SHORT COMMUNICATION

Effects of indomethacin on the metabolism of glycerol by rat-kidney tubules: an alternative explanation for the enhancement of glycerol-induced acute renal failure by indomethacin

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Summary

1. The metabolism of glycerol by isolated rat-kidney tubules was investigated.
2. Indomethacin, at a concentration of 0.1 mmol/l, markedly inhibited the utilization of glycerol and its conversion into glucose and CO₂.
3. The glycerol 3-phosphate production rose significantly when indomethacin was present, indicating a fall in the rate at which glycerol 3-phosphate was oxidized.
4. The results are discussed in relation to the observation that indomethacin increases the severity of glycerol-induced acute renal failure.

Key words: glycerol, indomethacin, kidney tubules, renal failure.

Introduction

Glycerol, when administered intramuscularly or subcutaneously in large doses, causes acute renal failure similar to that caused by circulatory injury (Flamenbaum, McNeil, Kotchen, Lowenthal & Nagle, 1973; Torres, Strong, Romero & Wilson, 1975a; Dach & Kurtzman, 1976; Hobbs, Chusilp, Kincaid-Smith & McIver, 1976; Churchill, Bidani, Fleischmann & Becker-McKenna, 1977), though the mechanism of this acute renal failure remains largely unknown.

In an attempt to define the role of prostaglandins in glycerol-induced acute renal failure, Papanicolaou, Callard, Barienty & Milliez (1975) and Torres, Strong, Romero & Wilson (1975b) co-administered glycerol and indomethacin, the latter being a powerful inhibitor of renal prostaglandin biosynthesis (Flower & Vane, 1974). In both studies, indomethacin increased the severity of the glycerol-induced renal failure, which led Papanicolaou et al. (1975) to suggest that renal prostaglandins had an important role in counteracting the effects of glycerol. Torres et al. (1975b) reached the same conclusion but pointed out that indomethacin might have other effects unrelated to the inhibition of prostaglandin biosynthesis.

Recently we showed that indomethacin is a potent inhibitor of l-glycerol 3-phosphate-flavin oxidoreductase (EC 1.1.99.5) in rat-kidney mitochondria (Cooney & Dawson, 1979). This enzyme has a vital role in the metabolism of glycerol (Berry, Kun & Werner, 1973; Werner, 1974). As the degree of glycerol-induced acute renal failure is related to the dose of glycerol (Torres et al., 1975b), it is possible that indomethacin increases the severity of acute renal failure by interfering with the metabolism of glycerol.

We report here our studies of the effect of indomethacin on the metabolism of glycerol by isolated rat-kidney tubules.
Methods

All chemicals were obtained from commercial sources.

Rat-kidney tubules were isolated and incubated by procedures described previously (Dawson, 1972, 1975). Each incubation system comprised a suspension of tubules (5–10 mg of protein) in 2 ml of medium containing the following ions (mmol/l): Na+ 146, K+ 4.7, Mg2+ 1–2, Ca2+ 2–5, Cl− 148, SO42− 1–2, PO43− 3–8, buffered at pH 7.4 and with [U-14C]glycerol present at 2.5 mmol/l. Test systems also contained indomethacin (0.1 mmol/l), which was introduced as 4 μl of an ethanolic solution, and systems containing 0.2% (v/v) ethanol served as controls. All systems were incubated for 1 h at 37°C. The choice of indomethacin at 0.1 mmol/l rested on the assumption that the doses of indomethacin administered to experimental animals by Papanicolaou et al. (1975) and Torres et al. (1975b) would, according to calculations based on the data of Yesair, Callahan, Remington & Kensler (1970a) and Yesair, Remington, Callahan & Kensler (1970b), lead to plasma indomethacin concentrations ranging from 0.05 mmol/l to 0.15 mmol/l throughout the experimental period.

Metabolic 14CO2 was collected over the course of the incubation and was estimated by liquid scintillation spectrometry (Dawson, 1977). Glucose was determined by the method of Krebs, Bennett, Degasquet, Gascoyne & Yoshida (1963). Lactate, glycerol 3-phosphate and glycerol were measured by standard enzymatic procedures (Gutmann & Wahlefeld, 1974; Michal & Lang, 1974; Weiland, 1974), under the modified conditions recommended by Engel & Jones (1978). Protein was determined by the method of Lowry, Rose-brough, Farr & Randall (1951) with application of the linear transform equation of Coakley & James (1978).

Results

The results presented in Table 1 show that, in the absence of indomethacin, [U-14C]glycerol was readily metabolized by kidney tubules, giving rise chiefly to glucose and 14CO2. Little lactate was formed but there was a slight accumulation of glycerol 3-phosphate. Indomethacin (0.1 mmol/l) caused glycerol utilization and 14CO2 formation to decrease by over 40%, and glucose production fell by more than 60%. In contrast, the production of glycerol 3-phosphate doubled, indicating that its utilization was lowered by indomethacin.

Discussion

In mammals, glycerol is metabolized mainly by the liver and kidney (Borchgrevink & Havel, 1963; Larsen, 1963; Lin, 1977). It is first phosphorylated to glycerol 3-phosphate, some of which may be used in the biosynthesis of glycerides while most is oxidized to dihydroxyacetone phosphate and enters either the gluconeogenic or the glycolytic pathway. Oxidation of glycerol 3-phosphate is catalysed by two enzymes, a mitochondrial L-glycerol 3-phosphate–flavin oxidoreductase and a cytoplasmic L-glycerol 3-phosphate–NAD+ 2-oxido-reductase (EC 1.1.1.8). However, the activity of the latter is limited by the rate at which cytosolic NADH can be reoxidized (Lin, 1977; Dawson, 1979) and, at high glycerol concentrations, the mitochondrial enzyme appears to play the major role (Williamson, Veloso, Ellington & Krebs, 1969; Berry et al., 1973; Werner, 1974; Lin, 1977).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Change in metabolite production (nmol h−1 mg−1 of protein)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Indomethacin</td>
</tr>
<tr>
<td>Glycerol</td>
<td>−214 ± 18</td>
<td>−124 ± 21</td>
</tr>
<tr>
<td>Glycerol 3-phosphate</td>
<td>21 ± 5</td>
<td>46 ± 9</td>
</tr>
<tr>
<td>Lactate</td>
<td>8 ± 2</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Glucose</td>
<td>87 ± 13</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>14CO2</td>
<td>131 ± 9</td>
<td>77 ± 6</td>
</tr>
</tbody>
</table>
It was the observation that indomethacin strongly inhibits the flavin-linked glycerol 3-phosphate dehydrogenase of rat-kidney mitochondria (Cooney & Dawson, 1979) that led us to investigate the effects of the drug on the metabolism of glycerol by isolated rat-kidney tubules. It was found that indomethacin inhibited the conversion of glycerol into glucose and CO₂ but caused a rise in the intracellular glycerol 3-phosphate concentration. The results indicated the partial blocking of glycerol 3-phosphate oxidation and were consistent with an inhibition of mitochondrial glycerol 3-phosphate dehydrogenase activity. Although there was no indication that indomethacin directly affected the phosphorylation of glycerol, elevation of the intracellular glycerol 3-phosphate concentration would be expected to inhibit glycerol kinase (Robinson & Newsholme, 1969), thereby causing the observed decrease in glycerol utilization.

There is reason to suggest that indomethacin would have similar effects on the hepatic metabolism of glycerol as the oxidation of glycerol 3-phosphate by liver mitochondria is also inhibited by indomethacin (A. G. Dawson, unpublished work).

The observations are consistent with the hypothesis that indomethacin increases the severity of glycerol-induced acute renal failure by inhibiting the metabolic disposal of glycerol and thus lowering the rate at which it is cleared from the blood. However, it should be borne in mind that indomethacin, even in the absence of glycerol, alters intrarenal blood flow, probably through an inhibition of prostaglandin synthesis (McGiff, Terragno & Itskovitz, 1974). Although the changes in blood flow are not known to cause acute renal failure, except in pathological states (Walshe & Venuto, 1979), the possibility remains that the changes have much more serious effects in the presence of high concentrations of glycerol.

References


