Note

Responses of Serum Lipids and Adipose Tissue Lipases to Lipopolysaccharide Administration in Normal and Hepatoma-bearing Rats

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Received September 8, 1995

The effects of in vivo lipopolysaccharide administration on serum lipid metabolism were studied in normal and hepatoma-bearing rats. Changes in serum lipid levels and adipose tissue lipase (lipoprotein lipase and hormone-sensitive lipase) activities following injection of lipopolysaccharide into normal rats resembled those in hepatoma-bearing rats. These results suggest the presence of some common factor(s) involved in the incidence of abnormal lipid metabolism upon lipopolysaccharide injection and hepatoma transplantation.

Key words: hepatoma; hyperlipidemia; lipoprotein lipase; hormone-sensitive lipase; lipopolysaccharide

Hepatomas, like other several kinds of cancers,1,2 induce abnormal serum lipid metabolism in humans3,4 and in rats.5,6 Donryu rats, implanted subcutaneously with an ascites hepatoma line of AH109A cells, have both hypercholesterolemia and hypertriglyceridemia during growth of the hepatoma.7,8 The hepatoma-induced hyperlipidemia is characterized by a slight but significant decrease in the high-density lipoprotein (HDL) fraction and a marked increase in the very-low-density lipoprotein (VLDL) plus low-density lipoprotein (LDL) fraction.9 Some specified amino acids9-11 and fatty acids12 in diets are shown to be remediable for the hepatoma-induced hyperlipidemia, indicating a possible application for the nutritional control of cancerous cachexia. Tumor necrosis factor-α (TNF), one of the cytokines, is mainly produced by activated macrophages and its secretion into the bloodstream is stimulated by the injection of lipopolysaccharide (LPS).13,14 TNF is documented to affect lipid metabolism, by increasing the serum triglyceride (TG) and cholesterol (Ch) levels15,16 and stimulating hepatic Ch and fatty acid (FA) synthesis17,18,19. Furthermore, TNF suppresses the lipoprotein lipase (LPL) activity and its mRNA level,15,16 and increases lipolysis20 in 3T3-L1 cells. Thus, it is likely for TNF to be one of the contributing factors for the incidence of the hyperlipidemia. In this study, we attempted to discover whether or not TNF is one of the contributing factors for the hepatoma-induced hyperlipidemia, by measuring the serum lipid levels and adipose tissue lipase activities following injection of various doses of LPS into both normal and hepatoma-bearing rats.

Male Donryu rats (4-week-old, NRC Haruna, Gunma, Japan) were kept on a stock pellet diet (CE-2, CLEA Japan, Tokyo) for 4 days, followed by a 20% casein (20C) diet for 7 days in an air-conditioned room with a temperature of 22 ± 2 °C, a relative humidity of 60 ± 5%, and an 8:00 a.m. to 8:00 p.m. light cycle. The composition of the 20C diet was as follows: 20% casein (Oriental Yeast Co., Tokyo, Japan), 5% corn oil (Hayashi Chemicals Co., Tokyo, Japan), 51.3% 2-carid starch (Nihon Nosan Kogyo Co., Yokohama, Japan), 17% sucrose (Nissin Sugar Manufacturing Co., Tokyo, Japan), 3.5% mineral mixture (AIN composition, Nihon Nosan Kogyo Co.), 1% vitamin mixture (AIN composition, Nihon Nosan Kogyo Co.), 0.2% choline bitartrate (Wako Pure Chemical Industries, Osaka, Japan), and 2% cellulose powder (Oriental Yeast Co.). The rats were then divided into two groups with equal body weights (Normal; 151 ± 2 g. Hepatoma-bearing; 151 ± 2 g) and one group received a subcutaneous injection of 5 × 10^7 AH109A cells (provided by SRL, Tokyo, Japan) in the back to produce a solid hepatoma (hepatoma-bearing group).20 and the other group received only a sham injection of phosphate-buffered saline (PBS; −), 0.5 ml rat and were regarded as normal rats (normal group). Rats of each group were kept for 14 more days on the 20C diet. Water and diet were available at all times. On the 14th day after AH109A implantation, rats of either the hepatoma-bearing group or normal group were again divided into four groups (3 rats/group) with equal body weights. Rats of each group received a intraperitoneal injection of 0.25, 2.5, or 25 μg 0.5 ml PBS (−) - r of LPS (from Salmonella typhimurium, prepared by phenolic extraction and gel filtration chromatography, obtained from Sigma Chemical Co., St. Louis, MO, USA.) 2 h before killing. Animals were deprived of their diet at 9:00 a.m. on day 14 but allowed free access to water until killing which was done by decapitation 4 h after stopping the diet supply. Blood was collected and left to clot at room temperature to obtain serum. The epididymal adipose tissue and solid hepatoma were quickly removed, washed with cold 0.9% NaCl, blotted on filter paper, and weighed. Samples of the epididymal adipose tissue were frozen in liquid nitrogen and stored at − 70 °C until analysis. Total lipids were extracted by the procedure of Folch et al.22 from the serum, and the TG level was measured as described previously.22 The serum nonesterified fatty acid (NEFA) level was measured by the method of Kushiro et al.16,17 The serum lipoproteins were separated into HDL and VLDL + LDL fractions by the precipitation method.23 The total Ch of unfractonated serum (T-Ch) and HDL (HDL-Ch) were measured by an enzymatic method using a Cholesterol C-Test Kit (Wako Pure Chemical Industries), and the difference between T-Ch and HDL-Ch was regarded as (VLDL + LDL)-Ch. LPL was extracted from epididymal adipose tissue by the method of Noguchi et al.,20 and the activity was then estimated21,22 as described previously.20 Hormone-sensitive lipase (HSL) activity in adipose tissue was also measured. From epididymal adipose tissue, HSL was extracted by homogenization,23 and the activity was then measured as described previously.20 One unit of lipase activity was defined as 1 μmol of FA released per hour. Results were expressed as means ± standard errors. Statistical analysis was done using Student’s t-test (between the normal and hepatoma-bearing groups at the same LPS dose) or Duncan’s multiple-range test (within the normal or hepatoma-bearing groups).24 The correlation coefficient was calculated by least square analysis.

Food intake and body weight gain for 14 days were lower in the hepatoma-bearing (H) rats than in the normal (N) rats (food intake; 192 ± 205 g (H) vs. 278 ± 285 g (N) and body weight gain;
62-70 g (H) vs. 110-114 g (N), respectively. Hepatoma weights were almost the same among four groups of hepatoma-bearing rats (38-42 g rat). Changes in the serum lipid levels and lipase activities are illustrated in Figure. With no LPS injection (0 μg LPS), the serum T-Ch, (VLDL + LDL)-Ch, TG, and NEFA levels, atherogenic index [(VLDL + LDL)-Ch: HDL-Ch], and HSL activity were higher in the hepatoma-bearing rats than in the normal rats, while the serum HDL-Ch level and LPL activity were lower in the former than in the latter. These parameters in the hepatoma-bearing rats were unaffected by the injection of various doses of LPS. However, in the normal rats, among these parameters, the serum (VLDL + LDL)-Ch, TG, NEFA levels, atherogenic index, and HSL activity increased but LPL activity decreased after injection of LPS of 2.5 or 25 μg/rat. The injection of LPS into normal rats did not influence the T-Ch and HDL-Ch levels appreciably.

This study showed that changes in serum lipid levels and adipose tissue lipase (LPL and HSL) activities following injection of LPS were similar to those observed in hepatoma-bearing rats, that is, serum (VLDL + LDL)-Ch, TG, and NEFA levels, atherogenic index, and adipose tissue HSL activity increased but adipose tissue LPL activity decreased. These results therefore suggest that there are some common factor(s) involved in the incidence of abnormal lipid metabolism when rats were injected with LPS or implanted with hepatoma AH109A cells. As reported previously, LPS administration in vivo stimulates TNF secretion into the blood stream.9,10 This cytokine stimulates hepatic lipase synthesis11,13,14 and lipolysis in 3T3-L1 cells,15,16 and supresses LPL activity and its mRNA level in 3T3-L1 cells,17,18 hence, causing hyperlipidemia.11,12 In a separate experiment, TNF productivity, in vitro, in resident peritoneal macrophages from AH109A-bearing rats was found to be much (85-fold) higher than that from normal rats (Komatsu et al., unpublished observation). The reduction in LPL activity and the elevation in HSL activity were correlated to the elevations in the serum TG (r = -0.86, p < 0.01) and NEFA (r = -0.90, p < 0.01) levels, respectively. These observations were consistent with previous studies reported by Noguchi et al.20 and by us.8 In summary, the results suggest that TNF contributes as a factor to induce hyperlipidemia in AH109A-bearing rats as well as in LPS-injected mice (a model for acute infection).14 Further studies including measurement of the serum TNF level of AH109A-bearing rats will clarify these questions.

References