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## Report

# Intraperitoneal injection of dextran sulfate as an anti-adherent drug for the prevention of peritoneal metastasis of cancer shows low toxicity in animals

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**Intraperitoneal dextran sulfate with a mean molecular weight of $5 \times 10^5$ has been developed for use in an anti-adherent therapy against peritoneal carcinomatosis. The present study examined acute toxicity of i.p. injection of dextran sulfate in mice and rabbits. The 10, 50 and 90% lethal dose values are 0.213 (0.146–0.252), 0.336 (0.291–0.405) and 0.530 mg/g (0.431–0.873 mg/g: 95% confidence interval) in mice, respectively. These are markedly larger than the efficacious dose of 0.005–0.01 mg/g obtained previously. Death or symptoms of intoxication were seen within 3 days after administration of toxic doses. Rabbits received i.p. injection of dextran sulfate at 0.02 mg/g, which was close to the efficacious dose. At 2, 4, 6, 8 and 13 days after administration, blood was taken for biochemical and hematological analyses. Dextran sulfate at 0.02 mg/g induced no remarkable abnormal findings. These results suggest that the i.p. dextran sulfate is safe as an anti-adherent agent against peritoneal metastasis of cancer. [© 2000 Lippincott Williams & Wilkins.]**

*Key words*: Anti-cancer-cell-adherent therapy, dextran sulfate, peritoneal metastasis, toxicity.

## Introduction

Peritoneal metastasis is one of the most common recurrent diseases after surgery for gastrointestinal malignancies. We have developed dextran sulfate with a high molecular weight as an anti-adherent drug for the prevention and treatment of peritoneal metastasis. It has been previously reported that i.p. dextran sulfate with a mean molecular weight of $5 \times 10^5$ effectively prevents cancer cells that cause peritoneal metastatic lesions in mice from adhering onto the peritoneum, thus improving the survival of mice bearing i.p. cancer cells.[1]

In the present study, we examined the lethal dose, autopsy, body weight changes, intoxication symptoms, and hematological and biochemical analyses of i.p. injected dextran sulfate in mice and rabbits.

## Materials and methods

Dextran sulfate (Dextran sulfate®; Sigma, St Louis, MO) of mean molecular weight $5 \times 10^5$ was dissolved in physiological saline. Concentrations of dextran sulfate ranging from 2 to 9.52 mg/ml in 10 increasing levels (1.19-fold increase/level) was used within 1 h of preparation.

### Toxicity in mice

Ninety-six male mice ($BDF_1$ strain, 5 weeks old) were purchased from the Shimizu Laboratory Animal Center (Kyoto, Japan). The mice were maintained under standard conditions (specific pathogen-free, room temperature of 22°C, relative humidity of 60%, day–night cycle of 12 h), and were allowed free access to standard mouse food and tap water from 5 days before drug administration until the end of the experiment. On day 0, after being acclimatized to the living conditions for 5 days, the mice (21–22 g body weight) were divided into 12 groups of eight mice each. Ten

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groups were given dextran sulfate solution at the aforementioned concentrations. The remaining two control groups were given either only physiological saline or nothing. The drugs were given in a volume of 0.05 ml/g body weight i.p. using a 23-G needle. In the 10 groups given dextran sulfate, dextran sulfate doses ranging from 0.1 to 0.476 mg/g were given in 10 dose levels, which were increased at a rate of 1.19-fold/level.

The mice were observed daily for 14 days after the drug administration, and intoxication symptoms, body weight changes and date of death were recorded. The LD($Y$) curve [$X$=LD($Y$), where $Y$ is the percentage of dead mice and $X$ is the dose of dextran sulfate] and the 95% confidence interval (CI) of $X$ for the respective $Y$ was calculated by the probit method[2] using computer software SAS/STAT (SAS Institute Inc, 1996).

The surviving animals were sacrificed on day 15. All animals, both dying of intoxication and surviving for the observation period of 14 days, were autopsied for macroscopic and microscopic changes in their body organs. The lungs, heart, liver, spleen, kidneys, thymus, testis, adrenal glands, small intestine and large intestine were removed and weighed. Then the absolute organ weight was used to calculate the relative organ weight with respect to the total body weight to better evaluate the change of organ weight due to toxicity. The weighed organs were then fixed with 10% buffered formalin, embedded in paraffin, sliced into 4 $\mu$m microscopic specimens and stained with hematoxylin & eosin. These microscopic specimens were examined microscopically for pathologic changes associated with drug toxicity.

### Toxicity in rabbits

In this part of the study dextran sulfate at 0.02 mg/g, which is close to the efficacious dose of 0.01–0.005 mg/g, was examined for hematological and biochemical effects. Four male rabbits (Japan-White strain, 2.5–2.7 kg in body weight) were purchased from the Shimizu Laboratory Animal Center. The rabbits were maintained under standard conditions, and were allowed free access to standard rabbit food and tap water from 7 days before the beginning of the study until the end of the experiment. On day 0, after being acclimatized to the living conditions for 7 days, blood was taken from the veins located near the margin of the ears. On day 3, the rabbits underwent general anesthesia with i.v. pentobarbital sodium at 25 mg/kg. A small laparotomy incision was made on the upper abdomen and a plastic tube was inserted through the incision into the peritoneal space so that dextran sulfate could be given precisely into the peritoneal space through the tube. Dextran sulfate at 20 mg/kg was administered. Then, the tube was removed and the laparotomy incision was closed by sutures.

Blood was taken on days 5, 7, 9 and 16 (2, 4, 6, 8 and 13 days after drug administration). Blood taken from each rabbit on day 0 (3 days before drug administration) and days 5, 7, 9 and 16 (2, 4, 6, 8 and 13 days after drug administration) was divided into two samples. One was used for hematological examination including erythrocyte count, leukocyte count, platelet count, hematocrit value, hemoglobin value, mean corpuscular volume of the red cells, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. The other blood sample was centrifuged at 6000 r.p.m. for 5 min, and the supernatant (blood plasma) was used for biochemical analysis of total bilirubin, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, lactic dehydrogenase, amylase, creatine phosphokinase, serum glucose, blood urea nitrogen, creatinine, sodium level, chloride level, potassium level, calcium level and C-reactive protein.

## Results

### Lethal dose values in mice

The LD($Y$) curve [$X$=LD($Y$), where $X$ is the dose of dextran sulfate and $Y$ is the rate of dead mice] and its 95% CI are shown in Figure 1. The $LD_{10}$, $LD_{50}$ and $LD_{90}$ values were 0.213, 0.336 and 0.530 mg/g, respectively, and the 95% CIs were 0.146–0.252, 0.291–0.405 and 0.431–0.873 mg/g, respectively (Table 1).

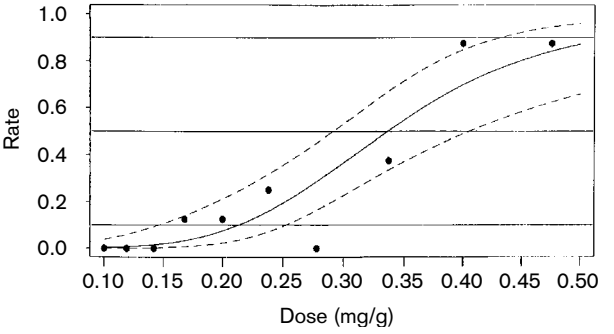


**Figure 1.** LD($Y$) curve and the 95% CI. $X$=LD($Y$), where $X$ is the dose of dextran sulfate in mg/g and $Y$ is the fitted rate of number of dead mice, was calculated by the probit method. The solid line shows the curve of $X$=LD($Y$), and broken lines indicates the lower and upper limits of the 95% CI. Closed circles indicate the observed rate of dead mice.

### Intoxication symptoms, date of death and body weight changes in mice

As the intoxication symptoms, weakness, lethargy and dishevelment were seen in all mice given dextran sulfate at doses more than 0.337 mg/g. These symptoms began on day 0 or 1 and disappeared by day 4.

The deaths were observed in groups given dextran sulfate at doses higher than 0.168 mg/g. All deaths were seen within 3 days after drug administration. There were no deaths in mice given physiological saline and in mice in the control group (Table 2).

Body weight changes are shown in Figure 2(A and B). In the mice given dextran sulfate at doses larger than the $LD_{50}$ value, weight loss continued for the first

**Table 1**. Lethal dose values of dextran sulfate

| Lethal probability (%) | Dose (mg/g) | 95% CI (mg/g) |
|---|---|---|
| 5 | 0.182 | 0.111–0.224 |
| 10 | 0.213 | 0.146–0.252 |
| 20 | 0.252 | 0.195–0.291 |
| 30 | 0.282 | 0.232–0.325 |
| 40 | 0.309 | 0.264–0.361 |
| 50 | 0.336 | 0.291–0.405 |
| 60 | 0.366 | 0.318–0.460 |
| 70 | 0.401 | 0.345–0.534 |
| 80 | 0.448 | 0.379–0.647 |
| 90 | 0.530 | 0.431–0.873 |
| 95 | 0.619 | 0.483–1.156 |

**Table 2**. Mortality of mice

| Dose of dextran sulfate (mg/g) | No. of dead mice/ no. of mice tested (mortality rate in %) | Date of death (days after administration) |
|---|---|---|
| 100 | 0/8 (0) | – |
| 119 | 0/8 (0) | – |
| 142 | 0/8 (0) | – |
| 168 | 1/8 (12.5) | 1 |
| 200 | 1/8 (12.5) | 1 |
| 238 | 2/8 (25.0) | 1,1 |
| 278 | 0/8 (0) | – |
| 337 | 3/8 (37.5) | 1,1,3 |
| 400 | 7/8 (87.5) | 1,1,1,1,3,3,3 |
| 476 | 7/8 (87.5) | 1,1,1,2,2,3,3 |
| 0[a] | 0/8 (0) | – |
| 0[b] | 0/8 (0) | – |

[a]Nothing was administered.
[b]Physiological saline without dextran sulfate was administered.

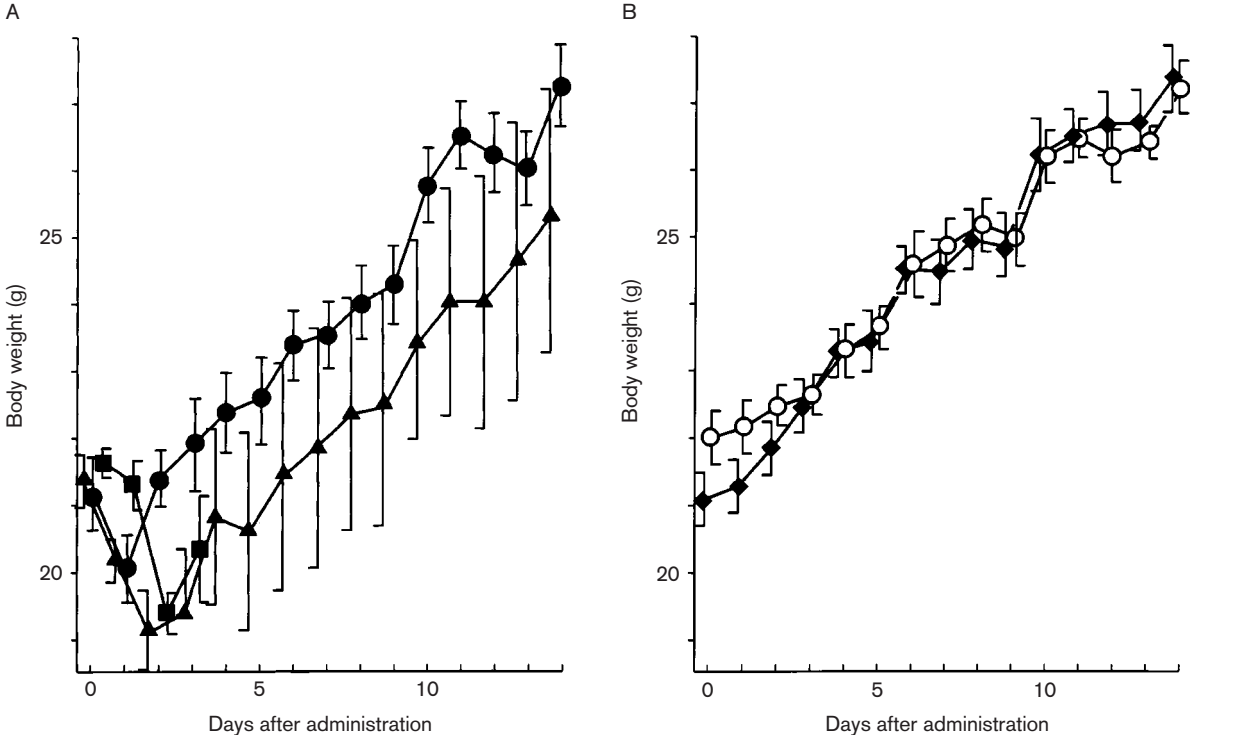


**Figure 2.** (A) Body weight changes in mice given dextran sulfate. Body weight changes are shown in the mice given dextran sulfate at doses of 0.278 (●), 0.337 (▲) and 0.400 (■) mg/g, which were close to the $LD_{30}$, $LD_{50}$ and $LD_{70}$ values. (B) Body weight changes in mice given nothing (◆) and in mice given physiological saline without dextran sulfate (○). The body weights increased to a similar extent in the two groups. Vertical bars represents the standard error.

2 days and began to increase on day 3 or 4. In the mice given dextran sulfate at a dose close to the $LD_{30}$ value, minimal weight loss was seen on day 1 and began to increase on day 2 (Figure 2A). In the mice given physiological saline without dextran sulfate and mice given nothing, body weights increased similarly (Figure 2B).

### Autopsy findings in mice

In dead mice, macroscopically the lungs appeared slightly congested, the liver and kidneys were anemic, and the mucosa of the small intestine seemed inflammatory, sometimes combined with erosion. Microscopically, the mucosa of the small intestine

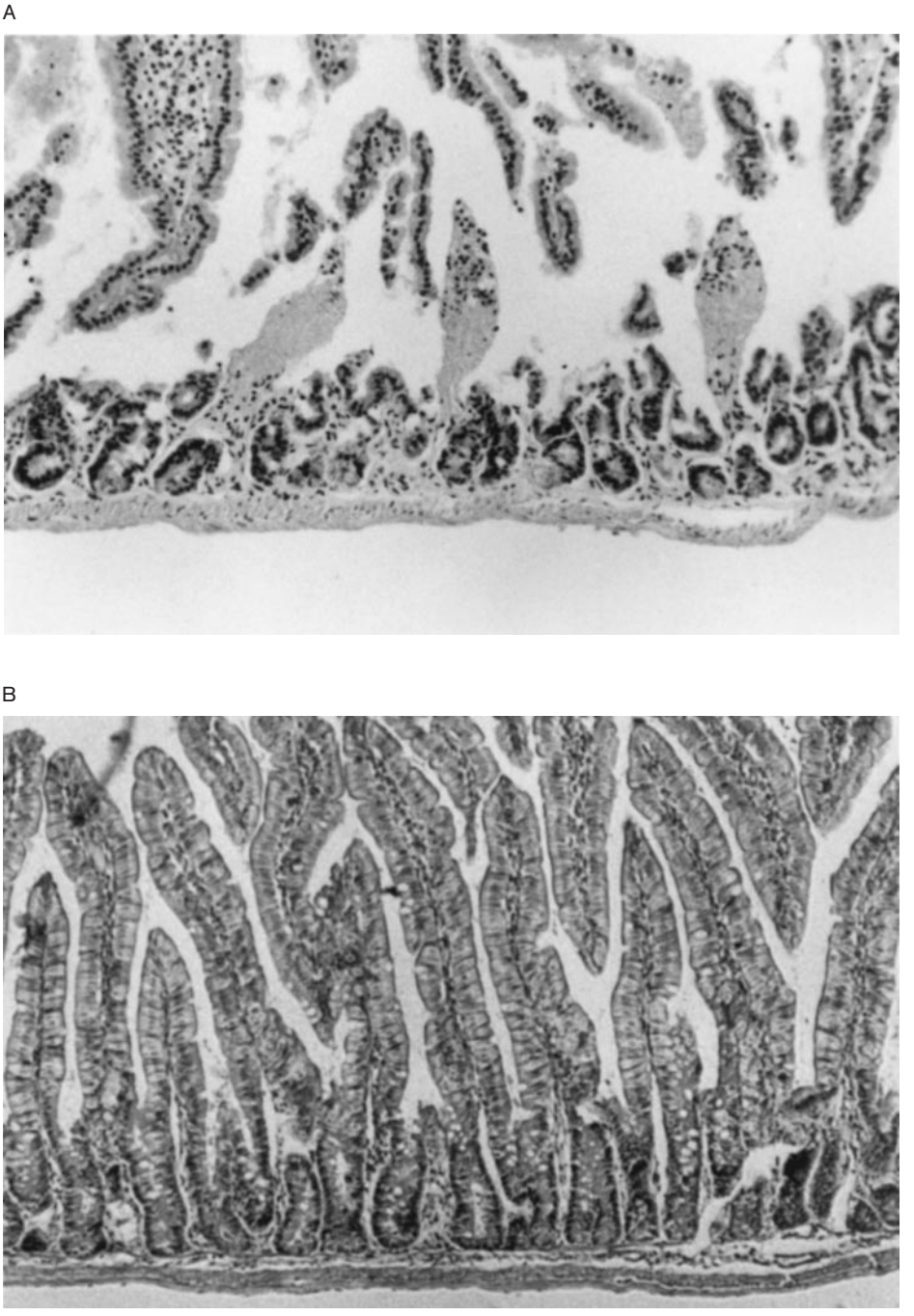


**Figure 3.** (A) Microscopic view of the small intestine of mice dying from intoxication by dextran sulfate. Erosion and degenerative changes are seen in the mucosal layer. (Original magnification $\times$100.) (B) Microscopic view of the small intestine of mice surviving for 14 days. Erosion and degenerative changes are not seen in the mucosal layer. (Original magnification $\times$100.)

was necrotic and/or degenerative (Figure 3A). However, these microscopic findings were not seen in mice surviving up to day 14 (Figure 3B).

Both absolute organ weight and relative organ weight (organ weight to body weight) are shown in Table 3. There were no remarkable differences in the weights of the organs between the mice that died from toxicity and the mice in the control group.

### Hematological and biochemical analyses in rabbits

Hematological data are shown in Table 4 and biochemical data are shown in Table 5. There were no significant changes in the hematological and biochemical data after drug administration, as compared to those before drug administration, except for C-reactive protein level. C-reactive protein, which indicates non-specific inflammatory response, was elevated significantly on day 5 (2 days after drug administration).

## Discussion

We have already reported that dextran sulfate with a high molecular weight is efficacious as an anti-cell-adherent agent against peritoneal carcinomatosis. Our study using mice revealed that dextran sulfate

**Table 3**. Organ weight changes in dead mice and in control mice

| Dose of dextran sulfate (no. of mice) | Mean weight of organ (g) [mean of organ weight/body weight (mg/g)] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Heart | Lung | Liver | Kidney | Testis | Spleen | Thymus |
| Control[a] ($n=8$) | 0.131 [6.09] | 0.180 [8.37] | 1.31 [61.1] | 0.382 [17.8] | 0.147 [6.84] | 0.076 [3.53] | 0.045 [2.09] |
| 0.168 mg/g ($n=1$) | 0.113 [5.95] | 0.398 [11.0] | 1.41 [74.3] | 0.347 [18.3] | 0.124 [6.53] | 0.060 [3.16] | 0.030 [1.58] |
| 0.200 mg/g ($n=1$) | 0.112 [5.09] | 0.234 [10.6] | 1.32 [60.0] | 0.390 [17.7] | 0.131 [5.95] | 0.065 [2.95] | 0.039 [1.77] |
| 0.238 mg/g ($n=3$) | 0.138 [7.05] | 0.200 [9.30] | 1.37 [63.5] | 0.438 [20.4] | 0.141 [6.57] | 0.082 [3.80] | 0.052 [2.43] |
| 0.337 mg/g ($n=3$) | 0.151 [7.81] | 0.199 [10.3] | 1.23 [63.7] | 0.377 [19.5] | 0.123 [6.36] | 0.080 [4.16] | 0.047 [2.43] |
| 0.400 mg/g ($n=7$) | 0.142 [6.65] | 0.209 [9.80] | 1.32 [61.6] | 0.403 [18.9] | 0.145 [6.81] | 0.080 [3.73] | 0.042 [1.99] |
| 0.476 mg/g ($n=7$) | 0.123 [6.09] | 0.217 [8.37] | 1.32 [61.1] | 0.411 [17.8] | 0.142 [6.84] | 0.070 [3.53] | 0.044 [2.09] |

[a]Nothing was administered.

**Table 4**. Hematological examination data

| Analysis items | Mean [95% CI] | | | | | |
|---|---|---|---|---|---|---|
| | 3 days before administration | Days after administration | | | | |
| | | 2 | 4 | 6 | 8 | 13 |
| Erythrocyte count ($\times 10^4$ cells/$\mu$l) | 633.2 [504–763] | 613 [417–810] | 594 [502–685] | 558 [507–610] | 570 [474–666] | 579 [498–658] |
| Hemoglobin value (g/dl) | 12.6 [10.0–15.3] | 12.3 [9.1–15.4] | 11.3 [9.9–12.6] | 10.8 [10.2–11.3] | 10.8 [9.0–12.5] | 11.0 [9.3–12.8] |
| Hematocrit value (%) | 42.1 [31.3–52.9] | 39.3 [26.8–51.8] | 37.8 [32.6–43.1] | 36.9 [32.5–41.2] | 37.7 [30.8–44.4] | 38.2 [31.1–45.3] |
| Thrombocyte count ($\times 10^3$ cells/$\mu$l) | 373 [189–556] | 375 [66–684] | 290 [26–553] | 490 [30–950] | 452 [54–849] | 317 [0–930] |
| Mean corpuscular volume (fl) | 65.8 [60.6–71.0] | 63.8 [58.5–69.0] | 63.5 [58.2–68.8] | 65.8 [62.6–68.9] | 65.5 [62.3–68.7] | 65.4 [60.7–70.0] |
| Mean corpuscular hemoglobin (pg) | 19.9 [18.9–20.9] | 20.0 [18.2–21.8] | 19.0 [16.7–21.3] | 19.2 [18.1–20.4] | 18.8 [17.7–20.0] | 19.1 [18.6–19.5] |
| Mean corpuscular hemoglobin concentration (%) | 30.0 [28.0–31.9] | 31.3 [28.0–34.6] | 29.8 [27.1–32.6] | 29.2 [27.0–31.4] | 28.6 [27.5–29.8] | 28.9 [27.8–30.1] |
| Leukocyte count ($\times 10^2$ cells/$\mu$l) | 101 [67–136] | 118 [57.9–178] | 117 [70.1–162] | 118 [96.7–139] | 117 [100–134] | 110 [84–137] |

**Table 5**. Biochemical analysis data

| Analysis items | Mean [95% CI] | | | | | |
|---|---|---|---|---|---|---|
| | 3 days before administration | Days after administration | | | | |
| | | 2 | 4 | 6 | 8 | 13 |
| Sodium (mEq/l) | 138 [134–142] | 140 [136–143] | 140 [137–143] | 141 [138–144] | 141 [138–144] | 138 [134–142] |
| Chloride (mEq/l) | 98 [94–102] | 98 [89–107] | 100 [91–108] | 101 [92–110] | 101 [96–106] | 98 [90–107] |
| Potassium (mEq/l) | 5.2 [3.1–7.4] | 6.5 [2.6–10.4] | 6.9 [4.8–9.0] | 5.9 [4.1–7.7] | 5.1 [3.6–6.5] | 6.8 [4.2–9.4] |
| Calcium (mg/dl) | 14.2 [12.8–15.6] | 13.8 [12.4–15.1] | 14.0 [12.6–15.4] | 13.5 [12.7–14.2] | 14.0 [13.1–14.9] | 14.2 [13.2–15.2] |
| Creatinine (mg/dl) | 0.78 [0.43–1.13] | 0.69 [0.58–0.80] | 0.76 [0.50–1.0] | 0.84 [0.57–1.1] | 0.75 [0.49–0.99] | 0.74 [0.61–0.87] |
| Blood urea nitrogen (mg/dl) | 22.9 [11.5–34.3] | 17.3 [9.3–25.3] | 17.1 [2.5–31.7] | 19.2 [15.4–23.1] | 20.8 [12.6–29.0] | 23.8 [16.5–31.0] |
| Total protein (g/dl) | 5.8 [4.9–6.7] | 6.0 [4.9–7.1] | 6.1 [5.3–6.9] | 6.0 [5.2–6.8] | 5.7 [5.4–6.2] | 5.9 [4.5–7.3] |
| Albumin (g/dl) | 4.6 [4.0–5.1] | 4.3 [3.0–5.5] | 4.3 [3.8–4.7] | 4.2 [3.7–4.7] | 4.3 [3.9–4.8] | 4.3 [3.5–5.0] |
| Serum glucose (mg/dl) | 95 [86–103] | 107 [68–146] | 104 [82–126] | 105 [89–122] | 97 [84–110] | 96 [88–104] |
| C-reactive protein (mg/dl) | 1.0 [0–3.8] | 5.2 [1.5–8.9] | 3.3 [0–8.3] | 2.3 [0–5.6] | 1.8 [0–3.6] | 1.3 [0–2.6] |
| Total bilirubin (mg/dl) | 0.1 [0.1] | 0.2 [0.2] | 0.2 [0.2] | 0.1 [0–0.2] | 0.2 [0.2] | 0.2 [0.2] |
| AST (IU/l) | 32 [9–54] | 35 [19–51] | 46 [21–71] | 45 [40–49] | 21 [9–32] | 31 [6–56] |
| ALT (IU/l) | 39 [31–47] | 38 [18–57] | 39 [25–53] | 37 [17–57] | 28 [14–42] | 34 [18–51] |
| LDH (IU/l) | 932 [232–1631] | 1005 [646–1364] | 1381 [0–2791] | 1372 [625–2118] | 660 [87–1233] | 965 [0–2100] |
| CPK (IU/l) | 1450 [623–2277] | 1426 [538–2314] | 2062 [0–6003] | 1943 [0–5076] | 1550 [467–2634] | 781 [378–1184] |
| Amylase (IU/l) | 241 [103–378] | 345 [188–501] | 331 [104–558] | 265 [168–361] | 266 [173–358] | 276 [217–335] |

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactic dehydrogenase; CPK, creatine phosphokinase.

at doses of 0.1–0.2 mg/mouse, which corresponded to less than 0.005–0.01 mg/g body weight, effectively prevents i.p. seeded malignant cells from causing peritoneal metastasis and improves the survival of mice bearing cancer cells i.p.[1] In the present study dextran sulfate was tested for its toxicity and revealed that the lethal doses are markedly larger than the efficacious dose, and the acute toxicity disappears after a relatively short period of time of 3–4 days. In rabbits, low-dose

dextran sulfate of 0.02 mg/g, which is close to the efficacious dose, was tested for the effects on hematological data of peripheral blood and biochemical data of blood plasma. Dextran sulfate at 0.02 mg/g induced no abnormal changes, except for an elevation of C-reactive protein level. C-reactive protein level, which indicates non-specific inflammatory response, was elevated significantly on day 5 (2 days after drug administration). In the present study, drug was administered after a laparotomy, which is known to cause elevation of C-reactive protein. The significant elevation of C-reactive protein level seems to be not due to the toxicity of dextran sulfate but due to the laparotomy with which dextran sulfate was administered.

We have reported that dextran sulfate has no lethal toxicity on normal cells and cancer cells: dextran sulfate at a concentration of 0.1 mg/ml induces neither necrosis nor apoptosis *in vitro* even when it detaches the cells.[3] In the present study, the subacute and chronic toxic effects were not examined. Although further studies for such toxicity must be performed, the present results suggest that dextran sulfate with a high molecular weight can be safely administered i.p. at a dose of 0.005–0.01 mg/g as anti-cancer-cell-adherent agent.

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