Simultaneous Measurement of Cortisol in Serum and Saliva after Different Forms of Cortisol Administration

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To prove the clinical usefulness of cortisol measurements in saliva for the exact assessment of a patient's corticoid status under therapeutic hormone substitution, we measured simultaneously total cortisol in serum and non-protein-bound cortisol in saliva after administration of different forms of hydrocortisone (cortisol) in eight cortisol-suppressed, healthy male volunteers. The intravenous and oral administration of 20 mg of cortisol exceeds the binding capacity of the corticosteroid-binding globulin (CBG), leading to an increase of the ratio between salivary and serum cortisol at the higher cortisol concentrations in blood. After rectal administration of 100 mg of cortisol acetate, the serum cortisol concentration does not exceed the binding capacity of CBG, so the ratio between salivary and serum cortisol remains nearly constant. However, this ratio was higher after rectal administration than after intravenous and oral administration, probably because of weaker binding of the acetate form of cortisol to CBG. Thus, the salivary measurement of the non-protein-bound (i.e., biologically active) cortisol offers a convenient way to monitor the effectiveness of various forms of systemic corticoid substitution.

Additional Keyphrases: corticosteroid-binding protein, variation, source of

Only the small fraction of cortisol in blood that is not bound to serum proteins such as corticosteroid-binding globulin (CBG) and albumin (1–15%) is considered biologically active (1). CBG binds cortisol with high affinity but low binding capacity, whereas albumin binds cortisol with low affinity but high binding capacity (2). Non-protein-bound, i.e., biologically active, cortisol enters saliva predominantly intracellularly (3). The cortisol concentration in saliva is independent of saliva flow rate and closely reflects the concentration of non-protein-bound cortisol in blood (4, 5), probably because of the absence of substantial amounts of CBG or albumin in saliva (6). In contrast to the measurement of cortisol in saliva, the measurement of non-protein-bound cortisol in serum requires a sophisticated technique that is not suitable for routine use (7). Thus, the measurement of salivary cortisol offers a convenient way to monitor the concentrations of biologically active cortisol in serum. In fact, various clinical investigations demonstrate that salivary cortisol measurement is a practical and convenient approach to assessing pituitary-adrenal function (5, 8).

Thus far, however, in comparison with our knowledge of serum cortisol concentrations, the manner in which the salivary cortisol concentration is immediately altered after various systemic application forms of cortisol has not been determined. Our objective in the present study was to monitor for as long as 480 min after different forms of systemic cortisol administration the cortisol concentrations in saliva and serum simultaneously.

Materials and Methods

Eight healthy male volunteers, mean age 28.4 (SD 8.6) years gave informed consent to participate in this study. On three different days at 0800, the volunteers took cortisol orally with some water [two 10-mg tablets of hydrocortisone (Hoechst, Frankfurt, FRG)] or intravenously [20 mg of hydrocortisone (Hoechst) dissolved in 4 mL of ethanol, then diluted to 20 mL with isotonic saline (NaCl 9 g/L) and delivered over 2 min into the arm not used for collecting blood], or rectally (100 mg of hydrocortisone acetate (Colifem®; Trommsdorff, Alsdorf, FRG). Endogenous cortisol production was suppressed by oral administration of 4 mg of dexamethasone (a dose that suppresses cortisol values to <10 μg/L) at 2200 h the day before the study.

Collection of samples. At least 1 h before blood sampling, an intravenous catheter was inserted into the cubital vein. The first blood sample was drawn just before the administration of cortisol. The other blood samples were obtained 10, 20, 30, 60, 120, 240, 360, and 480 min after the administration of cortisol. Each blood sample was centrifuged at 4°C, and the aspirated serum was stored at −20°C until hormone analysis.

Saliva samples, obtained without stimulation of salivary flow, were collected just before each blood sample, except for the sample at time zero. For this sample we used a "Salivette" device (Sarstedt, Nümbrecht, FRG). Without rinsing the mouth with water, small cellulose tampons were held in the mouth for 5 min to soak up the saliva and then were placed in the Salivette container, which was suspended inside a capped centrifuge vessel. After centrifugation of the tampons at 1800 × g for 10 min, about 1–2 mL of nonviscous, easily pipettable saliva was collected at the bottom of the centrifuge vessel. Cortisol does not adsorb to the cellulose tampons (9). All saliva samples were stored at −20°C until hormone analysis.

Cortisol assay. Serum and saliva cortisol concentrations were determined with a competitive solid-phase radioimmunoassay, applicable for measurements in se-

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rum and saliva (Cost-A-Count, Diagnostic Products Corp., obtained through H. Biemann GmbH, Bad Nauheim, FRG). We used nonextracted saliva in the assay because of the strong correlation between cortisol concentrations in extracted and nonextracted saliva, as measured with various kits (10, 11), including the kits in this study (P. Bodlaender, personal information). The unknown samples (25 µL of serum or 200 µL of saliva) were incubated for 45 min (serum) or overnight (saliva) with 125I-labeled cortisol in tubes coated with anticortisol antibodies. After aspirating the liquid from each tube, we measured the radioactivity in the tubes with a gamma counter (Multi-Crystal Counter LB 2104; Berthold, Bad Wildbad, FRG). The concentrations of the unknown samples were computed from standard curves (linear-log transformation). The between-assays coefficient of variation was 6.3% in serum at a mean cortisol concentration of 48 µg/L and 4.5% in saliva at a mean concentration of 5 µg/L (n = 8 each). Cortisol standards for serum and salivary cortisol assays were used in concentration ranges of 10–500 and 1–50 µg/L, respectively. The cross-reactivity of the assay for cortisone, cortisol acetate, and cortisone acetate was <1.0%, 4.7%, and <1.0%, respectively.

Pharmacokinetic analysis. The elimination rate constant ke was determined by linear regression of the terminal phase of a semilogarithmic plot of serum or salivary cortisol concentrations as a function of time. The half-life was calculated as 0.693/ke (12).

Statistics. The significance of correlation between serum and salivary cortisol concentrations was calculated by Spearman rank correlation coefficient.

Results

Figure 1 shows the time sequences of cortisol concentration in serum and saliva after intravenous, oral, and rectal administration of cortisol. After intravenous administration of 20 mg of cortisol (Figure 1A), serum and salivary cortisol show nearly identical first-order elimination kinetics. Salivary cortisol concentrations ranged from 18.2% to 2.3% of the serum values. The mean (SD) half-life of serum and salivary cortisol was 102 (30) and 72 (12) min, respectively. After oral administration of 20 mg of cortisol (Figure 1B), maximum serum concentrations of cortisol, 305 (SD 57) µg/L, were observed after 72 (SD 24) min. Salivary cortisol concentrations were relatively high over the first 30 min after administration (101.6–28.1% of the total cortisol values), probably because of residual contamination of the saliva by the ingested tablets. After this early period, salivary cortisol values ranged between 8.3% and 1.3% of the serum values. The half-life of serum and salivary cortisol was 108 (SD 30) and 66 (SD 6) min, respectively. After rectal administration of 100 mg of cortisol acetate (Figure 1C), serum cortisol values were generally low [maximum: 35 (SD 24) µg/L after 126 (SD 54) min]. However, salivary values were a relatively greater proportion of the serum values (27.6–9.3%) as compared with the proportion after the other modes of administration. The half-life of serum and salivary cortisol was distinctly higher than after intravenous and oral administration. However, the interindividual scattering of these values was relatively high. Therefore, mean values are not given.

Figure 2 shows scatterplots of the paired salivary-serum cortisol values after intravenous, oral, and rectal administration. Despite the significantly positive correlation between serum and salivary concentrations (P < 0.001) after intravenous and oral administration of cortisol (Figures 2A, B), the relationship over the whole concentration range was not linear. Rather, the plots displayed a marked change in slope at a serum concentration of ~200 µg/L (550 nmol/L) for cortisol. After rectal administration of cortisol acetate (Figure 2C), the ratio between salivary and serum values remained nearly constant.

Discussion

Concentrations of unbound, i.e., biologically active cortisol in blood are well known to vary with age and other clinical circumstances. Thus, the best assessment of the actual corticoid status of a patient will be obtained by measuring the unbound cortisol fraction. Given the lack of knowledge regarding the unbound fraction of cortisol shortly after systemic corticoid substitution, we
The decrease of serum and salivary cortisol after its intravenous and oral administration is greater than after rectal administration. This observation is confirmed by the half-life of cortisol in saliva and serum, being lower after intravenous and oral administration than after rectal administration. This is probably due to differences in the metabolism of cortisol and of its acetate form. Furthermore, the 1.6 times shorter half-life of salivary cortisol in comparison with serum cortisol confirms that cortisol in saliva represents the non-protein-bound fraction of this hormone (4, 5).

In this study, the relationship between salivary and serum cortisol concentration after both the intravenous and oral administration of cortisol displayed a clear-cut change in slope at ~200 μg/L (550 nmol/L) for cortisol in serum. At this cortisol concentration, the binding capacity of CBG in blood of healthy volunteers is completely saturated (2). Thus, higher cortisol concentrations exceed the binding capacity of CBG, leading to a disproportionately high increase of the cortisol concentration in saliva. Indirectly, this phenomenon is confirmed by studies with normal women taking oral contraceptives and with pregnant women, in whom an estrogen-dependent increase of CBG is well known. Compared with the case for males, in these women the time-matched samples of saliva and serum after intravenous administration of cortisol sodium succinate showed lower salivary cortisol concentrations at serum concentrations >200 μg/L (6).

After rectal administration of cortisol acetate, however, the serum cortisol concentration does not exceed the binding capacity of CBG. Consequently, the ratio between salivary and serum cortisol remains nearly constant over the whole period investigated—although the ratio between salivary and serum concentrations of cortisol after cortisol acetate administration was distinctly higher than after intravenous and oral administration of cortisol. We speculate that the acetate group at C-21 causes weaker CBG binding, as has been shown for testosterone (15).

References

Fig. 2. Relationship between serum and salivary cortisol concentrations in eight healthy male volunteers after intravenous (A), oral (B), and rectal (C) administration of cortisol. In Fig. 2B, only the values after 30 min after cortisol administration are presented.

Therefore measured cortisol simultaneously in serum and saliva after different forms of application. We measured salivary cortisol because in saliva only unbound cortisol is thought to be present (4, 5)—although small amounts of CBG have been determined in parotid fluids collected at resting flow rates (13).

Our study with corticoid-suppressed volunteers clearly indicated that the cortisol concentration in saliva and serum showed a nearly identical time course over the whole period tested after intravenous administration of cortisol. This is in line with the observation that cortisol in saliva appears within 5 min after an increase of cortisol in blood (5). Furthermore, 30 min after oral administration of cortisol, salivary cortisol and serum cortisol again showed a nearly identical time course. Finally, after rectal administration of cortisol acetate, serum cortisol values were about 10-fold less than after oral administration, even though the rectal dose was fivefold greater than the oral dose. This relatively low cortisol concentration in blood is probably attributable to a lower absorption of the acetate form of cortisol; a similar study of rectal administration of twice the amount of cortisol showed 10-fold greater serum cortisol concentrations (14).
Intact Parathyroid Hormone: Performance and Clinical Utility of an Automated Assay Based on High-Performance Immunoaffinity Chromatography and Chemiluminescence Detection

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The performance and clinical utility of an automated assay of intact parathyroid hormone (parathyrin, PTH) are evaluated. The method is based on the extraction of PTH from plasma by an HPLC column containing immobilized anti- (44–68 PTH) antibodies. The PTH retained is detected with a postcolumn reactor and use of anti- (1–34 PTH) chemiluminescent-labeled antibodies. The total cycle time of the assay is 6.5 min per injection after a 1-h incubation. The lower limit of detection for PTH in a 66-μL plasma sample was 0.5 pmol/L based on peak heights and 0.2 pmol/L based on peak areas. Mean analytical recovery for PTH added to plasma was 97%. The within-day precisions (CVs) for 4.2 and 30 pmol/L PTH plasma samples were 9.2% and 5.6% and the day-to-day precisions were 10.3% and 5.7%, respectively. No significant interferences from 1–34, 44–68, or 53–84 PTH fragments were noted, even at highly increased concentrations of fragments. The correlation of results with those of a manual assay of intact PTH was 0.97, and the results showed good agreement with disease state for patients with hypo- or hyperparathyroidism. The specificity of the assay for primary hyperparathyroidism was >95%. We discuss the advantages (speed and quality control) of this approach over current immunoassays and the potential use of this method for detecting other analytes.

Additional Keyphrases: hyperparathyroidism • chronic renal failure • hypercalcemia of malignancy

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3 Nonstandard abbreviations: BSA, bovine serum albumin; HPIAACL, high-performance immunoaffinity chromatography with chemiluminescence detection; ICMA, immunochemilumimetric assay; and PTH, parathyrin (parathyroid hormone).

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The measurement of parathyrin (parathyroid hormone; PTH), an 84-amino-acid peptide produced by the parathyroid glands, is a useful tool in the diagnosis of calcium disorders. The main function of PTH is to control the concentration of calcium in extracellular fluids, with the excretion of PTH increasing as the calcium concentrations decrease (1, 2). Normal concentrations of intact PTH in plasma range from ~1 to 5 pmol/L, with both the increased and decreased values being of clinical interest (2, 3). Besides intact PTH, various fragments representing the N-terminal, midmolecule, and C-terminal regions of PTH are also found in blood. Most of these fragments are formed after release of intact PTH into the circulation, but some are also produced within the parathyroid glands (4). Both intact PTH and its N-terminal fragment are biologically active, with intact PTH being the predominant active form under normal conditions (2).

PTH assays are used clinically in the differential diagnosis of disorders that produce hypercalcemia or hypocalcemia (e.g., primary hyperparathyroidism or hypercalcemia of malignancy) and in monitoring bone damage in patients with chronic renal failure (1, 2). The pathology and clinical importance of these disorders have been previously discussed (1–6).

Numerous methods are available for the clinical measurement of PTH in these disorders. These are typically based on immunoassays with antibodies directed toward the N-terminal, midmolecule, or C-terminal regions of PTH. One factor complicating the use and comparison of these assays is that they have different degrees of response to intact PTH and its circulating fragments (1, 2). Several methods for monitoring only intact PTH were recently reported, based on two-site immunometric assays (7–9). In these assays two sets of antibodies are used: one set is immobilized onto a solid support and...