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Selective segmental hepatic necrosis produced by the Shwartzman mechanism in rabbits

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Summary. In this study, a new experimental model for hepatic necrosis is presented using rabbits, and E. coli endotoxin as the Shwartzman reagent. A segment of the liver was chosen as a target site for the univisceral Shwartzman reaction. Endotoxin-Lipiodol emulsion was used as a preparative injection via the portal venous branch into the target segment by direct puncture. Provocation was made by an intravenous injection of endotoxin 24 h later. A marked and sharply demarcated necrotic area was produced selectively in the target segment; specific changes were not seen in other lobes of the liver or other parts of the body. This model, strongly enhanced by using Lipiodol, seems to be a subtype of the univisceral Shwartzman reaction of the liver.

Key words: Shwartzman reaction – Univisceral Shwartzman reaction – Endotoxin – Hepatic necrosis

Introduction

Shwartzman (1928) described a reaction that produced haemorrhagic necrosis of rabbit skin by an intravenous injection of bacterial endotoxin 24 h after an intradermal injection of endotoxin. Today we call this phenomenon the localized Shwartzman reaction. Four years before the Shwartzman's report, Sanarelli (1924) had delineated systemic haemorrhagic necrosis of organs by two intravenous injections of endotoxin at an interval of 24 h and this phenomenon is now called the generalized Shwartzman reaction, following the proposal of Apitz (1935). The first step is called the preparative injection and the second, the provocative injection. The process is suppressed by heparin (Cluff and Berthrong 1953; Good and Thomas 1953) and warfarin (Shapiro and McKay 1958) and under special conditions can be induced by a single injection of endotoxin. Pregnancy (Berghans and Ehry 1972) or a tumour-bearing state (Mori et al. 1986) are important factors that augment the response to the reaction.

We assumed that the Shwartzman mechanism play an important role in the pathogenesis of human fulminant hepatitis. We succeeded in producing an experimental model of massive hepatic necrosis (Mori et al. 1979) and later (Mori 1981) proposed univisceral or single organ changes as the third type of Shwartzman reaction.

Our study employed this univisceral Shwartzman reaction to produce selective segmental hepatic necrosis. The reaction was sharply localized by more selective venous cannulation and the use of Lipiodol emulsion as the endotoxin carrier was important in both localizing and enhancing the reaction.

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Materials and methods

Japanese albino adult rabbits weighing between 2.0 to 4.0 kg were used for the experiments. All were female, but none had been pregnant. Table 1 summarizes the experimental procedures.

E. coli endotoxin (EX) (0111:B4, Difco laboratories, Detroit, Michigan, USA) was used as the Shwartzman reagent. Lipiodol Ultra-Fluide (LPD) (ethyl esther of the fatty acid of poppy-seed oil, André-Gelbe Laboratory, France), an oily contrast medium for lymphography, was used as the carrier for EX. EX was dissolved, 3 mg/1 ml, in a mixture of 60% Urografin (Schering AG, Berlin/Bergkamen, West Germany) with one fifth volume of distilled water. Just before the preparation procedure, this solution was mixed with three times volume of LPD (termed EX-LPD emulsion). EX was dissolved in physiological buffered saline, 1 mg/10 ml, for use as provocation.

Rabbits were anesthetized with intravenous pentobarbital sodium (Somnopentyl, Pitman-Moore Manufacturing Co.,

Table 1. Experimental procedures

	1	2	3 Days
Group I ($n=6$)	LPD + Uro + EX (ipv)	EX (iv)	sacrifice
Group II ($n=5$)	LPD + Uro + EX (ipv)	Sa 0.6 ml (iv)	sacrifice
Group III ($n=5$)	LPD + Uro (ipv)	EX (iv)	sacrifice
Group IV ($n=5$)	LPD + Uro + EX (ipv) + Hp 5000U	EX (iv) + Hp 5000U	sacrifice
Group V ($n=3$)	LPD + Uro (ipv)		sacrifice

Abbreviations: LPD, Lipiodol; Uro, Urografin; EX, endotoxin; Sa, saline; Hp, heparin; ipv, portal venous injection; iv, intravenous injection

Washington Crossing, NJ, USA), 30 mg per kilogram of body weight. The abdomen was opened through a midline incision. A No. 27 gauge needle was inserted directly into a portal venous branch of either the right posterior or left anterior lobes of the liver. The EX-LPD emulsion was then injected manually. The dose was about 0.2 ml for the right posterior or about 0.4 ml for the left anterior lobe. Twice the dose was injected into the anterior lobe because of its greater size.

Twenty-four h after the preparation, EX dissolved in physiological buffered saline was injected through the ear vein, 0.02 mg per kilogram of body weight, as provocation.

The animals were divided into five groups. Group I was an experimental group, treated as described above, although Nos. 1–3 rabbits showed some difference in the preparative ratio and/or provocative dose. Groups II, III, IV and V served as controls. In group II, twenty-four h after the preparation, 0.6 ml of saline, instead of endotoxin, was injected as provocation. In the animals belonging to group III, a mixture of Lipiodol and diluted 60% Urografin without endotoxin was injected as preparation, followed by provocation with endotoxin. In the rabbits of group IV, both preparation and provocation were performed by the same method as in group I, but heparin (Novo Industry A/S, Denmark) was administered almost simultaneously with the endotoxin injection. At preparation, an intramuscular injection of 5000 units of heparin was given and, at provocation, an intravenous injection of 1000 units of heparin was given just before the endotoxin injection, and another 4000 units were injected intramuscularly 1 h and 15 min later. In group V, only preparation was done using a mixture of Lipiodol and diluted 60% Urografin without endotoxin.

All the animals were sacrificed twenty-four h after the provocation procedures by an intravenous overdose of sodium pentobarbital, and autopsied. All the organs were first examined carefully and some, including the liver, adrenals, kidneys, pancreas, spleen, lungs, and heart, were then fixed in formalin. They were embedded in paraffin, thin-sectioned, and stained with haematoxylin-eosin (HE) and phosphotungustic acid-haematoxylin (PTAH) for histological examination.

Low-KVP X-ray films of the resected livers by the Softex instrument were also taken to confirm the distribution of the EX-LPD emulsion.

Results

Table 2 summarizes the results. The severity of the necrosis was classified according to both macroscopical and microscopical findings. The hepatic changes were compared in the groups. In group I, all the rabbits showed selective segmental hepatic necrosis to a greater degree. However, the grade of hepatic necrosis in groups II, III and IV was less severe than that in group I. In groups II and III, a mild degree of hepatic necrosis was seen in every case. In group IV, a mild to moderate degree of necrosis was seen in three cases, but necrosis did not occur in two cases. Also, in group V, necrosis did not occur in every case.

Severe segmental hepatic necrosis occurred almost exclusively in the animals which received both the endotoxin preparation and provocation. Heparin administration was relatively effective in preventing the necrosis.

Figure 1 A shows the gross picture of the selective segmental hepatic necrosis in group 1. The necrotic lesion was bordered by a clear demarcation line and had diffuse, speckled necrotic foci that were grayish to yellowish white in color. In every case, haemorrhage was not seen macroscopically. The range of necrosis was completely coincident with the distribution of the EX-LPD emulsion on Low-KVP X-ray film (Fig. 1 B).

Figures 2–5 show the histological findings of the target segments in group 1. The hepatic necrosis occurred in the centrilobular or midzonal areas, with a higher frequency of the former (Fig. 2). However, in expansive necrotic areas, the necrosis showed a pattern of panlobular expansion. The change seen was acute, severe, and extensive necrosis including patterns of both coagulative and lytic necrosis. Segmented leukocytes frequently gathered around the area of necrosis but haemorrhage was not as common (Fig. 3). Fibrin thrombi existed chiefly in the portal veins and sinusoids. In particular, Lipiodol droplets surrounded by fibrin thrombi completely filled the portal venous branches (Fig. 4). In portal areas, mild periportal fibrosis, ductular proliferation, and inflammatory cell infiltration were also sometimes seen (Fig. 5).

In organs or lobes of the liver other than the target segment, neither necrotic nor haemorrhagic lesions were seen except rabbit No. 1, which was complicated by bilateral renal cortical necrosis and adrenal necrosis.

Discussion

There are various models of hepatic necrosis which have been made using carbon tetrachloride (Reck-

Table 2. Results of experiments

Group	No.	Dose of emulsion (ml)	Dose of endotoxin (mg)		Dose of Heparin (U)	Target segment	Grade of hepatic necrosis
			preparation	provocation			
I	1	0.33	0.25	0.12		R-P	+5
I	2	0.40	0.30	0.15		R-P	+4
I	3	0.40	0.30	0.20		L-A	+4
I	4	0.45	0.34	0.05		L-A	+5
I	5	0.45	0.34	0.05		L-A	+4
I	6	0.40	0.30	0.05		L-A	+5
II	7	0.30	0.23	Sa 0.6 ml		R-P	+2
II	8	0.40	0.30	Sa 0.6 ml		L-A	+2
II	9	0.40	0.30	Sa 0.6 ml		L-A	+1
II	10	0.40	0.30	Sa 0.6 ml		L-A	+3
II	11	0.40	0.30	Sa 0.6 ml		L-A	+2
III	12	0.40	0	0.06		L-A	+2
III	13	0.20	0	0.06		R-P	+1
III	14	0.20	0	0.06		R-P	+2
III	15	0.40	0	0.05		L-A	+1
III	16	0.20	0	0.05		R-P	+1
IV	17	0.40	0.30	0.05	10000	L-A	+2
IV	18	0.25	0.19	0.06	10000	R-P	−
IV	19	0.25	0.19	0.06	10000	R-P	−
IV	20	0.40	0.30	0.06	10000	L-A	+3
IV	21	0.20	0.15	0.05	10000	R-P	+4
V	22	0.40	0	0		L-A	−
V	23	0.35	0	0		R-P	−
V	24	0.40	0	0		L-A	−

Abbreviations: Sa, saline; R-P, right posterior lobe of the liver; L-A, left anterior lobe of the liver

Grade of necrosis: − negative; + microscopically positive; +2 macroscopically positive but slight; +3 less than half of the target segment of the liver; +4 over half of the target segment; +5 almost all the target segment

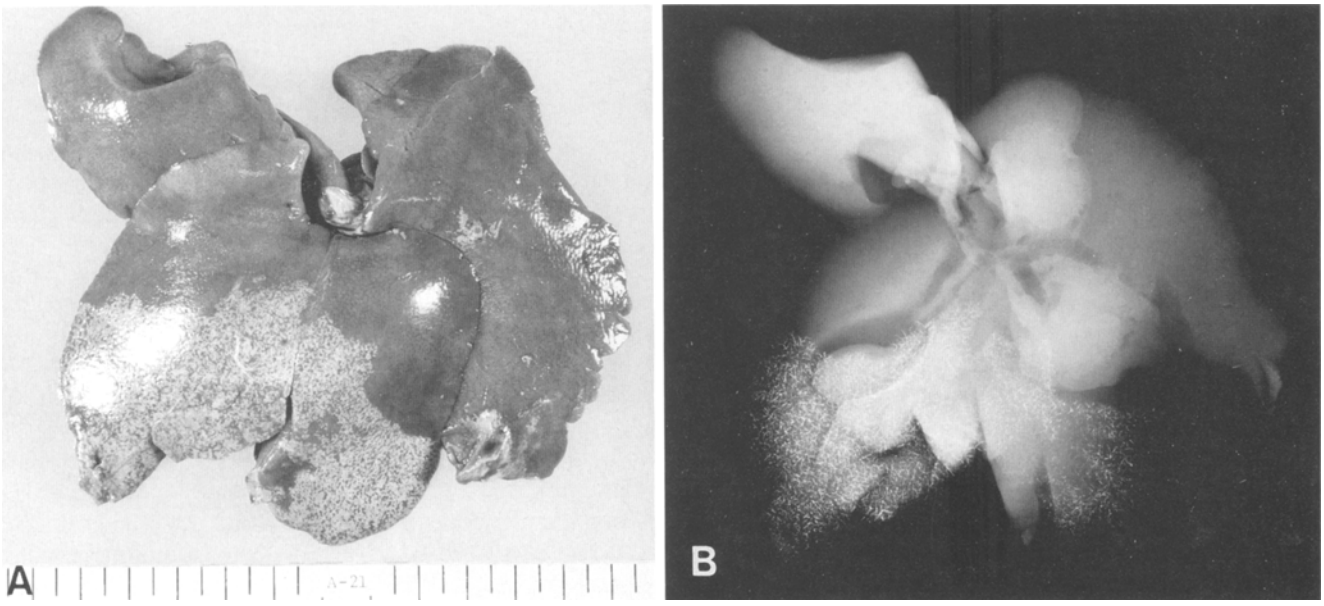

Fig. 1. (**A**) Gross appearance of the selective segmental hepatic necrosis of the left anterior lobe of the liver (group 1). The necrotic area, which had diffuse, speckled necrotic foci, is sharply demarcated. (**B**) Distribution of EX-LPD emulsion on Low-KVP X-ray film (same case). The distribution coincides with the range of necrosis precisely

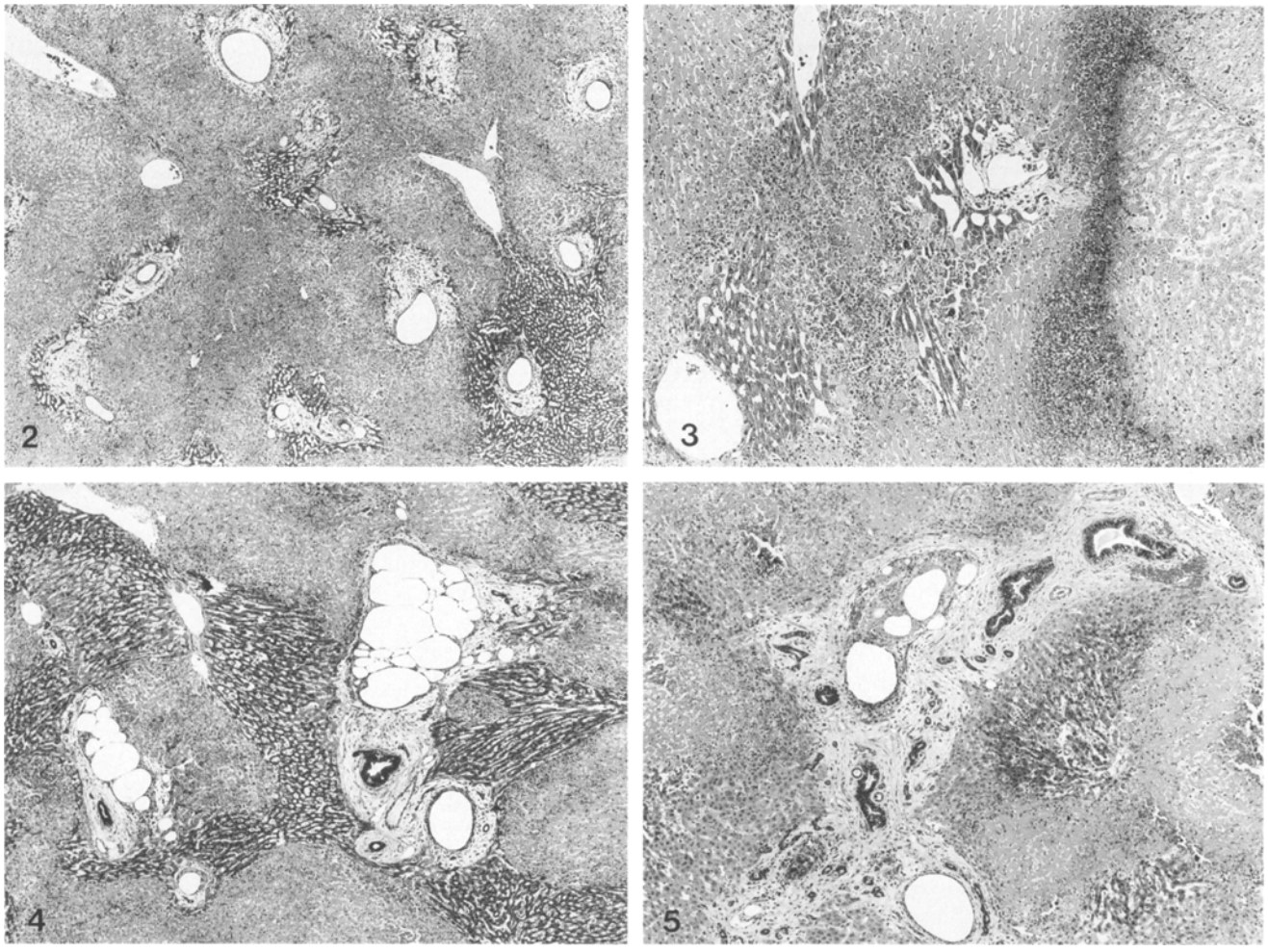

Fig. 2. Severe and extensive hepatic necrosis is shown (group 1), which is mainly coagulative and centrilobular. Note small viable areas in the periportal region. Portal venous branches are dilated and filled with Lipiodol droplets. (HE × 33)

Fig. 3. Leukocyte infiltration is seen around the area of necrosis (group 1). (HE × 66)

Fig. 4. In portal areas, the portal venous branches are filled completely with Lipiodol droplets surrounded by fibrin thrombi (group 1). (HE × 45)

Fig. 5. In portal areas, newly formed periportal fibrosis and ductular proliferation are also seen (group 1). Lipiodol droplets with fibrin thrombi are shown in the portal venous branches. (HE × 50)

nagel 1967) or D-galactosamine (Decker and Keppler 1972). However, in these models, the type of necrosis is diffuse rather than localized. The most important result of this study is the successful production of acute and severe necrosis of a limited segment of the liver selectively by the Shwartzman mechanism.

The univisceral Shwartzman reaction is different from the other two classical types in the following three respects. First, the reaction occurs almost exclusively in the target organ with minimal changes in the other parts of the body. Second, the change is extensive within the target site. Third, the involved viscus becomes severely disordered in function, causing a "disease" and often results in the host's death. There are some human diseases for which this univisceral Shwartzman reaction could be a useful model to clarify the pathogenesis. Attempts to produce the univisceral Shwartzman reaction have succeeded in various organs including the pancreas (Thal and Brackney 1954), liver (Mori et al. 1979), adrenal (Levin and Bluff 1965; Aoyama et al. 1987), intestine (Berry and Fraser 1968), renal papilla (Nakano et al. 1984), and lung (Shiga and Mori 1985).

As a rule, to produce the so-called "Shwartz-

man preparative condition" (Mori et al. 1981), it is very important that endotoxin be exposed to the target site for some period of time. If the target site can be exposed selectively by some means or other, stronger reaction will occur.

In our former experiment (Mori et al. 1979), endotoxin was given as a preparative injection through either the common bile duct or portal venous trunk (via the mesenteric vein) into the liver of rabbits. The hepatic necrosis thus produced was diffuse but limited to the liver. Animals receiving preparation through the common bile duct showed more severe hepatic necrosis than those via the portal vein. This seems to be due to an immediate washout of endotoxin in physiological buffered saline by the portal blood flow.

In the present study our model is proposed both to supplement the disadvantage of this portal route and to produce necrosis only in a limited segment selectively. This has been accomplished by applying the technique of drug injection in the transcatheter chemoembolization of hepatocellular carcinoma (Nakamura 1986) and using Lipiodol as the endotoxin carrier. Lipiodol injected into the portal vein remains in the peripheral portal venous branches for a long time. For example, it remains about 2 to 4 weeks in dogs and humans without specific side effects noted in either biochemical or pathological examinations (Idezuki et al. 1966).

Endotoxin was dissolved in diluted 60% Urografin, and this solution was mixed with Lipiodol as a preparation injection. Stable suspension could be obtained since Lipiodol and diluted 60% Urografin have the same specific gravity. Endotoxin is encapsulated in the Lipiodol and is retained within the peripheral portal venous branches. This sustained-release effect (Nakamura 1986) of endotoxin produces a potent Shwartzman preparative condition. The endotoxin administered directly into the portal venous branch is gradually absorbed into the circulation, quickly captured by the Kupffer cells and kept at the site. So the generalized Shwartzman reaction will not occur after the provocation in spite of two intravenous injections of endotoxin administered at an interval of 24 h.

In our experiment a unique lesion in a segment of the liver, characterized by severe coagulation necrosis, has been produced in rabbits. We compared the range of necrosis with the distribution of EX-LPD emulsion on Low-KVP X-ray film; both coincided completely. Many microthrombi were seen in the lesion, and the reaction was somewhat inhibited by heparin administration. There were no marked changes in other organs and we believe that this change represents a univisceral Shwartzman reaction in the liver. The target site was limited to a part of the liver and all the animal lived for 24 h after provocation.

To produce marked segmental hepatic necrosis, both the endotoxin preparation and provocation are necessary, and the grade of necrosis has no relation to the provocative dose of endotoxin, according to the results of group I.

In group II, contrary to our expectations, hepatic necrosis occurred in each case, although the grade of necrosis was milder than that in group I. This seems to be due to the sustained-release effect of endotoxin of the EX-LPD emulsion. The Shwartzman reaction must be induced, after the potent Shwartzman preparative condition, by the same endotoxin of EX-LPD emulsion. By the same reasoning, the inhibiting effect of heparin administration was not sufficient to prevent injury in group IV, in which hepatic necrosis occurred in three cases.

The surprising finding obtained from group III is that the Lipiodol-Urografin emulsion without endotoxin may produce the Shwartzman preparative condition in the target segment when injected into the portal venous branch, although the degree of necrosis was mild. In our preliminary experiment, we had already confirmed that Lipiodol itself has weak preparative powers in the local skin Shwartzman reaction. Also, according to the results of group V, it is clear that the Lipiodol-Urografin emulsion without endotoxin could not cause hepatic necrosis in the target segment without provocation and that, histologically, Lipiodol fat embolism had no relation to hepatic necrosis. Clinically, this emulsion is widely used as a carrier of anticancer agents in the transcatheter arterial embolization of hepatocellular carcinoma. It is well known that this solution remains both in the tumour and surrounding liver parenchyma for a long time. We speculated that the emulsion injected through this arterial route had the same preparative effect as that through the portal route. The Shwartzman mechanism may play an important role in liver dysfunction after transcatheter arterial embolization, probably being induced by the intraportal endotoxin absorbed from the intestine. The hepatoma-bearing state may also augment the effects of the Lipiodol-Urografin emulsion.

In the present study, we fulfilled our purpose in creating severe necrosis of a limited segment of the liver selectively. To our knowledge, our experiment is the first to produce the Shwartzman reaction in a part of a parenchymal organ selectively. (Berry and Fraser (1968) had produced local-

ized lesions in the gut). Our experimental model seems to be a subtype of the univisceral Shwartzman reaction of the liver. It is very interesting that, in the same rabbit, only the area of the liver fed by the injected portal venous branch becomes severely necrotic in the segmental unit, while the other parts of the liver remain unchanged. This model will be useful in some studies of the liver, for example, liver regeneration and experimental therapy of liver tumours.

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