Effect of Glucose Feeding on Regenerating Rat Liver Following Partial Hepatectomy

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Abstract: It has been reported that glucose feeding drastically potentiates the toxicity of several hepatotoxicants in rats. Accordingly, it was speculated that the potentiation mechanism involves the suppression of hepatocellular regeneration, i.e., the tissue healing process following initial damage. In this study, we quantitatively examined the effect of glucose feeding on hepatocellular regeneration following a partial hepatectomy (PH). Rats received a 15% glucose supplementation through drinking water for 7 days and control animals were supplied with tap water *ad libitum*. Following the glucose or tap water feeding, rats were subjected to a two-thirds PH. Glucose or tap water feeding was continued throughout the liver regenerative phase. On days 0, 1, 2, 4, and 7, serum and liver biochemical examinations, and measurements of liver weights, and immunohistochemical examinations on terminated animals, were conducted. The results demonstrated that liver weight gain was suppressed and the number of mitotic hepatocytes, an indicator of cell regeneration, was reduced by glucose feeding. These results support the speculation of hepatotoxicity potentiation by glucose feeding. Furthermore, changes of serum glucose and triglyceride levels, and hepatic glycogen and triglyceride contents were also observed in glucose-fed rats, indicating changes in glucose and triglyceride metabolism in the liver. However, the relationship between these biochemical changes and the suppression of liver regeneration remains to be clarified. (J Toxicol Pathol 1998; 11: 95~99)

Key words: Glucose feeding, Regeneration, Bromodeoxyuridine, Hepatocyte, Partial hepatectomy

Introduction

A large number of chemical agents are known to produce hepatic damage, including necrosis¹⁻³, steatosis⁴⁻⁶, or cholestasis⁷⁻⁹, in experimental animals. Consequently, numerous reports regarding the mechanism of cell injury, including hepatocytes and cholangiocytes, have been published. However, only a few have noted a tissue healing process following cell injury; Mehendale¹⁰ proposed the idea that hepatotoxicity can be divided into two stages: initial cell injury (Stage I) and hepatocellular regeneration (Stage II), which is considered to follow immediately after the initial cell insult. According to this so-called "two-stage model of toxicity", acceleration and suppression of cell regeneration (Stage II), respectively, result in the increased and decreased toxic effects of hepatotoxicants.

For instance, the protective effect of a small dose of CCl₄, against a second normally lethal dose of the agent administered 24 hr later, is well known¹¹⁻¹⁴. Recently Thakore and Mehendale^{15,16} indicated that the mechanism of the protective effect involves hepatocellular regeneration (Stage II). This phenomenon is called autoprotection. Furthermore, Mehendale and Chanda¹⁷ showed that hepatotoxicity induced by several structurally and mechanistically different hepatotoxicants was potentiated by a 15% glucose solution supplement (glucose feeding) through drinking water. They

speculated that the potentiation of hepatotoxicity due to glucose feeding was not attributable to potentiation of the initial hepatocyte damage (Stage I), but was attributable to the suppression of hepatocellular regeneration (Stage II) following the initial insult^{17,18}. However, the effect of glucose on cell regeneration is still controversial¹⁹⁻²³. Therefore, in the present study we evaluated the significance of glucose feeding on hepatotoxicity (Stage II), in partially hepatectomized (PH) rats during mitotic hepatocyte regeneration under the same experimental conditions as reported by Mehendale *et al.*^{17,18}.

Materials and Methods

Male Sprague-Dawley rats (6 weeks old) were purchased from Charles River Laboratory (Kanagawa, Japan). Animals were given standard diet and tap water *ad libitum* for 1 week. Thereafter the rats were divided into two groups and given either tap water or 15% glucose solution through their drinking water (pre-treatment). After the seven day pre-treatment, all rats were subjected to a two-thirds PH according to the method of Emond, *et al.*²⁴. The day of surgery was designated as Day 0. Glucose or tap water feeding was continued during the regenerative phase of the liver. Blood sampling and autopsies were performed on days 0, 1, 2, 4, and 7 after PH.

Biochemical examinations (measured parameters: glucose and triglyceride)

Blood samples were obtained from rats under ether anesthesia. Glucose and triglyceride levels in plasma samples from the collected blood were measured with an autoanalyzer (model 7250, Hitachi Ltd.).

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Measurements of the liver remnant weight

After blood sampling, the liver was removed and weighed. The relative liver weight (absolute liver weight/body weight) was calculated based on the final body weight of the animals.

Liver biochemical examinations (measured parameters: glycogen and triglyceride)

Biochemical examinations of the liver were conducted on each animal after termination. A part of the liver taken for pathological examinations was homogenized in 1.15% KCl solution. Then assay samples for glycogen measurements were extracted from the homogenate to which 30% KOH solution had been added. The measurement was performed according to the method of Montgomery²⁵. For triglyceride measurements of the liver, another aliquot of liver was homogenized in 90% isopropanol. Supernatant obtained by centrifugation (3,000 r.p.m., 10 min.) was applied to a Hitachi 105 (Hitachi Ltd.) spectrophotometer.

Pathological examinations: Evaluation of BrdU-labeling index

The number of mitotic hepatocytes was determined according to the method of Gratzner²⁶. In brief, 1 hour before termination, each animal was given bromodeoxyuridine (BrdU: Sigma Chemical Co., St Louis, MO) intraperitoneally at a dose level of 100 mg/kg body weight. Immediately after the autopsy, a part of the liver was fixed in 10% neutral buffered formalin. Paraffin sections were stained with an anti-BrdU antibody (Progen Biotechnik GmbH, Germany), using a LSAB kit (Dako, Japan). These sections were also counterstained with hematoxylin. For quantitative evaluation, the number of labeled cells was counted and the BrdU-labeling index (labeled cell number/unit area) was calculated for each animal.

Statistical analysis

Quantitative data are expressed as the mean value $\pm$ standard deviation (SD). The data obtained from the biochemical examinations, the ratio of liver remnant to body weight, and the labeling indices were analyzed by Student's *t*-test or Aspin-Welch's test. A *p* value < 0.05 was considered significant.

Results

Biochemical examinations (measured parameters: glucose and triglyceride)

Plasma glucose levels in glucose-fed and control rats decreased from Day 0 to Day 1 or 2, after which the values tended to increase up to Day 7. The values from glucose-fed rats were significantly higher than those of the controls on days 1, 4, and 7 (112, 112, and 110% of control on days 1, 4, and 7, respectively; Fig. 1A).

Plasma triglyceride levels of glucose-fed and control rats decreased remarkably from Day 0 to Day 1, and then showed a tendency to increase, reaching peak levels on Day 4. The values of glucose-fed rats were significantly different on days 0 and 4 from those of the controls (71 and 115% of control on days 0 and 4, respectively; Fig. 1B).

Fig. 1. The changes of plasma glucose (A) and triglyceride (B) levels in the control (open circles) and glucose-fed (closed circles) rats after PH. Each point represents the mean $\pm$ SD (vertical bars) for 5 animals. Values significantly different from the controls, *p* < 0.05 , are represented by an asterisk.

Relative liver weights

In both groups, the liver weights rapidly increased until Day 4 and thereafter remained relatively constant up to Day 7. However, the relative weights of glucose-fed rats were significantly lower than those of the controls on days 1, 2, and 4 (92, 94 and 89% of control on days 1, 2, and 4, respectively; Fig. 2).

Liver biochemical examinations (measured parameters: glycogen and triglyceride)

In both groups, glycogen content in the liver decreased from Day 0 to Day 1 or 2, and then gradually increase up to Day 7. The values for glucose-fed rats were significantly higher than those for controls on all days measured (157, 191, 213, 159, and 126% of control on Day 0, 1, 2, 4, and 7, respectively; Fig. 3A).

PH did not appear to influence triglyceride content in either group. The values for glucose-fed rats, however, were significantly higher than those for the control animals on days 2, 4, and 7 (143, 179, and 166% of control on days 2, 4, and 7, respectively; Fig. 3B).

Fig. 2. The changes of the ratio of liver remnant weight to body weight in the control (open circles) and glucose-fed (closed circles) rats after PH. Abbreviations are as in Fig. 1.

Fig. 3. The changes of liver glycogen (A) and triglyceride (B) contents in control (open circles) and glucose-fed (closed circles) rats after PH. Abbreviations are as in Fig. 1.

Pathological examinations: Evaluation of BrdU-labeling index

In the labeling study, the nucleus of mitotic hepatocytes was stained positively for BrdU-immunohistochemical examination. BrdU-labeled cells localized mainly in the periph-

eral to mid-zone in both groups on Day 1 (Figs. 4a and 4b), whereas the cells localized diffusely in the hepatic lobule on days 2 and 4 (Figs. 4c and 4d). On Day 7, labeled cells were rarely observed in either group, comparable to the liver on Day 0 (Figs. 4e and 4f).

The number of BrdU-labeled cells counted per area in both groups drastically increased after PH, showing the maximum level on Day 1. Thereafter the number of labeled cells decreased to the control level, i.e., the number in the non-treated liver, on Day 7. A comparison of the value between both groups revealed that the labeled cell number in glucose-fed rats was significantly below that of the control rats on days 1, 2, and 4 (54, 63, and 56% of control on days 1, 2, and 4, respectively; Fig. 5).

Discussion

Subsequent to a two-thirds PH, rat hepatocytes proliferate drastically through accelerated cell division, and the remnant liver mass is fully restored to its size prior to PH within 7 to 10 days^{27,28}. The present results also demonstrate that the liver weights following PH rapidly increased in both glucose-fed and tap-water supplied rats. However, on days 1, 2, and 4 after PH the remnant liver weights of glucose-fed rats was significantly lower compared with those of the control, indicating that hepatocyte regeneration in glucose-fed rats was inferior to that in the control. Furthermore, the BrdU-labeling examination revealed that on days 1, 2, and 4 the number of BrdU-labeled cells in glucose-fed rats was significantly lower compared with that in the control. These results clearly indicate that glucose feeding suppressed the liver regeneration under the present condition.

Mehendale and Chanda¹⁷ reported on several hepatotoxicants where the induced toxicity was potentiated by the same glucose supplemental regimen as in the present study, and speculated that the potentiation was attributable to the suppression of hepatocellular regeneration (Stage II), but not to the initial insult (Stage I). Although the present finding supports their speculation, glucose-feeding effects on the initial insult of hepatotoxicants under the present supplemental regimen have not been fully examined. Thus, additional studies are required to address the effect of glucose feeding on initial insults induced by the hepatotoxicants.

On the other hand, effects of glucose feeding on liver regeneration have already been investigated, though controversy still exists: some researchers have reported a suppressive effect¹⁹⁻²², while others have reported an accelerating effect²³ on hepatocyte regeneration. The suppressive effect has been speculated to be the result of the reduced ability to utilize glucose for adenosine triphosphate synthesis, of which increased amounts are needed for hepatocyte division²⁰, or that lipid mobilization and partial fatty acid oxidation are inhibited in the remnant liver tissue²¹. Chanda and Mehendale¹⁸ also proposed the possibility that glucose feeding causes increased insulin release. Conversely, one cause of acceleration is speculated to be the accelerated metabolism of exogenous glucose, an energy substrate used for hepatocellular regeneration²³. Thus, it is still controversial whether glucose feeding inhibits or accelerates hepatocellular regeneration. However, there are some differences in supplemental regimen among previous reports: 28%²⁰ or 20%²¹ glucose solution was infused, or 60%²³ was administered through drinking water. Therefore, it is conceivable that the effects of glucose feeding on liver regeneration depend on the sup-

Fig. 4. Photographs of BrdU immunohistochemistry in the liver from the control (a, c, e) and glucose-fed (b, d, f) rats on Day 1, 4, and 7 after PH. Note that numerous labeled cells are observed in the peripheral zone, compared with the control. $\times 80$.

plemental regimen.

In plasma and liver biochemical examinations, several significant differences between glucose-fed and control rats were observed for some of the data obtained. The hypoglycemia observed on Day 2, in particular, has also been reported elsewhere and was speculated to be a change in liver metabolism induced by PH^{23,29}. Furthermore, it is conceivable that glucose feeding itself alters the metabolic activity of the liver, and that both PH and glucose feeding affect the values of the parameters measured during the plasma- and liver biochemical examinations. Although differences in biochemical data between both groups, i.e., plasma triglyceride and glucose levels on Day 1 and 4, and Day 0 and 2,

respectively; hepatic glycogen contents on all examination days; and hepatic triglyceride on days 2, 4, and 7, are considered to be due to the influence of glucose feeding, the relationship between these changes and the suppression of the mitotic activity is unclear.

In conclusion, the present study revealed that glucose feeding under the present regimen suppressed the hepatocellular regeneration. This suggests that suppression of hepatocellular regeneration is attributable to the potentiation of hepatotoxicity by glucose feeding as hitherto described by Mehendale *et al.*^{17,18}. However, the present evidence regarding the mechanism of regenerative suppression is insufficient and behooves further investigation.

Fig. 5. The changes of labeled cell number in the control (open circles) and glucose-fed (closed circles) rats after PH. Abbreviations are as in Fig. 1.

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