DEPRESSIVE EFFECT OF T-2 TOXIN ON HEPATOCYTE PROLIFERATION UNDER TWO EXPERIMENTAL CONDITIONS

Min-Jae Lee, Makio Takeda, Kensuke Hirasawa, Shin-ichi Itagaki, and Kunio Doi

Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo

Abstract: The effect of T-2 mycotoxin on hepatocyte proliferation was investigated in mice using two models of hepatocyte regeneration, i.e. 2/3 partial hepatectomy (Ph) and CCl₄-intoxication (CCl₄). At 2 days after Ph or CCl₄, mice were given T-2 toxin (2 or 4 mg/kg, po). Five mice each were killed daily up to 7 days after Ph or CCl₄, and they were injected with BrdU (100 mg/kg, ip) at 1 hour before sacrifice. T-2 toxin significantly delayed the recovery of liver weight loss after Ph or CCl₄, and it also significantly delayed the reduction in size of centrilobular hepatocyte necrosis after CCl₄. In addition, T-2 toxin significantly depressed BrdU-uptake by hepatocytes in the early days after Ph or CCl₄. It can be said that T-2 toxin depressed the proliferation of hepatocytes, resulting in the delay of recovery of liver weight loss. This seems to be related to the inhibitory effect of T-2 toxin on nuclear acid synthesis of hepatocytes. (*J Toxicol Pathol* 7: 449~454, 1994)

Key words: BrdU, CCl₄, Hepatocyte proliferation, Mouse, Partial hepatectomy, T-2 toxin

Introduction

T-2 toxin [3 α -hydroxy-4 β , 15-diacetoxy-8- α -(3 methylbutyryloxy)-12, 13-epoxytrichothec-9-ene] is a member of trichothecene mycotoxins produced by *Fusarium* species^{1,2}. This toxin has radiomimetic³ as well as immunosuppressive effects⁴ and affects actively dividing cells in the gastrointestinal mucosa and lymphoid and hematopoietic tissues in many animal species⁴. In addition, it is well known that T-2 toxin could induce degenerative changes in the liver in some animal species including mice and rats⁵⁻⁷. However, there are only few reports on the effect of T-2 toxin on hepatocyte proliferation. In this regard, liver regeneration is proposed to be a good system for studying cell proliferation⁸.

This study was carried out to clarify the effect of T-2 toxin on hepatocyte proliferation using two

experimental models of liver regeneration, i.e. partial hepatectomy and CCl₄-intoxication. Administration of T-2 toxin after partial hepatectomy or CCl₄-intoxication was done 2 days after those treatments based on the results of preliminary examinations.

Materials and Methods

Animals

One hundred and ninety-five 5-week-old ICR: CD-1 male mice weighing 25-30 g were purchased from Charles River Japan Inc. The animals were housed in an isolator caging system (Niki Shoji Co.) in an air conditioned animal room (temperature; 23 $\pm$ 2°C, relative humidity; 55 $\pm$ 5%) and fed commercial pellets, MF (Oriental Yeast Co. Ltd.) and water *ad libitum* throughout the experimental period.

Chemicals

T-2 toxin (Sigma Chemicals) dissolved in 20% ethanol in phosphate buffered saline (PBS), CCl₄,

李 旻宰 武田真記夫 平澤健介 板垣慎一 土井邦雄

Accepted for publication: August 7, 1994

Mailing address: Min-Jae Lee, Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Bunkyo, Tokyo 113, Japan.

(Wako Pure Chemical Industry Co. Ltd.) in olive oil, and 5-bromo-2'-deoxyuridine (BrdU) (Sigma Chemicals) in 0.5N NaHCO₃ were used.

Experimental design and treatment

Experiment 1: Partial hepatectomy (Ph) was done on 90 animals (weighing 25.6 ± 0.98 g). Namely, two-third of the liver was surgically removed under ether anesthesia according to the method of Higgins and Anderson⁸. Ten animals which died during the first 2 days were excluded from the experiment. At 2 days after Ph, 50 animals were orally given T-2 toxin (2 or 4 mg/kg b.w.) and 30 animals solvent alone, respectively. Five animals each were killed by exanguination under ether anesthesia as scheduled up to 7 days after Ph. At one hour before sacrifice, the animals were given 100 mg/kg b.w. of BrdU intraperitoneally. In addition, 5 animals each were killed on the first and the last day of experiment and used as non-treated controls.

The dose of T-2 toxin was decided based on the results of preliminary acute toxicity studies, in which 4 mg/kg of T-2 toxin did not cause any degenerative changes in hepatocytes.

Experiment 2: Eighty five animals (Weighing 27.3 ± 1.21 g) were orally given 1 ml/kg b.w. of CCl₄. Two days later, 50 animals were orally given T-2 toxin (2 or 4 mg/kg b.w.) and 35 animals solvent alone, respectively. Five animals each were killed as scheduled in the same way. In addition, 5 animals each were killed on the first and last day of experiment and used as non-treated controls.

Liver histopathology and BrdU nuclear labeling

Immediately after macroscopic examination, the liver was weighed and then fixed in 10% neutral buffered formalin.

Paraffin sections (4 μ m) were stained with hematoxylin and eosin (HE) for histopathology or with BrdU immunohistochemistry.

Morphometry of hepatocyte proliferation and necrotic area

Morphometrical analysis was done using an image analyzer, SP500 (Olympus Co. Ltd.). Namely, in Experiment 1 and 2, randomly chosen

2,000 hepatocytes were scored for BrdU labeling on each specimen and the results were expressed as the percentage labeling index (LI), and mean LI was calculated.

In addition, in Experiment 2, the area of CCl₄-induced centrilobular hepatocyte necrosis in five hepatic lobules was calculated on each specimen, and mean area was calculated.

Statistical analysis

All values are expressed as mean $\pm$ SD, and statistical analysis was performed by use of the Student's *t*-test.

Results

Experiment 1

As compared with the Ph-group, the body weight gain was depressed in Ph+T-2 toxin groups throughout the experimental period (Fig. 1).

At 4 days after Ph, the liver weight almost returned to the preoperation value, showing the most steep increase from 3 to 4 days after Ph (Fig. 2). In contrast, the recovery of liver weight loss showed a significant delay of 2 to 3 days in the

Fig. 1. Changes in body weight.

Values are expressed as mean $\pm$ SD. *—*** Significantly different from Ph-group (*; $P < 0.05$, **; $P < 0.01$ ***; $P < 0.001$).

○: Control, ●: Ph-group, ▼: Ph+T-2 toxin (2 mg/kg)-group, ■: Ph+T-2 toxin (4 mg/kg)-group.

Fig. 2. Changes in liver weight.
(See the foot note of Fig. 1.)

Fig. 3. Changes in BrdU labeling index of hepatocytes.
(See the foot note of Fig. 1.)

Fig. 4. Liver at 3 days. BrdU immunohistochemistry, $\times 150$.
a) Ph-group. Many BrdU-positive hepatocytes (dark nuclei) are seen.
b) Ph+T-2 toxin (4 mg/kg)-group. BrdU-positive hepatocytes are fewer than those in a).

Ph+T-2 toxin-groups as compared with the Ph-group (Fig. 2).

As shown in Fig. 3, hepatocyte LI increased rapidly following Ph and reached its peak at 3 days. Then, it declined at 4 days to the level similar to that at 1 day. At 3 days after Ph, hepatocyte LI was apparently lower in a dose-dependent manner in the Ph+T-2 toxin-groups than in the Ph-group (Figs. 3 and 4). After that, there was no significant difference in hepatocyte LI between Ph-group and Ph+T-2 toxin (2 mg/kg)-group. However, in the Ph+T-2 toxin (4 mg/kg)-group, hepatocyte LI reincreased transiently at 5 days after Ph (Fig. 3).

There was no apparent evidence of degenerative changes of hepatocytes due to T-2 toxin.

Experiment 2

As compared with the CCl_4 -group, the body weight gain was depressed in the CCl_4 +T-2 toxin groups throughout the experimental period (Fig. 5).

The liver weight almost returned to the pretreatment value at 2 days after CCl_4 -intoxication and it reached a plateau one day later (Fig. 6). In the CCl_4 +T-2 toxin-groups, the recovery of liver weight loss showed a significant delay of 3 to 4 days as compared with the CCl_4 -group (Fig. 6).

Fig. 5. Changes in body weight.

Values are expressed as mean $\pm$ SD.

*-****Significantly different from CCl₄-group (*; $P < 0.05$, **; $P < 0.01$, ***; $P < 0.001$).

○: Control, ●: CCl₄-group, ▼: CCl₄+T-2 toxin (2 mg/kg)-group, ■: CCl₄+T-2 toxin (4 mg/kg)-group.

Fig. 6. Changes in liver weight.

(See the foot note of Fig. 5)

Histopathologically, marked centrilobular coagulative necrosis of hepatocytes developed at 1 day after CCl₄-intoxication. After that, necrotic area reduced day by day and almost disappeared at 7 days after CCl₄-intoxication (Fig. 7). In the CCl₄+T-2 toxin (4 mg/kg)-group, a reduction in

Fig. 7. Changes in necrotic area after CCl₄-intoxication.

(See the foot note of Fig. 5.)

necrotic area was significantly delayed throughout the experimental period (Figs. 7 and 8) as compared with the CCl₄-group.

On the other hand, in the CCl₄+T-2 toxin (2 mg/kg)-group, a reduction in necrotic area was delayed from 5 to 7 days after CCl₄-intoxication (Fig. 7). There was no apparent evidence of degenerative changes of hepatocytes due to T-2 toxin.

As shown in Fig. 9, hepatocyte LI increased rapidly and reached its peak already at 1 day after CCl₄-intoxication. After that, it declined rapidly to the level similar to that of non-treated controls at 5 days after CCl₄-intoxication. On the other hand, hepatocyte LI was lower in the CCl₄+T-2 toxin-groups at 3 and 4 days after CCl₄-intoxication (Figs. 9 and 10).

Discussion

In the present study, we, for the first time, examined the effect of T-2 toxin on hepatocyte proliferation using two models of hepatocyte regeneration, i.e. partial hepatectomy and CCl₄-intoxication in mice.

In Experiment 1, changes in the liver weight and hepatocyte LI after partial hepatectomy were similar to those previously reported in mice and rats¹⁰. Following T-2 toxin-treatment, the recov-

Fig. 8. Liver at 7 days. HE, $\times 150$.

- a) CCl_4 -group. Necrotic area is almost undetectable.
- b) CCl_4 -T-2 toxin (4 mg/kg)-group. Reduction in necrotic area is delayed.

Fig. 9. Changes in BrdU labeling index of hepatocytes.
(See the foot note of Fig. 5.)

ery of liver weight loss was delayed, and this delay seems to correlate with the prominent decrease in hepatocyte LI at 1 day after T-2 toxin-treatment, i.e. 3 days after Ph when hepatocyte LI reached its peak in Ph-group. However, the cause for re-increase of hepatocyte LI transiently observed at 3 days after T-2 toxin-treatment in the Ph+T-2 toxin (4 mg/kg)-group is still obscure.

In Experiment 2, hepatocyte LI very quickly increased and reached its peak already at 1 day after CCl_4 -intoxication and then decreased rapidly. This seems to contribute to the relatively fast recovery of liver weight loss after CCl_4 -intoxication. In addition, the area of centrilobular hepatocyte necrosis was reduced to a very small size at 7 days after CCl_4 -intoxication as reported previously¹¹.

Following T-2 toxin-treatment, the recovery of liver weight loss brought about by CCl_4 -intoxi-

Fig. 10. Liver at 4 days. BrdU immunohistochemistry, $\times 150$.

- a) CCl_4 -group. Many BrdU-positive hepatocytes are seen.
- b) CCl_4 +T-2 toxin (4 mg/kg)-group. BrdU-positive hepatocytes are fewer than those in a).

cation was delayed, and this delay correlated well with the delay in the reduction in area of centrilobular hepatocyte necrosis. These changes in turn seem to be based on the decrease in hepatocyte LI at 1 or 2 days after T-2 toxin-treatment, i.e. 3 or 4 days after CCl₄-intoxication.

Being common to Experiments 1 and 2, there was no apparent evidence of degenerative changes of hepatocytes due to T-2 toxin, and the delay in the recovery of liver weight loss both in the Ph+T-2 toxin-group and in the CCl₄+T-2 toxin-group is considered to be brought about by an inhibitory effect of T-2 toxin on nuclear acid synthesis of hepatocytes¹²⁻¹⁴, resulting in a depression of hepatocyte proliferation as indicated by the decrease in hepatocyte LI as mentioned above.

Now, we are investigating the effect of T-2 toxin on the normal cell cycle of hepatocytes in mice.

References

1. Bumburg, JR, Riggs, NV, and Strong, FM: The structures of toxins from two strains of *Fusarium tricinctum*. *Tetrahedron* **24**: 3329-3336, 1968.
2. Hsu IC, Samlley EB, Strong, FM: Identification of T-2 toxin in moldy corn associated with lethal toxicosis in dairy cattle. *Appl Microbiol* **24**: 684-690, 1972.
3. Ueno, Y.: Toxicology. In: *Trichothecenes-chemical, biological and toxicological aspects*, Y Ueno Ed, Elsevier, Amsterdam, 1983.
4. Ueno, Y, Ishii, K, Sakai, K, Knaeda, S, Tsunoda, H, Tanaka, T, and Enomoto, M.: Toxicological approaches to the metabolites of fusaria. IV. Microbial survey on "Bean-Hulls poisoning of horses" with the isolation of toxic trichothecenes, neosolaniol and T-2 toxin of *Fusarium solani* M-1-1. *Jpn Exp Med* **42**: 187-203, 1972.
5. Min-Jae Lee, Kawamura, S, Itagaki, S and Doi, K: Toxic effects of T-2 toxin on the primary hepatocyte culture from mice. *J Toxicol Pathol* **7**: 43-50, 1994.
6. Trusal, LR, and O'Brien, JC: Ultrastructural effects of T-2 mycotoxin on rat hepatocytes in vitro. *Toxicon* **24**: 481-488, 1986.
7. Quiroga, MA, Doi, C, Han, JS, Akai, H, Tamura, K, and Doi, K: Acute hepatotoxicity of T-2 toxin in mice. *J Toxicol Pathol* **4**: 35-44, 1991.
8. Lundy, B, Janet, EM, Marilyn, P, Ryoko, M, Graeme, JB, and Nelson, F: Transforming growth factor β m-RNA increases during liver regeneration: A possible paracrine mechanism of growth reguration. *Proc Natl Assoc Sci* **85**: 1539-1543, 1988.
9. Higgs, GM and Anderson, RM: Experimental pathology of the liver. *Arch Path* **12**: 186-202, 1931.
10. Noguchi, S, Ohba, Y, and Oka T: The role of transcription and messenger RNA stability in the regulation of epidermal growth factor receptor gene expression in regeneration mouse liver. *Hepatology* **15**: 88-96, 1992.
11. Bhathal, PS, Rose, NR, Mackay IR and S. Whittingham: Strain differences in mice in carbon Tetrachloride-induced liver Injury. *Br J Exp Path* **64**: 524-533, 1983.
12. Tashiro, F, Hirai, K and Ueno Y: Inhibitory effects of carcinogenic mycotoxins on deoxyribonucleic acid dependent ribonucleic acid polymerase and ribonuclease H.: *Appl Environ Microbiol* **38**: 191-196, 1979.
13. Thompson, WL and Wannemacher, JR: Detectin and quantitation of T-2mycotoxin with a simplified protein synthesis inhibition assay. *Appl Environ Microbiol* **48**: 1176-1180, 1984.
14. Rosenstein, Y and Lafarge-Frayssinet, C: Inhibitory effect of *Fusarium* T-2 toxin on lymphoid DNA and protein synthesis. *Toxicol Appl Pharmacol* **70**: 283-288, 1983.