arrow). Aggregation of foam cells was noted in the splenic marginal zone and its adjacent area in the red pulp in a MW-dependent manner (G, saline; H, DEX 40 kDa; I, DEX 200–300 kDa; PAS, ×200)

Fig. 3. Histopathological findings of the kidneys from SD rats intravenously administered DEXs for 28 days. Sparse foamy deposits in the glomeruli and sporadic foam cell infiltration in the cortical interstitial tissue were noted in the kidneys of the DEX 200–300 kDa group. PAS-positive granules were stained in the foamy deposits as in the case of foam cells (A, arrowheads; PAS, ×200). No changes were noted in the epithelium of the convoluted tubules. The foam cells in the cortical interstitial tissue and mesangial foamy deposits in the glomeruli were positive for ED1 in the kidneys (B, arrowheads; Immunohistochemistry for ED1, ×400).

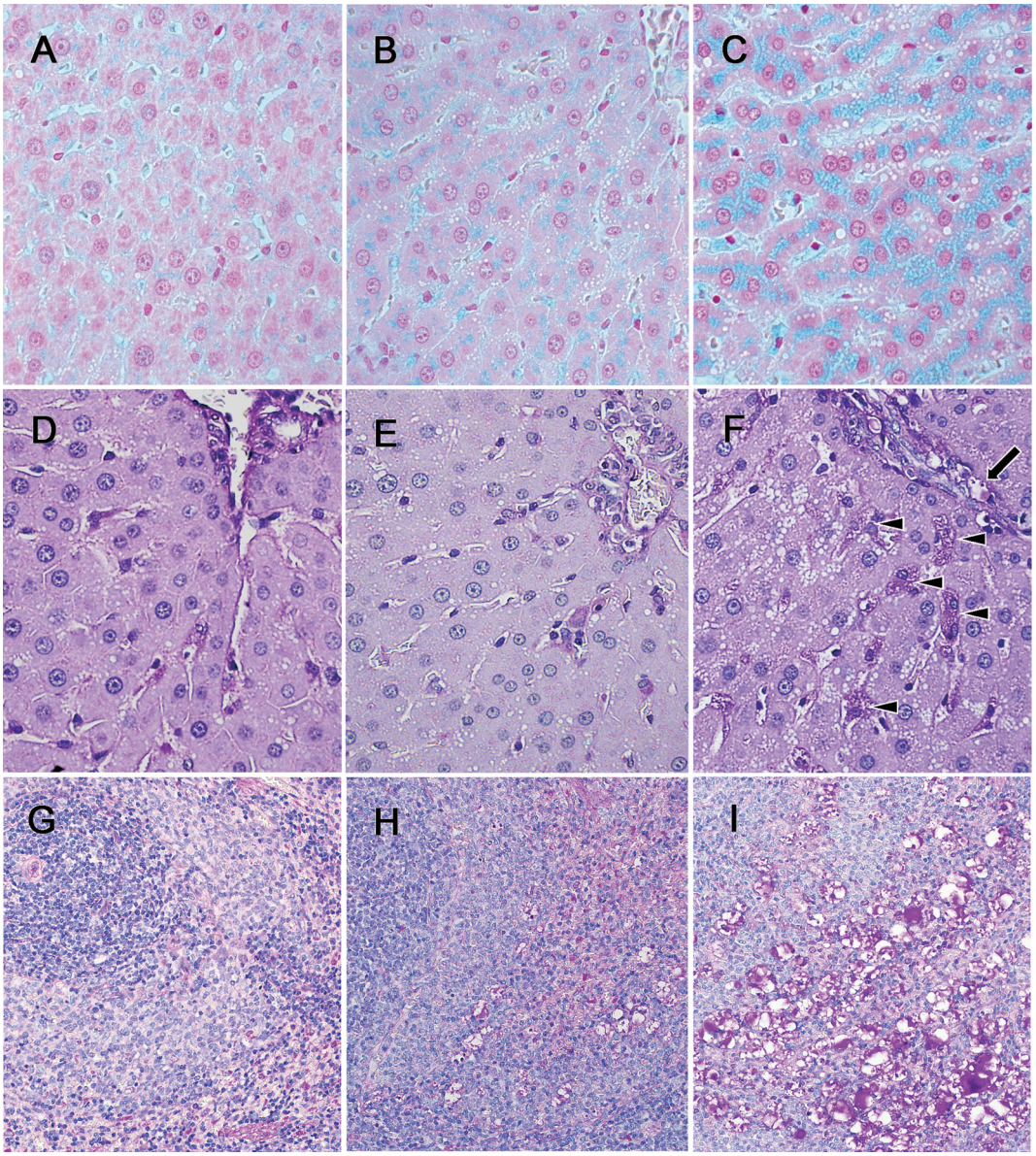

Fig. 2.

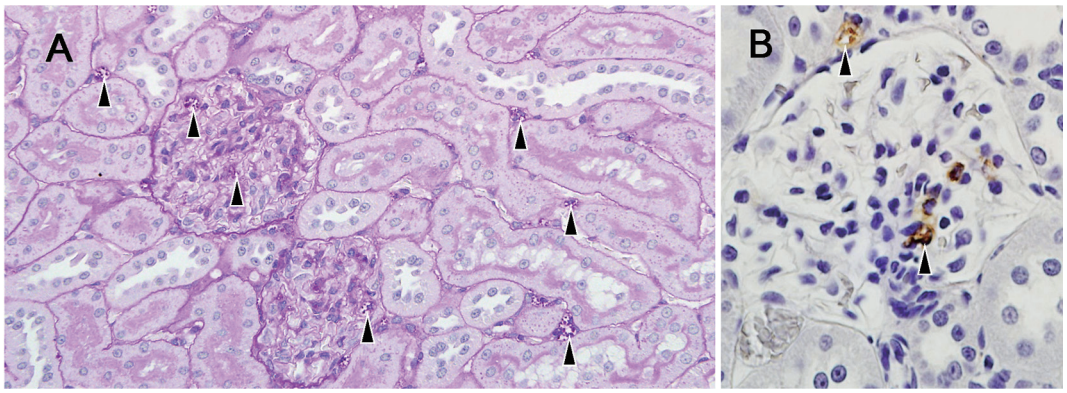

Fig. 3.

in rats, the tissue:serum AUC ratios for the liver increased from 0.346 for DEX 4 kDa to 28.8 for DEX 70 kDa, and decreased to 8.59 for DEX 150 kDa. Our 28-day repeated dose study showed remarkable accumulation of DEX 200–300 kDa in the liver, so it seems that repeated administration would impact tissue accumulation of high MW DEXs.

Osmotic nephrosis can be induced by a variety of substances, such as DEXs, sucrose, hydroxyethyl starch, imaging agents[16] and PEGylated substances[14, 18]. Vacuolization and swelling of the proximal tubular cells reflects accumulation of exogenously administered solutes in the proximal tubular lysosomes. Occasionally, the parietal epithelial cells lining the Bowman's capsule, the podocytes or interstitial histiocytes may be affected and show similar vacuoles[16]. In this study, no histopathological changes were noted in the proximal tubular cells, but sparse foamy deposits in the glomerular mesangial area and sporadic foam cell infiltration in the cortical interstitial tissue were noted in the DEX 200–300 kDa group, which might be related to an osmotic nephrosis that was induced by the experimental conditions.

In conclusion, histopathological changes indicating accumulation of DEXs in MPS and the liver were induced by 28 days of repeated intravenous administration. These changes were MW-dependent in the liver and spleen, but not in the lungs, and this might be the result of the difference in tissue distribution of parentally administered DEXs. These results are considered useful in interpreting data from preclinical studies, in which DEXs or their derivatives are administered as test or control substances.

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