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Plasma 6β-hydroxycortisol measurements for assessing altered hepatic drug metabolizing enzyme activity

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To study the usefulness of 6β-hydroxycortisol (6βOHF) measurements for assessing hepatic drug metabolizing enzyme activity, plasma 6βOHF and cortisol were measured in 22 patients with alcoholic liver disease after at least 2 weeks of alcohol abstinence, in 5 patients with severe Cushing's syndrome and in 12 healthy non-drinker subjects. Blood samples were drawn under resting conditions during midnight, in the morning at 0800 h, after a 1-mg overnight dexamethasone test and after ACTH administration. Plasma cortisol and 6βOHF were determined with radioimmunoassay. In patients with alcoholic liver disease, the plasma cortisol levels at midnight and 0800 h, as well as after the administration of dexamethasone and ACTH were not different from corresponding values measured in non-drinker controls. In addition, these patients with alcoholic liver disease had similar plasma 6βOHF levels at midnight, 0800 h and after dexamethasone administration as compared to corresponding values in controls. By contrast, ACTH administration in patients with alcoholic liver disease resulted in a significantly ($p<0.05$) larger increase of plasma 6βOHF (from 106±22 to 1102±106 ng/dl, mean ± SE) as compared to that found in controls (from 74±3 to 337±76 ng/dl). The markedly increased 6βOHF response to ACTH administration in patients with alcoholic liver disease was similar to that measured in patients with severe Cushing's syndrome, in whom increased and non-suppressible plasma cortisol levels were accompanied by markedly elevated plasma 6βOHF levels.

These results indicate that alcohol abstinence in patients with alcoholic liver disease is associated with an exaggerated 6βOHF response to ACTH and that this abnormality may prove to be a clinically useful parameter for a sensitive detection of altered drug metabolism present in these patients.

Keywords: 6β-hydroxycortisol, cortisol, cortisol metabolism drug metabolizing enzyme, Cushing's syndrome, chronic alcoholic liver disease, alcohol abstinence

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The unconjugated metabolite of cortisol, 6β-hydroxycortisol (6βOHF), is formed primarily in the hepatic endoplasmic reticulum by mixed function oxygenases (1–3) and is excreted into the urine in amounts corresponding to 1–2% of total cortisol metabolites (2). Despite its relatively low amounts, measurement of urinary or plasma 6βOHF has gained a wide acceptance in clinical pharmacological studies which used 6βOHF as a simple and non-invasive marker of the induction of the drug metabolizing enzyme activity (4). It has been shown that urinary excretion of 6βOHF is increased following administration of estrogens, phenobarbital, diphenylhydantoin, carbamazepin, spironolactone, 2,2-bis(2-chlorophenyl-4-chlorophenyl)-1,-1-dichloroethane (o,p'-DDD), and rifampicin, which are all known inducers of the hepatic metabolic enzymes (3, 5–10). Administration of high doses of cortisol or stimulation of endogenous cortisol production with ACTH was also associated with an increase of urinary 6βOHF excretion, suggesting that cortisol may be also an inducer of the metabolizing enzymes (5). In agreement with these findings, a few studies indicated elevated plasma 6βOHF concentrations (11) and urinary 6βOHF excretions in patients with Cushing's syndrome (5, 11–12).

Although liver dysfunction that occurs in patients with severe chronic liver diseases is likely to impair drug metabolism (13), a few studies which examined the hepatic drug metabolizing enzyme activity in patients with alcoholic liver disease indicated an increased, rather than decreased activity of the metabolizing enzymes (14). It seems that chronic ethanol consumption itself results in an increase of hepatic drug metabolizing activity (15–16) possibly due to an increase of hepatic cytochrome CYP3A (17) and other cytochrome P450 enzyme content (18). One study has indicated that increased urinary secretion of 6βOHF may be a useful marker of ethanol-induced increase of the metabolizing enzyme activity in patients with alcoholic liver diseases, regardless of the severity of liver lesion (14). According to this study, the increased 6βOHF excretion may return to normal within 2 weeks of abstinence, perhaps suggesting again that ethanol-induced increase of hepatic 6β-hydroxylase enzyme activity, rather than liver lesion itself may be responsible for the altered drug metabolizing activity in these patients (14). However, it still remains uncertain whether mild alteration in 6β-hydroxylase enzyme activity, unrevealed by urinary 6βOHF, could persist during ethanol abstinence. To address this question, in the present study we examined plasma 6βOHF and cortisol concentrations in 22 patients with alcoholic liver disease after at least 2 weeks of alcohol abstinence and in 12 healthy non-drinker subjects under resting conditions as well as after administration of 1-mg dexamethasone, which is a known inhibitor of cortisol and 6βOHF production, and after administration of ACTH, which stimulates cortisol and 6βOHF secretion. In addition, plasma 6βOHF and cortisol concentrations were also determined in 5 patients with severe Cushing's syndrome, in whom increased cortisol secretion has been shown to produce marked increases in 6β-hydroxylase enzyme activity.

Materials and Methods

The study included 22 patients with alcoholic liver disease (age, 53±1.5 years, mean ± SE), 12 apparently healthy non-drinker control subjects (age, 48±3.1 years), and 5 patients with Cushing's syndrome due to adrenal adenomas (age 47±3.78 years). Patients with alcoholic liver disease consumed more than 100 g ethanol daily for at least 5 years. They were investigated for some suspicion of hepatic Cushing's syndrome after at least 2 weeks of ethanol abstinence and they proved to have an apparently normal pituitary and adrenal function. Of the 22 patients, 5 had histologically proven cirrhosis of the liver, whereas the diagnosis of alcoholic liver disease in the other 17 patiens was based on history and clinical findings. All patients were classified as Child-Pugh class A. Control subjects were investigated for suspicion of endocrine abnormality but a detailed endocrine evaluation revealed normal pituitary, adrenal, thyroid and gonadal function. In all control subjects liver and kidney functions were normal and drug treatment was withheld for at least 6 weeks before the study. Adrenal adenomas causing Cushing's syndrome were diagnosed on the basis of suggestive clinical symptoms, adrenal computed tomography, increased plasma cortisol levels with absence of diurnal variation, absence of suppression of plasma cortisol levels after low and high doses of dexamethasone, and suppressed plasma ACTH levels. All patients with Cushing's syndrome underwent operation and in all cases histological evaluation revealed a benign adrenocortical adenoma. Informed consent was obtained from all individuals who participated in the study.

Blood samples were collected under resting conditions during midnight, in the morning at 0800 h, after a 1-mg overnight dexamethasone test, and after ACTH administration (2 mg Cortrosyn Depot i.m.; Organon, Oss, The Netherlands). Blood samples were centrifuged and the plasma was stored at –20 °C until used for hormone measurements. Plasma cortisol and 6βOHF concentrations were determined with radioimmunoassays (19–20) after extraction with ethylacetate followed by chromatographic separation using a modified paper chromatographic system. Plasma samples were extracted in 20 ml disposable tubes. For estimating recovery, ^{3}H-cortisol and ^{3}H-6βOHF (Amersham International, Buckinghamshire, UK, 7000 dpm each) in 0.1 ml of water were added to each tube containing 0.3 ml of plasma. Samples were mixed, then kept at room temperature for 15 min. Extraction was carried out with 15 ml of ethylacetate, then the tubes were centrifuged to separate the aqueous phase, as previously described (21). The extracts were evaporated and the residues were reconstituted in dichlormethane, then applied to a paper chromatographic system. Chromatography was performed in ethylacetate-chloroform-methanol-water (1 : 3 : 2 : 2) for 4 hours. Elution from paper following chromatography was done with 5 ml of 5% ethanol for cortisol and with 2 ml of 0.1 M phosphate buffer (pH 7.0) for 6βOHF. Aliquots of 0.3 ml of eluates were pipetted into counting vials for recovery estimation, while duplicate aliquots were used for cortisol and 6βOHF

radioimmunoassays. The recoveries for both ^{3}H-cortisol and ^{3}H-6βOHF were between 65 and 80%. The intra- and inter-assay variances were evaluated by duplicate measurements of the same samples in different assays. The intra- and inter-assay coefficients of variation of the cortisol assay were between 5% and 8%, and between 7% and 13%, respectively, whereas the intra- and inter-assay coefficients of variation of the 6βOHF assay were between 4% and 6%, and between 8% and 12%, respectively.

Statistical analysis included nonparametric tests (Mann-Whitney-Wilcoxon test, Spearman rank correlation). $P<0.05$ was considered to be statistically significant.

Results

In control subjects, morning plasma 6βOHF and cortisol concentrations (74.3±3.6 ng/dl and 8.5±0.5 μg/dl, respectively) were considerably higher than those measured during midnight (26.6±2.1 ng/dl and 1.0±0.4 μg/dl, respectively) and after dexamethasone administration (25.9±1.6 ng/dl and 0.8±0.08 μg/dl, respectively). In these subjects, administration of ACTH induced a large increase of both 6βOHF and cortisol (377±76 ng/dl and 45.2±12.6 μg/dl, respectively).

In patients with alcoholic liver disease, plasma 6βOHF and cortisol concentrations in the morning (106.0±22 ng/dl and 11.3±1.3 μg/dl, respectively), during midnight (44.4±6.3 ng/dl and 2.1±0.5 μg/dl, respectively) and following dexamethasone administration (48.2±6.5 ng/dl and 1.3±0.2 μg/dl, respectively) were similar to corresponding values measured in the control group (Table I). By contrast, the ACTH-induced increase of plasma 6βOHF (1102±106 ng/dl), but not that of plasma cortisol (55.5±2.7 μg/dl), was significantly higher as compared to corresponding values in the control group.

As expected, patients with Cushing's syndrome had significantly higher morning plasma cortisol concentration (25.4±5.1 μg/dl) with no significant decreases during midnight (23.1±3.4 μg/dl) and after dexamethasone administration (21.5±7.2 μg/dl). In addition, ACTH administration resulted in a larger increase of plasma cortisol (140±46 μg/dl) in patients with Cushing's syndrome as compared to ACTH-stimulated plasma cortisol values in the control group. As shown in Table I, these changes in plasma cortisol levels in patients with Cushing's syndrome were accompanied by large elevations of plasma 6βOHF levels, and the values attained under all conditions tested were significantly higher than those measured in the control group.

Table I

Plasma 6β-hydroxycortisol (6βOHF) and cortisol concentrations in the baseline state in the morning, midnight, and following dexamethasone and ACTH administration in control subjects, in patients with alcoholic liver disease and in patients with Cushing's syndrome

		Plasma 6βOHF (ng/dl, mean ± SE)	Plasma cortisol (μg/dl, mean ± SE)
Control subjects	(n=12)		
Morning		74.3 ± 3.6	8.5 ± 0.5
Midnight		26.6 ± 2.1	1.0 ± 0.4
Dexamethasone		25.9 ± 1.6	0.8 ± 0.08
ACTH		337.0 ± 76	45.2 ± 12.6
Patients with alcoholic liver disease	(n=22)		
Morning		106.0 ± 22	11.3 ± 1.3
Midnight		44.4 ± 6.3	2.1 ± 0.5
Dexamethasone		48.2 ± 6.5	1.3 ± 0.2
ACTH		1102.0 ± 106*	55.5 ± 2.7
Patients with Cushing's syndrome	(n=5)		
Morning		726.0 ± 225*	25.4 ± 5.1*
Midnight		632.0 ± 127*	23.1 ± 3.4*
Dexamethasone		413.0 ± 153*	21.5 ± 7.2*
ACTH		5095.0 ± 1392*	140.0 ± 46*

*$p<0.05$ vs corresponding values in the control group.

Discussion

The main new finding of the present study is that after an at least 2 weeks of ethanol abstinence patients with alcoholic liver disease exhibit an exaggerated increase of plasma 6βOHF in response to ACTH stimulation. As plasma 6βOHF concentration appears to be a reliable marker of the hepatic drug metabolizing enzyme activity, it seems likely that the exaggerated 6βOHF response to ACTH indicates an induction of the metabolizing enzyme. Perhaps more importantly, these changes in ACTH-induced increases of plasma 6βOHF were not accompanied by an exaggerated plasma cortisol response, as plasma cortisol responses to ACTH were similar in patients with alcoholic liver disease and in the control group. It seems therefore likely that the disproportionally larger increase of plasma 6βOHF after ACTH stimulation was due to an increase of enzyme activity rather than an ACTH-induced increase of cortisol secretion, which results in an increased amount of substrate for 6βOHF production.

Earlier studies indicated, that patients with alcoholic liver disease after 2 weeks of ethanol abstinence had normal urinary 6βOHF secretion (14), suggesting that increased hepatic drug metabolizing enzyme activity, which is present during ethanol consumption, remained undetectable by urinary 6βOHF measurements after this period of abstinence. In agreement with these earlier findings, plasma 6βOHF concentrations measured either in the morning or midnight in our study were similar in patients with

alcoholic liver disease after an at least two weeks of ethanol abstinence and in non-drinker control subjects. Thus, ACTH-stimulated plasma 6βOHF concentration appears to be a more sensitive marker of ethanol-induced changes of metabolizing enzyme activity than baseline plasma or urinary 6βOHF levels, although its possible clinical usefulness for the detection of altered drug metabolizing activity remains to be established.

Our results confirm previous observations showing that increased cortisol secretion in patients with Cushing's syndrome is associated with a large increase of 6βOHF production. Since increased cortisol secretion may induce hepatic drug metabolizing enzyme activity (5, 21), it is possible that the large increase of plasma 6βOHF in these patients was due to an induction of the enzyme. Alternatively, it is also possible that the increased plasma 6βOHF concentrations in patients with Cushing's syndrome was, at least partly, the consequence of an increased secretion of 6βOHF by adrenal cells, as already proposed (1, 11, 22). In any case, plasma 6βOHF concentrations appear to be an appropriate marker of increased adrenocortical activity present in patients with Cushing's syndrome. Earlier studies have already indicated that plasma and urinary 6βOHF measurements can be used as a diagnostic test for chronic hypercortisolism in a way comparable (11, 12) or even superior to the measurement of urinary cortisol excretion (5).

In summary, the results of the present study indicate that alcohol abstinence in patients with alcoholic liver disease is associated with an exaggerated 6βOHF response to ACTH and that this abnormality may prove to be a clinically useful parameter for a sensitive detection of altered drug metabolism present in these patients. In addition, the results confirm previous observations showing that due to a possible induction of the metabolic enzyme activity by cortisol, increased plasma 6βOHF concentration may be an appropriate marker of increased adrenocortical activity in patients with Cushing's syndrome.

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