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Effect of *Escherichia coli* Free-Endotoxin on Hepatic Function in Rats

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ABSTRACT

In rats intraperitoneally injected with small doses of free-endotoxin daily for several successive days, significant decrease in serum aspartate aminotransferase activity was in contrast to significant increase in hepatic enzyme activities of alanine aminotransferase, oxidized glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase and hepatic total sulfhydryls content, whereas no distinct change was noted for levels of serum and hepatic total lipid, cholesterol and triglyceride when compared to control group.

In rats treated with cellular-endotoxin, significant increase in hepatic thiobarbituric acid-reactive substances, total sulfhydryls, non-protein sulfhydryls, and oxidized glutathione reductase activity and serum asparate aminotransferase activity was noted, in addition to significant decrease in hepatic triglyceride as compared with those of the control group.

In rats treated with enterotoxin, the hepatic total sulfhydryl and nonprotein sulfhydryl levels were significantly increased, whereas hepatic thiobarbituric acid-reactive substances and activities of oxidized glutathione reductase, and glutathione peroxidase, and total lipid significantly decreased.

Therefore, free-endotoxin is a toxic substance when administered in large doses, whereas in small doses it may serve to promote the hepatic function of the host such as amino acid metabolism and glutathione-associated enzyme activities.

*Key words: Free-endotoxin, **Escherichia coli,*** Hepatic function, Glutathione-associated enzyme.

In our previous study it was reported that both free- and cellular-endo-toxin of *Escherichia coli* had similar acute toxicity to rats in causing hypotension, leukopenia, thrombocytopenia, hemoconcentration, as well as elevated activities of serum aspartate aminotransferase,

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alanine aminotransferase, and alkaline phosphatase. Increased blood urea nitrogen, uric acid and creatinine were also noted, whereas blood sugar concentration dropped. In contrast, enterotoxin showed no above toxic effect[1].

Escherichia coli is at present the main causative agent of nosocomial infection and opportunistic infection. It is worthy to study the effect of *E. coli* produced enterotoxin and free/cellular endotoxin on the host via enteric or extraenteric infections notably on the physiological function and immunity capacity of the host. The frequency of endogenous endotoxemia caused by gram-negative enteric bacteria in patients with liver cirrhosis has lately received much attention in the clinical study of endotoxin. To clarify the reason for such high frequency, the indices of phagocytic activity by reticuloendothelial system, alpha 2-macroglobulin and endotoxin were studied in patients with chronic liver disease and in normal controls. In the case of liver cirrhosis, the phagocytic activity of RES was markedly diminished, and a negative correlation between the phagocytic activity of RES and alpha 2-macroglobulin was recognized in endotoxemia. These results suggest that endotoxemia in liver cirrhosis is based on a spillover of endotoxins via portal flow[2-6].

The present report is concerned with the comparative effect of *E. coli* free-endotoxin, cellular-endotoxin and enterotoxin on the physiological mechanisms of rats when administrated with adequate dose injections

MATERIALS AND METHODS

Experimental animals: Female rats of Wister strain of body weight 250 g were bought from the Animal Center, National Taiwan University. They were supplied with standard food (Taiwan Sugar Co.) and deionized water *ad libitum* and put in a air conditioned room for two weeks. Then, a daily small dose of *Escherichia coli* free-endotoxin, cellular-endotoxin (10ug/rat/time) or enterotoxin (20ug/rat/time) was intraperitoneally injected[7].

Tissue preparation: The rats were anesthetized with sodium pentobarbitol. Blood was collected from the femoral arteries, and allowed to clot at room temperature for 30-60 min and then centrifuged at 2,300 g for 10 min at 4° C for serum collection. The liver was perfused *in situ* with ice-cold 1.15% KCl until it is uniformly pale, then it was blotted and minced. The liver homogenate was prepared in suitable amount of ice-cold 1.15% KCl by motor-driven teflon pestle in a glass homogenizing vessel. These preparations were used for the determinations of enzyme activity levels of alkaline phosphatase (ALP; EC 3.1.3.1), aspartate aminotransferase (GOT; EC 2.6.1.1), alanine aminotransferase (GPT; EC 2.6.1.2), glutathione (GSH) peroxidase (EC 1.11.1.9), oxidized glutathione (GSSG) reductase (EC 1.6.4.2) and glucose-6-phosphate dehydrogenase (G-6-PD; EC 1.1.1.49), and thiobarbituric acid (TBA)-reactive substances. The liver homogenate prepared in ice-cold 0.02 M EDTA (Na_2) (16 ml/g) was used for the determination of total sulfhydryl(TSH) and non-protein sulfhydryl (NPSH) levels, according to the method described by Sedlak and Lindsay [8].

Extraction of hepatic lipid: The lipid was extracted by the procedure of Folch *et al*[9]. One g of liver was homogenized with 19 ml of 2:1 chloroformmethanol mixture, the homogenate being left at 37° C for 3 hrs and then filtrated. The organic phase was shaken with 1/5 volume of 0.01 M NaCl, centrifuged, then evaporated

to dry with N_2 gas. For the determinations of total lipid (TL), cholesterol (Cs) and triglyceride (TG) levels, the lipid was dissolved immediately into isopropylalcohol containing 10% Triton X-100.

Biochemical analysis: Protein was determined with the Folin phenol reagent according to the method fof Lowry *et al*[10] using bovine serum albumin as standard.

Determinations of ALP was measured as described by Frankel *et al*[11], and of GOT and GPT were according to the Reitman-Frankel method[11].

Determinations of TL was measured as described by Frankel et al [12], and of Cs was measured by the principle of Liebermann-Burchard reaction[13] and the TG was determined with reagent kit (TG-5, product of The International Reagent Co., Ltd., Kobe, Japan) as described by Henry *et al*[14].

The level of TBA-reactive substances in organs was measured by the method described by Uchiyama *et al*[15]. The hepatic activities of GSH peroxidase and GSSG reductase were determined by measuring the disappearance of NADPH at 340 nm with a Hitachi 139 spectrophotometer at 37°C. The GSH peroxidase assay was conducted by a modification of the method of Paglia *et al*[16]. The assay procedure used for the determination of GSSG reductase activity was based on the method described by Pinto *et al*[17]. The values of GSH peroxidase and GSSG reductase activities were defined as the amount of NADPH oxidized per milligram protein per munite. And the activity of G-6-PD was determined by a modification of the method described by Bergmeyer *et al* [18].

Data analysis: All results were given as mean ± SD values. Differences between experimental and control results were evaluated for significance by Student's t-test and analysis of variance. A p value of < 0.05 was considered as significant.

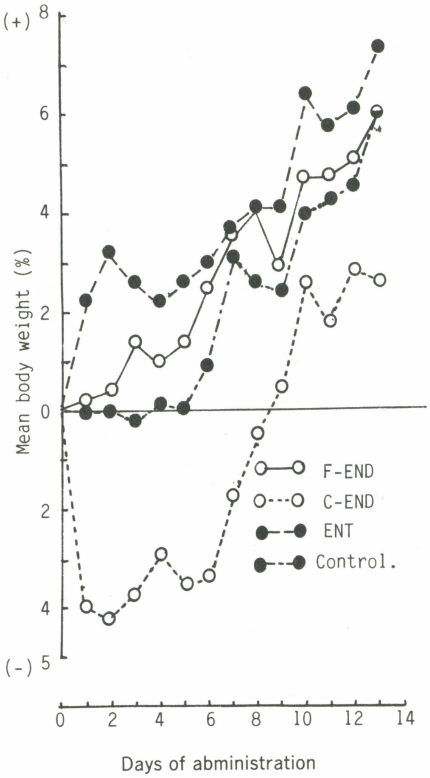

Fig. 1. Changes in the body weight of female Wister rats intreperitoneally injected with toxins. Each point represents mean of 10 animals. F-END: Free-endotoxin, C-END: Cellular-0endotoxin and ENT: Enterotoxin.

RESULTS

Effect on the body weight of the rats (Fig. 1): On the second day of cellular-endotoxin injection the body weight began to decrease, until gradual recovery to normal on the 9th day. In contrast, there was no distinct change in body weight for rats injected with free-endotoxin and enterotoxin. As shown in Table 1, when comparing the C-END group to the control group, the

Table 1. Effect of Escherichia coli Free Endotoxin on the Organ Weight of Rats

Treated with	Time (day)	Organ Weight# Liver	Spleen
F-END	4	2.90±0.09	0.28±002
	14	2.94±0.04	0.29±0.01 *
C-END	4	2.89±0.09	0.36±0.02 * *
	14	3.22±0.06 * *	0.48±0.02 * * *
ENT	4	2.63±0.09 *	0.26±0.01
	14	2.56±0.03 * * *	0.34±0.02 * * *
Control	0	2.87±0.07	0.25±0.01

#The organ weight is corrected for body weight (organ weight/body weight) X 100. Each datum is the mean± SE value expressed on a wet basis. Significantly different from Control group (df=18): * p< 0.05, * * P<0.01 and * * * P<0.001. Each group consisted of 10 rats.
Abbreviation: F-End: Free-endotoxin, C-END: Cellular-endotoxin, and a ENT: Enterotoxin.

spleen weight of rats with 4-day treatment, and the liver and spleen weight of the rats with 14-day treatment all significantly increased, In the F-END group, except for significant increase in the spleen weight of rats with 14-day treatment, no distinct change was noted. In the enterotoxin group, the liver weight of rats significantly dropped whereas apparent increase was noted for the spleen weight of rats with 14-day treatment as compared to the control group.

Effect on the liver function (Fig. 2): In comparing to normal controls, the serum GOT activity for rats receiving a 14-day F-END treatment were significantly decreased, whereas their hepatic GPT activity significantly increased. For the C-END group, the serum GOT

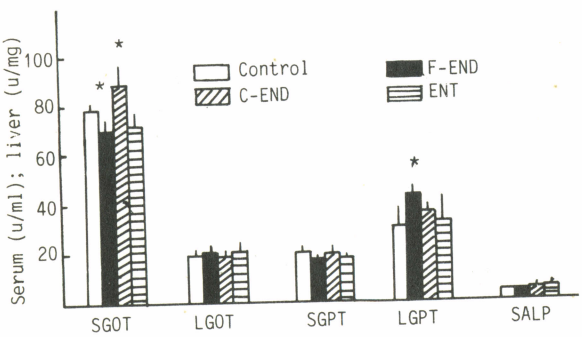

Fig. 2. Changes in the activities of GOT, GPT and ALP in the serum and liver after intraperitoneal injection 14 days of free-endotoxin (F-END), cellularendotoxin (C-END) or enterotoxin (ENT), respectively. * Significantly different from control group (P<0.05). Each group consisted of 5 rats.

Table 2. Effect of Escherichali Free

Treated with	TBA-reactive substances (n mole/mg protein/ml; X 10^{-4})
F-END	1.273±0.130#
C-END	1.430±0.111*
ENT	1.183±0.182 *
Control	1.330±0.186

#mean±DS. Each group consisted of 5 rats. Significantly different from Control group (df=8): * P<0.05.

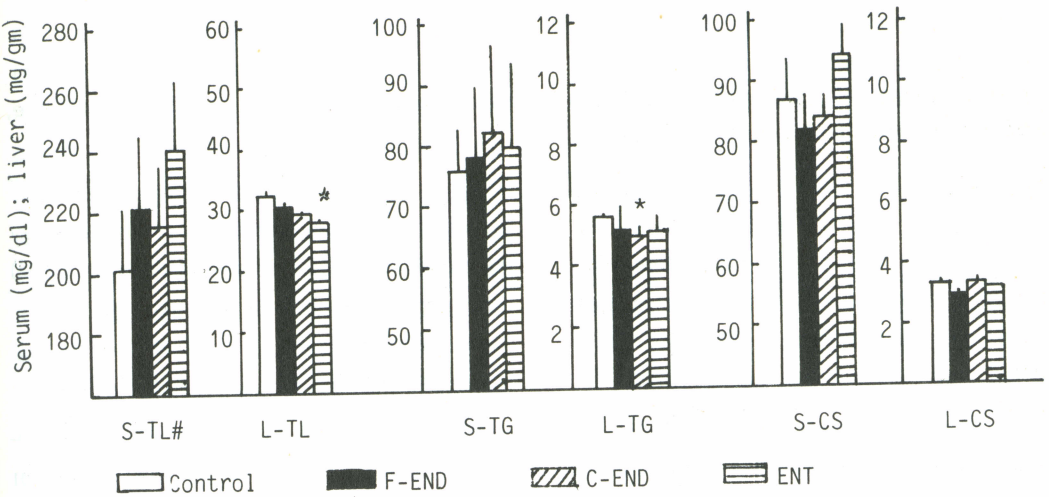

Fig. 3. Changes in the levels of total lipid (TL), cholesterol (Cs) and triglyceride (TG) after intraperitoneal injection (14 days) of free-endotoxin (F-END), cellular-endotoxin (C-END) or enterotoxin (ENT), respectively. * Significantly different from Control group ($p < 0.05$).Each group consisted of 5 rats.

Table 3. Effect of Escherichia coli Free Endotoxin on TSH and NPSH in Rats

Treated with	TSH	NPSH
	(Cysteine; mcg/mg protein/min; mean ± SD)	
F-END	6.567 ± 0.861 *	0.174 ± 0.032
C-END	7.121 ± 0.955 * *	0.214 ± 0.032 * *
ENT	6.569 ± 0.962 *	0.245 ± 0.091 * *
Control	5.855 ± 1.109	0.156 ± 0.066

Significantly different from Control group (df = 8): * $P < 0.05$, * * $P < 0.01$ and * * * $P < 0.001$. Each group consisted of 5 rats. Abbreviation: See foot note in Table 1.

activity was significantly increased when compared to the control group. Except for a significant decrease in the level of hepatic total lipid in the ENT group and hepatic triglyceride level in the C-END group. The three toxins showed no distinct effect on the levels of TL, TG and cholesterol in treated rats(Fig.3).

Effect on liver lipid peroxidation (Table 2): The TBA-reactive substances in rats treated with ENT significantly decreased than the control group and the C-END group showed the contrary result.

Effect on the quantity of total sulfhydryls (TSH) and non-protein sulfhydryls (NPSH) in liver (Table 3): Quantity of TSH in F-END group and quantities of TSH and NPSH in C-END group and ENT group were significantly increased as compared to the control group.

Effect on the activities of GSH-peroxidase, GSSG-reductase and G-6-PD in rats (Table 4):

Table 4. Effect of Escherichia coli Free-Endotoxin on the Activities of GSH-Peroxidase, GSSG-reductase and G-6-PD in Rats

Treated with	GSH-peroxidase (NADPH oxid. u mole/ mg protein/min)	GSSG-reductase (NADPH oxid. u mole/ mg protein/min; $x10^{-3}$)	G-6-PD (NADPH red. u mole/ mg protein/min; $X10^{-3}$)
F-END	182.568±36.842 * #	13.670±2.022 * * *	13.474±4.720 *
C-END	158.197±20.391	11.728±1.570 *	10.088±1.271
ENT	131.270±26.998 *	8.948±1.864	10.480±1.660
Control	154.964±27.916	9.984±2.150	10.748±1.690

#mean±SD. Each group consisted of 5 rats. Significantly different from Control group (df=8): * $p<0.05$, * * $p<0.01$ and * * * $P<0.001$.
Abbreviation: See the foot note in Table 1.

Significant increase was noted for the activities of GSH-peroxidase, GSSG-reductase and G-6-PD in the F-END group, and GSSG-reductase activity in the C-END group, but GSH-peroxidase acdtivity was decreased in the ENT group when compared to the control group. Therefore, F-END distinctly promoted the glutathione-associated enzyme activities in rat's liver.

DISCUSSION

The development and wider use of the limulus amoebocyte lysate assay for the detection of endotoxin has led to renewed interest in the link between gutderived endotoxin, liver injury, and extra-hepatic manifestations of clinical liver disease [19-20].

When a large quantity of endotoxin is absorbed through intestine, it causes liver damage, which in turn affects the detoxicating ability of liver, thereby causing the subsequent absorption of endotoxin which further damages liver before entering the blood circulation. Many reports pointed out that after entering host body, either lipopolysaccharide or endotoxin may rapidly accumulate in liver and spleen (in the presence of a larger quantity of reticularendothelial cells). Therefore, when the host is attacked by endotoxin and assumes the state of shock, liver happen to be the most important final target organ [21-23]. Administration of endotoxin can promote the release of hydrolytic enzymes, lipid peroxidase or TBA-reactive substances from liver, whereas inhibiting NPSH and superoxide dismutase [24-26]. It also causes increased levels of serum total lipid, lipoprotein (especially VLD-lipoprotein), and liver triglyceride to accumulate in large quantities[22].

From the results of our experiment, it was found that in rats administered with multiple small doses of free-endotoxin, the serum aspartate aminotransferase activity apparently decreased, whereas the liver alanine aminotransferase activity apparently increased. Therefore, free-endotoxin may serve to promote amino acid metabolism in rat's liver. Free-endotoxin also

had excellent promoting effect on the quantity of NPSH and TSH in liver which contained abund ant glutathione, and on raising the activities of glutathione-associated enzymes, glutathione peroxidase, oxidized glutathione reductase and glucose-6-phosphate dehydrogenase. Cellular-endotoxin had significant increased on the activity of oxidized glutathione reducatase, but no any effect on the activities of glutathione peroxidase and glucose-6-phosphate dehydrogenase in rat's liver. The effect that free-endotoxin administration can promote glutathione-associated enzyme activities in liver or increased total sulfhydryls and non-protein sulfhydryls levels may serve to explain the important mechanism of detoxication in host against chronic organic/inorganic mercury intoxication[27]. And free-endotoxin also gives very good protection against acute intoxication of mercury nitrate [28–29].

Daily administration of low, non-lethal doses of bacterial endotoxin to mice and rats has been shown to induce a tolerant effect to a sudden challenge dose of endotoxin, decreased hepatic-microsomal drug metabolizing activity, decreased cytochrome p-450 activity in liver and increased hemooxygenase activity [30–31]. The rats pretreated with free-endotoxin was found with increased resistance to lethal bacterial infection, enhanced phagocytosis of macrophage against *Candida albicans* and promoted ability of plaque-forming cell formation (the effect being more distinct to pretreatment with cellular-endotoxin)[32].

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大腸桿菌釋放性內毒素對鼠體肝功能的影響

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連續數日以低劑量之釋放性內毒素(free endotoxin)注射老鼠腹腔內，血清中 aspartate aminotransferase 之活性顯著地隆低，肝臟內之 alanine aminotransferase, oxidized glutathione reductase, glutathione peroxidase, glucose 6-phosphate dehydrogenase 的活性及 total sulfhydryl 含量增加，而血清及肝內的總脂肪、膽固醇及三酸甘油脂無明顯改變。

若以構造性內毒素(cellular-endotoxin)投與老鼠、肝內 thiobarbituric acid reactive substances, total sulfhydryls, non-protein sulfhydryls, 及 oxidized glutathione reductase 活性上升，血清中 aspartate aminotransferase 活性也增加，肝內三酸甘油脂卻明顯下降。

腸毒素(enterotoxin)造成鼠肝之 total sulfhydryl and non-protein sulfhydryl 增加，肝內之 thiobarbituric acid-reactive substances 及總脂肪之含量及 oxidized glutathione reductase and glutathione peroxidase 之活性均下降。

總括而言，釋放性內毒素大量攝取，會造成成毒性，少量則可改善肝功能。

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民國七十九年十二月六日受理