EFFECTS OF ROKITAMYCIN ON BILIRUBIN-ALBUMIN BINDING: A STUDY IN VITRO

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ABSTRACT — The effects of rokitamycin, a macrolide antibiotic, on the binding of bilirubin to albumin were examined in vitro using blood plasma from young rats with hyperbilirubinemia, bilirubin-supplemented serum separated from human cord blood, and the human serum from a newborn infant with icterus gravis neonatorum. Rokitamycin at concentrations from 1 to 500 µg/ml (the entire range over which experiments could be conducted) had little or no effect on the concentration of unbound bilirubin in any of the incubation mixtures used. This result suggests that rokitamycin has no effect on the binding of bilirubin to albumin.

KEY WORDS: Rokitamycin, Unbound bilirubin, Rat plasma. Human serum.

INTRODUCTION

Most bilirubin exists in a harmless form, being bound to albumin in the blood. Some drugs compete with bilirubin for binding to albumin and promote the transfer of bilirubin from the blood to brain tissue (Brodersen, 1980). Therefore, drugs that may be administered to neonates, mothers during lactation, pregnant women, or women immediately before delivery, should be tested to confirm that they neither increase levels of unbound bilirubin in the blood or of bilirubin in the brain nor induce kernicterus. Rokitamycin is a macrolide antibiotic. In the previous study (Yamamura et al., 1988), we demonstrated that rokitamycin has little or no effect on the plasma total bilirubin concentration, plasma unbound bilirubin concentration and cerebellar bilirubin level for up to 24 hrs after oral administration at a dose of 1,000 mg/kg to 14-day-old rats with hereditary, non-hemolytic hyperbilirubinemia (homozygous Gunn rats). However, it remains unclear whether or not rokitamycin has the potential ability to displace bilirubin from albumin, since the ability of some drugs to displace bound bilirubin from albumin increases with the concentration of the drugs (Bratlid, 1972; Windorfer and Karitzky, 1975). In addition, it is not clear whether rokitamycin itself, or its metabolite, has some effects. Furthermore, it is clinically important to examine whether or not rokitamycin affects the bilirubin-albumin binding in human neonatal blood. Therefore, in the present study, the effects of rokitamycin on the binding of bilirubin to albumin were examined in...
vitro using plasma from young rats with hyperbilirubinemia, bilirubin-supplemented serum from human cord blood and serum from a newborn infant with icterus gravis neonatorum.

MATERIALS AND METHODS

1. Test drug

Rokitamycin is 3'-propionyl-leucomycin A5 (code number: TMS-19-Q) and is produced by propionylation of the 3'-position of leucomycin A5, which is a component of kitasamycin, a macrolide antibiotic. The chemical structure is shown in a previous paper (Yamamura et al., 1988). The drug was dissolved in dimethyl sulfoxide (DMSO).

2. Plasma and serum

Blood plasma from young rats with hyperbilirubinemia: Homozygous Gunn rats, which develop lifelong hyperbilirubinemia soon after birth as the result of a deficiency in hepatic bilirubin-UDP-glucuronosyltransferase (Weatherill et al., 1980; Iyanagi et al., 1989; Sato et al., 1991; Roy-Chowdhury et al., 1991), were bred in the Second Department of Anatomy, Mie University School of Medicine. A detailed description of breeding conditions is given in a previous paper (Yamamura et al., 1988). It has been estimated that a rat pup of 7–14 days of age can be considered to be equivalent to a human newborn (PMA Guidelines, 1981), and so rats were used on postnatal day 14. Under ether anesthesia, blood was collected from the heart through a silicon capillary tube into a heparinized test tube and was then centrifuged at 2,500 rpm for 10 minutes. Plasma from four animals was pooled.

Bilirubin-supplemented serum from human cord blood: Blood was collected from umbilical cords of human newborns (gestational age, 38–40 weeks; body weight, 2,740–3,600 g) and it was centrifuged at 2,500 rpm for 10 minutes to obtain serum. Because the total bilirubin level of the serum was low, bilirubin was added to the serum as follows: two mg of crystalline bilirubin (Sigma, St. Louis, MO, USA) were dissolved in a mixture of 99 µl of 0.1 N NaOH and 1 µl of 0.1 N disodium ethylenediaminetetraacetate, and an aliquot of the solution was mixed with serum at a ratio of 1 to 200 (v/v).

Human serum from a newborn infant with icterus gravis neonatorum: Upon exchange transfusion, blood was collected from a human newborn (gestational age, 33 weeks; body weight at birth, 1,440 g) with icterus gravis neonatorum on day 7 after birth. Blood was centrifuged at 2,500 rpm for 10 minutes to obtain serum.

3. Incubations

Each solution of rokitamycin was mixed with plasma or serum at a ratio of 1 to 100 (v/v). As a preliminary experiment, human sera from a newborn infant with icterus gravis neonatorum containing rokitamycin at a concentration of 0 (DMSO only), 50, 100 or 500 µg/ml were incubated in a water bath for 0, 30 or 60 minutes at 37°C, and levels of unbound bilirubin were measured. The difference in levels of unbound bilirubin was small between three different incubation times in each concentration of rokitamycin (data not shown). Therefore, the incubation time of 30 minutes was selected.

4. Determination of concentrations of total and unbound bilirubin in plasma or serum

Total bilirubin was quantitated by the diazo method of Malloy and Evelyn (1937). The amount of unbound bilirubin was measured by the method of Jacobsen and Wennberg (1974). The peroxidase rate constant (Kp) was 4.1 ΔA/min/μM of bilirubin at 440 nm. The oxidation rate of albumin-bound bilirubin was 0.0011 and 0.0012 ΔA/min/μM of bilirubin bound to bovine (instead of rat) and human serum albumin at 460 nm, respectively.

5. Statistical analysis

The statistical significance of differences between mean values was evaluated by the two-tailed Student’s t-test.

RESULTS

Rokitamycin was dissolved at a maximal concentration of 500 µg/ml in rat plasma and human serum, and was assayed at 0, 1, 5, 10, 50, 100 and 500 µg/ml.

Table 1 shows total bilirubin levels of rat plasma and human sera. When bilirubin was added to human serum that had been separated from cord blood, the total bilirubin level changed from 1.46±0.34 (mean±SD) to 10.16±0.47 mg/100 ml. The pH changed from 8.19±0.15 to
8.28±0.15 and no significant difference was apparent between the pH before and after the addition of bilirubin.

Table 2 shows levels of unbound bilirubin in rat plasma and human sera. Rokitamycin at concentrations from 1 to 500 μg/ml gave no significant difference in terms of levels of unbound bilirubin from results without the drug in the case of both plasma from young rats with hyperbilirubinemia and bilirubin-supplemented human serum separated from cord blood. In addition, in human serum from a newborn infant with icterus gravis neonatorum, the unbound bilirubin level in the samples treated with rokitamycin at 500 μg/ml was only 1.1 times that in the control. These results suggest that rokitamycin has little or no effect on the binding of bilirubin to albumin.

DISCUSSION

Drugs that bind strongly to proteins are most effective in displacing bilirubin from albumin (Bratliid, 1976). A study in vitro revealed that 85.7% and 91.5% of rokitamycin bound to protein in rat plasma (concentration of rokitamycin: 6.25 μg/ml) and human plasma (ibid., 6.25 μg/ml), respectively, and 72.8% of rokitamycin bound to human serum albumin (ibid., 6.24 μg/ml). The binding of rokitamycin to human serum albumin was shown linearly at the concentration below 12.49 μg/ml and nonlinearly at the concentration of 24.98 – 399.6 μg/ml (unpublished data of Asahi Chemical Industry, Co., Ltd.). Therefore, the possible displacement of bilirubin from albumin must be investigated to assure safe use of the drug. We attempted to assay the effects of rokitamycin at the highest concentrations possible since the ability of some drugs to displace bilirubin from albumin increases with the dose (Bratliid, 1972; Windorfer and Karitzky, 1975). However, even at 500 μg/ml in rat plasma or human serum, rokitamycin had little or no effect on the concentration of unbound bilirubin. This result suggests that the affinity of rokitamycin for albumin is lower than that of bilirubin for albumin, or that the binding site of rokitamycin on albumin is different from that of bilirubin.

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**Table 1.** Total bilirubin levels in plasma or sera.

<table>
<thead>
<tr>
<th>Plasma from young rats with hyperbilirubinemia</th>
<th>Bilirubin-supplemented human serum separated from cord blood</th>
<th>Human serum from a newborn infant with icterus gravis neonatorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples pooled</td>
<td>mg/100 ml (mean ± SD)</td>
<td>No. of samples</td>
</tr>
<tr>
<td>5</td>
<td>7.47±0.42</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 2.** Effects of rokitamycin on binding of bilirubin to albumin.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Plasma from young rats with hyperbilirubinemia</th>
<th>Bilirubin-supplemented human serum separated from cord blood</th>
<th>Human serum from a newborn infant with icterus gravis neonatorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples pooled</td>
<td>Unbound bilirubin level (μg/100 ml) (mean ± SD)</td>
<td>No. of samples</td>
<td>Unbound bilirubin level (μg/100 ml) (mean ± SD)</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>20.83±7.19</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>21.05±8.43</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>21.40±7.12</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
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<td>50</td>
<td>5</td>
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<tr>
<td>100</td>
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<td>24.05±9.34</td>
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<tr>
<td>500</td>
<td>5</td>
<td>24.52±9.01</td>
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</table>
ACKNOWLEDGMENTS

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REFERENCES


