Effect of Methionine and Threonine on the Hypercholesterolemia Induced by Polychlorinated Biphenyls in Rats Fed a Nonprotein Diet

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(Received July 3, 1989)

Summary It was previously reported that the hypercholesterolemia induced by polychlorinated biphenyls (PCB) was influenced by dietary protein quantity and quality. On the other hand, the supplementation of methionine and threonine to a nonprotein diet ameliorated the body weight loss and decreased the urinary urea excretion in rats. We examined the effect of methionine and threonine supplements on the hypercholesterolemia induced by PCB in rats fed a nonprotein diet. The administration of PCB increased plasma cholesterol concentration and the supplements of methionine and threonine to the nonprotein diet significantly accelerated the elevation of plasma level of cholesterol due to PCB feeding. Liver microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in rats fed the nonprotein diet was also elevated by PCB administration and the supplementation of methionine and threonine caused further inducing effect.

Key Words hypercholesterolemia, PCB, nonprotein diet, methionine, threonine, HMG-CoA reductase

Administration of polychlorinated biphenyls (PCB) to rats caused hypercholesterolemia (1). This hypercholesterolemic effect became more pronounced when dietary protein quantity and quality were increased (2, 3). 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [EC 1.1.1.34] is the rate-limiting enzyme for cholesterol biosynthesis (4) and liver microsomal HMG-CoA reductase activity was shown to be elevated by PCB treatment (5, 6).

On the other hand, methionine and threonine supplements to a nonprotein diet reduced body weight loss and urinary urea excretion. This nitrogen-sparing action was specific only for methionine and threonine and other essential amino acids had no effect (7). Methionine and threonine are considered to be the endogenously most limiting amino acids in rats fed a nonprotein diet.
In this paper we examined the effect of methionine and threonine on the PCB-induced hypercholesterolemia in rats fed a nonprotein diet.

EXPERIMENTAL

Animals and diets. Five-week-old male Wistar rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were individually housed and fed a 25% casein-sucrose diet for 5 to 7 days. The basal nonprotein diets contained 3.5% mineral mixture (8), 1% vitamin mixture (9), 0.2% choline chloride, and 2% corn oil. Sucrose was used as a source of carbohydrate to make 100%. And 0.3% of L-methionine and L-threonine and 0.03% of PCB (Aroclor 1248, Mitsubishi Monsanto Co. Ltd., Tokyo, Japan) (10) were added at the expense of sucrose. Feed and tap water were supplied ad libitum. The room temperature was kept at 22°C with a 12-h cycle of light (0800–2000 h) and dark.

Experiment 1. On feeding days 0, 3, 7, 10, and 14, 50 μl of blood was drained from the tail vein into the heparinized micro-hematocrit tube and plasma was obtained. After feeding the experimental diets for 21 days, rats were sacrificed by decapitation (0845–0945 h) and blood was collected into a heparinized tube. After gastrointestinal contents were removed, the carcass and liver were stored at −20°C until analysis. To determine urinary urea content, urine was collected into 0.1 N HCl on days 12 to 13.

Experiment 2. Rats were sacrificed by decapitation (2230–2330 h) after 10 days of feeding period. Livers were quickly removed and aliquots were used for the measurement of liver microsomal HMG-CoA reductase activity. For the analysis of fecal bile acids, feces were collected on days 7 to 10 and dried at 60°C under air stream for overnight.

Analytical methods. Minced carcass and liver lipids were extracted by the procedure of Folch et al. (11) and total lipids were measured gravimetrically. Cholesterol in carcass, liver, and plasma was measured using a commercially available kit (Boehringer Mannheim Yamanouchi, Tokyo, Japan). Urinary urea was determined by the urease-indophenol method (12). Liver microsomal HMG-CoA reductase activity was assayed with a modification of the procedure of Brown et al. (13) as follows. NADP⁺, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase in the reaction mixture were replaced by 5 mM of NADPH. Fecal bile acids were extracted and measured according to the methods by Malchow-Møller et al. (14) and Bruusgaard et al. (15) respectively.

Statistical analysis. Statistical evaluations of the means were carried out by two-way analysis of variance (ANOVA) and Student’s t-test (16).

RESULTS

Body weight loss was observed in rats fed the nonprotein diet and the supplements of methionine and threonine significantly prevented the body weight...
Table 1. Effect of methionine and threonine supplement on body weight change, liver weight, and urinary urea excretion in rats fed a nonprotein diet with or without PCB for 21 days (Experiment 1).

|                | Initial body weight (g) | Body weight change (g/21 days) | Liver weight (g/100 g B.W.) | Urinary urea days 12–13 (mg/100 g B.W./day) |
|----------------|-------------------------|--------------------------------|----------------------------|---------------------------------------------|
| 1. Nonprotein  | 130.8 ± 2.7             | −34.5 ± 1.3                    | 4.94 ± 0.22                | 29.6 ± 4.4                                 |
| 2. 1 + Met, Thr| 130.2 ± 1.6             | −25.7 ± 1.2                   | 4.38 ± 0.15                | 6.9 ± 0.5                                  |
| 3. 1 + PCB     | 130.2 ± 1.1             | −39.7 ± 1.4                   | 6.68 ± 0.21                | 33.9 ± 3.1                                 |
| 4. 2 + PCB     | 130.5 ± 0.6             | −25.8 ± 0.6                   | 7.89 ± 0.44**              | 10.8 ± 1.3                                 |

ANOVA
- Met, Thr
- PCB
- Interaction

Values are M ± SE (n = 5). B.W., Body weight. NS, Not significant. ††† means significantly different from the values of rats fed the nonprotein diet without PCB at p < 0.001 using Student’s t-test. ** and *** mean significantly different from the values of rats fed the nonprotein diet with PCB at p < 0.01 and p < 0.001, respectively, using Student’s t-test.

The nonprotein diet feeding greatly decreased plasma cholesterol concentration below the half level of the 25% casein diet group level within 3 days irrespective of the dietary treatment (Fig. 1). After 3 days of feeding the addition of methionine and threonine to the basal diet containing PCB continuously increased plasma level of cholesterol throughout the experimental period. However, the supplements of methionine and threonine to the basal diet without PCB only slightly elevated the plasma level of cholesterol.

The addition of PCB decreased liver cholesterol content when the experimental period was 10 days (Table 3), however, cholesterol was accumulated in livers of rats fed PCB for 21 days (Table 2). Administration of PCB deposited lipids in the liver and more significant deposition was observed in the methionine and threonine-supplemented group (Table 2). Total lipids and cholesterol level in carcass were not affected by dietary PCB, methionine, or threonine.

The active form of HMG-CoA reductase was induced by PCB administration and methionine and threonine treatment further increased the activity when expressed as units per relative liver weight (Table 4). Total activity showed a similar tendency.

Fecal excretion of bile acids was lowered by the addition of methionine and...
Fig. 1. Effect of supplementation of methionine and threonine on plasma level of cholesterol in rats fed a nonprotein diet with or without PCB. Each point represents M±SE (n=5). △, 25% casein; ○, Nonprotein (N); □, N+Met, Thr (MT); ●, N+PCB; ■, MT+PCB.

Table 2. Effect of methionine and threonine supplement on liver and carcass lipids in rats fed a nonprotein diet with or without PCB for 21 days (Experiment 1).

|                | Liver total lipids (mg/g) | Liver cholesterol (mg/g) | Carcass total lipids (mg/g) | Carcass cholesterol (mg/g) |
|----------------|---------------------------|--------------------------|----------------------------|----------------------------|
| 1. Nonprotein  | 99 ± 2                    | 2.58 ± 0.16              | 99 ± 7                     | 2.23 ± 0.05                |
| 2. 1 + Met, Thr| 106 ± 3                   | 3.28 ± 0.37              | 103 ± 11                   | 2.11 ± 0.03                |
| 3. 1 + PCB     | 158 ± 8                   | 3.43 ± 0.24              | 93 ± 12                    | 2.41 ± 0.03                |
| 4. 2 + PCB     | 226 ± 5***                | 5.41 ± 0.35***           | 100 ± 8                    | 2.15 ± 0.04                |
| ANOVA          |                           |                          |                            |                            |
| Met, Thr       | <0.01                     | <0.01                    | NS                         | NS                         |
| PCB            | <0.01                     | <0.01                    | NS                         | NS                         |
| Interaction    | <0.01                     | <0.01                    | NS                         | NS                         |

Carcass does not contain serum and liver. Values are M±SE (n=5). *** means significantly different from the values of rats fed the nonprotein diet containing PCB at p<0.001 using Student’s t-test.
Table 3. Effect of methionine and threonine supplement on body weight change, liver weight, and plasma and liver cholesterol in rats fed a nonprotein diet with or without PCB for 10 days (Experiment 2).

| Initial body weight (g) | Body weight change (g/10 days) | Liver weight (g/100 g B.W.) | Plasma cholesterol (mg/100 ml) | Liver cholesterol (mg/g) |
|------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------|
| 1. Nonprotein          | 118.2±1.2                     | 21.3±0.8                      | 4.32±0.03                     | 45±2                     | 4.34±0.28               |
| 2. 1+Met, Thr          | 118.0±1.1                     | 16.8±0.8                      | 4.43±0.11                     | 55±2†                    | 4.27±0.17               |
| 3. 1+PCB               | 118.0±0.9                     | 23.8±0.5                      | 5.63±0.24                     | 56±3                     | 3.71±0.16               |
| 4. 2+PCB               | 118.2±0.9                     | 17.2±0.4                      | 6.49±0.1†***                  | 114±3***                 | 3.29±0.09               |

ANOVA
- Met, Thr: <0.01
- PCB: <0.01
- Interaction: <0.01

B. W., Body weight. Values are M±SE (n=5). † means significantly different from the values of rats fed the nonprotein diet not containing PCB at p<0.05 using Student’s t-test. *** means significantly different from the values of rats fed the nonprotein diet containing PCB at p<0.001 using Student’s t-test.

threonine but not affected by PCB administration (Table 4).

DISCUSSION

It has been shown that serum cholesterol concentration was affected by dietary protein level (2). Nonprotein diet feeding caused significant reduction in plasma cholesterol level (Fig. 1); the comparison between present data (Table 4) and data in the literature (5) (Active form was 2.83 units per 100 g of body weight in rats fed the 20% casein diet) shows this reduction might be attributed to the low HMG-CoA reductase activity.

PCB administration caused hypercholesterolemia in rats (1) and this hypercholesterolemic effect was more pronounced when dietary protein quality or quantity was increased (2, 3). The increased activity of liver microsomal HMG-CoA reductase seems to be responsible for the hypercholesterolemia induced by PCB administration (5) and Jenke (6) showed dietary PCB acts at the transcriptional level to induce HMG-CoA reductase. In agreement with the previous reports, the addition of PCB increased plasma cholesterol level in rats fed the nonprotein diet (Fig. 1) and this increment in plasma level of cholesterol was significantly stimulated by the supplementation of methionine and threonine to the basal nonprotein diet. When rats were fed a diet containing normal level of protein with PCB, continuous increment in serum cholesterol concentration was observed (17); however, plasma cholesterol was elevated by the addition of PCB only between 3 to 7 days and thereafter plasma cholesterol level was almost constant (Fig. 1). This is probably
Table 4. Effect of methionine and threonine supplement on liver HMG-CoA reductase activity and fecal bile acid excretion in rats fed a nonprotein diet with or without PCB for 10 days (Experiment 2).

|               | HMG-CoA reductase | Fecal bile acids  |
|---------------|-------------------|-------------------|
|               | Active            | Total<sup>1</sup> | days 7–10               |
|               | (units/g liver)  | (units/g liver)  | (μmol/100 g B.W./day)  |
|               | (units/liver/100 g B.W.) | (units/liver/100 g B.W.) |                         |
| 1. Nonprotein | 0.09 ± 0.01      | 0.31              | 5.2 ± 0.7                |
| 2. 1 + Met, Thr | 0.05 ± 0.01 | 0.20              | 3.3 ± 0.4                |
| 3. 1 + PCB    | 0.21 ± 0.04      | 1.33              | 5.7 ± 0.4                |
| 4. 2 + PCB    | 0.30 ± 0.05      | 1.90 ± 0.31*     | 2.6 ± 0.5                |

ANOVA

|          | Met, Thr | PCB | Interaction |
|----------|----------|-----|-------------|
|          | NS       | <0.01 | NS          |
|          | NS       | <0.01 | NS          |

<sup>1</sup> Pooled sample. One unit is one nmol of mevalonate formed per min. B. W., Body weight. Values are M ± SE (n = 5). * means significantly different from the values of rats fed the nonprotein diet containing PCB at p < 0.05 using Student's t-test.
due to the deficiency of amino acids for the synthesis of HMG-CoA reductase. The main cause of hypercholesterolemia in rats fed the nonprotein diet containing PCB together with methionine and threonine appears to be elevated activity of liver microsomal HMG-CoA reductase. Reduced fecal excretion of bile acids might further confirm this hypercholesterolemic effect. It is noteworthy that methionine and threonine supplements to the nonprotein diet greatly stimulated the elevation of plasma level of cholesterol due to PCB feeding, just like the protein diet.

Activity of HMG-CoA reductase is regulated by dephosphorylation and phosphorylation (4). The percentage of the dephosphorylated form (active) of HMG-CoA reductase to the total (active and inactive forms) was 16 to 30% (Table 4) and these values are comparable to the values, 10 to 25%, reported previously (5,13). Because the ratio of the active and inactive forms of the enzyme was not changed appreciably, the changes in HMG-CoA reductase activities would probably be due to changes in enzyme protein mass.

NADPH is required for cholesterol biosynthesis and we showed PCB administration induced NADPH-generating enzymes such as glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase, and malic enzyme (Hitomi and Yoshida, in preparation) and these results would explain the elevated demand of NADPH for cholesterogenesis as well as for the drug-metabolizing enzyme system.

Fatty liver is one of the typical PCB-induced metabolic changes (2,3). Hinton et al. (18) demonstrated that fatty liver induced by PCB resulted from increased half-life of lipids but not from increased lipid synthesis. However, the mechanism of why the supplementation of methionine and threonine caused more severe fatty liver is unknown. It is also unknown why liver cholesterol content was different depending on the feeding period of PCB-containing diet.

These results suggest that methionine and threonine are the endogenously limiting amino acids in rats fed a nonprotein diet and the supplements of methionine and threonine to the nonprotein diet containing PCB stimulated the elevation of plasma cholesterol, just like the protein diet.

This study was supported in part by a grant from the Elizabeth Arnold Fuji Foundation.

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