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ASSESSMENT OF URINARY 6β-HYDROXYCORTISOL AS AN *IN VIVO* INDEX OF MIXED-FUNCTION OXYGENASE ACTIVITY

Introduction

The hepatic mixed-function oxygenases are responsible for the metabolism of many drugs and therefore changes in the activity of these enzymes may lead to changes in drug action and changes in drug toxicity (Park & Breckenridge, 1981). Assessment of factors which influence the activity of these enzymes is therefore an important part of our overall understanding of drug action. Several methods have been suggested for investigating the activity of the drug-metabolising enzymes *in vivo* (reviewed by Breckenridge, 1975), including the measurement of metabolites from endogenous and exogenous substances. 6β-Hydroxycortisol is a minor metabolite of cortisol formed primarily in the endoplasmic reticulum of hepatocytes by the mixed-function oxygenases and excreted unconjugated in urine (Burstein, Dorfman & Nadel, 1954; Frantz, Katz & Jailer, 1961). Conney (1967) suggested that measurement of urinary 6β-hydroxycortisol, in relation to total 17-hydroxycorticosteroids, may reflect changes in the activity of the mixed-function oxygenases and, subsequently, many workers have used urinary 6β-hydroxycortisol as an index of enzyme induction. The purpose of this review is to discuss the scope and limitations of 6β-hydroxycortisol as an index of mixed-function oxygenase activity in the light of current concepts of drug metabolism.

Measurement of urinary 6β-hydroxycortisol

In the past extensive investigations of urinary 6β-hydroxycortisol have been restricted by the complexity of the methods of measurement. Assays developed previously have employed either a long and tedious chromatographic separation prior to a non-specific colour reaction (Frantz *et al.*, 1961; Thrasher *et al.*, 1969) or oxidation of the sample prior to gas-liquid chromatography (Chamberlain, 1971). More recently studies have been facilitated by the development of a radioimmunoassay (Park, 1978) and a high performance liquid chromatography assay (Roots *et al.*, 1979). The normal range of 6β-hydroxycortisol excretion measured by these new assays is similar to, or slightly lower than, values obtained with assays developed previously (Roots *et al.*, 1979) and there is a good correlation between the two methods ($r = 0.99$, $P < 0.0001$) when a direct comparison is made (Gerber-Tarras, Park & Ohnhaus, 1981). Using radioimmunoassay, 6β-hydroxycortisol may be measured directly in a fraction of a microlitre of urine (Park, 1978).

Prediction of drug oxidation rates

Urinary 6β-hydroxycortisol excretion cannot be used to predict inter-individual differences in the rate of drug oxidation in man. Smith & Rawlins (1974) found that there was no correlation between 6β-hydroxycortisol excretion and the clearances of warfarin, phenylbutazone and antipyrine, in healthy volunteers. Combining results from several studies we found no correlation between baseline 6β-hydroxycortisol excretion (corrected for 17-hydroxycorticosteroids) and baseline antipyrine clearance. However these findings are not surprising as many factors may influence 6β-hydroxycortisol excretion including sex (Thrasher *et al.*, 1969), thyroid status (Yamaji *et al.*, 1969), liver disease (Eade *et al.*, 1977), adrenal status (Frantz *et al.*, 1961) and hypertension (Kornel *et al.*, 1975) without necessarily affecting the rate of drug metabolism. Furthermore it has been suggested that there may be some adrenal production of 6β-hydroxycortisol (Burstein *et al.*, 1967; Thrasher *et al.*, 1969) and this also may show inter-individual variation. In patients with high plasma cortisol concentrations there is a disproportionate increase in the excretion of 6β-hydroxycortisol relative to other 17-hydroxycorticosteroid metabolites which may indicate that cortisol itself induces cortisol 6β-hydroxylase (Voccia *et al.*, 1979) or may simply reflect the high capacity of the enzyme relative to other enzymes involved in the metabolism of cortisol. The normal diurnal variation in cortisol production (Rose *et al.*, 1972) does not appear to influence cortisol 6β-hydroxylase activity as we have found that the diurnal variation in the excretion of 6β-hydroxycortisol parallels the diurnal variations in the excretion of 17-hydroxycorticosteroids (Figure 1).

Enzyme induction

The normal urine output of 6β-hydroxycortisol may be increased by a number of agents including drugs and environmental chemicals which also stimulate drug metabolism (Table 1). This increase in 6β-

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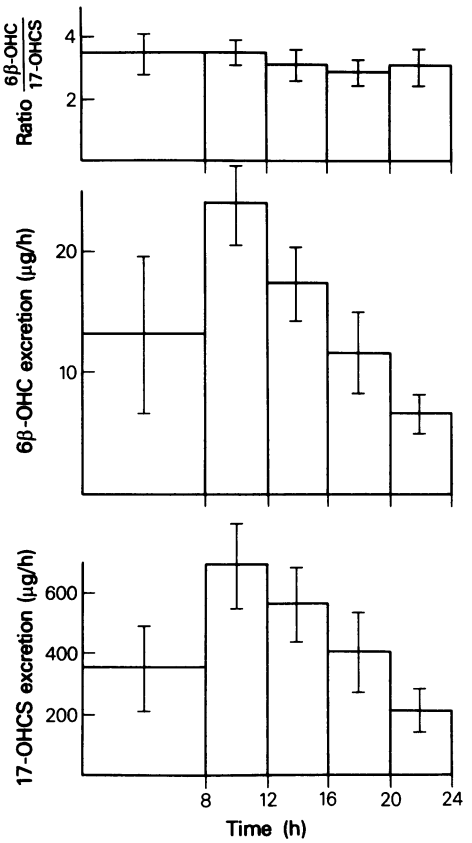

Figure 1 Diurnal variation in the excretion of 6β-hydroxycortisol and 17-hydroxycorticosteroids in normal volunteers. Results are means ± s.d. ($n = 5$).

hydroxycortisol excretion has been shown to reflect an increase in 6β-hydroxycortisol production without change in cortisol production (Burstein *et al.*, 1967). The net increase in cortisol 6β-hydroxylation is accompanied by a relative decrease in the production of conjugated, tetrahydro-derivatives of cortisol (Werk, MacGee & Sholiton, 1964).

We have investigated 6β-hydroxycortisol as an *in vivo* parameter of enzyme induction in man by measuring the 24-h urinary output from volunteers before and after antipyrine, phenobarbitone and rifampicin (Ohnhaus & Park, 1979). The increase in 6β-hydroxycortisol excretion was corrected for changes in 17-hydroxycorticosteroid excretion, to allow for any minor changes in adrenal corticoid production (Figure 2) and compared with changes in antipyrine clearance, plasma γ-glutamyltranspeptidase concentration and D-glucaric acid excretion in the same volunteers.

All three drugs produced significant increases in 6β-hydroxycortisol excretion without affecting urinary 17-hydroxycorticosteroids; the effect of rifampicin was dose-dependent. When expressed in relation to total 17-hydroxycorticosteroids, the increase in 6β-hydroxycortisol excretion showed a significant correlation ($r = 0.69$; $P < 0.001$) with the increase in antipyrine clearance but no correlation with either changes in plasma γ-glutamyltranspeptidase or urinary D-glucaric acid. Cortisol hydroxylation and antipyrine clearance are both directly dependent on the activity of the hepatic mixed function oxygenases and are therefore probably better indices of enzyme induction than either urinary D-glucaric acid or plasma γ-glutamyltranspeptidase. However 6β-hydroxycortisol excretion cannot be used to predict antipyrine clearance because the predictive value $r^2 = 0.48$. Vesell (1979) has recently drawn attention to the misuse of the correlation coefficient r for the prediction of drug metabolism rates.

Table 1 Substances which increase the urinary excretion of 6β-hydroxycortisol in man

Substance	*Reference*
Antipyrine	Ohnhaus & Park (1979)
Carbamazepine	Roots *et al.* (1979)
DDD	Bledsoe *et al.* (1964)
DDT	Poland *et al.* (1970)
DL-473 (rifampicin analogue)	Birmingham *et al.* (1978)
Endrin	Jager (1970)
Methaqualone + diphenhydramine	Ballinger *et al.* (1972)
Pentobarbitone	Berman & Green (1971)
Phenobarbitone	Burstein & Klaiber (1965)
Phenylbutazone	Kuntzman *et al.* (1966)
Phenytoin	Werk *et al.* (1964)
Phetharbital	Southren *et al.* (1969)
Rifampicin	Ohnhaus & Park (1979)
Spironolactone	Huffman *et al.* (1973)

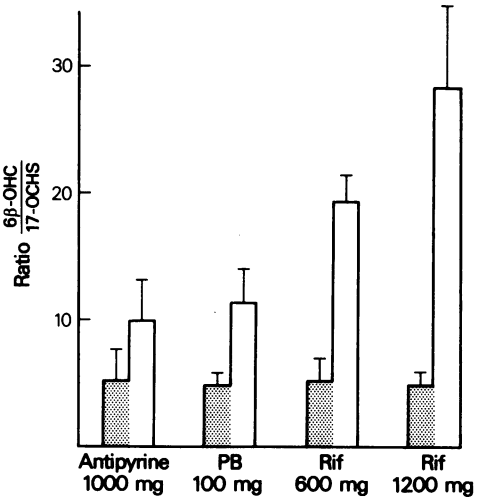

Figure 2 The effect of treatment with antipyrine (1000 mg) phenobarbitone (100 mg, PB) and rifampicin (600 and 1200 mg, Rif) on urinary 6β-hydroxycortisol excretion (from Ohnhaus & Park, 1979). ▨ before, □ after treatment.

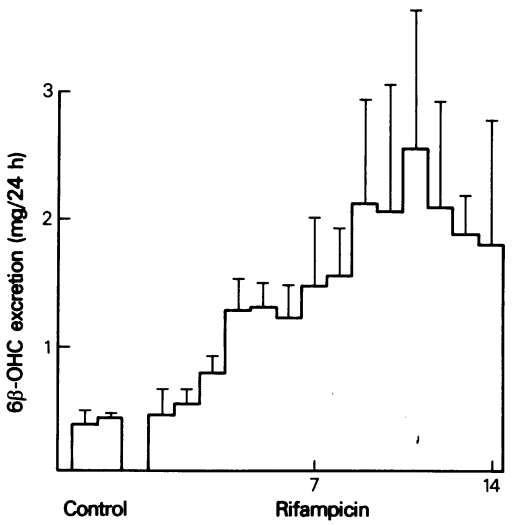

Figure 3 The effect of treatment with rifampicin (1200 mg) on urinary 6β-hydroxycortisol excretion in normal volunteers (Ohnhaus & Park, unpublished data).

On a molar basis rifampicin and phenobarbitone were more potent enzyme inducing agents than antipyrine, and Roots *et al.* (1979) have noted that other anti-epileptiform drugs such as phenytoin and carbamazepine produce a large increase in 6β-hydroxycortisol excretion. Carbamazepine also produced a significant increase in 6β-hydroxycortisol excretion and in antiyprine clearance in children (Moreland, Park & Rylance, 1981). The minimum dose of phenobarbitone required to increase 6β-hydroxycortisol excretion is 30 mg/day. This dose also increased the clearance of lignocaine and antipyrine but did not change either plasma γ-glutamyltranspeptidase or urinary D-glucaric acid (Perucca *et al.*, 1981).

Urinary 6β-hydroxycortisol may be used to study the time-course of enzyme induction. In a study in which volunteers were given rifampicin (600 mg for 14 days) it was found that the excretion rate of 6β-hydroxycortisol had returned to the original control values within a further 14 days (Brodie *et al.*, 1980). Significant changes in the urinary excretion of 6β-hydroxycortisol may be observed after 48 h and the maximum increase appears on about day 10 (Figure 3). The time-course may reflect, in part, fluctuations in rifampicin clearance as rifampicin is an autoinducer (Acocella *et al.*, 1971). Nevertheless the rate of change, which is theoretically only a function of enzyme turnover, is similar to the rate of change of drug clearance obtained by other workers (Lai, Levy & Cutler, 1978).

There is now direct evidence for multiple forms of cytochrome P-450 in human liver (Kahn *et al.*, 1980) but it is not known which form(s) is responsible for cortisol 6β-hydroxylation and it is therefore not possible to predict which type(s) of drug biotransformation will be induced in concert with cortisol 6β-hydroxylation.

Enzyme inhibition

Little attention has been paid to the effect of enzyme inhibitors on 6β-hydroxycortisol excretion. We have found that cimetidine, a known inhibitor of hepatic mixed function oxygenases (Serlin *et al.*, 1979) significantly ($P < 0.05$) reduced the excretion of 6β-hydroxycortisol from 3.65 ± 1.00 (mean ± s.d.) (expressed as a ratio of total 17-hydroxycorticosteroids) to 2.66 ± 0.72 after 14 days administration. Similar results were obtained with patients taking cimetidine for various periods (unpublished data).

Isoniazid (300 mg daily) produced a significant reduction in 6β-hydroxycortisol excretion when given to volunteers (Brodie *et al.*, 1981) and also reduces the effect of rifampicin on 6β-hydroxycortisol excretion in patients (Roots *et al.*, 1979). It is not established whether the mechanism involves enzyme inhibition or is associated with the known hepatotoxicity of isoniazid (Scharer & Smith, 1969).

At present we would not advocate the use of 6β-hydroxycortisol as an *in vivo* index of enzyme inhibition, but these data do confirm that urinary 6β-hydroxycortisol excretion reflects mixed-function oxygenase activity *in vivo*.

Animal studies

In vivo experiments in man have shown that there is an empirical relationship between enzyme induction and 6β-hydroxycortisol excretion, but the qualitative changes in the microsomal enzymes associated with increased urinary 6β-hydroxycortisol have not been determined. We have therefore attempted to develop a suitable animal model with which to study the biochemical changes associated with enhanced cortisol 6β-hydroxylation. Obviously the species used for this purpose must have cortisol as the major circulating corticosteroid which immediately rules out commonly used laboratory animals such as the rat, mouse and the rabbit. The guinea pig has been suggested as a suitable model although apparently contradictory results have been obtained using phenobarbitone as an inducing agent (Burstein & Bhavnani, 1967; Roots *et al.*, 1977). We studied this species using a specific combined h.p.l.c. radioimmunoassay for 6β-hydroxycortisol and found that there was no direct relationship between enzyme induction and urinary 6β-hydroxycortisol excretion. However metabolic studies revealed that 6β-hydroxycortisol itself undergoes extensive and variable metabolism in this species and that this further metabolism may be induced by phenobarbitone (Challiner & Park, unpublished results).

The effect of drugs on the metabolism of 6β-hydroxycortisol in man has not been studied. Burstein and co-workers (1967) administered tritiated 6β-hydroxycortisol to two volunteers and found that within 48 h, 59% of the dose was excreted as unconjugated steroids of which the unmetabolised steroid constituted the major portion. Nevertheless it is still possible that administration of drugs may affect (inhibition or induction) the further metabolism of 6β-hydroxycortisol in man and thereby complicate the relationship between excretion of the steroid and the activity of the mixed-function oxygenases.

In contrast to the guinea pig the marmoset monkey (*Callithrix jacchus*) does not metabolise 6β-hydroxycortisol extensively and has been shown to be a suitable animal model for investigating 6β-hydroxycortisol excretion (Challiner *et al.*, 1980). A comparative study of the effects of phenobarbitone and 3-methylcholanthrene administration indicated that cortisol 6β-hydroxylase is associated with cytochrome P-450 rather than cytochrome P-448 mixed-function oxygenases (Challiner *et al.*, 1981).

Conclusion

Measurement of urinary 6β-hydroxycortisol excretion cannot be used to predict drug metabolism rates in man. Nevertheless changes in the rate of steroid excretion provide a simple non-invasive method for detecting enzyme induction, although an increase in urinary 6β-hydroxycortisol excretion cannot be used as proof *per se* of enzyme induction. Changes in 6β-hydroxycortisol excretion may also be used to monitor the time-course of enzyme induction. Studies using an animal model indicate that cortisol 6β-hydroxylase is associated with cytochrome P-450 rather than cytochrome P-448 mixed-function oxygenases. However the effect of P-448 mixed-function oxygenase inducers on urinary 6β-hydroxycortisol excretion in man has not been determined.

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