EFFECT OF ACTIVATED CARBON BEADS ON SERUM LIPID LEVELS AND FECAL BILE ACID EXCRETION IN RATS

YOSHITERU HONDA,* MASAHIRO NAKANO* AND NAOMI I. NAKANO**

Department of Pharmacy, Kumamoto University Hospital,* 1-1-1 Honjo, Kumamoto 860, Japan and Ginkyo College of Medical Science,** 819 Ohkubo, Shimizu-machi, Kumamoto 860, Japan

(Received April 2, 1987)

The effects of oral activated carbon beads on the total serum cholesterol and triglyceride levels and on the fecal bile acid excretion in rats fed a basal or high cholesterol diet containing 5% activated carbon beads for 6 weeks were investigated. The beads appeared to exert little effect on the growth rate of rats and no tendency towards constipation was also observed. Although treatment with activated carbon beads gave little influence on the total serum cholesterol and serum triglyceride levels in rats fed either a basal or high cholesterol diet, the beads did produce a significant rise in the total fecal bile acid excretion. The extra fecal bile acids resulted chiefly from an increase in lithocholic acid. Furthermore, the beads showed a tendency to inhibit absorption of dietary cholesterol from the gastrointestinal tract in rats fed a high cholesterol diet. These results suggested the possibility of activated carbon beads as a hypocholesterolemic agent through binding with bile acids in the intestine.

**Keywords** — activated carbon; activated carbon bead; activated charcoal; serum cholesterol; serum triglyceride; fecal bile acid excretion; bile acid composition; hypolipidemic agent; hypocholesterolemic agent; intestinal adsorbent

INTRODUCTION

Since activated carbon has long been known to have great adsorptive power and also to be relatively free from adverse effects when administered orally,1) the development of a palatable oral dosage form would find application in the wide fields of medicine as an intestinal adsorbent. Thus, in the past few years, we have been directing our efforts to the utilization of powdered activated carbon in the development of an easily swallowable formulation without a concomitant decrease in adsorbing capacity. Activated carbon beads prepared for trial in our laboratories are the palatable fine granules of agar in which activated carbon powder is entrapped2) and are free from many handling problems associated with the fine black powder. Their in vitro and in vivo adsorption characteristics have been evaluated in various drugs.3) As the results, the adsorption capacity of the activated carbon powder was found to be essentially retained in the beads. Moreover, these beads were demonstrated to inhibit effectively the adsorption of two neutral drugs, theophylline and phenytoin, one basic drug, quinidine,4) and one acidic drug, nalidixic acid,5) from the gastrointestinal tract in human subjects, and thus were suggested to be a promising candidate as a gastrointestinal adsorbent for the early treatment of acute drug intoxication.

In addition, we have previously compared the bile salt adsorption characteristics of the beads with those of cholestyramine, a quaternary ammonium anion exchange resin, in vitro4) from the standpoint of possible usefulness in the treatment of hypercholesterolemia,5) in the relief of pruritus,6) etc., where adsorption of bile acids in the intestinal tract is desired. These beads have been found to be capable of adsorbing bile salts in a sufficient quantity to exert a hypocholesterolemic effect. Based on these findings, the present study was intended to examine whether activated carbon beads can stimulate the fecal excretion of bile acids and subsequently reduce the serum lipid levels by a mechanism similar to cholestyramine, using rats.

MATERIALS AND METHODS

**Materials** — Activated carbon powder of Japanese Pharmacopeial grade was obtained from Inuhinode Pharmaceutical Co., Osaka, Japan, and activated carbon beads, 2/3 parts of which consist of activated carbon, were prepared as reported previously2) by drying spherical beads in which activated carbon powder was dispersed. Cholesterol E-Test Wako, Triglyceride G-Test Wako and Total Bile Acids-Test Wako for serum lipids, fecal cholesterol and fecal total
bile acids measurements were purchased from Wako Pure Chemical Industries, Osaka, Japan. Three % QF-1 on Chromosorb WAW DMCS (80—100 mesh) for gas chromatographic measurement of the individual bile acids in the feces was also purchased from Gasukuro Kogyo Inc., Tokyo, Japan. The basal diet was purchased from Clea Japan Inc., Tokyo, Japan. All other chemicals used were of the highest purity commercially available.

Animal Experiments — Wistar strain male rats (Kuroda Co., Kumamoto, Japan), weighing 204—220 g, were used. Rats were housed in an individual cage with a stainless steel net floor which allowed collection of feces for 6 weeks. Sixteen rats were randomly divided into 4 equal groups (groups A—D), which consist of 4 rats. Group A was fed the basal diet and group B was fed the basal diet supplemented with 5% of activated carbon beads. Group C was given a high (2%) cholesterol diet and group D received the high cholesterol diet supplemented with 5% of activated carbon beads. Diet and water continued ad libitum throughout the study. Body weight was recorded every week and blood samples (200 μl) were collected from a tail vein at weekly intervals for 6 weeks. The blood was centrifuged immediately after collection and the sera were preserved at −40 °C until analysis. In addition, the feces were collected weekly from each rat and the daily amounts of feces excreted were calculated from their weights.

Fecal bile acids and cholesterol were extracted according to the procedure of Grundy et al.7) Feces were disintegrated in water and subjected to mild saponification with 1 N NaOH in 90% (v/v) ethanol for 2 h. After cooling, fecal cholesterol was extracted with petroleum ether. To the remaining aqueous phase was added 10 N NaOH and the mixture was rigorously saponified for 4 h at 120 °C in an autoclave. The saponification mixture was acidified to pH 2—3 with concentrated HCl and subsequently, fecal bile acids were extracted with chloroform-methanol (2:1). Then, total fecal bile acids and cholesterol were determined enzymatically by means of Total Bile Acids-Test Wako and Cholesterol E-Test Wako respectively. The individual bile acids were determined by the gas chromatographic method of Imai et al.8) The evaporated residue of final extracts were allowed to react with hexafluoroisopropanol and trifluoroacetic anhydride at 37 °C for 30 min. The resulting derivatives were gas chromatographed on QF-1 as the stationary phase. A gas chromatograph (Hitachi 063, Tokyo, Japan) equipped with a flame ionization detector was used. The operating conditions were as follows: injection port temperature, 240 °C; column temperature, 220 °C; detector temperature, 240 °C; carrier gas, nitrogen; 45 ml/min. 5β-Cholanic acid was used as an internal standard for the correction of losses during the extraction procedure.

RESULTS

Body Weight and Daily Amounts of Feces

Figure 1 shows body weight gain for 6 weeks. The activated carbon bead treatment did not show any significant difference in body weights between groups A and B, and also between groups C and D throughout the study. Therefore, activated carbon beads appear to exert little effect on growth rate of rats.

Figure 2 presents effects of activated carbon beads on the daily amounts of feces excreted. Although the only possible side effect of activated carbon is a constipation, no tendency towards constipation was noted when activated carbon was taken in a form of beads. Conversely, administration of the beads produced an increase in the

![Graph](image-url)

**FIG. 1. Effect of Activated Carbon Beads on Body Weight Gain in Rats**

Each point represents the mean of 4 rats.

Key: ○ A (basal diet); — B (basal diet + activated carbon beads);

△ C (high cholesterol diet); — ▲ D (high cholesterol diet + activated carbon beads).
amounts of feces by 1–2 g per day in groups B and D comparing with groups A and C, respectively. These increases, however, were mainly due to the fecal excretion of activated carbon powder, which in itself was not absorbed from the gastrointestinal tract, in each diet containing activated carbon beads. Consequently, the beads also do not seem to produce any change in the excretion of feces.

**Total Serum Cholesterol and Triglyceride**

The feeding of activated carbon beads gave little effect on total serum cholesterol and triglyceride levels in rats fed a basal diet during the test periods (Fig. 3). The beads showed a slight tendency to lower the total serum cholesterol after the second week, but no definite lowering action was observed. In rats fed a high cholesterol diet, no cholesterol elevation occurred contrary to expectation. Consequently, this study failed to confirm an effectiveness of the beads in the treatment of the hypercholesterolemic rats.

**Fecal Bile Acid Excretion**

Figure 4 shows the influence of activated carbon beads on fecal bile acid excretion. Total

---

**FIG. 2. Effect of Activated Carbon Beads on the Amount of Feces Excreted in Rats**

Each point represents the mean ± S.E. of 4 rats.

Key: The same as in Fig. 1.

**FIG. 3. Effect of Activated Carbon Beads on Total Serum Cholesterol (A) and Serum Triglyceride (B) Levels in Rats**

Each point represents the mean ± S.E. of 4 rats.

Key: The same as in Fig. 1.

**FIG. 4. Effect of Activated Carbon Beads on the Total Fecal Excretion of Bile Acids in Rats**

Each point represents the mean ± S.E. of 4 rats.

Key: The same as in Fig. 1.

a) significantly different from group A, p < 0.01.
b) significantly different from group A, p < 0.05.
c) significantly different from group C, p < 0.01.
d) significantly different from group C, p < 0.05.
FIG. 5. **Effect of Activated Carbon Beads on the Individual Bile Acids in the Feces in Rats**

Results are the mean of 4 rats.

Key: A, group A (basal diet); B, group B (basal diet + activated carbon beads); C, group C (high cholesterol diet); D, group D (high cholesterol diet + activated carbon beads). a—d): The same as in Fig. 4.

Fecal bile acid output was increased on the average 1.71-fold (1.56-, 1.41-, 1.82-, 1.79- and 1.96-fold at the 2nd, 3rd, 4th, 5th and 6th week, respectively) by addition of activated carbon beads to the basal diet groups. On the other hand, in the high cholesterol diet groups, the average magnitude of their increases by the beads was 1.54-fold (1.45-, 1.78-, 1.49-, 1.34- and 1.63-fold at the 2nd, 3rd, 4th, 5th and 6th week, respectively). Accordingly, it was concluded that the bead feeding resulted in an about 1.3−2.0 fold stimulation of total fecal bile acid excretion. Especially, as is shown in Fig. 5, the fecal excretion of lithocholic acid significantly increased with respect to the composition (1.60- and 1.87-fold on the average in the basal and high cholesterol diet groups, respectively) as well as the amounts excreted (2.85- and 2.94-fold on the average) by the beads, and thus the increases in the lithocholic acid portion were more marked than was the increase in total excretion. Deoxycholic acid also showed a tendency to increase quantitatively. Furthermore, although dietary cholesterol caused a significant increase in cholic acid excretion in rats fed a high cholesterol diet, treatment with activated carbon beads led to a normal level.

**Fecal Excretion of Cholesterol**

The amount of cholesterol excreted in the feces was not influenced by feeding with activated carbon beads in the basal diet groups (Fig. 6). On the other hand, in the high cholesterol diet groups, the amount of cholesterol excreted increased more than 10-fold comparing with the

---

FIG. 6. **Effect of Activated Carbon Beads on the Fecal Excretion of Cholesterol in Rats**

Each point represents the mean ± S.E. of 4 rats

Key: The same as in Fig. 1.

a) significantly different from group C, p < 0.02.
basal diet group and was further promoted by the addition of the beads into the diet; i.e., the beads tended to cause an increase in the fecal excretion of cholesterol to a certain extent.

DISCUSSION

The hypercholesterolemic state has received considerable clinical attention in connection with atherogenesis in recent years. A hypolipidemic effect of activated carbon was reported by Friedman et al. in patients with renal insufficiency and in uremic and diabetic rats. Although little is known about the mechanism by which this effect is accomplished, it seems to be roughly similar to that of cholestyramine. That is to say, oral administration of activated carbon increases fecal bile acid excretion. The loss of bile acids by the interruption of normal enterohepatic circulation elicits a compensatory increase in cholesterol catabolism and results in a subsequent decrease in serum cholesterol levels in which the rate of cholesterol catabolism appears to exceed its rate of biosynthesis.

In this experiment, the total serum cholesterol levels were not affected by treatment with activated carbon beads even though fecal bile acid excretion was increased up to 1.3—2.0-fold. This may account for the ability of the rat to compensate for increased cholesterol catabolism via bile acid loss. The similar effects, the lack of effect on serum cholesterol levels, were reported with cholestyramine treatment. According to Gallo et al., feeding cholestyramine to rats did not lower blood cholesterol levels as it did in other species, notably the chicken, dog and man. This species difference is mainly ascribed to a greater ability of rats to synthesize hepatic cholesterol. By the way, inclusion of cholesterol in the diet, contrary to expectation, could not render the rat hypercholesterolemic in this study. The reason is probably that the hepatic de novo cholesterol synthesis would be markedly reduced due to the dietary sterol thus inhibiting compensation. Also, our data obtained from the determination of individual bile acids showed that the bile acid spectrum of the cholesterol-feeding rat differed from that of the control rat in only one way: the cholic acid concentration was much higher. Cholic acid, therefore, should be formed from the dietary cholesterol to a greater extent than chenodeoxycholic acid and thus, if the synthesis of cholic acid is inhibited by the addition of cholic acid with cholesterol to the diet, the hypercholesterolemic state may result.

In the present study, it was demonstrated that activated carbon beads did produce a significant rise in total fecal bile acid excretion. In particular, the increase of fecal excretion in lithocholic acid was notable. There are at least two reasons for this. First, since activated carbon favors adsorption of hydrophobic molecules, lithocholic acid, which is a monohydroxy bile acid, is more effectively adsorbed than the di- and trihydroxy analogs. Second, since lithocholic acid is usually hard to be reabsorbed from the intestine, it tends to remain in the intestine and to have more chance to be adsorbed by activated carbon. On the other hand, the amount of deoxycholic acid in the feces also exhibited a tendency to increase. Consequently, these findings suggest that the beads may chiefly affect on the fecal excretion of secondary bile acids and thus, a depressed enterohepatic circulation of the bile acid may be accompanied by metabolism by the intestinal micro-flora. As a matter of fact, as shown in Fig. 5, treatment with the beads brought about the small decreases in the fecal excretion of primary bile acids; namely cholic acid and chenodeoxycholic acid.

In regard with the fecal excretion of cholesterol, the endogenous cholesterol was not influenced by treatment with activated carbon beads but the beads had a tendency to impair the absorption of exogenous dietary cholesterol. It seems likely that an increase in fecal cholesterol excretion in rats fed a high cholesterol diet supplemented with the beads resulted solely from a direct adsorption of dietary cholesterol onto the beads and is almost not associated with a diminished concentration of bile acids in the intestine due to the binding of those. Therefore, the inhibitory effect by the beads on cholesterol absorption is moderate, and thus a more extensive malabsorption of cholesterol seems less likely by treatment with the beads.

The results obtained in this study verified that activated carbon beads had an accelerating effect on the fecal excretion of bile acids, while its capacity was somewhat smaller than that of cholestyramine. The beads, however, have some definite advantages as described previously. First, the beads show a greater adsorption capacity for trihydroxy bile acids than the resin
preparation and second, the encapsulation of the powder by agar apparently permits selective adsorption in the presence of the lipid. In addition, the side-effects such as constipation, intestinal obstruction and hypercholesteremic acidosis associated with cholestyramine therapy are unlikely to occur with the agar-entrapped activated carbon. It is aware that activated carbon beads will bind a broad range of organic and inorganic compounds and that the daily ingestion of activated carbon may alter the absorption of essential nutrients. However, since the rats dosed with the beads gained body weight normally and produced normal stools during the present test periods, the beads do not seem to disturb seriously the absorption of essential nutrients such as fat soluble vitamins. Also, the beads may be effective in preventing constipation because of a laxative action of agar.

In conclusion, the principal findings of our study are that: (1) activated carbon beads interrupted the enterohepatic circulation of bile acid; (2) the beads also impaired the absorption of dietary cholesterol from the intestinal tract; however (3) the beads had little effect on the total serum cholesterol and triglyceride levels in normal rats. These and previous findings suggest that activated carbon beads may be a safe, promising and useful therapeutic agent in the treatment and prevention of atherosclerosis. Recently Kuusisto et al. reported that activated carbon reduced plasma levels of total and LDL-cholesterol by 25% and 41%, respectively, in patients with hypercholesterolemia resistant to therapy with fibrin acid derivatives or nicotinic acid, and that its effects on HDL-cholesterol (a slight rise) and triglycerides (no change) were opposite to those usually reported for cholestyramine. Although further studies are required to assess more precisely the role of these beads as a lipid-modulating agent, a palatable preparation of carbon powder such as the beads reported here may serve as an alternative to cholestyramine therapy. Particularly, in the management of some azotemic diabetic and nephrotic hyperlipidemia and primary hypercholesterolemia resistant to conventional therapy, the beads may find applicability and play an important role.

REFERENCES