EFFECT OF CATECHIN ON INTESTINAL LIPID METABOLISM

A. K. VALSA, S. K. ASHA AND N. R. VIJAYALAKSHMI*

Department of Biochemistry,
University of Kerala, Kariavattom,
Trivandrum – 695 581

(Received on August 28, 1997)

Abstract: On analysing the effect of catechin on intestinal lipid metabolism, an increase in the concentration of cholesterol in the duodenum and jejunum was observed along with an increase in the HMGCoA reductase activity. In the in vitro experiments also it was found that cholesterol and free fatty acid (FFA) levels were increased in these two regions. Binding of catechin with cholesterol in the lumen, reduces the availability of cholesterol for absorption which may in turn stimulate cholesterol biosynthesis and a rise in the HMGCoA reductase activity. These alterations produced by catechin may also be related to the degradation of cholesterol to bile acids, as endogenous cholesterol is the preferred substrate for bile acid synthesis.

Key words: HMG COA activity cholesterol intestinal lipids catechin

INTRODUCTION

Hyperlipidemia is a potent risk factor in the development of cardiovascular diseases. Liver, adipose tissue and small intestine are the major contributors of lipids and lipoproteins in the circulation., (1, 2) For many years, it was considered that liver is the main organ which maintained the homeostasis of blood cholesterol (3). Now it has been shown that small intestine synthesizes even higher amounts of cholesterol than the liver (4).

The hypocholesterolemic effect of tannins has been demonstrated by several workers (5–11). Catechin, a monomeric form of condensed tannin, also called flavan–3–ol, has been reported to reduce micellar solubility and intestinal absorption of cholesterol in rats (12). A concentration-response related study carried out in our laboratory showed that the hypocholesteremic activity of catechin was maximum at a dose of 10 mg/kg, BW/day, and that the hypolipidemic activity was due to the higher rate of degradation and excretion (13). We studied the effect of catechin at a dose of 10 mg/kg BW/day on the intestinal lipid metabolism.

METHODS

Male rats of the Sprague-Dawley strain weighing 100–120 g, were divided into two groups comprising 6 rats in each group. Animals of group I were controls of group II. The rats were fed normal laboratory diet
and water ad-libitum. Animals of group II received catechin at a dose of 10 mg/kg BW/day by gastric intubation. At the end of 60 days, rats were deprived of food overnight, and sacrificed. Small pieces (2–3 cm in length) from the three regions of the intestine, namely duodenum, jejunum and ileum, were removed to ice cold containers, extracted with appropriate solvents/buffers for the various estimations. Lipids were extracted from the tissues by the method of Radin (14). Cholesterol was estimated by the method of Abell et al. (15), triglycerides by the method of Van Handel and Zilversmit (16) with the modification that florisil was used to remove phospholipids, phospholipids by the method of Stewart (17). Activity of HMGCoA reductase (EC 1.1.1.34) was estimated by the method described earlier (18).

In vitro synthesis of lipids from $^{14}$C glucose

Approximately 5-7 cm of duodenum, jejunum and ileum were removed, the lumen was washed with the cold oxygenated Krebs Ringer Bicarbonate buffer (KRB). The entire length was bent in the form of 'U' and 7.5 ml of KRB containing 7.5 uCi of $^{14}$C glucose was injected in to the lumen. Incorporation of $^{14}$C was detected by counting the activity in a scintillation counter after extraction (19) and separation of lipids by TLC (Silica gel G, solvent system Hexane: ether: acetic acid in the ratio 80:20:1). Statistical significance was calculated using student's 't' test (20).

RESULTS

Concentrations of cholesterol, triglycerides and Phospholipids (Table I): Concentration of cholesterol was significantly increased in the duodenum and jejunum of rats administered catechin. In the ileum, there was no significant change. The triglyceride concentration was increased in the three regions of the intestine of experimental animals receiving catechin when compared to the respective regions in the normal group. The concentration of phospholipids was significantly reduced in

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters analysed</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Cholesterol</td>
<td>191.4 ± 4.7</td>
<td>178.3 ± 4.4</td>
<td>221.0 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>239.9 ± 6.0*</td>
<td>214.1 ± 5.4*</td>
<td>227.0 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>401.1 ± 10.0</td>
<td>264.7 ± 6.6</td>
<td>215.3 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>787.7 ± 19.7*</td>
<td>449.4 ± 11.2*</td>
<td>406.0 ± 10.1*</td>
</tr>
<tr>
<td></td>
<td>Phospholipids</td>
<td>1276.4 ± 31.9</td>
<td>2041.0 ± 51.0</td>
<td>1778.9 ± 44.47</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>916.9 ± 22.9*</td>
<td>1766.6 ± 44.1</td>
<td>1537.8 ± 38.4</td>
</tr>
</tbody>
</table>

Average of the values of 6 rats in each group ± SE.
Group II (experimental) is compared with Group I (control) a = P <0.01.
TABLE II: *In vitro* Synthesis of lipids from $^{14}$C glucose.
(Values expressed as counts/mg tissue).

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Free fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>$2498 \pm 49.9$</td>
<td>$692 \pm 13.8$</td>
<td>$19705 \pm 433.5$</td>
<td>$250 \pm 5.0$</td>
</tr>
<tr>
<td>II</td>
<td>$5064 \pm 101.3^a$</td>
<td>$2109 \pm 42.2^a$</td>
<td>$21807 \pm 479.7$</td>
<td>$673 \pm 13.5$</td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>$3721 \pm 81.8$</td>
<td>$2596 \pm 62.3$</td>
<td>$18162 \pm 363.2$</td>
<td>$508 \pm 13.7$</td>
</tr>
<tr>
<td>II</td>
<td>$4681 \pm 102.9^a$</td>
<td>$3213 \pm 77.1^a$</td>
<td>$22634 \pm 452.7^a$</td>
<td>$1128 \pm 30.5^a$</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>$5542 \pm 149.6$</td>
<td>$3727 \pm 100.6$</td>
<td>$58485 \pm 1754.6$</td>
<td>$502 \pm 11.5$</td>
</tr>
<tr>
<td>II</td>
<td>$6974 \pm 163.9$</td>
<td>$4324 \pm 116.7^a$</td>
<td>$27565 \pm 826.9^a$</td>
<td>$1067 \pm 24.5^a$</td>
</tr>
</tbody>
</table>

Average of the values of 6 rats in each group ± SE.
Group II is compared with Group I

- $a = P < 0.01$,
- $b = 0.01 < P < 0.05$.

TABLE III: Activity of HMGCoA Reductase (Activity expressed as the ratio of HMG CoA to Mevalonate$^a$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$2.7 \pm 0.09$</td>
<td>$1.9 \pm 0.04$</td>
<td>$1.5 \pm 0.04$</td>
</tr>
<tr>
<td>II</td>
<td>$1.3 \pm 0.03^a$</td>
<td>$1.5 \pm 0.04$</td>
<td>$1.3 \pm 0.03$</td>
</tr>
</tbody>
</table>

Average of the values of 6 rats in each group ± SE.
Group II is compared with group I.

- $a - p < 0.01$,
- $^a$Smaller ratio indicates higher activity.

Activity of HMGCoA reductase
(Table III): Activity of HMGCoA reductase, the rate limiting enzyme of cholesterol biosynthesis was found to be increased in duodenum and jejunum of experimental animals. In the ileum, there was no significant change in the activity of this enzyme when compared to control animals.

DISCUSSION

The above results clearly indicate that intestinal lipid metabolism is deranged by catechin administration. In humans, *de novo* synthesis appears to contribute two or three times more cholesterol to the body pool than does the absorption of dietary cholesterol.
Mucosa of the gastrointestinal tract is responsible for the cholesterol absorption and is an active site of choledrogenesis (22). In the present study, cholesterol levels were increased in the duodenum and jejunum of rats administered catechin compared to their pair fed controls. Activity of HMGCoA reductase was also higher in these regions than the control group. In the gut mucosa, as in other tissues, the enzyme 3-hydroxy-3 methylglutaryl coenzyme A reductase is the rate determining step in cholesterol synthetic rate (23, 24). Significant activity of HMGCoA reductase is present throughout the human gut (25). Intestinal cholesterol synthesis can be regulated by luminal factors such as, cholesterol and bile salts and may also be subject to feed back regulation by circulating LDL cholesterol (26, 29). The increase in cholesterol levels in the duodenum and jejunum may be due to the increase in activity of HMGCoA reductase. Small intestinal cholesterol synthesis is regulated by the flux of bile acids through the mucosa (30). Dietschy emphasized the profound stimulatory effect that diversion of bile acids had on intestinal choledrogenesis (27). There was an increase in free fatty acid and triglyceride levels upon catechin administration. During absorption of long chain fatty acids, apoprotein and cholesterol are contributed by the mucosal cells for chylomicron formation (26). Recently, it has been reported that some of the flavonoids bind glycine and taurine conjugates of bile salts, cholate, chenodeoxycholate and deoxycholate and free forms of cholate (31). Catechin may bind cholesterol in the lumen of duodenum and jejunum whereby exogenous cholesterol becomes low, so that cholesterol synthesis might have been necessary to meet the demands. Earlier studies revealed that fatty acids of the C-18 series stimulated intestinal HMGCoA reductase (26). This finding is consistent with the hypothesis that the cholesterol requirement for packaging and transport of fatty acids was the mechanism producing rise in the reductase activity (26). Venugopala Rao and Ramakrishnan (4) reported an increase in the rate of cholesterol synthesis in the middle segment (jejunum) of the intestine than the first and third segments. Though catechin enhances lipid synthesis, the overall effect of catechin at this concentration is to lower lipid levels in serum and tissues and the lipid lowering action is mainly attributable to decreased absorption, a higher rate of degradation and elimination of lipids. (13).

The following conclusions have been made on analysing the results of the above experiment. Catechin was shown to exert a stimulatory effect on the synthesis of cholesterol and triglycerides in various regions of the intestine. Cholesterol synthesis was significantly increased in the duodenum and jejunum as evident from the higher activity of HMGCoA reductase. Higher incorporation of 14C glucose in the cholesterol, triglyceride and fatty acid fractions provide ample evidence for the higher rate of synthesis of these lipid components in the intestine, although the net effect of catechin is to alleviate dyslipidemia.

REFERENCES


