Influence of Bile Acids on Rat Gut Microflora Deterioration Induced by Oral Ampicillin Treatment

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ABSTRACT

Background: Metabolic capacity of gut microflora is huge and this “microb” organ can be considered as second biggest metabolic organ in body. The potential for an antibiotic to influence gut microflora is related to its spectrum of activity, pharmacokinetics, dosage and length of administration. In terms of pharmacokinetics, the rate of intestinal absorption plays a fundamental role. Apart from basic physiological functions, bile acids and their analogues are recognized as transport promoters for other substances, in potentiating their action. The aim of this study was to demonstrate potential protective effect of monoketocholic bile acid on rat intestinal microflora from oral ampicillin.

Materials, Methods & Results: Eighteen Wistar rats were divided into three groups (n = 6). The experimental protocol was approved by Ethics Committee on Animal Use of the University Novi Sad. All animals received 10 mL/kg of body weight of drugs solutions per os by oral intubations. The animals have been treated twice daily for three days, with saline, ampicillin 500 mg/kg and ampicillin 500 mg/kg + monoketocholic bile acid (MKH) 4 mg/kg. The fecal pellets were collected twice, before and after the treatment was completed. Within 2 h of collection, samples of whole pellets were processed microbiologically. Weighed portions of feces were suspended 1:10 in sterile 0.9% NaCl and further diluted with same solutions up to 1: 1015. The number of colony forming units (CFU) was determined by direct counting. Only the plates containing 30 to 300 CFU were considered as valid. The ampicillin treated group, showed significant reduction of CFU number compared to value before treatment under aerobic (P = 0.019) and anaerobic (P = 0.00) conditions. Concomitant use of ampicillin and MKH did not show statisticaly significant reduction (P > 0.05) of CFU number compared to value before treatment in both cultivation conditions. There is significant reduction of CFU number (P = 0.02) only in group treated with ampicillin comparing to control group under the aerobic condition. Statistical analysis was performed by single test ANOVA and Kruskal- Wallis test with Monte Carlo exact test to test significant differences in CFU reduction between groups. Paired two-tailed t-test was performed on the log-transformed data of the CFU/g fecal sample to test for significant differences between counts before and after treatments inside the group.

Discussion: Oral antibiotic applications can change the composition of normal gut microflora. Modification of normal microflora can change metabolism of many compounds. Longer local retention of ampicillin in the gut, due to poor absorption of antibiotic has led to a significant reduction in the number of intestinal microorganisms. Although the fecal flora does not exactly represent the gut flora, comparison of number of CFU from feces specimen before and after antibiotic treatment indirectly reflects the effect of the antibiotic on the bacteria in the gut. Co-administration of ampicillin and MKH, due to promoting effect of bile acid on to absorption of ampicillin, led to less disruption of CFU than in ampicillin group. Based on these results, it is concluded that concomitantly use of ampicillin and MKH, could be useful for reducing the harmful effects of ampicillin on the intestinal flora. These results are consistent with the results obtained in pharmacokinetics study with the same substances.

Keywords: gut microflora, monoketocholic bile acid, oral ampicillin
**INTRODUCTION**

It is believed that microflora is composed of over 50 genera of bacteria and over 400 different species [24] but only 30-40 of them have dominant role in gut homeostasis in every single human being [9,11]. The microflora plays many critical roles in the body and there are many areas of host health that can be compromised with alternations of gut flora composition. Factors such as age [5], antibiotics [21], psychological and physical stress, and certain dietary components have been found to contribute to intestinal dysbiosis. Antibiotic use is the most common and significant cause of major alterations in normal microflora.

The potential for microbial alternation is related to antibiotics spectrum of activity, pharmacokinetics, dosage and length of administration [3,19]. Apart from basic physiological functions, bile acids and their analogues are now recognized as having major therapeutic applications in the treatment of cholelithiasis, as transport promoters for other substances, in potentiating the action of other substances (analgesic, antibiotics, antiviral, hypoglycaemic) and as hypoglycaemic and hypolipidemic agents [7,8,10,18,25,26]. Permeation enhancement through the tissue- solubilising effect of bile salts was found to be one of several mechanisms by which bile salts can facilitate drug absorption. Other mechanisms involve bile salts’ effect on efflux and afflux protein transporters on the cell wall of various tissues [12].

This study was created to demonstrate potential protective effect of MKH on rat intestinal microflora deterioration induced by oral ampicillin.

**MATERIALS AND METHODS**

**Materials**

Ampicillin\(^1\) commercial preparation for oral application in form of capsule by ICN Galenika, Serbia. Monoketocholic\(^2\) bile acid (MKH) was synthesized and purified in the Department of Pharmacy, Novi Sad by the method of Miljkovic et al.\(^{14}\)

**Subjects and design**

Eighteen Wistar rats body weights 200 g to 250 g were used. Animals were housed in climatisating condition, with regular changes of nights and days. The animals were divided in three groups; group treated with saline, one with ampicillin and one with ampicillin and MKH. All animals have been treated for three days. The fecal pellets were collected in sterile sampling tubes, before the treatment and after the treatment. Within 2 h of collection, samples of whole pellets were processed microbiologically. A weighed portions of feces was suspended 1:10 in sterile 0.9% NaCl and furthered diluted with same solutions up to 1:10\(^3\). One hundred microliters of appropriate dilutions were aseptically plated in triplicate onto nutrient agar. Plates were incubated on 37°C for 72 h, under aerobic and anaerobic conditions. The number of colony forming units (CFU) was determined by direct counting. As a valid for enumeration, we took plates with 30 to 300 CFU [6].

**Drug administration and dosages**

All animals received 10 mL/kg of body weight of drugs solutions per os by oral intubations. The animals have been treated twice daily for three days, with saline, ampicillin 500 mg/kg and ampicillin 500 mg/kg + MKH 4 mg/kg.

**Statistical analysis**

Single test ANOVA and Kruskal- Wallis test with Monte Carlo exact test were used to test significant differences in CFU reduction between groups. Paired two-tailed \(t\)-test was performed on the log-transformed data of the CFU/g fecal sample to test for significant differences between counts before and after treatments inside the group. Differences were considered significant at \(P < 0.05\) and the results were expressed as means ± SD. Statistical analysis of collected data was performed by SPSS 11.5 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

Under the aerobic condition (Table 1) there is statistical significant reduction of CFU inside group only in group treated with ampicillin \((P = 0.019)\). The groups treated with ampicillin+MKH and saline did not showed significant changed of CFU number compared to value before treatment \((P > 0.05)\).

The number of CFU after anaerobic cultivation, showed statistical significant reduction only in ampicillin group \((P = 0.00)\) compared to value before treatment (Table 2).

Under the aerobic condition, there is significant change \((P = 0.02)\) in group treated with ampicillin comparing to control group (Table 3).

The number of CFU after anaerobic cultivation (Table 4) did not show statistical significant reduction in any treated group compared to control group \((P > 0.05)\).
Table 1. Logarithmic transformed data of the CFU/g fecal sample cultured for each group in aerobic cultivation condition.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean</th>
<th>N</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controle</td>
<td>Before</td>
<td>14.262</td>
<td>6</td>
<td>0.50457</td>
<td>0.20599</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>14.371</td>
<td>6</td>
<td>0.55386</td>
<td>0.22611</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Before</td>
<td>14.537</td>
<td>6</td>
<td>0.19223</td>
<td>0.07848</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>13.398*</td>
<td>6</td>
<td>0.98769</td>
<td>0.40322</td>
</tr>
<tr>
<td>Ampicillin+MKH</td>
<td>Before</td>
<td>13.848</td>
<td>6</td>
<td>0.64742</td>
<td>0.26431</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>12.839</td>
<td>6</td>
<td>0.87627</td>
<td>0.35781</td>
</tr>
</tbody>
</table>

*Indicate significant ($P < 0.05$) reduction of CFU.

Table 2. Logarithmic transformed data of the CFU/g fecal sample cultured for each group in anaerobic cultivation condition.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean</th>
<th>N</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controle</td>
<td>Before</td>
<td>14.6426</td>
<td>6</td>
<td>0.480751</td>
<td>0.196266</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>14.5580</td>
<td>6</td>
<td>0.479693</td>
<td>0.194709</td>
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<tr>
<td>Ampicillin</td>
<td>Before</td>
<td>14.19350</td>
<td>6</td>
<td>0.990472</td>
<td>0.404358</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>12.67017*</td>
<td>6</td>
<td>1.218533</td>
<td>0.497464</td>
</tr>
<tr>
<td>Ampicillin+MKH</td>
<td>Before</td>
<td>14.12767</td>
<td>6</td>
<td>0.407276</td>
<td>0.166270</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>13.18950</td>
<td>6</td>
<td>0.731028</td>
<td>0.298500</td>
</tr>
</tbody>
</table>

*Indicate significant ($P < 0.05$) reduction of CFU.

Table 3. Change of log CFU number between the groups under aerobic condition.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controle</td>
<td>6</td>
<td>0.109</td>
<td>0.44854</td>
<td>0.18312</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>6</td>
<td>-1.398*</td>
<td>0.99642</td>
<td>0.40679</td>
</tr>
<tr>
<td>Ampicillin+MKH</td>
<td>6</td>
<td>-1.009</td>
<td>0.80432</td>
<td>0.32843</td>
</tr>
</tbody>
</table>

*Indicate significant ($P < 0.05$) change of CFU number.

Table 4. Change of log CFU number between the groups under anaerobic condition.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controle</td>
<td>6</td>
<td>-0.0847</td>
<td>0.3619</td>
<td>0.1478</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>6</td>
<td>-1.5233*</td>
<td>0.3892</td>
<td>0.1589</td>
</tr>
<tr>
<td>Ampicillin+MKH</td>
<td>6</td>
<td>-0.9382</td>
<td>0.9828</td>
<td>0.4013</td>
</tr>
</tbody>
</table>

*Indicate significant ($P < 0.05$) change of CFU number.

DISCUSSION

It is well known that gut microflora present complex but relatively stable ecosystem. There is great variety in the composition of gastrointestinal microflora among animals, as well as varying degrees of sensitivity among these microbes to the variety of antibiotics that can be used to inhibit their growth. Lots of studies involving temporary gut sterilization of rodent digestive tracts have been carried out on laboratory rats and mice.

Although the fecal flora does not exactly represent the gut flora, comparison of number of CFU from feces specimen before and after antibiotic treatment indirectly reflects the effect of the antibiotic on the bacteria in the gut [23]. Direct counts indicated that the feces contained $10^{12}$ cells/g, a count similar to that reported in
the feces of humans and previous study of rats [1]. However, the value may be higher, as considerable portions of the bacteria were difficult to disperse and remained associated with the particulate matter within the feces. More than 99.9% of bacteria in gastrointestinal tract are facultative anaerobes [20]. Fastidious and oxygen-sensitive obligate anaerobes may not have survived the non-invasive sampling methods employed here. As a result, the cultivable bacteria would consist largely of aerotolerant obligate and facultative anaerobes. As little as a 2 h delay in processing fecal samples can result in a reduction of 24% in the counts, compared to samples processed immediately after collection [15].

In our investigation, we used ampicillin since it is safe and widely used antibiotic. Ampicillin alone caused reduction of CFU number after aerobic cultivation, which was statistical significant in comparison to pretreatment values and the value of control group. This may be explained by ampicillin low bioavailability and prolonged time of absorption. After the anaerobic cultivation in ampicillin group, we observed statistically significant reduction in comparison to pretreatment number of CFU. In the ampicillin + MKH group, change in the number of CFU before and after treatment was not statistically significant either with aerobic or anaerobic cultivation condition.

As a consequence of their amphiphilic properties, bile acids can interact with biological membranes, thus disturbing their functioning. At lower concentrations, bile salts may cause an increase in membrane permeability due to the function of mixed micelles and cell swelling, whereas at concentrations above their critical micellar concentration (CMC) may associate with phospholipids of cell membranes, causing a membranolytic effect which is directly related to the intensity of their enhancer effect [22,27]. We assume that bile acid in this case showed promotional effects and amplified apical pass from gut lumen through biological membranes. Our results are consistent with the results obtained in many pharmacokinetics studies with the same and similar substances [2,4,13,16,17].

CONCLUSION

Concomitantly usage of ampicillin and MKH may be useful for reducing the harmful effects of ampicillin on the intestinal flora.

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