ASPIRIN HYPOCHOLESTEROLEMIA ASSOCIATED WITH INCREASED MICROSMAL COPPER IN LIVER

Leslie M. Klevay, M.D., S.D. in Hyg., United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota 58202

ABSTRACT

Aspirin was fed to rats (2.4 g/kg of diet) in a diet deficient in copper because of an hypothesis linking the etiology of ischemic heart disease to copper metabolism and because of the apparently protective effect of aspirin in this disease. Cholesterol was measured by fluorometry and copper was measured by atomic absorption spectrometry. Aspirin decreased cholesterol in plasma approximately 18% (p<0.003) in each of two experiments. Copper in liver microsomes was doubled by aspirin (p<0.002). The effects of aspirin were compared to those of clofibrate in a similar experiment (Drug-Nutrient Interactions 2:131,1983) and to other chemicals that alter both cholesterol metabolism and copper metabolism. Effects on copper may be central to the action of cholesterotropic agents.

KEY WORDS: aspirin, copper, copper deficiency, hypcholesterolemia, cholesterotropic chemiosis, cuprotropic chemicals

INTRODUCTION

Hypercholesterolemia is predictive (1) of risk of ischemic heart disease, the leading cause of death in the United States (2). The etiology of this disease is unknown; many characteristics of the environment have been implicated. A new hypothesis on the etiology of ischemic heart disease has been in development (3-11). It is hypothesized that "a metabolic imbalance in regard to zinc and copper is a major factor" in the etiology of ischemic heart disease. This imbalance, either a "relative or absolute deficiency of copper characterized by a high ratio of zinc to copper," results in hypercholesterolemia, damage to hearts and arteries, and increased mortality. The hypothesis, called the zinco/copper hypothesis, is based on experiments with animals, epidemiologic observations, iatrogenic maneuvers, and experiments of nature. Recently, emphasis has been placed on the several similarities between people with ischemic heart disease and animals deficient in copper (7,10,11) the most important being hypercholesterolemia (3),
abnormalities of the electrocardiogram (12), hyperuricemia (13), and glucose intolerance (14). The realization (11,15) that only about 25% of daily diets in the United States contain the 2mg of copper generally thought to be required by adults (16) has been useful in the development of this hypothesis.

Aspirin, acetylsalicylic acid, is one of many salicylates in medical use. In general, these compounds are easily hydrolyzed to salicylic acid in vivo, and it is from this parent compound that therapeutic benefits are obtained (17). Salicylic acid is well known as a chelating agent (18) capable of binding cations of transition and other elements.

Regular consumption of aspirin often is associated with lower risk of ischemic heart disease (19-25). Sometimes apparent benefits of aspirin consumption were minimal (23,24). Some studies have shown no benefit (26,27).

The dose of aspirin presumably was high in the early epidemiologic studies because patients with rheumatoid arthritis were included (19,21,23). More recent therapeutic trials were done with the lower doses presumed adequate in minimizing thrombosis (20,22,24,25).

The realization that aspirin interacts with transition elements and has been implicated in the epidemiology of ischemic heart disease prompted the idea that aspirin may affect both copper and cholesterol metabolism as has been found for clofibrate (28). With the discovery (28) that as clofibrate lowers cholesterol in plasma, copper increases in liver, it was suggested that a new class of chemicals --- those both cholesterolotropic and cuprotropic --- had been identified. Nearly a dozen of these widely diverse chemicals is known (29). Some of these chemicals, such as clofibrate, induce hypocholesterolemia and enhance copper metabolism; others, such as histidine, produce hypercholesterolemia and inhibit copper metabolism. The effects on copper metabolism are variable (29) and include decreased intestinal absorption and increased concentrations in organs.

This experiment was designed to determine whether or not aspirin is cholesterolotropic and cuprotropic. The hypotheses that (a) aspirin can lower the concentration of cholesterol in plasma of rats fed a diet deficient in copper, and (b) that the hypocholesterolemic effect was mediated by an enhancement of hepatic copper were tested.

On the assumption, based on review of more than two dozen references (11), that many people eat approximately 1mg of copper per day and that an effective dose of aspirin is 3.2g/day, the dose of aspirin selected for testing was 1150 times the amount of dietary copper on a molar basis.
METHODS

Weanling male rats obtained from Harlan Sprague-Dawley (Madison, WI) were matched into two groups of 15 differing in mean weight by approximately 0.1g. They were fed a diet deficient in copper based on sucrose (62% by weight), egg white (20%), and corn oil (10%). The diet has been in use for over a decade (3); it rapidly produces copper deficiency in rats by several criteria. The only modifications have been an increase in the amount of biotin (30) to 2.00 mg/kg and a change from cane sugar to beet sugar (American Crystal Sugar Co., Moorhead, MN). As the diet is also deficient in zinc, all rats were given a drinking solution containing 10 µg Zn/ml (as acetate) (3). The diet contains all other nutrients known to be essential for rats. Animals were maintained under conditions similar to those described (31).

Copper was measured in an aliquot of control diet for each of two experiments. After the dose of aspirin was calculated based on the analytical results, the experimental diets (containing aspirin) were prepared using dietary components obtained from the same containers as were used in preparation of the control diets. The amounts of copper and the amounts of aspirin are shown in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Experiment One</th>
<th>Experiment Two</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (mg)</td>
<td>0.870</td>
<td>0.630</td>
</tr>
<tr>
<td>Aspirin^a (g)</td>
<td>2.84</td>
<td>2.05</td>
</tr>
</tbody>
</table>

*All diets were deficient in copper. In each experiment half of the animals were fed the diet with aspirin and half, without it.

Aspirin was obtained from a local pharmacy. Tablets were ground in a mortar shortly before being mixed with the other dietary components. Weighing of several tablets revealed that they were only 79% aspirin; this finding was considered when aspirin was weighed for dietary addition.

Aspirin and plasma were collected under pentobarbital sodium anesthesia. Hepatic microsomes were prepared according to a standard method (32,33) protein in microsomes was measured by the biuret method (34). Copper and zinc were measured by atomic absorption spectrometry (35). Organic matter in diet, heart, liver, and liver microsomes was destroyed with nitric and sulfuric acids augmented with hydrogen peroxide (36). Copper and zinc were measured in plasma following dilution with distilled, deionized water. Statistical comparisons of results were done using
Student's t test and linear regression (37). Blood was collected from the tail vein for measurement of cholesterol by fluorometry (38).

RESULTS

In the first experiment, three rats fed aspirin had ventricular aneurysms. One rat was found dead with evidence of excessive blood loss after blood was collected for cholesterol measurement. No cardiovascular pathology was found in the second experiment.

The concentration of cholesterol in plasma was lower in each experiment after 35 and 32 days of dietary exposure, respectively, if aspirin was consumed (Table 2). The results of the second experiment confirmed those of the first.

<table>
<thead>
<tr>
<th></th>
<th>Experiment One</th>
<th></th>
<th>Experiment Two</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
<td>Copper</td>
<td>Zinc</td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td>(mg/dl)</td>
<td>(μg/dl)</td>
<td>(μg/dl)</td>
<td>(mg/dl)</td>
</tr>
<tr>
<td>No aspirin^a</td>
<td>132±5.0</td>
<td>3.9±0.38</td>
<td>96±4.7</td>
<td>118±4.1</td>
</tr>
<tr>
<td>Aspirin</td>
<td>106±4.2</td>
<td>2.8±0.06</td>
<td>140±13.9</td>
<td>98±3.6</td>
</tr>
<tr>
<td>N</td>
<td>15,15</td>
<td>15,14</td>
<td>15,14</td>
<td>15,15</td>
</tr>
<tr>
<td>p</td>
<td>0.0012</td>
<td>0.020</td>
<td>0.010</td>
<td>0.0027</td>
</tr>
</tbody>
</table>

^aMean ± SE
^bNumber of samples in the groups without and with aspirin, respectively.

The hematocrit of rats fed aspirin was decreased (31 vs 42%, p=0.0019) in the first experiment. In the second experiment, hematocrits were similar (44 vs 42%, p>0.05). Mean weight of rats fed aspirin was increased 24% at kill in the first experiment and decreased 19% in the second experiment.

The concentration of copper in plasma (Table 2) was significantly lower in rats fed aspirin in the first experiment. The apparent increase due to aspirin in the second experiment was insignificant (p>0.10). Plasma zinc increased in both experiments, but statistical significance was attained only in the first experiment. Liver zinc (Table 3) was slightly higher when aspirin was consumed, but statistical significance was attained only in experiment one (p=0.0003). Liver copper values in both groups in experiment one were similar; the increase in liver copper in experiment two was insignificant (p>0.05). Consumption of aspirin did not affect copper or zinc in heart.
TABLE 3
Organ Analyses (µg/g) Dry Weight*

<table>
<thead>
<tr>
<th></th>
<th>Copper</th>
<th>Zinc</th>
<th>Copper</th>
<th>Zinc</th>
<th>Copper</th>
<th>Zinc</th>
<th>Copper</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>No aspirin</td>
<td>1.30±0.09</td>
<td>33.8±0.63</td>
<td>1.84±0.43</td>
<td>13.1±2.89</td>
<td>2.10±0.19</td>
<td>69.1±2.30</td>
<td>8.3±0.67</td>
<td>77.7±2.14</td>
</tr>
<tr>
<td>Aspirin</td>
<td>1.32±0.11</td>
<td>40.7±1.22</td>
<td>1.74±0.22</td>
<td>23.7±4.15</td>
<td>2.80±0.33</td>
<td>76.5±2.65</td>
<td>10.2±0.85</td>
<td>82.1±4.58</td>
</tr>
<tr>
<td>N</td>
<td>14,13</td>
<td>15,14</td>
<td>15,15</td>
<td>15,15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.0003</td>
<td>0.84</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
<td>&lt;0.09</td>
<td>&lt;0.06</td>
<td>&lt;0.10</td>
<td>&lt;0.3</td>
</tr>
</tbody>
</table>

*See footnotes to Table 2.
Copper in liver microsomes was measured only in experiment two. Consumption of aspirin produced a significant increase (ng Cu/mg protein): 3.80 (0.44, S.E.) vs. 1.80 (0.19) (p<0.002).

DISCUSSION

The rats in these experiments were deficient by several criteria. Three ventricular aneurysms were found. Similar aneurysms often are found in copper deficiency (39). Only a minority of rats deficient in copper will have ventricular aneurysms even in a lengthy experiment (39). Hypoopuremia has been found frequently in deficiency; the highest mean values for plasma copper (Table 2) were approximately 3% of normal (35). Similarly, the highest mean value for liver copper (Table 3) is indicative of copper depletion (35).

The hypercholesterolemia of copper deficiency (35) has been found in at least seven independent laboratories (for references see 40). Its mechanism remains obscure but seems associated with low liver copper. This phenomenon has been studied in several species including man (41).

The effect of aspirin on the concentration of cholesterol was similar in both experiments: a decrease of approximately 18%. The decrease was unaffected by body weight. The hypotheses were tested successfully. This hypocholesterolemic action of aspirin apparently has escaped general notice (42), although Nakagawa, et al. (43) found similar results. They also found aspirin increased the activity of lecithin-cholesterol acyltransferase. This effect of aspirin on enzyme activity may have been mediated via copper, as enzyme activity is decreased in copper deficiency (44).

The effect of aspirin on copper metabolism was more subtle than the effect on cholesterol. The lack of effect of aspirin on liver copper in experiment one prompted study of liver microsomes in experiment two because of the inverse correlation between plasma cholesterol and copper in liver microsomes (45). Aspirin had an inconsistent effect on liver copper (small, insignificant increase in experiment two) but its consumption more than doubled copper in liver microsomes. In this sense, the coprotropic effect of aspirin seems more specific than that of clofibrate.

Clofibrate (28) had no significant effect on zinc in heart, liver, or plasma but did increase copper in both liver and plasma without an effect on cardiac copper. Clofibrate increased liver copper by more than 45%; its effect on liver microsomes was not studied.

The source of the increased copper in microsomes associated with the consumption of aspirin has not been identified. It probably did not come from heart; it may have come from the plasma pool or from some other liver compartment. Perhaps intestinal absorption of copper was increased.
The inverse correlation between plasma cholesterol and microsomal copper \( (r=-0.36, n=28, p<0.06) \) was similar to that of Jacob, et al. \(^{(45)}\), but the probability of chance was slightly greater. The inverse correlation in experiment two between plasma cholesterol and liver copper \( (r=-0.42, n=30, p<0.02) \) also has been found \(^{(28,35)}\).

As there was no difference in the color (melena) of the intestinal contents in either group in the experiments, the difference in hematocrits (experiment one) probably is not because of blood loss. It is more likely to be from some change in copper utilization induced by aspirin.

Roe \(^{(46)}\) reviewed nutrient and drug interactions, but no enhancement of nutriture by drugs was mentioned. The rats fed aspirin required less dietary copper to maintain a normal cholesterol concentration in plasma. This apparently beneficial effect of a drug on the utilization of a nutrient may be unique.

Copper may be unique in that copper nutriture can be enhanced by dietary chemicals. Several agents have been identified: calcium, clofibrate, guaran and sodium phytate (for references see 29). Although the chemical structures are diverse (Figure 1) --- a benzoic acid derivative, a complex carbohydrate, an ether, a phosphorylated cyclohexane, and a salt --- all have the similar biological effect of being hypocholesterolemic and enhancing copper metabolism. In contrast (legend, Figure 1), an amino acid, some other salts, simple carbohydrates, and steroids are hypercholesterolemic and inhibit copper metabolism. Perhaps effects on copper are central to the action of cholesterotropic agents.

**CHOLESTEROTROPIC AND CUPROTROPIC CHEMICALS**

![Chemical structures](image)

**FIG. 1**

Several other chemicals are hypercholesterolemic and are copper inhibiting: ascorbic acid, cadmium, cholesterol plus cholic acid, fructose, glucose, histidine, sucrose, and zinc.
Aspirin is the most frequently mentioned analgesic in office-based medical care; nearly one fourth of all analgesic agents used contain aspirin (47). Industrial capacity for aspirin production in the United States is 43 million pounds per year (48). At least 200 products sold in the United States contain aspirin (49,50). People have ample opportunity to consume aspirin; everyone in the United States (51) could have consumed 3.2g every day for 27 days if all of that produced here in a year were consumed here. This cornucopia can complicate clinical trials of antilipemic drugs, dietary trials, and epidemiologic studies of determinants of heart disease risk. Whether or not lower doses of aspirin are effective in lowering cholesterol is unknown.

Aspirin apparently enhanced the delivery of copper to a cellular locus with a beneficial effect on cholesterol metabolism. The arrival at that locus had the accuracy of Ehrlich's magic bullet (52,53). The mechanism by which this delivery occurred, the way in which copper and aspirin interacted, and the source of the copper, whether extrinsic (dietary) or intrinsic (from within the rat), remain obscure. Perhaps the copper was bound to a ligand which, although not beneficial itself, relieved a metabolic block or permitted a reaction to proceed. If aspirin transported and relinquished the copper, this action is different from that postulated by Sorensen (54) who suggests that some anti-inflammatory agents are active because they are converted to stable copper complexes in vivo. This action may be typical of the other chemicals that lower cholesterol and enhance copper metabolism.

The chemicals that increase cholesterolemia and inhibit copper metabolism (legend, Figure 1) such as histidine or cholesterol plus cholic acid probably remove copper from this cellular locus. This metabolic removal of copper may result in blocking an enzyme. As these chemicals are not toxic themselves, they become toxic because of the effect on copper. This effect is conceptually similar to the lethal synthesis of Peters (55).

**PROPRIETARY STATEMENT**

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

**REFERENCES**


Accepted for publication September 8, 1986.