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Normal pathways for glucuronidation, sulphation and oxidation of paracetamol in Gilbert's syndrome

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Abstract. A group of eleven subjects with Gilbert's syndrome was characterized by conventional tests and determination of bilirubin and its conjugates in plasma by alkaline methanolysis and thin layer chromatography. After a 1 g dose of paracetamol h.s. the drug and its metabolites were measured by high performance liquid chromatography (HPLC) in the overnight 8-h urine sample. The amounts of paracetamol and of its metabolites recovered in urine were almost identical with those found in the control group ($n = 10$). The glucuronide : paracetamol ratio, which is considered to be an index of glucuronidation, was not correlated with the fraction of bilirubin present in plasma as glucuronides. These data do not suggest that in subjects with Gilbert's syndrome therapeutic doses of paracetamol are associated with an increased risk for hepatic or systemic toxicity.

Keywords. Gilbert's syndrome, paracetamol, glucuronidation, sulphation, oxidation.

Introduction

Although Gilbert's syndrome is considered to be a benign disorder of bilirubin conjugation, it has been well established that abnormalities are not limited to hepatic handling of bilirubin [1–8]. Clearances of paracetamol, sulphobromophthaleine, indocyanine green, tolbutamide, josamycin, and rifamycin were found to be reduced in some or all of the investigated individuals (Table 1). These abnormalities are thought to be due to associated defects. Within this context the problem of paracetamol glucuronidation has remained controversial, since in one of the early studies Schmid and Hammaker [10] suggested that paracetamol glucuronidation was normal, whereas Douglas *et al.* [14] found the paracetamol clearance to be decreased.

Since these studies, the criteria for the diagnosis of Gilbert's syndrome have evolved. Determination of monoconjugated and diconjugated bilirubin has become possible [23] and Gilbert's syndrome may now be diagnosed by the elevation of only the unconjugated bilirubin [24,25]. In addition, high performance liquid chromatography (HPLC) has rendered methods for the determination of paracetamol and its metabolites more specific, and a new paracetamol test has recently been evaluated [26]. It appeared pertinent, therefore, to apply these new techniques for a re-examination of paracetamol glucuronidation in a group of well-defined subjects with Gilbert's syndrome.

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Patients and methods

Patients

Eleven persons (ten males, and one female) with Gilbert's syndrome, aged between 15 years and 35 years, were studied. All of them gave a history of periods with intermittent jaundice. At the time of the present study total bilirubin was elevated, i.e. above 17 μmol l^{-1} in all subjects. Hepatic diseases were excluded by history, physical examination and normal values of conventional biochemical liver tests such as transaminases, alkaline phosphatase, τ-glutamyltranspeptidase, cholinesterase, serum protein electrophoresis and serological tests for hepatitis-A and -B. Sonographic examination of the liver was unremarkable. None of the persons showed signs of haemolytic disease: normal findings were obtained for haematocrit, haemoglobin, red cell indices, haptoglobin, reticulocyte count and lactate dehydrogenase. Osmotic fragility and seventeen erythrocyte enzymes (including hexokinase, glucose-phosphoisomerase, pyruvate kinase, glucose-6-phosphate dehydrogenase, glutathion reductase) were within normal limits. Haemoglobin electrophoresis was investigated and was normal in five patients.

Gilbert's syndrome was confirmed in the patients by fasting for 24 h (zero calories). During this period total serum bilirubin increased to $62{\cdot}9 \pm$ SD $9{\cdot}9$ μmol l^{-1} or at least to 143% of the pre-fasting value. None of the subjects was a smoker.

Ten healthy volunteers (five males, five females), aged between 18 years and 38 years, served as controls.

Table 1. Drug disposition in Gilbert's syndrome

Process	Drug	Disposition	Reference
Glucuronidation	Menthol	d*	8
	Salicylamide	n	9
	Paracetamol	n	10
Oxidation	Antipyrine	n	11
	Aminopyrine	n	12
N-Acetylation	Sulphadimidine	†	13
Clearance	Paracetamol	d	14
	Sulphobromophthalein	d‡	15
	Indocyanine green	d§	16,17
	Tolbutamide	d	18
	Josamycin	d	19
	Rifamycin	d	20
	Oxazepam, lorazepam	n	21
	Oestradiol benzoate	d¶	22

* n = normal, d = decreased.
† More slow acetylators.
‡ Gilbert's syndrome types II and III.
§ Gilbert's syndrome type III.
¶ Great variability.

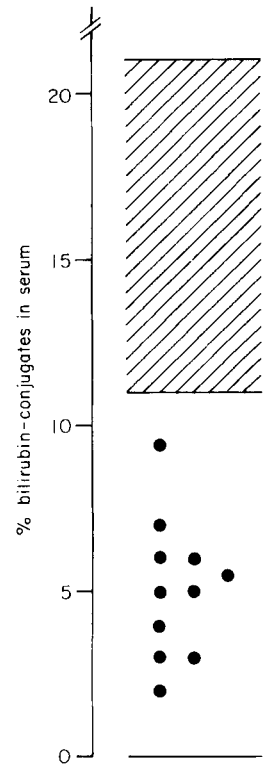

Figure 1. Relative proportion of the sum of bilirubin mono- and diconjugates (determined by thin layer chromatography after alkalin methanolysis) in Gilbert's syndrome. Shaded area represents the mean ± SD for healthy controls [24].

Their histories were unremarkable regarding liver or haematological diseases and none was a smoker. Physical examination and total bilirubin were normal. The following laboratory tests revealed normal findings: transaminases, τ-glutamyltranspeptidase, alkaline phosphatase, haematocrit, haemoglobin and lactate dehydrogenase.

Methods

Written informed consent was obtained before the study from all subjects. The procedure of the paracetamol test, the chromatographic separation by HPLC and the detection of paracetamol and its metabolites were performed as described in detail elsewhere [26]. Briefly, after ingestion of 1 g of paracetamol h. s. an 8-h urine sample was collected overnight. From the amount of paracetamol-metabolites and parent compound, the metabolite:paracetamol (M:P) ratio was formed. This ratio may be regarded as an equivalent of a partial clearance of paracetamol in regard to the formation of the investigated metabolite. Judged by creatinine excretion, patient compliance concerning urine collection appeared to be excellent. Collected urine volumes ranged from 200 to 700 ml (median 485 ml).

Total and direct serum bilirubin concentrations were determined by the diazo method [27]. In addition the relative amounts of unconjugated and conjugated bilirubin were evaluated by thin layer chromatography (TLC) after alkaline methanolysis according to Blanckaert [23].

All data were expressed as means ± standard deviation. Differences were analysed by the Student's *t*-test for unpaired values. Correlations were evaluated by Spearman's rank order correlation [28].

Results

Bilirubin and its conjugates in serum

Serum total bilirubin was significantly lower in the ten healthy volunteers ($9{\cdot}6 \pm 4{\cdot}1$ μmol l^{-1}) than in the eleven subjects with Gilbert's syndrome ($25{\cdot}8 \pm 5{\cdot}6$ μmol l^{-1}). Conjugated bilirubin (mono- plus diconjugates) was lower in subjects with Gilbert's syndrome ($4{\cdot}7 \pm 1{\cdot}6$% total bilirubin) then in the controls, which were within the reference range of 16 ± 5% ($P < 0{\cdot}01$; Fig. 1).

Paracetamol metabolism

In both groups, i.e. subjects with Gilbert's syndrome and controls, total recovery of paracetamol and of its metabolites was approximately 60% of the 1·0 g oral dose (Table 2). The relative proportion of the various metabolites were also the same in both groups.

Analysis of the individual conjugation capacities by means of the M:P ratios for glucuronidation and

Table 2. Recovery of paracetamol and its metabolites in an overnight 8-h urine sample after an oral dose of 1000 mg. All data are given in molar percentages of the dose (mean ± SD)

	Controls ($n=10$)	Gilbert's syndrome ($n=11$)
Paracetamol glucuronide	32·4 ± 2·4	37·5 ± 4·7
Paracetamol sulphate	21·0 ± 3·40	24·1 ± 3·4
Paracetamol cyst./mercap.*	4·6 ± 1·2	5·2 ± 1·8
Paracetamol	1·8 ± 0·6	1·8 ± 0·5
Total recovery	60·0 ± 4·0	67·0 ± 12·0

* Sum of cystein- and mercapturic-acid adducts.

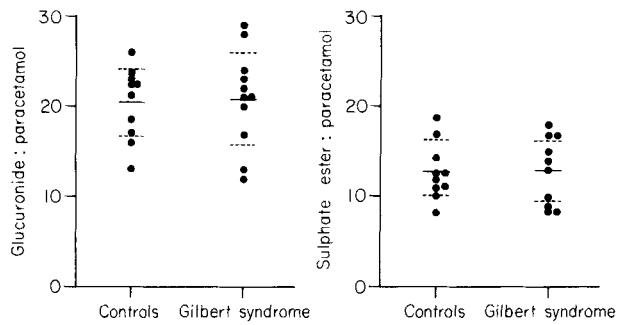

Figure 2. Individual M:P values for paracetamol-glucoronidation and -sulphation in Gilbert's syndrome and controls. Solid and dotted bars represent the mean ± SD.

sulphation revealed no differences between subjects with Gilbert's syndrome and healthy individuals (Fig. 2). The M:P ratio for glucuronidation in Gilbert's syndrome was 20·9 ± 5·1 and in controls 20·5 ± 3·7. The mean values for sulphation in Gilbert's syndrome and in controls were 13·0 ± 3·6 and 13·1 ± 3·1, respectively. Metabolite:paracetamol ratios for the formation of cystein and mercapturic acid adducts in Gilbert's syndrome and in controls were comparable (2·84 ± 1·51 and 3·26 ± 1·16, respectively). Metabolite:paracetamol ratios for paracetamol-glucuronidation were not correlated with the relative amounts of bilirubin conjugates in serum ($r_s = 0{\cdot}027$, NS).

Discussion

The findings of this study suggest that in Gilbert's syndrome metabolism of paracetamol is normal. This conclusion is based on a group of investigated subjects in whom the condition has been characterized by classical [1,3,29–32] as well as modern [24,25] diagnostic criteria. These methods, however, do not exclude genetic heterogeneity of the hyperbilirubinemia in the investigated subjects, because the molecular basis of the condition is not as yet established. In view of the negative findings of the study, genetic homogeneity is not critical. Unfortunately, the applied procedures do not assure that the present study group is comparable with the subjects investigated in other studies [10,14].

The newly developed paracetamol test is based on a highly sensitive and specific HPLC method. It gives information not only about paracetamol glucuronidation, but also measures sulphate esters and GSH-derived mercapturic acid adducts. The test was found to give reproducible results to indicate enzyme induction by phenytoin, rifampicin and by heavy smoking [26], consequently, it appeared to be a suitable tool for the study of subjects with Gilbert's syndrome. The data of this study have not produced evidence that any of the metabolic processes involved in paracetamol disposition is abnormal in Gilbert's syndrome.

The lack of a defect in paracetamol glucuronidation is consistent with the findings of Schmid and Hammaker [10] but has to be reconciled with the abnormalities observed by Douglas *et al.* [14], who described in six subjects with Gilbert's syndrome a 28% reduction in paracetamol clearance and a similarly reduced volume of distribution. The latter investigators have not measured urinary output of paracetamol and of its metabolites. In the paracetamol test, as applied in this study, a smaller volume of distribution and a reduction in clearance should be reflected in corresponding increases in urinary output of unchanged paracetamol, a finding which could not be confirmed by our data. It should be noted, however, that sampling took place overnight in the present study, whereas Douglas *et al.* presumably investigated their subjects during the ordinary working hours. Possibly, chronopharmacological phenomena might explain the differences; alternatively, the study group of Douglas *et al.* was different from ours as discussed above.

The lack of a correlation between paracetamol and bilirubin glucuronidation is compatible with the concept that different UDP-glucuronyltransferases are responsible for the two reactions [32–36].

The present findings are reassuring in regard to the administration of paracetamol to patients with Gilbert's syndrome. It appears that ordinary doses are not associated with increased risks of hepatic or systemic toxicity [37]. The presumably toxifying mercapturic acid pathway appears to be normal [38]. In view of the considerable therapeutic range of paracetamol even a 30% reduction in paracetamol clearance is unlikely to lead to problems. Nevertheless, in view of the genetic heterogeneity of subjects with Gilbert's syndrome, the presented data do not exclude that in some subgroups abnormalities in paracetamol metabolism might occur.

Acknowledgment

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