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Alleviating Effects of Vitamin C on the Gramoxone Toxicity in Rat Liver

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Abstract

The behaviour of glycogen and histological changes of hepatic tissues in the liver of rats, aged 6 to 7 weeks, fed 18% casein diet under control, gramoxone and gramoxone + vitamin C (Vt. C) diets has been investigated in a combined histopathological and histochemical studies. Cloudy swelling and fat changes of hepatic cells were observed in the gramoxone group with the duration of feeding time. Fat changes of hepatic cells were observed more obviously than cloudy swelling, especially in the hepatic cells of periportal area. The number of Kupffer's cells increased significantly in the gramoxone group fed for 4 weeks. The cloudy swelling and fat changes decreased obviously in the gramoxone + Vt. C group. Glycogen content of hepatic cells tended to increase slightly in the gramoxone group as compared with the control group. Moreover, glycogen depositions were higher in the hepatic cells where fat changes were obvious. Glycogen content appeared to decrease in the gramoxone + Vt. C group as compared with the gramoxone group. It seems to be that Vt. C has alleviating effects on the gramoxone toxicity in the patterns of glycogen distribution and histological structure of hepatic tissues.

Key words : gramoxone toxicity, Vt. C, rat liver

INTRODUCTION

Gramoxone (paraquat ; 1,1'-dimethyl-4,4'-bipyridinium dichloride) is the most commonly used herbicide for destruction of noxious weeds. It has been, however, reported that gramoxone caused the necrotic effects in animal lung, liver and kidney, the suppressed activities of alkaline and acid phosphatases, and the alterations of mucosubstances in the intestinal tract of rats[1-4]. Moreover, significant morphological changes such as fat changes of hepatic cells and increases in the number of Kupffer's cells, were found in rats fed the gramoxone-treated diets[5]. Suggested mechanism of gramoxone toxicity is related to the formation of superoxide, hydrogen peroxides and NADPH dependent lipid peroxides, damage to hemoglobin by free radical, and the catabolism of protein and hemolysis by lipid peroxides in cell membrane[6,7].

Vitamin C (L-ascorbic acid) is well known as a antiscurvic agent, and plays an important role in numerous biological reactions including collagen synthesis, the healing of wounds, the union of bone fractures, the regeneration of nerve cells, the metabolism of folic acid, the absorption of iron, carnitine synthesis, norepinephrine synthesis, degradation of cholesterol, and the metabolism of tyrosine[8-11]. Vitamin C caused an increase in the mobility of white blood cells, the serum levels of immunoglobulins, and in antibody formation[10]. According to previous reports[12], it seemed to be that vitamin C has ameliorating effects on the gramoxone toxicity in rats with regard to the body weight gain, feed efficiency ratio, lipid contents and TBA value in liver. In addition, the changes of the liver protein patterns, such as the decrease of high molecular weight protein and the increase of low molecular weight protein were observed in rats fed the gramoxone + Vt. C-treated diet. This study is designed to examine the effect of dietary supplementation of vitamin C on the gramoxone toxicity in hepatic tissues of rats.

MATERIALS AND METHODS

In order to determine the effects of dietary supple-

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mentation of Vt. C on the gramoxone toxicity, 32 Wistar-strained male rats, aged 6 to 7 weeks, were divided into a control group (12) and an experimental group (20). The experimental group was divided into gramoxone-treated group (toxic group) and gramoxone+Vt. C-treated group (alleviating group). Rats in control group and gramoxone-treated group were fed 18% casein diet and 18% casein+0.04% gramoxone diet for 4 weeks, respectively. In case of the gramoxone+Vt. C-treated group, rats were fed 18% casein+0.04% gramoxone+Vt. C diet for 2 weeks after feeding 18% casein+0.04% gramoxone diet for 2 weeks. Experimental diets were prepared according to the composition shown in Table 1.

For the tissue preparation, at the end of desired experimental days, rats of each group fasted for 18 hours and sacrificed under ether anesthesia. The liver was removed immediately and then fixed in the solution of 10% neutral buffered formalin for 24 hours, dehydrated, and embedded in paraffin according to routine methods. Sections were cut in series at 5 to 6μm.

For the histological and histopathological structure of liver tissue and histochemical demonstration of glycogen in hepatocytes, deparaffinized and hydrated section were stained as following methods :

1. Hematoxylin and eosin (H-E) staining for the general observation of histological and histopathological structure.

2. Periodic acid-Schiff, Schiff's reaction for the distribution of glycogen[13].

The staining degrees of the histological and histopathological characteristics of hepatic cells were classified into 6 groupings : −, absent ; ±, trace ; +, weak ; ++, moderate ; +++, intense ; ++++, very intense. The staining degrees of the glycogen distribution of hepatic cells were classified into 5 groupings : ±, trace ; +, weak ; ++, moderate ; +++, intense ; ++++, very intense.

Table 1. Composition of the experimental diets

Constituents (%)	CG	GG	GVG
Corn starch	72	72	69
Casein	18	18	18
Corn oil	5	5	5
Salt mixture[1]	4	4	4
Vitamin mixture[2]	1	1	1
L-ascorbic acid	−	−	3
Gramoxone	−	0.04	0.04

Abbreviations : CG, 18% casein diet (control group) ; GG, 18% casein + 0.04% gramoxone diet (gramoxone group) ; GVG, 18% casein + 0.04% gramoxone + 3% L-ascorbic acid diet (gramoxone-vitamin C group)

[1]Salt mixture : Purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

[2]Vitamin mixture : Vitamin diet fortification mixture ; purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

RESULTS AND DISCUSSION

Histological and histopathological findings of the hepatic tissue of rat fed experimental diets are presented in Table 2.

Examination of H-E stained sections of liver of the control group revealed no significant histological abnormalities. In a H-E section examined under lower power, the liver tissue is seen to be composed of masses of epithelial, hepatocytes arranged in anastomos-

Table 2. Histological findings of hepatic cells of rats fed the experimental diets

Dietary group		Epithelial change			Stromatic change		
		CS	FC	KC	ICI	F	PC
CG	2 wks	−	±	±	−	−	−
	3 wks	−	−>±	+	−	−	−
	4 wks	−	−>±	+	−	−	−
GG	2 wks	±	+	+++	−	−	−
	3 wks	+	+−++	+++	−	−	−
	4 wks	+−++	+++−++++	+++,++++	−	−	−
GVG*	3 wks	±−+	±>+	+++,++++	−	−	−
	4 wks	±	±−+	+++			

Abbreviations : CS, cloudy swelling ; FC, fat change ; KC, cell ; ICI, inflammatory cells infiltration ; F, fibrosis ; PC, passive congestion ; −, absent ; ±, trace ; +, weak ; ++, moderate; +++, intensive; ++++, very intensive ; >most marked
Others are the same as those in Table 1

ing and branching plates that form a three-dimentional lattice. The portal areas are so arranged as to delineate lobules of liver tissue. Hepatic lobule has several portal canals at its periphery, and in its center is a central vein from which plates of parenchymal cells radiate like the spokes of a wheel from a central hub. And the sinusoidal spaces between liver plates are lined by endothelial cells and satellite cell of Kupffer (Fig. 1).

Fig. 1. The liver of rat showing the hepatic cells and central vein (CV) in the control group fed for 4 weeks.
PAS stain.×400. Glycogen of hepatic cells distributed in minimal amounts.

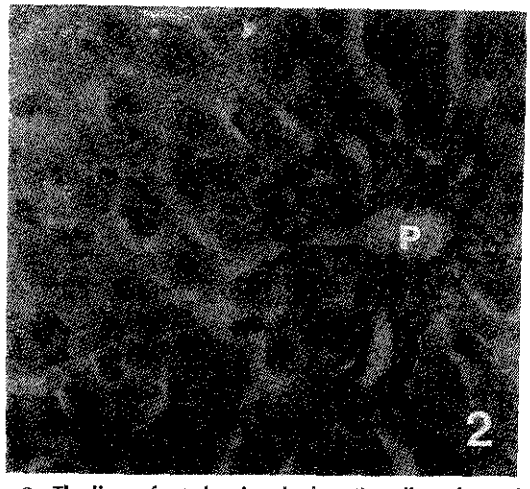

Fig. 2. The liver of rat showing the hepatic cells and portal area (P) in the control group fed for 4 weeks.
PAS stain.×400. Glycogen of hepatic cells distributed in minimal to small amounts.

No stromatic changes were observed in the liver tissue of the control, gramoxone and gramoxone+Vt. C groups (Fig. 1~6). There were, however, epithelial changes in the liver tissue with duration of feeding time in all experimental groups, even though slight differences existed. Cloudy swellings were not observed in the liver cells of the control group, while fat drople-

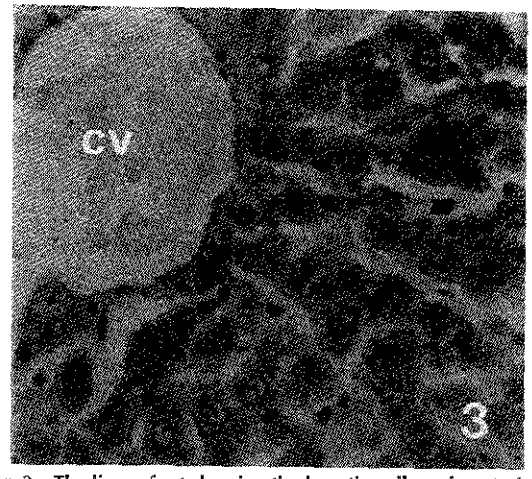

Fig. 3. The liver of rat showing the hepatic cells and central vein (CV) in the gramoxone group fed for 3 weeks.
PAS stain.×400. Glycogen of hepatic cells distributed in minimal to small amounts. Cloudy swelling and fat changes of hepatic cells occured slightly in the centrolobular zone.

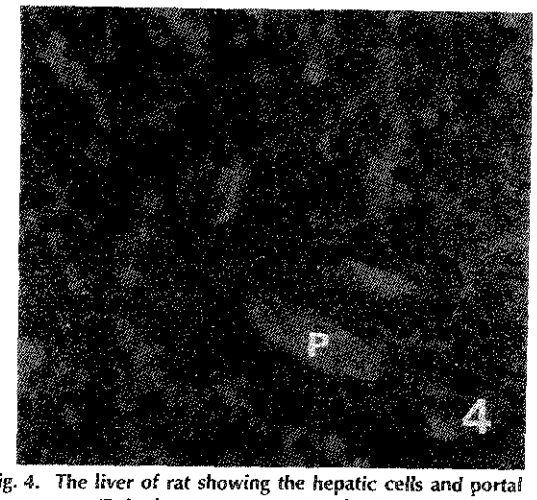

Fig. 4. The liver of rat showing the hepatic cells and portal area (P) in the gramoxone group fed for 4 weeks.
PAS stain.×400. Glycogen of hepatic cells increased than those of control group. Cloudy swelling and fat changes of hepatic cells occured severely in the periportal zone.

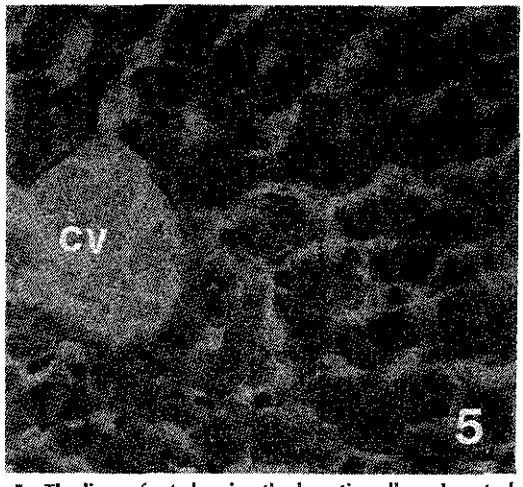

Fig. 5. The liver of rat showing the hepatic cells and central vein (CV) in the gramoxone + vitamin C group fed for 3 weeks.
PAS stain. × 400. Glycogen of hepatic cells distributed in minimal amounts.

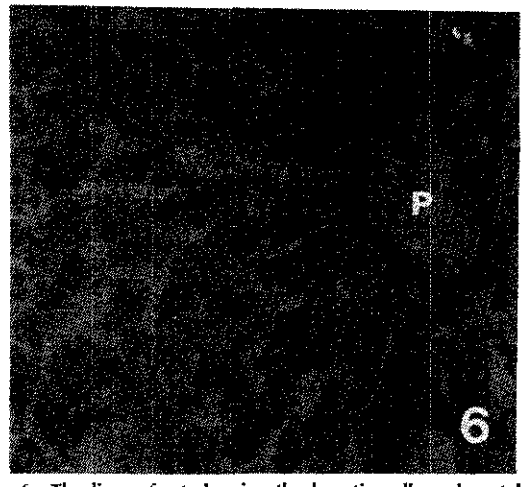

Fig. 6. The liver of rat showing the hepatic cells and portal area (P) in the gramoxone + vitamin C fed for 4 weeks.
PAS stain. × 400. Glycogen of hepatic cells decreased than those of gramoxone group. Cloudy swelling and fat changes of hepatic cells decreased obviously in the periportal zone than those of gramoxone group.

ts were seen in a few hepatic cells of the liver tissue of the control group. Additionally, minimal increases of Kupffer's cell appeared in sinusoidal space with the duration of feeding time (Fig. 1 and 2).

In the gramoxone group, cloudy swelling and fat changes of the hepatocytes were revealed in hepatic lobules with the duration of feeding time, and fat droplets showed especially abundant in a large number of the hepatocytes in the periportal area of the hepatic lobules (Fig. 3 and 4).

Also, the number of the phagocytic Kupffer's cells tended to increase in these groups, and was more prominent in 4 week-gramoxone group than the other groups. Cloudy swelling and fat changes of the hepatic cells tended to decrease remarkably in the hepatic lobules of the gramoxone+Vt. C group. However, a decrease in number of Kupffer's cells was not obvious(Fig. 5 and 6).

Administration of foreign compounds into animals caused toxicities in the liver even though differences exisited with the kinds of the compounds. The toxicities reported were cloudy swelling, vesicular degeneration and fat accumulations of hepatocytes[14-18]. Administration of carbon tetrachloride caused cloudy swelling and hydropic degeneration in parenchymal cells of experimental animals[14-16], and necrosis and fat accumulation of hepatocytes[17]. In addition, fat changes or necrosis were occured by interruption of lipoprotein formation because of a injury in endoplasmic reticulum of liver cells. Also, administration of parathion resulted in vesicular degeneration, fat accumulation and necrosis of hepatocytes in the intermediate and central zones of the hepatic lobules in the mouse[18]. The results obtained in this study were in accordance with other researchers[14-18]. However, there were differences in the process of fat accumulation into each region of the hepatic lobules by the kinds of foreign compounds. Adriamycin[19] and EPN[20] caused fat accumulation in the hepatic cells of the midlobular and centrilobular regions of hepatic lobules. Parathion caused fat accumulation in the centrilobular and midlobular regions at the initial stage and fat accumulations were observed in the hepatic cells of the periportal regions with the duration of time[21]. Carbon tetrachloride caused fat accumulation in the hepatic cells of the centrilobular regions and in sucession all area of hepatic lobules[22]. However, administration of cycloheximide resulted in fat accumulation in the hepatocytes of the periportal regions[23]. In this study, administration of gramoxone into the diet caused fat changes in the hepatic cells from the periportal regions at first and next, from the midlobular and the centrilobular regions. Especially, the de-

Table 3. Glycogen distribution in the liver of rats fed the experimental diets

Zone	CG			GG			GVG	
	2 wks	3 wks	4 wks	2 wks	3 wks	4 wks	3 wks	4 wks
Zone 1	±>+	±−+	±−+	±-+	±-+>++	+>++-+++	±,+	±,+
Zone 2	±	±	±,+	±,+	±,+	+	±>+	±−+
Zone 3	±	±	±	±	±,+	±−+	±	±−+>++

Abbreviations : Zone 1, periportal region of hepatic lobule ; Zone 2, midlobular region of hepatic lobule; Zone 3, centrilobular region of hepatic lobule. Others are the same as those in Table 1

gree of fat changes was serious in the hepatic cells of periportal regions.

Several researchers reported that ascorbic acid had alleviating effects on the toxicities of various foreign compounds. Chung[24] demonstrated that the ATPase activity in liver mitochondria was interrupted by Hg^{2+} *in vitro*, and that the interruptive action was prevented by 10^{-2}M of ascorbic acid. According to Suzuki and Yoshida[25], 50ppm of cadmium containing diet caused to decrease the contents of iron in liver, kidney, spleen, testis, intestine and tibia of rat and caused to increase the activities of sGOT and sGPT. In addition, they suggested that the increase of activities of sGOT and sGPT was due to the damage of liver and was prevented by the supplementation of iron and ascorbic acid. Cadmium-containing diet resulted in the decrease of iron level in liver, due to the interruption of intestinal absorption of iron[26]. And the supplementation of both ascorbic acid and D-isoascorbic acid had ameliorating effects on the toxicity. Moreover, Rajini and Krishnakumari[27] reported that the administration of primiphos-methyl suppressed the activities of cholinesterase in the brain and blood serum of rat, and to increase the level of ascorbic acid and glucuronic acid in urine remarkably. They reported that the supplementation of L-ascorbic acid appeared to have alleviating effects on the toxicity of the pesticide partially. In this study, decreases of cloudy swelling and fat accumulation in hepatic cells were observed in the gramoxone+Vt. C group (Table 1).

From these results, it was suggested that ascorbic acid has ameliorating effects on gramoxone toxicity in liver. In addition, the increase of Kupffer's cells in the gramoxone group was due to the defense mechanism of hepatic tissue against the foreign materials.

Glycogen distribution in the hepatic cells of rats fed the experimental diets is shown in Table 3.

Glycogen distribution of hepatic cells was minimal or small amounts in the control group and the glycogen contents were remarkable in hepatocytes of the periportal zone rather than in the centrilobular and midlobular zones (Fig. 1 and 2). Glycogen contents of hepatic cells in each area of hepatic lobules tended to increase slightly in the gramoxone group as compared with the control group. Glycogen contents of hepatocytes increased obviously in rats fed gramoxone diet for 4 weeks. Moreover, the amount of glycogen was distinctly marked in the hepatic cells of the periportal area where fat changes arised significantly (Fig. 3 and 4). Under the gramoxone+Vt. C group, the amount of glycogen in the hepatocytes of the hepatic lobules decreased slightly except for the 4 week-group, as compared with the hepatic lobules of gramoxone group. In the case of rats fed gramoxone+Vt. C diet for 4 weeks, glycogen distribution tended to decrease significantly in hepatic cells of every zone except a few of hepatic cells in centrilobular zone (Fig. 5 and 6).

According to Suzuki and Yoshida[25], the contents of glucose in rat plasma and glycogen contents in liver remained unchanged with the administration of 50 ppm of cadmium containing diet for 180 days. However, Singhal *et al.*[28] reported that daily infusion of either 0.25mg of cadmium chloride for 21days or 1mg of cadmium chloride for 45 days resulted in the increases of blood glucose and serum urea, and in the decrease of glycogen contents in the liver.

According to Min *et al.*[29], administration of chlorambucil caused a decrease in glycogen content significantly in hepatocytes of hepatic lobules. The glycogen content in hepatocytes was restored when 0.5mg of saponin was added into the chlorambucil compared with the chlorambucil treated group. Also, a decr-

ease in the amount of hepatic glycogen was marked after the administration of compound 48/80, and glycogen decreased in the midlobular and periportal zones noted rather than the centrilobular zone[30].

The administration of dimethyl sulfoxide (DMSO) caused a decrease in glycogen content of hepatic cells of rat initially, but the glycogen content tended to increase with the duration of time as compared with the control group[31]. It was also suggested that the glycogen accumulation effects by DMSO in the cultured hepatocytes resulted from the delay of cell life span by suppressing cell growth and the decrease of energy expenditure required in the process of the activity of hepatocytes as a secretory cell[32].

In this study, glycogen distribution of hepatocytes in the gramoxone group increased slightly, and the increase of glycogen was remarkable in the periporatl regions, especially where fat changes arised significantly. The results that the glycogen content decreased in the hepatic cells of gramoxone+Vt. C group were likely to have the alleviating effects of saponin on the chlorambucil toxicity, even though the alleviating mechanism is different[29].

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흰쥐 간조직에 미치는 제초제 Gramoxone 독성에 대한 비타민 C의 완화 효과

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요 약

흰쥐의 간조직에 미치는 gramoxone 독성과 비타민 C의 완화효과에 대해 조사하였다. Gramoxone처리군에서 간세포의 혼탁종창과 지방변화가 사육기간이 증가함에 따라 현저하고 혼탁종창보다는 지방변화가 더 현저하였으며, 특히 간소엽의 문맥야주변대 간세포에서 지방변화가 심하였다. Kupffer세포의 수도 증가하여 4주 사육군에서 제일 많이 증가하였다. Gramoxone-비타민 C 처리군에서 간세포의 혼탁종창과 지방변화가 현저히 감소하였다. 간세포의 glycogen 함량은 정상대조군에 비해 gramoxone처리군에서 다소 증가하는 경향을 나타내었으며 특히 지방변화가 많이 일어난 간세포에서 glycogen 함량이 더 많았다. Gramoxone-비타민 C 처리군에서 gramoxone처리군에 비해 간세포의 glycogen 함량이 감소하는 경향을 나타내었다.