Drug conjugation in clinical toxicology

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Introduction
Conjugation plays a vital role in terminating the biological action of many drugs and promoting their rapid elimination from the body. Nowhere is this more important than in the severely poisoned patient. Although conjugation is primarily a mechanism of detoxification, pharmacological activity may occasionally be retained or even increased, as for example with N-acetylprocainamide and morphine-6-glucuronide, respectively (Kalant & Macleod, 1980). In addition, rare instances are known of toxicity mediated by unstable conjugates (Mulder et al., 1978; Van Bladeren et al., 1980).

Acetylation and conjugation with glucuronide and sulphate are major routes of metabolism for many drugs. Thus toxic for analgesics, aspirin and paracetamol. Glucuronyl transferases are ubiquitous and glucuronide conjugation is large, saturation may occur with some drugs at doses within or close to the therapeutic range. The phenolic glucuronidation of salicylate may be cited as an example (Levy et al., 1972), but this is only a minor pathway in man and of little toxicological significance.

The position is different with paracetamol. This drug is commonly taken in overdosage, and without specific therapy severe liver damage occurs in 8–10% of patients. Glucuronide conjugation is the major route of metabolism, accounting for about 60% of a therapeutic dose and there is no decrease in this fraction after overdosage (Prescott, 1980). The large capacity for glucuronide conjugation of paracetamol is a vital safety factor because its rapid removal by this pathway restricts the fraction which undergoes toxic metabolic activation by cytochrome P-450 dependent mixed function oxidase (Mitchell et al., 1974). It has been claimed that liver damage following overdosage results from saturation and glucuronide conjugation with diversion to the toxic pathway, and a pharmacokinetic model has been proposed on this assumption (Slattery et al., 1979). However, in poisoned patients who had not received specific therapy the plasma paracetamol half-life did not shorten progressively as concentrations fell below the therapeutic range and there is thus no evidence of saturation in this fraction after overdosage (Prescott et al., 1971; Prescott, 1980). Indeed, the reverse may occur.

In rare cases of exceptionally severe poisoning, such as the case shown in Fig. 1, glucuronide conjugation probably does become saturated. This patient absorbed about 900mg/kg of paracetamol, and would almost certainly have died from hepatic failure without treatment. Fortunately,

Abbreviations used: PAPS, adenosine-3-phosphate 5-sulphato- phosphate; UDPGA, uridine diphosphoglucuronic acid.

Fig. 1. Plasma concentrations of paracetamol and its glucuronide and sulphate conjugates following massive overdosage in a 43 year old woman.

Treatment with intravenous N-acetylcysteine was started 5h after ingestion.
intravenous N-acetylcysteine was given early and she suffered no hepatic or renal damage. The plasma paracetamol concentration with saturation at concentrations above 430 mg/l, but even so, other explanations are possible. Absorption could have been delayed, and initially liver blood flow was undoubtedly reduced because the patient was severely acidotic and hypotensive. In addition, the rate of disappearance of paracetamol from the plasma often increases several hours after treatment with N-acetylcysteine because sulphate conjugation is restored (see below). The elimination of paracetamol is delayed in patients who develop severe liver damage, but the half-life is prolonged from the outset and this seems to be due to immediate impairment of hepatocyte function with the first passage of the drug through the liver during absorption. Subsequently, conjugation may virtually cease in patients who suffer fatal liver damage (Prescott & Wright, 1973).

The capacity for glucuronide conjugation of paracetamol after overdosage is thus very large unless the liver is badly damaged. It is not known whether conjugation in man is limited by the availability of UDPGA, but there is evidence that this may occur in mice (Galatulas & Montanari, 1976). The glucuronide conjugation of paracetamol is enhanced in patients receiving chronic treatment with anticonvulsants or rifampicin (Prescott et al., 1979; Galinsky & Levy, 1977), and sodium sulphate and N-acetylcysteine have been suggested that administration of inorganic sulphate would maintain sulphate conjugation after overdosage and thus protect against toxicity by enhancing its elimination. Sodium sulphate increased the acute LD50 of paracetamol in mice from 425 to 575 mg/kg (Slattery et al., 1979); and sulphate and N-acetylcysteine increased the formation of paracetamol sulphate in rats (Galinsky & Levy, 1979; Galinsky et al., 1979). Similarly, in man N-acetylcysteine increases the plasma concentrations and urinary excretion of paracetamol sulphate following overdosage (Prescott, 1980). N-Acetylcysteine clearly stimulates the sulphate conjugation of paracetamol, but this effect is unlikely to be of significance in protecting against liver damage in man. Its primary action is to stimulate glutathione conjugation of the hepatotoxic metabolite (see below).

Glutathione conjugation

Pulmonary, hepatic and renal toxicity may be caused by metabolic activation and the formation of reactive intermediates which bind covalently to essential proteins and enzymes. In some cases, reduced glutathione plays a vital protective role by combining with and inactivating these toxic metabolites (Mitchell & Jollow, 1975; Boyd et al., 1982; Trush et al., 1982). The reactions are usually catalysed by glutathione S-transferases and the resulting conjugates are excreted into the bile and eventually appear in the urine as mercapturic acid and cysteine conjugates (Chasseaud, 1976). Glutathione and other sulphydryl compounds also protect against the toxicity of heavy metals by forming a stable complex with the SH group. This forms the basis for the use of thiols in the treatment of poisoning with gold, arsenic, lead, mercury and copper (Sharma et al., 1981; Mitchell et al., 1982).

Glutathione-dependent hepatotoxicity has been established for a variety of agents including halogenated hydrocarbons, furans, paracetamol and cocaine. There is a direct relationship between depletion of hepatic glutathione, formation of glutathione conjugate, covalent binding of the compound to hepatic proteins and liver cell necrosis. Toxicity can be prevented or reduced by glutathione precursors and related sulphydryl compounds which protect against its hepatotoxicity are thought to act primarily by providing cysteine to facilitate glutathione synthesis and the subsequent glutathione S-

Table 1. Dose-dependent sulphate conjugation of paracetamol in man

<table>
<thead>
<tr>
<th>Subject Type</th>
<th>No. of subjects</th>
<th>Dose (mg/kg)</th>
<th>% of total excrated as sulphate conjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers</td>
<td>5</td>
<td>5</td>
<td>35.2 ± 7.9</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>5</td>
<td>20</td>
<td>30.6 ± 10.1</td>
</tr>
<tr>
<td>Poisoned</td>
<td>9</td>
<td>150</td>
<td>9.3 ± 3.0</td>
</tr>
</tbody>
</table>

The saturation of sulphate conjugation following large doses of paracetamol is due in part to depletion of inorganic sulphate (Lin & Levy, 1981; Morris & Lees, 1977). It has been suggested that administration of inorganic sulphate would maintain sulphate conjugation after overdosage and thus protect against toxicity by enhancing its elimination. Sodium sulphate increased the acute LD50 of paracetamol in mice from 425 to 575 mg/kg (Slattery et al., 1979); and sodium sulphate and N-acetylcysteine increased the elimination of paracetamol in rats (Galinsky & Levy, 1979; Galinsky et al., 1979). Similarly, in man N-acetylcysteine increases the plasma concentrations and urinary excretion of paracetamol sulphate following overdosage (Prescott, 1980). N-Acetylcysteine clearly stimulates the sulphate conjugation of paracetamol, but this effect is unlikely to be of significance in protecting against liver damage in man. Its primary action is to stimulate glutathione conjugation of the hepatotoxic metabolite (see below).
transferase catalysed conjugation of the reactive metabolite (Rollins & Buckpitt, 1979; Moldeus, 1981). In keeping with this hypothesis, N-acetylcysteine increases the urinary excretion of salicylurate under all conditions, despite in young children. At low therapeutic doses, the major route of salicylate metabolism is conjugation to glycine to form salicylurate. This conjugate has a renal clearance of about 500 ml/min in adults and is thus rapidly excreted. However, salicylurate formation is saturated well within the therapeutic dose range. In adults, the $K_m$ value is about 5 mg/kg and the maximum rate of salicylurate formation is about 0.9 mg/h per kg (Levy, 1978).

Satisfaction of the glycine conjugation of salicylate has important toxicological implications. Most of a low therapeutic dose of salicylate is converted to salicylurate and rapidly eliminated with a half-life in adults of 3-4 h. At high therapeutic doses and following overdosage, salicylurate formation is completely saturated and the half-life is prolonged to about 30 h. Thus not only is recovery from overdosage very slow, but there is a serious danger of cumulative and toxicity with repeated high therapeutic doses. Such 'therapeutic' overdosage is often unrecognized and carries a high mortality, especially in young children (Anderson et al., 1976; Done, 1978).

The other routes of salicylate metabolism are of little quantitative significance, and the only remaining mechanism for its elimination is renal excretion. Fortunately, the renal clearance of salicylate is highly pH-dependent, and urinary alkalization is employed routinely to enhance its removal following overdosage. As shown in Table 3, alkalization of the urine has a striking effect on the renal excretion of salicylate and the salicylurate to salicylate ratio, but only a modest effect on the plasma salicylate half-life in healthy volunteers given a single oral dose of 20 mg/kg of aspirin. This is because most of the dose is converted to salicylurate under all conditions, despite partial saturation. Following overdosage, urinary alkalization has a proportionately much greater effect on the elimination of salicylate. Salicylurate formation is now fully saturated and if renal function is not impaired, plasma concentrations remain low (<10 µg/ml) and constant, irrespective of urine pH or flow rate. The maximum rate of removal by this route in adults is only 50-60 mg/h (Prescott et al., 1982). Alkalization of the urine dramatically increases the renal clearance of salicylate, and this becomes the dominant route of elimination with a corresponding marked increase in the rate of elimination of salicylate from the body. Indeed, the ratio of the percentages recovered as salicylurate and salicylate is completely reversed by making the urine alkaline (Table 3).

But for the pH-dependent renal clearance of salicylate, intoxication would be prolonged and the risk of serious complications and a fatal outcome would be considerably increased. In a recent study of patients with aspirin poisoning, the administration of glycine reduced the time taken to recover 50% of the total overdosage of salicylate, suggesting that salicylurate formation might be limited by the availability of glycine. However, the fraction recovered as salicylurate was not increased (Notarianni et al., 1983). The glycine conjugation of salicylate is inducible, and this may be relevant during long-term high dose therapy (Day et al., 1983).

Summary

Conjugation is an important mechanism for the rapid inactivation and extensive removal of drugs and poisons.

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Table 2. Intravenous N-acetylcysteine in the treatment of severe paracetamol poisoning

<table>
<thead>
<tr>
<th>Ingestion-treatment interval (h)</th>
<th>No. of patients</th>
<th>Max. ALT (units/l)</th>
<th>No. with severe liver damage</th>
<th>No. with renal failure</th>
<th>No. of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8</td>
<td>40</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8-12</td>
<td>31</td>
<td>810</td>
<td>4 (13%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12-24</td>
<td>30</td>
<td>4296</td>
<td>18 (60%)</td>
<td>5 (17%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Controls</td>
<td>57</td>
<td>&gt;2022</td>
<td>33 (58%)</td>
<td>6 (11%)</td>
<td>3 (5%)</td>
</tr>
</tbody>
</table>

Table 3. Effects of urinary alkalization on salicylate half-life and renal excretion of salicylic and salicyluric acids in healthy volunteers given 20 mg/kg aspirin and in patients with aspirin overdosage

<table>
<thead>
<tr>
<th>Urinary recovery</th>
<th>Salicylate (%)</th>
<th>Salicylurate (%)</th>
<th>Salicylurate/salicylate ratio</th>
<th>Salicylate half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Volunteers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>6.3</td>
<td>0.9</td>
<td>81 (8%)</td>
</tr>
<tr>
<td>Alkaline urine</td>
<td>6</td>
<td>7.5</td>
<td>4.2</td>
<td>390 (39%)</td>
</tr>
<tr>
<td>Aspirin overdosage</td>
<td>16</td>
<td>6.1</td>
<td>1.4</td>
<td>376 (25%)</td>
</tr>
<tr>
<td>Alkaline urine</td>
<td>16</td>
<td>8.1</td>
<td>2.6</td>
<td>3871 (76%)</td>
</tr>
</tbody>
</table>

1984
from the body, and as such it is of major significance in clinical toxicology. The capacity for conjugation of some drugs is large, but with others, saturation may occur with overdosage or even high therapeutic doses. Glutathione conjugation is an important mechanism of protection against toxicity produced by heavy metals, alkylating agents and some compounds which undergo metabolic activation.


Cloning genes that encode inducible forms of P-450

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Introduction

The Ah locus controls the induction, by polycyclic aromatic compounds such as TCDD and MC, of a few of the total number of forms of P-450. Cytochrome P-450 is defined as all forms of CO-binding membrane-bound haemoproteins having NADPH- and sometimes NADH-dependent mono-oxygenase activities. 'P-450' is defined as that form of polycyclic aromatic-inducible P-450 most closely associated with polycyclic aromatic-inducible aryl hydrocarbon hydroxylase activity. 'P-450' is defined as that form of isosafrole-induced enzyme which metabolizes isosafrole best. 'P-450' is defined as that form of polycyclic aromatic-induced enzyme most closely associated with polycyclic aromatic-inducible acetanilide 4-hydroxylase activity. 'P-450' is one of these forms. The incubation of 3H-4-hydroxy-3-methylcholanthrene (3H-MC) with liver microsomes (900 µg of microsomes) and NADPH produces a peak at 448 nm when reduced and combined with CO. We have chosen to rename 'mouse P-448' (Negishi & Nebert, 1979) as P-450. P-450 is one of these forms. The incubation process is mediated by the cytosolic Ah receptor (reviewed in Eisen et al., 1983). Numerous relatively planar foreign chemicals bind avidly (apparent Kd approx. 1 nM) to the Ah receptor in direct proportion to their potency as inducers of