Hydrocortisone Succinate and Hydrocortisone Simultaneously Determined in Plasma by Reversed-Phase Liquid Chromatography, and Their Pharmacokinetics in Asthmatic Children

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Hydrocortisone succinate and hydrocortisone are chromatographed with a mobile phase consisting of sodium acetate/acetonitrile (77/23, by vol), the effluent being monitored at 254 nm. p-Hydroxybenzoate-N-propyl is used as the internal standard. Detection limits are 0.5 mg/L for hydrocortisone succinate, 0.2 mg/L for hydrocortisone. For five different concentrations the respective mean analytical recoveries were 88.2% and 100.5%, the mean intra-assay CVs for slope 3.9% and 2.1%, and the inter-assay CVs 3.3% and 1.6%. Simultaneous measurement of hydrocortisone and its succinate ester may be useful for pharmacokinetic study. Concentration-time profiles for plasma after administration of hydrocortisone sodium succinate are presented.

Additional Keyphrases: corticosteroids - pediatric clinical chemistry

Bioavailability is not a consideration when a drug is given intravenously, because the dosage and the rate of administration can be precisely controlled. However, certain drugs are modified chemically to produce more water-soluble derivatives, and the clinical response depends on their conversion in vivo to the parent drug. Slow conversion can result in poor bioavailability of the active form of the drug.

A case in point is hydrocortisone sodium succinate, a water-soluble ester of hydrocortisone (sodium 11β,17α-dihydroxy-3,20-dioxopregn-4-en-21-yl succinate). After intravenous administration of this ester, it is hydrolyzed to the parent corticosteroid, hydrocortisone, the presumed active compound.

Studies on the pharmacokinetics of synthetic corticosteroids require specific and reproducible analytical techniques. Several methods have been described for simultaneously determining hydrocortisone and some synthetic corticosteroids: "high-performance" liquid chromatography (HPLC) (1–3) and isotope dilution–mass spectrometry (4). These same techniques can be used for simultaneously determining parent corticosteroids and their ester derivatives (5–7), but are time-consuming and expensive. Therefore we developed a rapid, reliable, and simple method for measuring concentrations of hydrocortisone and hydrocortisone succinate in plasma.

Corticosteroids play an important role in the treatment of asthma (8). We wished to determine the rate of conversion of hydrocortisone sodium succinate to hydrocortisone and to establish the pharmacokinetics of the two compounds in plasma after intravenous injection of hydrocortisone sodium succinate to asthmatic children.

Materials and Methods

Apparatus. The chromatographic apparatus consisted of a continuous-flow, constant-volume Model 6000A solvent delivery system with a U6K universal loop injector and a Model 440 absorbance detector (all from Waters Associates, Milford, MA, as "ALC Model 244"). The 4 mm × 300 mm column was packed with μBondapak C18.

Reagents. Hydrocortisone and hydrocortisone sodium succinate were from Nikken Chemicals Co., Ltd., Tokyo, Japan. Hydrocortisone succinate was from Hoechst Japan Ltd., Tokyo, Japan. Methanol, p-hydroxybenzoate-N-propyl, acetone, sodium acetate, sodium chloride, and glacial acetic acid were from Wako Pure Chemical Industries Ltd., Osaka, Japan. Solvents and all other chemicals were analytical grade.

We stored the stock solution of p-hydroxybenzoate-N-propyl in methanol (20 mg/L) at 4 °C, added 2 mL of glacial acetic acid to 10 mL of this methanolic solution, and diluted this to give a final concentration of 2 mg/L for the working solution of internal standard.

Sample treatment. Plasma was extracted with methanol as follows, which presumably denatured the native binding proteins. To 200 μL of methanol containing p-hydroxybenzoate-N-propyl as internal standard, add 100 μL of plasma sample, vortex-mix for 1 min, centrifuge at 1500 × g for 10 min, and transfer the aqueous layer to a test tube for chromatographic analysis. Inject 50 μL onto the column.

Chromatographic conditions. For reversed-phase chromatography, prepare a mobile phase by mixing 0.05 mol/L sodium acetate buffer (adjusted to pH 2.8 with acetic acid, and containing 0.1 mol sodium chloride per liter) with acetonitrile (77/23, by vol). This mixture is delivered at a flow rate of 2.5 mL/min at room temperature. Monitor the column effluent at 254 nm, a wavelength that is common to essentially all HPLC instruments containing a mercury lamp and that yields nearly optimum absorbance of several corticosteroids (1). Set the sensitivity of the detector to 0.01 A full scale, and the recorder chart speed to 5 mm/min.

Calibration and reproducibility. As a reagent for the calibration curve and analytical recovery, we used hydrocortisone succinate; hydrocortisone sodium succinate is hygroscopic and the pure material can be recrystallized only with difficulty. Methanolic solutions of hydrocortisone succinate and hydrocortisone were each diluted with distilled water to give concentrations from 0.5 to 10 mg/L. To 1 mL of the solution we added 2 mL of methanolic solution containing the internal standard (2 mg/L) and injected 50 μL onto the column. Calibration curves were plots of peak-height ratios of individual hydrocortisone succinate and hydrocortisone peaks vs the amount injected.

We evaluated analytical recovery and reproducibility by adding 100 μL of a solution containing 0.51 to 10.20 mg/L (for hydrocortisone succinate) or 0.53 to 10.68 mg/L (for hydrocortisone) to lyophilized pooled serum and assaying. Samples were taken through the whole procedure and results calculated by use of calibration curves.

Subjects and plasma sample collections. We studied three asthmatic children (13 to 14 years) admitted with asthmatic attack.

At the time of their mild to moderate asthmatic attacks, each received an intravenous injection of 5 mg of hydrocorti-
sone (as hydrocortisone sodium succinate) per kilogram of body weight without any other bronchodilators over 12 h, and corticosteroids three days before the study. No adverse effects were observed during or after the injection. Blood was sampled from an indwelling needle in the opposite antecubital vein before and 5, 10, 15, 30, 60, 120, and 240 min after the drug injection. The blood samples were dispensed into tubes containing tetrasodium EDTA as anticoagulant. The tubes were centrifuged at 1500 \( \times g \) for 10 min, and the separated plasmas were stored at \(-20^\circ C\) until analysis.

Other procedures. To compare the performance of the HPLC method with that of radioimmunoassay (RIA), we measured the concentrations of hydrocortisone in plasma samples from asthmatic children treated with intravenous hydrocortisone by a specific radioimmunoassay with a SPAC Cortisol kit (Daiichi Radioisotope Labs, Ltd., Tokyo, Japan). Antiserum to \( \alpha \)-hydroxy cortisol \( \beta \)-hemisuccinate-bovine serum albumin conjugate was raised in rabbits (9); its cross reactivity with hydrocortisone succinate was <2% (10).

**Results**

Figure 1 shows representative chromatograms for a reconstituted extract of a plasma sample to which a standard solution of anti-asthmatic drugs—theophylline, salbutamol, orciprenaline, terbutaline, noscapine, tipepideine hibenzone, \( \beta \)-hydroxybenzoate-\( N \)-propyl, hydrocortisone succinate, and bromhexine—had been added; for an extract of a blank plasma sample; and for an extract of a plasma sample from one of the asthmatic children treated with the single intravenous injection described. Blank plasma assayed by this procedure showed no peaks that might noticeably interfere with the analysis. Under the conditions specified, the respective retention times for hydrocortisone, the internal standard, and hydrocortisone succinate were 4.8, 7.2, and 8.4 min.

Typically, calibration curves for hydrocortisone succinate and hydrocortisone are linear over the concentration range studied (Figure 2). The intercepts were not significantly different from zero. The detection limit of the method was about 0.5 mg/L for hydrocortisone succinate and 0.2 mg/L for hydrocortisone. Values for hydrocortisone are corrected for blank serum response, representing endogenous hydrocortisone, the concentration of which, in a pooled specimen of serum, was calculated to be 0.10 mg/L.

As shown in Figure 3, the relationships between measured \( y \) and added \( x \) hydrocortisone succinate and hydrocortisone were linear: \( y = 0.924x - 0.085 \) \((r = 0.9992)\) for hydrocortisone succinate, and \( y = 1.001x - 0.004 \) \((r = 0.9997)\) for hydrocortisone. Mean analytical recoveries were 88.2% for hydrocortisone succinate, 100.5% for hydrocortisone. The intra-assay CVs were 3.9% for hydrocortisone succinate, and 2.1% for hydrocortisone. Inter-assay CVs for 10 plasma samples with concentrations of 2.5 mg/L were 3.3% for hydrocortisone succinate, 1.6% for hydrocortisone.
We measured hydrocortisone in 13 plasma samples by both RIA (y) and HPLC (x). The regression equation for the results was: \( y = 0.913x + 0.300; r = 0.929 \) (Figure 4).

Figure 5 shows data for a typical concentration–time curve after intravenous administration of hydrocortisone sodium succinate to the three asthmatic children. Table 1 summarizes the peak plasma concentrations and biological half-lives. The mean peak concentration attained by hydrocortisone succinate was 27.53 (SD 1.83) mg/L, ranging from 25.87 to 29.49 mg/L. The concentration in plasma decreased rapidly, with a mean half-life of 5.40 (SD 0.60) min, range 4.76 to 5.94 min. The mean concentration of hydrocortisone in plasma increased to a peak value of 4.21 (SD 0.92) mg/L, range 3.51 to 5.26 mg/L after 10 min, then decreased gradually with a mean half-life of 84.8 (SD 22.6) min (range 68.3 to 110.5 min).

### Discussion

Our procedure is rapid, specific, and reproducible. Of the several procedures described for extracting corticosteroids from plasma, all recent methods are relatively time-consuming, involving several solvent-extraction steps before liquid-chromatographic quantification. In the present method, plasma samples were simply extracted once with methanol. The choice of p-hydroxybenzoate-N-propyl as internal standard, which has a retention time between those of the two compounds being measured, also contributes to the rapid analysis time. The detection limits were not adequately sensitive to monitor hydrocortisone at the low concentrations normally found in plasma. However, our method allows simultaneous assay of the drug and its metabolite in plasma after injection. It is sensitive enough for routine application in pharmacokinetic studies and drug monitoring. Indeed, a hydrocortisone peak appears in chromatograms of plasma samples as the hydrocortisone succinate concentration approaches its maximum and then decreases rapidly. This confirms the observation of Smith (6), and provides further evidence of the metabolism of hydrocortisone succinate to hydrocortisone.

Although relatively large doses of corticosteroids are usually recommended for the treatment of acute severe asthma, the precise optimal amount required has not been clearly delineated (8). Early studies recommended the hydrocortisone concentration of 1 mg/L in plasma as the minimum necessary to achieve a therapeutic response. The Section on Allergy and Immunology of the American Academy of Pediatrics recommended (11) that an initial loading dose of corticosteroid equivalent to 1–2 mg of prednisone per kilogram of body weight be administered intravenously, followed by an equivalent dosage over the next 24 h. Elimination half-lives of hydrocortisone in plasma in the present study were in agreement with values found by other investigators (12 13). The hydrocortisone concentrations in plasma could be kept above 1 mg/L for >2 h after the administration. These findings indicate that adequate concentrations of the drug in plasma can be achieved by an intravenous injection of hydrocortisone at 5 mg/kg body weight. Further study is needed to elucidate pharmacodynamics (the relationship between hydrocortisone concentration in plasma and anti-asthmatic effects) in the treatment of acute severe asthma.

### Table 1. Pharmacokinetic Parameters of Hydrocortisone Succinate and Hydrocortisone in Plasma after Administration of Hydrocortisone Sodium Succinate (Hydrocortisone at 5 mg/kg Body Weight)

<table>
<thead>
<tr>
<th>Patient's age (years)</th>
<th>Hydrocortisone succinate</th>
<th>Hydrocortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak concn, mg/L</td>
<td>Half-life, min</td>
</tr>
<tr>
<td>13 M</td>
<td>27.23 (SD 0.5)</td>
<td>4.21 (SD 0.3)</td>
</tr>
<tr>
<td>14 M</td>
<td>25.67 (SD 0.5)</td>
<td>5.94 (SD 0.5)</td>
</tr>
<tr>
<td>13 M</td>
<td>29.49 (SD 0.5)</td>
<td>5.50 (SD 0.5)</td>
</tr>
<tr>
<td>Mean</td>
<td>27.53 (SD 0.5)</td>
<td>5.40 (SD 0.5)</td>
</tr>
<tr>
<td>SD</td>
<td>1.83 (SD 0.5)</td>
<td>0.60 (SD 0.5)</td>
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References