Acta Medica et Biologica
Vol. 43, No. 4, 229-232, 1995

Specific Effects of 15-Deoxyspergualin on the Fat Metabolism in Partially Hepatectomized Rats

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Received May 30 1995; accepted August 2 1995

Summary. **Dynamic changes in fatty parameters in the blood and body were examined after 15-deoxyspergualin (DSG) or FK506 (FK) administration in 65% hepatectomized rats. The body composition of fatty parameters, the Lee-index and retroperitoneal white adipose tissue weight (RPWT) were estimated at 0, 1, 3, 5 and 7 days after hepatectomy. The serum total cholesterol concentration (Tch) and other valves including liver and kidney functions were also estimated. DSG 5 mg/kg increased Tch at 3 days after hepatectomy, then returned to the control level within an additional 2 days. On the other hand, RPWT decreased gradually following DSG injection, and a significanct decrease was observed 7 days after hepatectomy. No difference in Lee-index was seen among the groups. The food and water intake over 9 days and body weight gain after hepatectomy were similar among the three groups. DSG and FK increased blood urea nitrogen (BUN) levels compared with the control.**

Because the doses of agents utilized have been shown to stimulate liver regeneration, our results suggest that DSG at a dose stimulating liver regeneration produces a higher cholesterol level and promotes the catabolism of adipose tissue more slowly and continuously than FK.

Key words—lipid, cholesterolemia, FK506, liver.

INTRODUCTION

Although liver transplantations have come to be widely performed, the liver plays a highly complex role in whole body cholesterol homeostasis, which is achieved by the balance of *de novo* synthesis, the uptake and secretion of lipoproteins, acyl CoA: cholesterol acyltransferase activity and bile acid formation.[1] After partial hepatectomy, the demand for cholesterol for membrane formation in regenerating the liver and the decrease in cholesterol synthesis are compensated by an increase in lipoprotein uptake from the blood.[1] Glende et al.[2] found that adipose tissue fat is the source of the fatty acids incorporated into hepatic lipids, which suggests a close relation between the extrahepatic cholesterol supply and the adipose tissue. Recently we observed that FK-506 (FK), an immunosuppressant, at a dose stimulating liver regeneration has the potential to catabolize the intrinsic fat store[3] at the time of liver regeneration, indicating the possibility that FK increases the cholesterol supply from the body store.

On the other hand, another immunosuppressant, 15-deoxyspergualin (DSG), enhances liver regeneration after partial hepatectomy.[4] DSG shows its immunosuppressions differently from FK.[4]

This study is designed to investigate whether DSG influences serum cholesterol concentration and lipid metabolism in consideration of FK.

MATERIALS AND METHODS

Sixty-six male Wistar rats were used. The animals were housed individually and allowed free access to laboratory chow (MF, Oriental Yeast, Osaka, Japan) and tap water throughout the experiments. The room temperature was controlled at 24±2℃ with 12 h: 12 h light: dark cycles (lighting from 08:00-20:00 h). The estimation of the individual body weight, food and water intake and surgery were done between 10:00 and 12:00 h to eliminate diurnal variation.

When the animals reached about 180 g, a partial hepatectomy was performed under ether anesthesia

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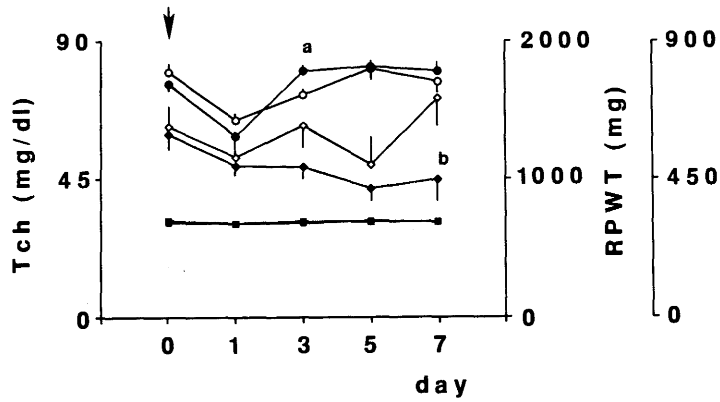

Fig. 1. Changes in fatty parameters in rats with DSG. Tch (○——●), RPWT (◇——◆) and Lee-index (□——■) were estimated following saline (○——◇——□) and DSG 5 mg/day (●——◆——■) administration. The arrow shows the day of hepatectomy. Values are the mean±SEM (n=6). [a]P<0.05 vs ○. [b]P<0.01 vs ◇.

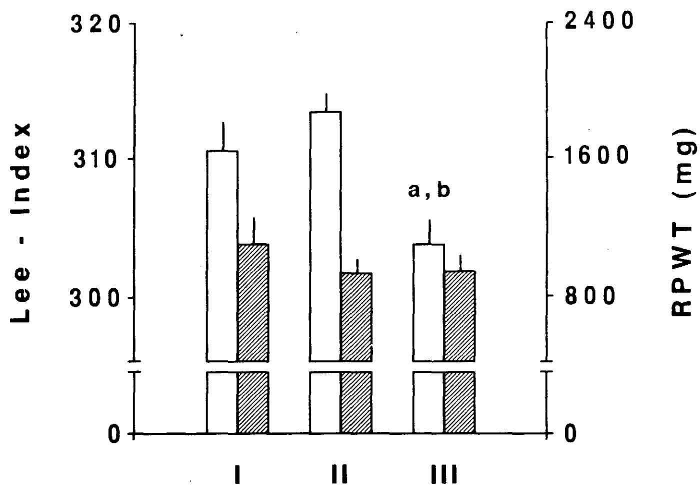

Fig. 2. Changes in fatty parameters 5 days after DSG and FK administration. Saline (I), DSG 5 mg/day (II) and FK 0.1 mg/day (III) have been injected, and the Lee-index (opened bars) and RPWT (shaded bars) estimated. Values are the mean±SEM (n=6). [a]P<0.05 vs I. [b]P<0.01 vs II.

by the method previously described.[5-7] In brief, the median and left lateral lobes of the liver, constituting two-thirds of the total liver mass, were removed. The abdominal wall was closed in layers. After surgery, the animals were returned to their cages and allowed free access to food and water.

The animals were anesthetized by an intraperitoneal injection of pentobarbital sodium 40 mg/kg at 10 : 00 h at 0, 1, 3, 5 and 7 days following hepatectomy. Blood was collected from the tail vein and the animals were killed to estimate the body composition of fatty parameters, Lee-index [body wt (g)$^{0.33}$/nasoanal length (mm)$\times 10^4$] and retroperitoneal white adipose tissue weight (RPWT).[8,9]

The blood collected was cooled immediately on ice and centrifuged at 2,200 rpm for 20 min. Separated serum was stored at −20℃ until the measurement of the following parameters with an autoanalyzer (Hitachi-736, Hitachi, Tokyo, Japan):[4,7] total cholesterol (Tch, cholesterol oxidase colorimetric method); total protein (TP, Biuret method); glucose (Glc, glucose oxidase method); glutamic alanin transaminase (ALT, ultraviolet method); lactate dehydrogenase (LDH, Wroblewski-LaDue method); alkaline phosphatase (Alp, Bessey-Lowry method); total bilirubin (TB, azobilirubin method), blood urea nitrogen (BUN, urease ultraviolet method) and creatinine (Cre, Jaffe method).

DSG (5 mg/kg/day, Nippon Kayaku, Tokyo, Japan) and FK (0.1 mg/kg/day, Fujisawa, Osaka, Japan) were administered once a day for three days, starting one day before surgery. Saline was injected in the control group. Each dose was adjusted to 0.1 ml and given by intramuscular injection. The doses of the agents used in this study have been shown to stimulate liver regeneration.[3,4,10]

The statistical siginificance of the differences among the values was evaluated by ANOVA and Duncan's multiple range test: P<0.05 was defined as a significant difference.

Table 1. Food and water intake, and body weight gain over a 9-day period following administration of DSG or FK

Treatment	Food intake (g)	Water intake (ml)	Body weight gain (g)
Saline (control)	159.5± 7.0	189.3±12.0	37.2±3.9
DSG 5.0 mg/kg	167.5±12.2	196.0±13.0	28.5±5.3
FK 0.1 mg/kg	174.5± 7.1	198.8± 6.4	30.8±3.5

Values are the mean±SEM (n=6).

Table 2. Serum concentrations of blood parameters at 7 days after partial hepatectomy

	Saline (control)	DSG (5 mg/kg)	FK (0.1 mg/kg)
TP (g/dl)	5.4±0.1	5.5±0.2	5.2±0.1
Glc (mg/dl)	165±3	156±7	160±6
ALT (U)	22±3	31±5	26±3
LDH (U)	404±69	378±159	308±74
Alp (U/1)	885±84	1102±86	926±111
TB (mg/dl)	0.1±0.0	0.2±0.1	0.2±0.0
BUN (mg/dl)	16.1±1.0	20.9±1.5[a]	20.1±1.2[a]
Cr (mg/dl)	0.4±0.0	0.4±0.0	0.4±0.0

Values are the mean±SEM (n=6).
[a]$P<0.05$ compared with the control.

RESULTS

The administration of DSG 5 mg/kg increased Tch at 3 days after hepatectomy. RPWT was decreased gradually after DSG injection, and a significant decrease was observed 7 days after hepatectomy. The Lee-index was unchanged throughout the experimented periods (Fig. 1).

On comparing the values 5 days after hepatectomy, the Lee-index in the FK-treated rats had decreased to the control and DSG-treated animals. However, no significant difference was seen between the control and DSG-treated animals. RPWT was not significantly different among the three groups of animals (Fig. 2).

Food and water intake over 9 days and body weight gain following hepatectomy were similar for all three groups of animals (Table 1).

Table 2 shows the serum parameters 7 days after hepatectomy. No significant differences in the liver functional parameter were seen among the groups. Concerning the kidney functional parameters, BUN had increased in the DSG- and FK-treated animals compared with the control.

DISCUSSION

The present study shows that DSG at a dose stimulating liver regeneration produces a higher cholesterol level and promotes the catabolism of the intrinsic fat store because DSG administration of 5 mg/kg increased Tch at 3 days after hepatectomy and decreased RPWT at 7 days (Fig. 1). DSG could act to induce hyperlipidemia as cyclosporin; hyperlipidemia continues to be very common after transplantation and is related to the cyclosporin currently used for immunosuppression.[12]

The finding that a partial hepatectomy decreases Tch was consistent with an earlier report.[13] The demand for membrane formation in the regenerating liver and the decrease in cholesterol synthesis are compensated by an increase in low-density lipoprotein uptake.[1,14] We observed previously that FK at the dose stimulating liver regeneration did not produce any difference in Tch but decreased the Lee-index and RPWT 3 days after hepatectomy.[3] FK kept low levels in the Lee-index at 5 days (Fig. 2). Although DSG started to reduce RPWT at 3 days after surgery, no significant changes were observed in Tch, the Lee-index and RPWT at 5 days when the stimulating effect of DSG on liver regeneration was observed,[4] and DSG resulted in a significant decrease in RPWT at 7 days (Fig. 1). These observations suggest that DSG could act to promote the mobilization of fatty acids from adipose tissue to the liver more slowly and continuously than FK.

The present study demonstrated that DSG increased Tch 3 days following hepatectomy (Fig. 1). The amount of cholesterol supplied from the intrinsic stores by DSG would be larger than that consumed in the liver at this time.

Takeuchi et al. suggested that cholesterol feeding did not inhibit the regeneration mechanism of the liver.[14] Both DSG and FK induced no differences in food and water intake or body weight gain (Table 1), implying that the difference observed in lipid metabolism is not due to the oral intake.

The serum levels of TP and Glc suggested that no unusual nutritional event occured in the animals; neither was any significant effect of DSG or FK observed on the hepatic scores. Increments of the BUN level, indicating renal toxity, might have been induced by the administration of both DSG and FK (Table 2).[4,11] However, the difference in lipid metabolism caused by DSG and FK was not due to the renal dysfunction, because the renal scores were almost at the same levels.

This study showed that DSG has a specific action on the lipid metabolism in regenerating liver. Recently it has been reported that cardiovascular disorders, including myocardial infarction, stroke, and complications of peripheral vascular disease, have surpassed infectious diseases as the major causes of morbidity and mortality following renal transplantation.[12] The development of hyperlipidemia, a risk factor for atherosclerosis, is promoted by corticosteroids and cyclosporine.[12] DSG should be considered as to whether it may cause hyperlipidemia when used in liver transplantation. Since changes in other lipid scores such as triacylglycerol or lipoprotein were not investigated in the present study, further studies are awaited.

From these observations, we conclude that DSG at a dose stimulating liver regeneration after partial hepatectomy produces a higher cholesterol level and promotes the catabolism of adipose tissue more slowly and continuously than FK.

Acknowledgements. The authors are grateful to Nippon Kayaku Co., Ltd. for supplying DSG; to Fujisawa Pharmaceutical Co., Ltd. for FK; and to Mr. T. Hatano (Dept. of Surg.) and Mr. K. Kunihara (Dept. of Physiol.) for their assistance.

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