Effect of Dietary Conjugated Linoleic Acid on Liver Regeneration after a Partial Hepatectomy in Rats

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Received March 12, 2003

Summary We examined the effect of dietary conjugated linoleic acid (CLA) on liver regeneration after a partial hepatectomy (PH) in Sprague-Dawley rats. PH was performed on rats fed a 0 or 1 wt.% CLA diet for 3 wk. Average liver weight in the CLA fed rat population was heavier than the control rat population at the time of PH and 1-d after PH. Conversely, CLA fed rats’ liver weight was significantly lower than control rats at 7-d after PH. This suggests that dietary CLA reduced liver weight gain after PH. Dietary CLA did not affect serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) activities. However, CLA significantly reduced serum albumin levels at 1-d but not at 7-d after PH. 5-Me- and 5-iododeoxyuridine incorporation into hepatocytes 1-d post PH was lower in the CLA group. In conclusion, the data suggests that dietary CLA inhibits DNA synthesis after PH, which results in hepatocyte proliferation inhibition.

Key Words conjugated linoleic acid, liver regeneration, hepatectomy, Sprague-Dawley rats, DNA synthesis

Conjugated linoleic acid (CLA) is a generic term for the positional and structural isomers of octadecadienoic acid. CLA has various beneficial physiological functions such as anti-carcinogenic (1), anti-obesity (2), and anti-atheroscleotic activities (3). Belury et al. reported that 1.0 wt.% CLA diet induced an elevation of ornithine decarboxylase (ODC) activity in SENCAR mice (4). ODC is a rate-limiting enzyme for the synthesis of polyamines, which are known to play a key role in DNA synthesis in various cells including rat hepatocytes (5). In addition, we previously showed that 9c, 11t-CLA could promote the proliferation of Donryu rat derived hepatoma cells (6). Moreover, we also reported that dietary CLA (2 wt.%) promoted the growth of transplanted hepatoma dRLh-84 cells, which was the same cell line used in Ref. 6) (7). These data imply that dietary CLA acts as a rodent hepatocyte proliferator in vitro and in vivo. Here, we study the effect of CLA on normal hepatocytes in vivo.

At normal conditions, hepatocytes and other non-parenchymal cells in the liver are in the G0 phase and are at rest. However, when cell death or liver dissection occurs, the liver has the ability to maintain mass by inducing the proliferation of hepatocytes and other nonparenchymal cells. Higgins and Anderson established an evaluation procedure for liver regeneration after surgically removing part of the liver, which results in a 2/3 partial hepatectomy (PH) (8). Thus, we examined the effect of CLA on the liver regeneration after PH.

MATERIALS AND METHODS

Experimental animal and partial hepatectomy. Dietary CLA and safflower oil (SAF) were supplied from Rinoru Oil Mills Co., Ltd. (Nagoya, Japan). Composition of CLA was as follows: 9c,11t; 46.2%, 10t,12c; 47.5%, 9c,11c and 10c,12c; 3.1%, 9t,11t and 10t,12t; 3.3%. Male, 4-wk-old Sprague-Dawley rats were purchased from Seac Yoshitomi (Fukuoka, Japan) and were given a nonpurified diet and water ad libitum for 6-d after arrival. Rats were kept at the Biotron Institute of Kyushu University in a 12h light/12h dark (8:00 to 20:00) cycle at 20°C under specific pathogen free conditions. Experimental diets contained 7 wt.% SAF (control) or 6 wt.% SAF with 1 wt.% CLA (CLA) and were prepared according to the AIN-93G type diet. After an acclimatization period, rats were separated into 2 groups with 10 or 11 rats each. After 3 wk, 2/3 PH was performed according to the method described by Higgins and Anderson under light anesthesia using sodium 5-ethyl-5-(1-methylbutyl) barbiturate (8). The dissected liver weight was measured immediately after PH and then the rats were maintained at the same experimental condition. Rats were killed by withdrawing blood from abdominal aorta at 1-d or 7-d after PH. Body weight was measured once a week before PH, at PH, and the time of sacrifice. The

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weight of the spleen, liver and adipose tissues were measured immediately after the excision. Serum was prepared soon after the blood was collected and was analyzed within a day. This experiment was carried out under the guideline for Animal Experiments at the Faculty of Agriculture’s Graduate Course, Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government.

Immunohistochemistry of BrdU. One hour before sacrifice, 50 mg/kg body weight 5-bromo- and 5-iododeoxyuridine (BrdU) was intraperitoneally administered. DNA synthesis was measured by assessing the percentage of BrdU positive cells (9). Briefly, the samples of the remnant liver were fixed in 70% ethanol one day and then embedded in paraffin. Five micrometer-thick sections were deparaffinized and immunohistochemically stained by the avidin-biotin-peroxidase complex method using monoclonal anti-BrdU antibodies made from clone BU33 (Sigma, Saint Louis, MO) following the recommended procedure. BrdU positive cells were expressed as the ratio of 100 hepatocytes.

Analysis of the liver function. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using commercial kits according to the appended protocol (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum albumin level was also measured using a commercial kit (Wako).

Statistical analysis. Data were analyzed by Student’s t-test and Mann-Whitney’s U-test to evaluate the significance of difference.

RESULTS

Table 1 shows the effect of CLA on growth and tissue weight. No significant difference was seen between the control and CLA groups for body weight at PH, 1-d and 7-d after PH. CLA fed rats showed an ability to metabolize food more efficiently than the control rats at 7-d post PH. Remarkable spleen weight gain was recognized for both dietary groups from 1-d to 7-d post PH. However, we could not detect any significant difference between the control and CLA groups. Weights of epididymal and perirenal fats were significantly lower in CLA fed rats except for epididymal fat weight at 7-d post PH. Epididymal fat weight loss was confirmed in both dietary groups from 1-d to 7-d post PH, but only control rats showed positive perirenal fat weight gain.

Table 2 shows the effect of CLA on the liver weight at various points. At the time of PH, the dissected liver weight, which corresponds to 2/3 of total organ mass at PH, was slightly higher in CLA fed rats than control rats. One-day post PH also showed similar results. However, liver weight was significantly lower for the CLA group than control at 7-d post PH. When we calculated the liver weight gain from 1-d to 7-d, it was revealed that regenerated liver weight was significantly lower for the CLA-fed rats compared to the control rats. Recovery rate compared to the estimated liver weight at the time of PH, which is shown as the regeneration rate in Table 2, was also significantly lower in the CLA group. As also shown in Table 2, we examined the effect of CLA on DNA synthesis in hepatocytes using the BrdU incorporation method. BrdU was injected (i.p.) 1 h before the animal was sacrificed. Comparing samples from both groups at 1-d post PH, the percent of positive BrdU cells in the CLA fed group was significantly lower than the control. At 7-d post PH, both dietary groups showed extremely lower results compared to 1-d post PH. Finally, serum albumin level was significantly lower for the CLA-fed rats (87.2% of non-CLA fed rats level) at

| Table 1. Effect of dietary conjugated linoleic acid on the growth of Sprague-Dawley rats after partial hepatectomy. |
|---------------------------------------------------------------|
|                  | Control | CLA     |
| Body weight (g)   |         |         |
| Initial           | 131±12  | 130±9   |
| At PH             | 283±24  | 293±12  |
| 1-d after PH      | 258±31  | 273±28  |
| 7-d after PH      | 261±38  | 281±26  |
| Food efficiency (weight gain/feeding amount)                  |
| At PH             | 0.353±0.032 | 0.383±0.025 |
| 1-d after PH      | 0.294±0.031 | 0.321±0.031 |
| 7-d after PH      | 0.263±0.046 | 0.299±0.044* |
| Spleen weight (% of body wt.)                                |
| 1-d after PH      | 0.20±0.06 | 0.22±0.05  |
| 7-d after PH      | 0.54±0.24 | 0.54±0.08  |
| Epididymal fat weight (% of body wt.)                         |
| 1-d after PH      | 2.13±0.49 | 1.45±0.43* |
| 7-d after PH      | 1.35±0.28 | 1.03±0.16  |
| Perirenal fat weight (% of body wt.)                          |
| 1-d after PH      | 1.41±0.34 | 1.18±0.22* |
| 7-d after PH      | 1.94±0.47 | 1.09±0.34* |

Data are means±SD for five or six rats in each group. Values containing an asterisk mark are significantly different from the control value at *p<0.05. PH: partial hepatectomy.

| Table 2. Effect of dietary conjugated linoleic acid on the liver regeneration and hepatic function of Sprague-Dawley rats after partial hepatectomy. |
|---------------------------------------------------------------|
|                  | Control | CLA     |
| Dissected liver (% of body wt.)                              |
| 4.05±0.38        | 4.26±0.37 |
| 1-d after PH (% of body wt.)                                |
| 1.21±0.20        | 1.39±0.27  |
| 7-d after PH (% of body wt.)                                |
| 3.54±0.32        | 2.94±0.39*  |
| Weight gain (% of body wt.)                                 |
| 2.33±0.32        | 1.55±0.39** |
| Regeneration rate (%)                                      |
| 52.5±4.0         | 44.2±5.5*  |
| BrdU-positive cells (%)                                     |
| 1-d after PH      | 29.8±4.5  | 24.3±3.7* |
| 7-d after PH      | 6.4±1.1   | 5.2±1.9   |
| Serum albumin level (g/dl.)                                 |
| 1-d after PH      | 3.45±0.38 | 3.01±0.17* |
| 7-d after PH      | 3.06±0.32 | 3.00±0.24 |

Data are means±SD for five or six rats in each group. Values containing an asterisk mark are significantly different from the control value at *p<0.05 and **p<0.01. *Weight gain=(Liver weight after 7-d PH)–(Liver weight after 1-d PH). **Regeneration rate=(Regeneration liver weight)/(Estimated whole liver weight at the time of operation)x100.
Table 3. Effect of dietary conjugated linoleic acid on the liver injury of Sprague-Dawley rats after partial hepatectomy.

|                  | Control           | CLA               |
|------------------|-------------------|-------------------|
| Serum AST activity (Karmen U) |                  |                   |
| 1-d after PH     | 1,008±500         | 694±95            |
| 7-d after PH     | 216±82            | 366±199           |
| Serum ALT activity (Karmen U) |                |                   |
| 1-d after PH     | 137±39            | 178±26            |
| 7-d after PH     | 15±13             | 24±11             |

Data are means±SD for five or six rats in each group. PH: partial hepatectomy. AST: aspartate aminotransferase, ALT: alanine aminotransferase.

1-d post PH.

We measured serum AST, ALT activities as indicators of hepatic injury and liver function (Table 3). Serum AST and ALT activities were high in both dietary groups at 1-d post PH. We could not detect any significant difference between the control and CLA group (Mann-Whitney’s U-test).

DISCUSSION

We previously demonstrated that dietary CLA brought about slight liver enlargement in rats with the reduction of adipose tissue weight (6). In this report, we also confirm the reduction of fat weight at the time of PH in CLA group. We have shown that perirenal fat is more sensitive to dietary CLA than epididymal fat from an anti-obesitical standpoint (10). As shown in Table 3, perirenal fat weight slightly decreased in the CLA group, but increased in the control group between 1-d to 7-d post PH. One of the putative mechanisms for reducing this body fat is through the promotion of lipolysis (11). Mobilization of fatty acids from adipose tissue into the liver is an indispensable event for liver regeneration. Thus, perirenal fat might be preferentially mobilized over epididymal fat in CLA-fed rats after undergoing PH. These data suggest that dietary CLA regulates adipose tissue lipid metabolism post PH.

Most of the liver cells at normal state are in the quiescent G0 state. However, PH initiates the induction of various cell cycle related proteins followed by DNA synthesis. After these sequential events have been completed, it is believed that DNA synthesis in hepatocytes reaches a peak around 1-d after PH, and then substantial cell proliferation begins. Within 3–14 d, organ weight has been shown to be restored to the original mass (12–14). In this experiment, in spite of the slight liver enlargement at the time of PH, liver weight for the CLA fed group was significantly lower than control rats at 7-d post PH. This means that the liver weight gain between 1-d to 7-d is significantly lower in CLA-fed rats. It has been reported that dietary CLA increases liver weight in mice, hamsters and rats (7, 15, 16), and this effect was explained by induction of hypertrophy but not hyperplasia caused by lipid accumulation into hepatocytes. Thus, it is considered that present data shown in Table 1 also support these previous reports. Furthermore, the BrdU positive cell population was shown to be low in CLA-fed rats 1-d after PH. Taken together, our data indicate that dietary CLA inhibits liver cell proliferation and recovery of the liver weight after PH by inhibiting or delaying DNA synthesis.

In addition, we showed the reduction of serum albumin level by CLA (Table 2). As a whole, it is considered that inhibition of overall hepatic protein synthesis by dietary CLA involved in the reduction of liver cell proliferation post PH. The CLA used in this study contained 10t,12c and 9c,11t-CLA as the major compounds (details are shown in Materials and Methods). Recently, highly purified isomers have been commercially available, and this resource is expected to facilitate the clarification of each isomer’s physiological activity. Little is known about the isomer specific effect of CLA on the hepatic protein synthesis in rats. Therefore, it remains an important subject to identify a specific isomer, which reduces the serum albumin levels.

10t,12c-CLA has been shown to exert a potent body fat reducing effect accompanied with induction hepatic steatosis and reduction of liver function in rats and mice (15, 17, Yamasaki et al. unpublished data). So far, it has been reported that steatosis itself does not impair liver regeneration after PH (18). Thus, it is considered that hepatic steatosis induced by 10t,12c-CLA is not an essential cause to bring the reduction of DNA synthesis and substantial weight gain in the liver after PH. After PH, temporary elevation of serum AST and ALT activities are recognized, and this result is consistent with previous reports (19). In this report, we could not detect a significant difference in serum AST and ALT activities between the two dietary groups. Thus, it was considered that dietary CLA did not induce remarkable hepatic injury.

In this study, a 3-wk feeding of CLA before PH induces suppression of liver regeneration in Sprague-Dawley rats. More study is needed to examine the effects of timing, dose and duration of CLA on liver regeneration. Moreover, determining the effect and specificity of each isomer remains an important area of study.

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