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Mineralocorticoid activity of carbenoxolone: contrasting effects of carbenoxolone and liquorice on 11β-hydroxysteroid dehydrogenase activity in man

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SUMMARY

1. 11β-Hydroxysteroid dehydrogenase is an enzyme complex consisting of 11β-dehydrogenase and 11-oxoreductase responsible for the interconversion of cortisol to cortisone in man. Inhibition of 11β-dehydrogenase (e.g. after liquorice ingestion) results in cortisol acting as a potent mineralocorticoid. We have evaluated the effect of the synthetic liquorice derivative, carbenoxolone, on this enzyme complex.

2. Carbenoxolone given to six volunteers in metabolic balance produced sodium retention with suppression of the renin–angiotensin–aldosterone system. Plasma potassium fell, although there was no kaliuresis. This was associated with inhibition of 11β-dehydrogenase (as measured by a rise in the plasma half-life of [11α-3H]-cortisol). Unlike liquorice, however, carbenoxolone also inhibited 11-oxoreductase (as measured by the generation of cortisol after oral cortisone acetate).

3. The mineralocorticoid activity of carbenoxolone, like liquorice, is mediated via cortisol by inhibition of 11β-dehydrogenase. Carbenoxolone, however, also inhibits 11-oxoreductase activity and this may relate to its effect on renal potassium excretion.

Key words: carbenoxolone, cortisone, cortisol, 11β-hydroxysteroid dehydrogenase, liquorice, mineralocorticoid.

Abbreviations: allo-THF, 5α-tetrahydrocortisol; ANG I, angiotensin I; CBX, carbenoxolone; 11β-DH, 11β-dehydrogenase; GE, glycyrrhetinic acid; 11β-OHSD, 11β-hydroxysteroid dehydrogenase; THE, tetrahydrocortisone; THF, tetrahydrocortisol.

INTRODUCTION

Carbenoxolone (CBX; 3-(3-carboxy-1-oxopropoxy)-11-oxoolean-12-en-29-oic acid) has been used as an effective treatment for peptic ulceration since the 1960s [1, 2], but its use is limited by the occurrence of sodium retention, hypertension and hypokalaemia in up to 50% of cases [3], and thought, until recently, to be due to a direct affinity of CBX for the type 1 mineralocorticoid receptor [4].

CBX is a derivative of glycyrrhetinic acid (GE), which, along with glycyrrhizic acid, is the active apparent mineralocorticoid of liquorice (Fig. 1). Recently, we have shown that the mineralocorticoid activity of liquorice is due to inhibition of 11β-dehydrogenase (11β-DH), which along with 11-oxoreductase, forms the enzyme complex 11β-hydroxysteroid dehydrogenase (11β-OHSD; EC 1.1.1.146) responsible for the interconversion of cortisol and cortisone [5].

Congenital deficiency (the syndrome of ‘apparent mineralocorticoid excess’) or acquired inhibition of 11β-DH results in cortisol acting as a potent mineralocorticoid and is associated with a prolonged plasma cortisol half-life despite normal plasma levels, raised urinary free cortisol and a reduced daily cortisol production rate. The ratio of urinary cortisol metabolites [tetrahydrocortisol (THF) and 5α-tetrahydrocortisol (allo-THF)] to cortisone metabolites [tetrahydrocortisone (THE)] is increased. This study was carried out to evaluate the effect of CBX on 11β-OHSD activity.

MATERIALS AND METHODS

Six normal male volunteers, aged 27±2 years (mean ±SEM), were established on a fixed sodium/potassium diet (120/80 mmol/day) from the metabolic kitchen. When in balance (mean duration 5 days), CBX (300 mg/day in divided doses; Biogastrone; Sterling Winthrop) was given for 14 days. Daily 24 h urine collections were analysed for sodium, potassium and urinary free cortisol (Amerlex Kit, Amersham International) [6]. A 24 h
Plasma potassium, cortisol (Amerlex Kit, Amersham International), cortisone (in-house radioimmunoassay), and aldosterone (radioimmunoassay, Diagnostic Products Ltd) were measured at 09.00 hours on days 1, 2, 4, 8, 11, and 14. Plasma potassium fell from 3.9 ± 0.2 mmol/l on day −1 to a nadir of 3.4 ± 0.2 mmol/l on day 14 (P < 0.05), there was no kaliuresis (Fig. 2).

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Steroid data

There was no change in plasma cortisol at 09.00 hours during CBX administration despite a significant increase in urinary free cortisol (Fig. 2), compatible with inhibition of 11β-DH. The excretion of the principal urinary cortisol metabolites (THF, allo-THF, THE, cortols and cortolones) also fell significantly during CBX administration, suggesting a reduced daily cortisol secretion rate (Table 1). Inhibition of 11β-dehydrogenase was confirmed by studying the metabolism of [11α-3H]cortisol, the plasma half-life of this isotope rising from 39 ± 3 min to 123 ± 13 min on CBX (n = 3).

Despite inhibition of 11β-DH, there was no change in the (THF + allo-THF)/THE ratio during CBX administration (Table 1). Similarly, at 09.00 hours plasma cortisone showed little if any suppression after CBX. This was in marked contrast to our findings in volunteers taking liquorice, where we observed an increase in the (THF + allo-THF)/THE ratio [5] and a marked fall in plasma cortisone (Table 2). To account for this discrepancy we evaluated the effect of CBX on 11-oxoreductase activity. Fig. 3 shows the plasma cortisol response after 25 mg of cortisone acetate given orally at 09.00 hours to three dexamethasone-suppressed volunteers before and 3 days after CBX (300 mg/day). The peak cortisol response was significantly lower when taking the CBX (e.g. at 11.00 hours plasma cortisol was 671 ± 43 nmol/l off CBX compared with 432 ± 54 nmol/l on CBX, P < 0.01), suggesting coexistent inhibition of 11-oxoreductase.

DISCUSSION

It is not surprising that CBX is associated with mineralocorticoid side-effects, being a synthetic triterpenoid derived from liquorice root (Fig. 1). GE has been known to act as an apparent mineralocorticoid since the late 1940s [9–11]. Many explanations have been put forward to account for the mineralocorticoid activity of CBX, including a direct effect on adrenal steroid secretion [12], displacement of aldosterone from non-specific protein-binding sites by the highly protein-bound CBX and

Fig. 1. Structures of the active mineralocorticoids in liquorice, glycyrrhizic acid and GE, and their similarity with CBX.

urinary steroid metabolic profile measured by gas–liquid chromatography [7] for THF, allo-THF, THE, cortols and cortolones was performed on days −1, 2, 4, 8, 11, and 14. Plasma potassium, cortisol (Amerlex Kit, Amersham International), cortisone (in-house radioimmunoassay), renin activity [8], and aldosterone (radioimmunoassay, Diagnostic Products Ltd) were measured at 09.00 hours on days −1, 2, 4, 8, 11, and 14. Subjects were supine for 30 min before blood was taken. On days −1 and 7, the half-life of [11α-3H]cortisol (14.6 Ci/mmol) (which when acted on by 11β-dehydrogenase forms cortisone and 3H2O) was assessed in three volunteers using previously described methodology [5]. The normal plasma half-life of [11α-3H]cortisol is 40.9 ± 1.2 min (n = 17). Unpublished data from a similar earlier metabolic balance study [5] in which volunteers took liquorice (200 g/day) have been included for comparison.

In a separate experiment, three volunteers were dexamethasone suppressed by giving dexamethasone (1.5 mg) at 23.00 hours, 0.5 mg at 09.00 hours and 0.5 mg at 13.00 hours. Plasma cortisol and cortisone were measured throughout that day (from 09.00 hours to 17.00 hours), after 25 mg of oral cortisone acetate at 09.00 hours. This was performed before and 3 days after CBX (300 mg/day). Statistical analysis was performed using Student’s paired t-test, with the point of maximum effect taken by comparison with baseline values.

RESULTS

Metabolic balance study

As shown in Fig. 2, CBX produced marked sodium retention maximal on days 2–7 (urinary sodium 61.0 ± 5.0 mmol/24 h on day 3 compared with 114.0 ± 4.7 mmol/24 h on day −1, P < 0.001). This produced suppression of the renin-angiotensin-aldosterone system, with plasma renin activity falling from 1.64 ± 0.28 pmol of angiotensin I (ANG I) h−1 ml−1 on day −1 (reference range 0.4–1.3 pmol of ANG I h−1 ml−1) to 0.15 ± 0.05 pmol of ANG I h−1 ml−1 on day 8 (P < 0.01), and plasma aldosterone falling from 510 ± 67 pmol/l on day −1 (reference range 100–550 pmol/l) to 87 ± 21 pmol/l on day 8 (P < 0.01) (Fig. 2). Plasma renin activity was high, and plasma aldosterone at the upper limit of normal, in our volunteers on day −1 and this presumably reflects activation of the renin–aldosterone axis on reducing their daily sodium intake to 120 mmol/day.

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Mineralocorticoid activity of carbenoxolone

Fig. 2. Urinary sodium (a), urinary potassium (b), plasma renin activity (PRA) (c), plasma aldosterone (d), urinary free cortisol (e) and plasma cortisol (f) in six volunteers after CBX (100 mg, three times daily). Results are expressed as means ± SEM. Urinary sodium and urinary potassium are plotted from a fixed intake of 120 mmol/24 h and 80 mmol/24 h, respectively. Day – 1 refers to the last day of the run-in balance period (approximately 5 days). Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001.

Potentiation of the action of aldosterone [13, 14]. However, both plasma aldosterone levels and the renin–angiotensin systems are suppressed [15], and it has been widely accepted from receptor studies by Armanini et al. [4] that CBX acts as an aldosterone agonist on the type 1 mineralocorticoid receptor. Its affinity for this receptor, however, is only 1/15 000th that of aldosterone.

It was thought that the same was true of liquorice, i.e. that the direct action of GE on the type 1 receptor accounted for its mineralocorticoid activity [16]. There
were, however, several pointers in the literature suggesting that this was not the case, principally the finding by Molhuysen et al. [5] that mineralocorticoid activity in Addisonian or adrenalectomized patients was found principally in the liver and kidney, responsible for the interconversion of the active steroid cortisol to the inactive steroid cortisone. Activity in these sites differs markedly, the kidney containing predominantly \(11\beta\)-DH converting cortisol to cortisone, the liver \(11\alpha\)-oxoreductase converting cortisone to cortisol. The role of \(11\beta\)-DH has come to light from the understanding of congenital deficiency of the enzyme (the syndrome of ‘apparent mineralocorticoid excess’), a rare cause of low-renin hypertension found, until recently, only in children [17, 18]. Patients have a prolonged plasma cortisol half-life but normal plasma levels as a result of an adenocorticotropic hormone-mediated fall in the daily cortisol production rate. The ratio of urinary cortisol metabolites to cortisone metabolites is increased and many cases have a concomitant deficiency of \(5\beta\)-reductase [19].

In describing the first adult case of \(11\beta\)-DH deficiency we showed that cortisol acted as a potent mineralocorticoid and put forward the novel hypothesis that renal \(11\beta\)-DH acted as a paracrine mechanism dictating specificity of the type 1 mineralocorticoid receptor [20]. In normal subjects, \(11\beta\)-DH shuttles cortisol to cortisone, thus allowing sole access of aldosterone to the type 1 receptor [21]. In \(11\beta\)-DH deficiency (either congenital or acquired), intrarenal cortisol levels are high and cortisol swamps the non-specific mineralocorticoid receptor [22].

The aim of this paper was to determine whether CBX, the hemisuccinate derivative of GE, had the same effects on the renin-angiotensin system in six volunteers treated with the maximum recommended dose of CBX, i.e. 300 mg/day for 14 days. This was associated with inhibition of \(11\beta\)-DH as shown by a rise in urinary free cortisol, a fall in the daily cortisol production rate (as inferred from a reduction in the excretion of urinary cortisol metabolites) and a marked increase in plasma \([11\alpha-\text{3H}]\text{cortisol half-}

### Table 1. Urinary steroid metabolite profile in six subjects receiving CBX (300 mg/day)

Results are means ± SEM. Statistical significance: *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\).

<table>
<thead>
<tr>
<th>Steroid ((\mu g/24) h)</th>
<th>Day...</th>
<th>-1</th>
<th>+2</th>
<th>+8</th>
<th>+13</th>
</tr>
</thead>
<tbody>
<tr>
<td>THF</td>
<td>2410±260</td>
<td>2040±215</td>
<td>1872±129*</td>
<td>1878±208</td>
<td></td>
</tr>
<tr>
<td>Allo-THF</td>
<td>2055±278</td>
<td>1858±100</td>
<td>1687±209</td>
<td>1795±306</td>
<td></td>
</tr>
<tr>
<td>THE</td>
<td>5063±756</td>
<td>4643±348</td>
<td>3927±270</td>
<td>4207±591</td>
<td></td>
</tr>
<tr>
<td>(\alpha)- and (\beta)-cortols</td>
<td>908±43</td>
<td>606±70**</td>
<td>568±38**</td>
<td>638±109**</td>
<td></td>
</tr>
<tr>
<td>(\alpha)- and (\beta)-cortolones</td>
<td>1600±82</td>
<td>1202±163*</td>
<td>992±44**</td>
<td>1132±119*</td>
<td></td>
</tr>
<tr>
<td>Total metabolites</td>
<td>12 352±1191</td>
<td>10 362±655</td>
<td>9027±411**</td>
<td>9576±1189</td>
<td></td>
</tr>
<tr>
<td>(THF+ allo-THF)/THE ratio</td>
<td>0.90</td>
<td>0.86</td>
<td>0.92</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Cortols/cortolones ratio</td>
<td>0.57</td>
<td>0.52</td>
<td>0.58</td>
<td>0.58</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Plasma cortisol in subjects receiving liquorice (200 g/day) or CBX (300 mg/day)

Results are means ± SEM. Statistical significance: *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\).

<table>
<thead>
<tr>
<th>Day</th>
<th>Liquorice study†</th>
<th>CBX study†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=6)</td>
</tr>
<tr>
<td>0</td>
<td>42.8±6.4</td>
<td>46.6±5.0</td>
</tr>
<tr>
<td>+2</td>
<td>26.7±3.5**</td>
<td>37.4±7.8</td>
</tr>
<tr>
<td>+4</td>
<td>18.1±3.4***</td>
<td>35.2±7.1*</td>
</tr>
<tr>
<td>+8</td>
<td>14.1±1.7***</td>
<td>38.8±10.3</td>
</tr>
<tr>
<td>+11</td>
<td>10.2±0.5***</td>
<td>39.6±10.8</td>
</tr>
<tr>
<td>+14</td>
<td>–</td>
<td>51.0±5.8</td>
</tr>
</tbody>
</table>

†From [5].
life. However, as shown in Tables 1 and 2, there was no change in either the (THF+allo-THF)/THE ratio or 09.00 hour plasma cortisol (cf. liquorice ingestion) during CBX administration, suggesting a coexistent inhibition of 11-oxoreductase. This was confirmed in our three dexamethasone-suppressed volunteers taking cortisone acetate on and off CBX. Cortisone taken orally relies on hepatic metabolism to cortisol for its activity. The capacity for this conversion is immense, as exemplified by the fact that after oral cortisone, plasma cortisol levels do not rise [23]. In our three volunteers plasma cortisol levels were blunted after cortisone administration when taking CBX, indicating a defect in the conversion of cortisone to cortisol and hence defective 11-oxoreductase. This would explain why the urinary (THF+allo-THF)/THE ratio was unaltered. 11-Oxoreductase activity in the kidney is minimal [24], but the inhibition of the abundant 11-@-DH in the kidney results in sodium retention.

The absence of a kaliuresis in the face of significant hypokalaemia is an interesting observation, in obvious contrast to our observations from the liquorice study, but has been noted previously [15]. Although CBX produces a marked increase in rectal transmucosal electrical potential difference [25] (an index of mineralocorticoid activity on the rectum), there is no gastrointestinal potassium loss to account for the hypokalaemia [26]. More recently CBX has been shown to stimulate cellular Na+/K+-adenosine triphosphatase [27] and it is suggested that the hypokalaemia is simply a 'redistribution hypokalaemia' with no nett change in total body potassium.

We conclude therefore that the sodium retention associated with CBX ingestion is, like liquorice, mediated via cortisol by inhibition of 11-@-DH. The urinary (THF+allo-THF)/THE ratio and plasma cortisol are unaltered because of a concomitant inhibition of 11-oxoreductase, which does not appear to occur with liquorice ingestion. A normal (THF+allo-THF)/THE ratio does not therefore exclude a defect in 11-@-OHSD.

In spite of avid renal sodium retention and plasma hypokalaemia, there was no kaliuresis, suggesting a dissociated mineralocorticoid effect. It remains to be seen whether this relates in any way to the associated 11-oxoreductase defect.

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REFERENCES


