Comparison of effects of cholecystokinin and erythromycin on bile chemistry and gallstone formation in aged guinea pigs

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Background: There has been considerable interest in gall bladder motility in recent years. We compared the effects of cholecystokinin (CCK) and erythromycin on bile chemistry and gallstone formation in aged guinea pigs. Methods: Two groups of guinea pigs (1-mo and 3-y old; n=40 each) were studied. Each group was divided into four subgroups of 10 animals each; one subgroup received lithium diuretics, one each received CCK or erythromycin daily in addition to lithium diuretics for 4 weeks, and one received normal diet. After 4 weeks, the presence of gallstones or sludge was recorded and bile composition including concentrations of bile acid, cholesterol, lecithin and protein concentrations was studied. Results: No gallstones were observed in the 1-mo-old animals. In the 3-year-old animals, 9 of 10 guinea pigs on lithogenic diet and 4 of 10 in each treatment subgroup and the normal diet subgroup developed gallstones. CCK and erythromycin had similar effects on bile chemistry and stone formation. Conclusions: Aging increases the formation of gallstones in guinea pigs. Erythromycin is as effective as CCK in reducing gallstone formation by improving gall bladder motility. [Indian J Gastroenterol 2002;21:4-6]

Key words: Gall bladder motility

Cholesterol gallstones, composed predominantly of cholesterol monohydrate crystals, are a result of alterations in lipid composition of gall bladder (GB) bile.1 At least three defects play a role in their causation: unphysiologic supersaturation of bile with cholesterol, accelerated nucleation, and GB hypomotility. Defective GB and intestinal motility may reduce enterohepatic cycling and hence hepatic secretion of bile salts, and lead to cholesterol supersaturation of bile. GB stasis could further contribute to stone formation. Xu et al2 found that a high-cholesterol diet significantly impaired GB motility and prolonged small intestinal transit in the Richardson ground squirrel model.

GB contraction and emptying are the net result of several stimulatory and inhibitory influences. Vagal cholinergic and cholecystokinin (CCK)-mediated hormonal mechanisms, particularly the latter, are the most important controls of GB emptying under physiological conditions.3,4 Poston et al showed that, in guinea pigs, the sensitivity of the GB to CCK decreases with age because of diminution in the number of CCK receptors.5 This may be a major factor in the increased incidence of gallstone formation in aged guinea pigs.6

Erythromycin, a macrolide antibiotic, has been reported to induce premature migrating motor complexes in humans and dogs when administered at low, microbiologically ineffective doses.7 Its action resembles that of motilin: erythromycin inhibits motilin binding in membrane by activating gastrointestinal motilin receptors.8,9 It also enhances GB motility and hastens intestinal transit, promoting more rapid enterohepatic cycling of bile salts in an animal model.2

We evaluated the effects of age, CCK-8 and erythromycin on bile chemistry, biliary sludge and gallstone formation in guinea pigs placed on a cholelithogenic diet for 4 weeks.

Methods

Male guinea pigs in two age groups, 40 one-month-old weanlings and 40 three-year-old adults (average body weight 180 g and 1300 g, respectively) (Gulhane Research Laboratories, Ankara, Turkey) were studied. None of them had gallstones or biliary sludge on pre-study ultrasound. All guinea pigs were housed in a temperature-regulated environment with 12-h light-dark cycle. Care was provided in accordance with the procedure outlined in the Guide and Use of Laboratory Animals.10

Animals in each group were divided into 4 subgroups of 10 animals each; one subgroup each received (a) regular guinea pig chow (control subgroup); (b) a high-cholesterol (7%), low-vitamin C (300 mg/Kg) cholelithogenic diet (ICN Biochemicals, Cleveland, Ohio, USA) with access to tap water ad libitum; (c) cholelithogenic diet and CCK-8 sulfate (Peninsula Laboratories, Belmont, CA, USA) in 0.1% bovine serum albumin (BSA) saline each day by intraperitoneal injection; (d) cholelithogenic diet and erythromycin stearate (2 mg/Kg/day) by nasogastric feeding tube.

After 4 weeks, the guinea pigs were sacrificed, and

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the GB were immediately excised intact. The GB were examined macroscopically for the presence of gallstones by an experienced physician, who was unaware of the animal age and treatment. GB bile was examined microscopically for the presence of cholesterol crystals. Bile was then frozen at -40°C for subsequent chemical analysis. Gallstones were considered to be present if gallstones, regardless of size and number, or biliary sludge was seen.

Bile cholesterol concentration was measured by the method of Alain et al11 using an enzymatic assay based on cholesterol esterase (Bio Analytics, Palm City, FL). Bile salt concentration was measured by the method of Turley and Ditschky12 using an enzymatic assay based on 3-α-hydroxy-steroid dehydrogenase (Wormorton Biochemical Corp, Freehold, NJ, USA). Bile phospholipid was measured by the method of Gomori13 and bile protein concentration by the method of Lowry.14

**Statistical analysis**

Inter-group comparisons were done by the one-sided Fisher's exact test and Student's t test, as appropriate, at the 0.05 level of significance.

**Results**

All animals tolerated the 4-week therapeutic interventions well. They all gained weight of average 10% during the study period. No gallstone or biliary sludge was found in any of the 40 weanlings. In the adult group, 4 of 10 guinea pigs receiving normal diet and 9 of 10 guinea pigs on lithogenic diet developed gallstones. The gallstones were mostly millimeter in size and multiple in number. The relative risk for developing gallstones in animals on lithogenic diet was 2.8 (95% CI 1.17-6.65). Fewer animals receiving lithogenic diet and CCK-8, and those receiving lithogenic diet and erythromycin stearate (4/10 each) developed gallstones as compared to adult guinea pigs receiving only lithogenic diet (p<0.05); there was no difference in the frequency of gallstone formation between CCK-8 and erythromycin treatment subgroups.

Animals on lithogenic diet in both the weanling and adult groups had significantly higher cholesterol concentrations as compared to the respective groups on normal diet (Table). Cholesterol concentration was significantly lower in the CCK and erythromycin treatment subgroups among weanlings, but not among adults; there was no difference between CCK and erythromycin treatment in both age groups.

Bile lecithin concentrations in the weanlings who received lithogenic diet were significantly lower than those in controls (p<0.01); weanlings treated with CCK or erythromycin had higher lecithin concentrations (CCK: p<0.01). Adult guinea pigs in the CCK and erythromycin treatment groups had higher lecithin concentrations than those receiving lithogenic diet alone; there was no difference between the effects of CCK and erythromycin in both age groups.

Bile protein concentrations in weanlings were similar in the four subgroups. In adult animals, protein concentration was significantly higher with lithogenic diet as compared to controls; in the CCK and erythromycin treatment subgroups, it was significantly lower as compared to that in the lithogenic diet group. There was no difference between the effects of each treatment.

Bile salt concentrations were significantly lower in the lithogenic diet subgroup in both age groups. They were similar in the CCK and erythromycin treatment groups. Bile salt-to-cholesterol concentration ratio was lower in the weanling and adult groups by 70% and 34%, respectively with lithogenic diet when compared to those on normal diet. In the CCK and erythromycin treatment groups, the ratio was higher by 84% and 75% as compared to those on lithogenic diet, respectively, in the weanling group.

**Discussion**

We have shown that in adult guinea pigs, gallstone formation increases with high-cholesterol diet. Single daily administration of CCK-8 or erythromycin reduced the frequency of gallstones in adult guinea pigs.

In weanlings, lithogenic diet was associated with significantly lower bile salt and lecithin and bile salt/bile cholesterol ratio, and higher bile cholesterol and protein concentration; however, we did not observe gallstones in this group. This finding could be attributable to intact GB contractility.

Table: Effects of diet, cholecystokinin and erythromycin on bile chemistry of guinea pigs

<table>
<thead>
<tr>
<th></th>
<th>Normal diet</th>
<th>HCD</th>
<th>HCD+CCK-8</th>
<th>HCD+E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weanling group</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bile acid (mmol/L)</td>
<td>45.9 (4.5)</td>
<td>20.8 (1.9)</td>
<td>20.9 (1.9)</td>
<td>20.2 (1.3)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.8 (0.1)</td>
<td>1.2 (0.0)</td>
<td>0.7 (0.1)</td>
<td>0.7 (0.1)</td>
</tr>
<tr>
<td>Lecithin (mmol/L)</td>
<td>2.4 (0.4)</td>
<td>0.6 (0.3)</td>
<td>1.4 (0.2)</td>
<td>1.5 (0.2)</td>
</tr>
<tr>
<td>Protein (mg/mL)</td>
<td>2.2 (0.2)</td>
<td>2.3 (0.1)</td>
<td>2.4 (0.2)</td>
<td>2.2 (0.2)</td>
</tr>
<tr>
<td>Bile salt-cholesterol ratio</td>
<td>56.4 (5.9)</td>
<td>16.8 (1.5)</td>
<td>31.0 (3.4)</td>
<td>29.6 (0.9)</td>
</tr>
<tr>
<td><strong>Adult group</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Bile Acid (mmol/L)</td>
<td>35.0 (3.0)</td>
<td>23.1 (2.1)</td>
<td>22.9 (2.1)</td>
<td>23.1 (2.1)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.7 (0.1)</td>
<td>0.9 (0.1)</td>
<td>0.9 (0.1)</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>Lecithin (mmol/L)</td>
<td>1.8 (0.2)</td>
<td>2.0 (0.2)</td>
<td>2.3 (0.2)</td>
<td>2.4 (0.3)</td>
</tr>
<tr>
<td>Protein (mg/mL)</td>
<td>1.6 (0.4)</td>
<td>3.2 (0.3)</td>
<td>2.6 (0.3)</td>
<td>2.5 (0.5)</td>
</tr>
<tr>
<td>Bile salt-cholesterol ratio</td>
<td>48.0 (5.4)</td>
<td>22.6 (4.0)</td>
<td>25.5 (3.4)</td>
<td>25.5 (3.3)</td>
</tr>
</tbody>
</table>

All values are as mean (SD)

*: p<0.005 as compared to guinea pig of corresponding age on normal diet

**: p<0.005 from bile of guinea pigs of corresponding age on HCD

HCD: High cholesterol diet; CCK-8: Cholecystokinin; E: Erythromycin

*All values are as mean (SD)
In adult guinea pigs, on the other hand, lithogenic diet led not only to these biochemical abnormalities, but also increased gallstone formation. Treatment with CCK-8 and erythromycin led to increase in biliary lecithin and protein concentration, and prevented this increase in development of gallstones. Our findings about the effects of CCK-8 on gallstone formation were similar to those reported earlier in guinea pigs with similar age and prairie dogs of unknown age. However, Poston et al. found no change in bile protein and lecithin concentrations with CCK treatment. Animal studies have demonstrated an age-related diminution in gall bladder sensitivity to CCK, the primary hormonal stimulus for GB contraction. Some studies suggest that this may contribute to the higher incidence of gallstones observed with aging. Increased lecithin concentration improves the solubility of excess cholesterol in bile, and thus prevents stone formation. High biliary protein is also a possible risk factor for the pathogenesis of cholesterol gallstones.

In conclusion, our study showed that erythromycin to be as effective as CCK in altering bile composition and reducing gallstone formation. These results suggest a possible use of erythromycin in prevention of gallstones in high-risk patients.

References


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