Short communication

Sequence of and Regional Difference in Apoptotic Index in the Mouse Gastrointestinal Mucous Epithelia after T-2 Toxin Inoculation

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Abstract: Apoptotic index (AI) of the gastrointestinal mucous epithelia was examined in mice at 8, 16, 24 and 48 hours after oral inoculation (HAI) of T-2 toxin (10 mg/kg). In the fundic region of the stomach, AI prominently increased at 8 HAI and decreased thereafter in the foveolae gastricae while it started to increase slowly and became prominent at 24 HAI in the glandular base. AI in the glandular neck was constantly low. On the other hand, in the intestinal crypt epithelia, AI prominently increased at 8 HAI in all regions examined (duodenum, ileum, cecum and colon), and thereafter decreased with the lapse of time. AI was clearly higher in the small intestine than in the large one especially in the early stage. Mitotic index (MI) examined was drastically decreased at 8 HAI when AI prominently increased, and thereafter turned to recover in the lapse of time. However AI after T-2 toxin inoculation was not always proportional to MI before T-2 toxin inoculation. The sequence of and the regional difference in AI in the gastrointestinal mucous epithelia after T-2 toxin intoxication was first clarified in the present study. (J Toxicol Pathol 2000; 13: 193~196)

Key words: apoptotic index, gastrointestinal, mitotic index, mouse, T-2 toxin

T-2 toxin, a fungal metabolite produced by species of the genus *Fusarium*, is one of the most toxic members of trichothecene mycotoxin group^{1,2}. This toxin has a radiomimetic as well as an immunosuppressive effects, and it affects lymphoid and hematopoietic tissues, gastrointestinal mucosa, and skin in many animal species³⁻⁸. Recently it was clarified that T-2 toxin-induced lymphoid and hematopoietic damages were brought about by apoptotic cell death of lymphocytes and hematopoietic cells^{9,10}, and Shin-ozuka *et al.*¹¹ showed that *c-fos* mRNA induction is closely associated with T-2 toxin-induced lymphocyte apoptosis.

As to the gastrointestinal mucosa, Li *et al.*¹² first reported the T-2 toxin-induced apoptotic cell death in the ileal crypt epithelial cells. They described that, following T-2 toxin inoculation, dead cells showing pyknosis were sporadically observed in the ileal crypt epithelia and the nuclei of these cells were strongly stained by the modified TUNEL method which detects fragmented DNA *in situ* and that the dead cells showed ultrastructural characteristics of apoptosis. Therefore, they concluded that pyknotic cells are following apoptotic pathway. Similar findings were also detected in the case of lymphocyte and hematopoietic cell apoptosis in mice after T-2 toxin inoculation^{9,10}.

In this study, we examined the sequence of and regional difference in apoptotic index in the the gastrointestinal mucous epithelia of mice after T-2 toxin inoculation.

Fifty-six 5-week-old specific-pathogen-free ICR: CD-1 male mice (Charles River Japan Inc., Kanagawa) were used. The animals were maintained under controlled conditions (temperature: 22±2°C; humidity: 55±5%) and fed commercial plettes (MF, Oriental Yeast Co., Tokyo) and water *ad libitum*.

T-2 toxin (Lot No. 117F4078, Sigma Chemical Co., St. Louis, MO) was dissolved in 20% ethanol in 0.01 M phosphate buffer to obtain 10 mg/10 ml solution. After overnight fasting, 28 mice were orally inoculated with T-2 toxin (10 mg/kg b.w.), and 7 mice were killed under ethel anesthesia at 8, 16, 24 and 48 hours after inoculation (HAI), respectively. The remaining 28 mice were orally inoculated with 20% ethanol solution alone and killed in the same way.

The gastrointestinales collected from each mouse were fixed in 10% neutral buffered formalin. Coronal sections of the stomach (fundic region), duodenum (proximal portion), ileum (distal portion), cecum (middle portion) and colon (proximal portion) were embedded in paraffin. Four-μm paraffin sections were stained with hematoxylin and eosin (HE). Some of the serial sections were subjected to *in situ* detection of fragmented DNA according to the modified TUNEL method previously reported¹² in order to confirm the relationship between pyknotic and apoptotic cells.

In the present study, almost all of the nuclei of pyknotic cells observed on HE-stained sections were positively stained by modified TUNEL method, and these cells were considered to be under apoptotic cell death as mentioned above. In addition, any other cells than pyknotic ones were not stained by the method. Therefore, apoptotic index (AI) was calculated using the number of pyknotic cells in the present study. Namely, AI (pyknotic cells/total cells counted × 100%) in the

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intestine was calculated on randomly chosen¹⁰ crypts/section $\times$ 5 sections/mouse and expressed as mean $\pm$ standard error (SD) of 7 mice. As to the fundic region of stomach, AI in the foveolae gastricae, glandular neck and glandular base was calculated on randomly chosen 5 fields/section $\times$ 5 sections/mouse under light microscope with magnification of $\times$ 200 and expressed as mean $\pm$ SD of 7 mice.

In addition, mitotic index (MI) in the intestinal crypts was also calculated on HE-stained sections in the same way. Statistical analysis was performed by Student's and Welch's *t*-test.

As a result, in the fundic region of the stomach, the AI was negligible in any portions of the control animals. In the T-2 toxin-inoculated animals, as shown in Fig. 1, the AI in the glandular neck was less than 5% throughout the experimental period. The AI in the foveolae gastricae was about 18% at 8 and 16 HAI, and decreased thereafter ($2.5 \pm 0.3\%$ at 48 HAI). The AI in the glandular base started to increase slowly at 8 HAI (about 5%), peaked at 24 HAI ($26.5 \pm 2.8\%$), and decreased at 48 HAI ($16.5 \pm 1.5\%$).

In the intestinal crypts of control animals, the AI was also negligible in any regions of the intestine. In addition, the surface epithelial cells of the intestinal mucosa looked almost normal even in the T-2 toxin-inoculated animals throughout the experimental period.

In the intestinal crypt epithelia of the T-2 toxin-inoculated animals, as shown in Fig. 2, the AI in the duodenum and ileum was highest at 8 HAI (duodenum: $33.5 \pm 43.0\%$; ileum: $35.8 \pm 4.0\%$), and then decreased with the lapse of time and reached the level of about 12% at 48 HAI. In the cecum, the AI slightly increased from 8 ($9.8 \pm 1.3\%$) to

16 HAI ($13.3 \pm 1.5\%$), and then decreased to the level of $3.3 \pm 0.5\%$ at 48 HAI. In the colon, the AI also slightly increased from 8 ($18.3 \pm 2.0\%$) to 16 HAI ($21.0 \pm 2.3\%$), and then decreased to the level of $12.3 \pm 1.5\%$ at 48 HAI.

As to the MI in the intestinal crypt epithelia (Fig. 3), it prominently decreased at 8 HAI in all regions of the intestine (duodenum: $11.0 \pm 0.8\%$; ileum: $5.3 \pm 0.5\%$; cecum: $9.8 \pm 1.0\%$; colon: $5.3 \pm 0.3\%$) in comparison with the control values (duodenum: $34.4 \pm 3.5\%$; ileum: $22.8 \pm 2.8\%$; cecum: $20.4 \pm 2.5\%$; colon: $19.7 \pm 2.9\%$). Thereafter it turned to increase with the laps of time, but it did not recover to the control values even at 48 HAI (duodenum: $30.8 \pm 3.0\%$; ileum: $23.3 \pm 2.8\%$; cecum: $16.3 \pm 2.3\%$; colon: $16.2 \pm 3.0\%$).

Judging from the above-mentioned sequence of the AI in the fundic region of the stomach, the epithelial cells of the foveolae gastricae which come into direct contact with orally inoculated T-2 toxin first was first clearly affected, and then they turned to recover after 16 HAI. On the other hand, the epithelial cell damage in the glandular base started slowly, but it became prominent at 24 and 48 HAI. This may suggest that this portion is exposed to T-2 toxin *via* blood circulation after absorption of T-2 toxin through the small intestinal mucosa and that this portion contains epithelial cells with high mitotic activity. However why the epithelial cells of the glandular neck, which are located between the foveolae gastricae and the glandular base, were most resistant is still obscure.

In the intestine, the crypt epithelial cells in the small intestine were more sensitive than those in the large intestine, and those in the cecum was most resistant. This may be

Fig. 1. Apoptotic index in the stomach (fundic region) after T-2 toxin inoculation in mice.

—◆—: Foveolae gastricae; —□—: Glandular neck; —▲—: Glandular base; —○—: Control

***p* < 0.01: Significantly different from controls.

Fig. 2. Apoptotic index in the intestine after T-2 toxin inoculation in mice.

—◆—: Duodenum; —□—: Ileum; —▲—: Cecum; —×—: Colon; —○—: Control
 ** $p < 0.01$: Significantly different from controls.

Fig. 3. Mitotic index in the intestine after T-2 toxin inoculation in mice.

—◆—: Duodenum; —□—: Ileum; —▲—: Cecum; —×—: Colon

Value at 0 HAI indicates the mean of control values at 4 time points in each intestinal region.

* $p < 0.05$, ** $p < 0.01$: Significantly different from controls.

related with a repeated exposure of the small intestinal mucosa to T-2 toxin and its glucuronide through both mucosal absorption and biliary excretion by enterohepatic circulation¹³. The surface epithelial cells of the small intestinal mucosa, however, looked almost normal throughout the experimental period as mentioned above.

Mitosis is normally seen in the intestinal crypt epithelia in which stem cells of enterocytes exist. As mentioned above, the MI in the T-2 toxin-inoculated animals clearly decreased at 8 HAI, and thereafter increased with the lapse of time. This indicates that mitotic activity of intestinal crypt epithelial cells is markedly suppressed in the early stage of T-2 toxin-intoxication, probably because T-2 toxin is a potent inhibitor of protein and DNA synthesis in eukaryotic cells¹⁵. However the AI after the T-2 toxin inoculation was not always proportional to the MI before the inoculation. This may suggest that the increase in AI is not directly related with the suppression of mitotic activity.

Further studies on the mechanism of T-2 toxin-induced apoptosis and on the relationship between the cell cycle and the apoptotic cell death in the intestinal crypt epithelia should be done.

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