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Correlates of Plasma Cortisol and DNA Repair in Human Peripheral Lymphocytes: Suppression of Repair in Women Taking Estrogen

Key Words
DNA repair
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Abstract
We have examined the relationship between total plasma cortisol concentration and DNA repair capacity in human peripheral lymphocytes cultured in vitro; the data indicate that high concentrations of cortisol (>20 μg/dl) inhibit DNA repair. The inhibitory effect can be abrogated by the addition of RU38486, a cortisol antagonist. In addition, we compared plasma cortisol concentration and in vitro DNA repair capacity in 52 healthy individuals. Females on therapeutic estrogen (oral contraceptive or estrogen replacement therapy) had significantly elevated plasma cortisol and suppression of DNA repair capacity.

Introduction

Cortisol, the major human glucocorticoid, affects almost every organ system in the body, as evidenced by the presence of glucocorticoid receptors on every nucleated cell [1]. Physiological concentrations of the hormone are required for modulation of metabolism. Cortisol insufficiency or excess can lead to the development of disease [2].

Since cortisol levels are increased during physical, physiological, or psychological stress, we have investigated the effects of cortisol on DNA repair capacity measured by an in vitro assay in human peripheral lymphocytes. We have also examined the relationship between DNA repair capacity in vitro and total plasma cortisol concentration in healthy males and females. An association between serum estrogen and cortisol concentrations is observed in pregnant women [3] or nonpregnant women who have been administered estrogen [4]. Since high estrogen levels in these individuals result in elevated plasma cortisol concentrations, we included females taking therapeutic estrogen.

Genetic repair systems insure that aberrations in DNA resulting from exposure to chemically or physically damaging agents are repaired. The integrity of DNA must be maintained to allow replication and transcription to proceed normally. Unrepaired or misrepaired lesions can become the somatic mutations that lead to the initiation of carcinogenesis [5–7]. Hence, inhibition of repair processes can have deleterious effects.

DNA repair capacity was assayed in isolated human lymphocytes damaged by ultraviolet radiation. Repair of

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UV-induced lesions was measured by incorporation of tritiated thymidine during unscheduled DNA synthesis. Plasma cortisol concentration was quantitated by radioimmunoassay.

DNA repair experiments were performed in a way which approximates as closely as possible the in vitro environment of the cells [8]. Lymphocytes were isolated and exposed to mutagenic UV within 2 h of collection, to avoid the changes in cells which can occur during long-term culture in vitro (e.g. fibroblasts). Cells were cultured in autologous plasma to avoid artifacts which can result when they are transferred to different concentrations of biological response modifiers from those present in the plasma from which the cells were obtained. Previous work in our laboratory has shown that this is particularly important when one is looking for correlations between DNA repair capacity and hormone levels among individuals [8]. The mutagen used causes specific DNA damage as opposed to chemical carcinogens which can also damage enyzymes, proteins, and the cell membrane.

Materials and Methods

Tissue Acquisition and Lymphocyte Isolation

Patients scheduled for health maintenance examinations at the Anderson Family Practice were selected as research subjects. Seven patients were used for the hydrocortisone dose-reponse experiments. Fifty-two patients comprised the comparison study of in vitro DNA repair capacity and plasma cortisol concentration. Male and female donors ranged in age from 19 to 65. Female subjects were not pregnant. All donors were free of infectious, neoplastic, and autoimmune disease.

Venous blood was collected between 10 and 12 a.m. from healthy donors by antecubital venipuncture in a sodium heparin vacutainer. Mononuclear cells were separated by layering whole blood over polysucrose-sodium diatriazoate gradients (Histopaque 1077, Sigma Chemical Co.). The cells were washed with phosphate-buffered saline (PBS) and the volume adjusted to a concentration of 2×10^6 cells/ml.

In vitro DNA Repair Assay

Cell suspensions were divided into aliquots. Each aliquot was irradiated for a different time with a GE 15T8 germicidal lamp that emitted ultraviolet (UV) radiation having an intensity maximum at 254 nm. The fluence rate was 1 W/m^2. Doses of 0, 10, 20, and 30 J/m^2 were used.

The aliquots were centrifuged and the cell pellets resuspended in medium containing 15% autologous plasma, 1% gentamycin sulfate, 5 μgCi/ml ^{3}H-thymidine, and 4 m*M* hydroxyurea. Over 97% of the unstimulated mononuclear cells were in the G_0 phase of the cell cycle [9]. Hydroxyurea inhibits any residual replicative synthesis.

Hydrocortisone Dose-Response Experiments. In seven experiments, the medium was supplemented with concentrations of cortisol (hydrocortisone 21-hemisuccinate, Sigma) ranging from 2.5 to 60 μg/dl. All steroids were dissolved in a 4% solution of dimethyl sulfoxide.

In five experiments, RU38486 (mifepristone, Roussel-Uclaf), a cortisol antagonist, was added to one of the aliquots supplemented with 40 μg/dl hydrocortisone to give a final RU38486 concentration of 400 μg/dl. Another aliquot to be used as a control was supplemented with RU38486 alone to verify that there was no agonistic activity. All samples were incubated for 4 h at 37°C in an atmosphere of 5% CO_2.

Following incubation, the samples were centrifuged at 200 g for 10 min. Cell pellets were washed twice with PBS. Lysing solution (2% sodium lauryl sulfate, 0.1 *M* EDTA, and 2.0 *M* NaOH) was added and the mixture allowed to sit at room temperature overnight. Formaldehyde was added to a final concentration of 2% and the pH adjusted to approximately 7 as determined by a phenol red indicator. Each sample was diluted with 10 ml PBS and filtered through a 0.22 μm nitrocellulose filter (BA85, Schleicher & Schuell). The filters were air-dried and analyzed by scintillation spectrometry. Duplicate values were averaged for each aliquot. DNA repair capacities (C_{max}) were calculated by subtracting from the amount of radioactivity incorporated at the saturating UV dose (30 J/m^2) that incorporated by nonirradiated cells.

Comparison Studies of in vitro Repair Capacity and Plasma Cortisol Concentration. DNA repair assays were performed as described above but cultures were not supplemented with hydrocortisone or RU38486. Aliquots of plasma from these assays were frozen for later analysis of cortisol.

Total plasma cortisol concentration was measured by radioimmunoassay, using a method [10] that eliminated the ether extraction step. Plasma samples and standards (100 μl) were heated for 30 min at 70°C to remove corticosteroid-binding globulin (CBG), the major plasma binding protein for cortisol. After cooling, 200 μl of 1:800 cortisol antiserum (Endocrine Sciences) and 100 μl dual-labeled tritiated cortisol ([1,2-H(N)], New England Nuclear, sp. act. 80–100 Ci/m*M*) were added to each tube. The unbound portion of antiserum was removed by charcoal absorption. The samples were analyzed by scintillation spectrometry. Averages of triplicate values were used to construct standard curves by logit-log analysis. Assay sensitivity was 0.125 μg/dl.

Statistical Analyses

Simple linear regression analyses and Pearson correlation coefficients were calculated to determine the relationships between variables. Two-tailed Student's t test was used to determine differences between means of unequal variances.

Results

Effects of Hydrocortisone on DNA Repair in Cultured Lymphocytes

A dose-dependent relationship was observed between DNA repair capacity and the amount of hydrocortisone added to the medium (fig. 1). Although low concentrations of added cortisol increased DNA repair capacity, significant decreases in DNA repair were noted when cortisol concentrations were 20 μg/dl or above.

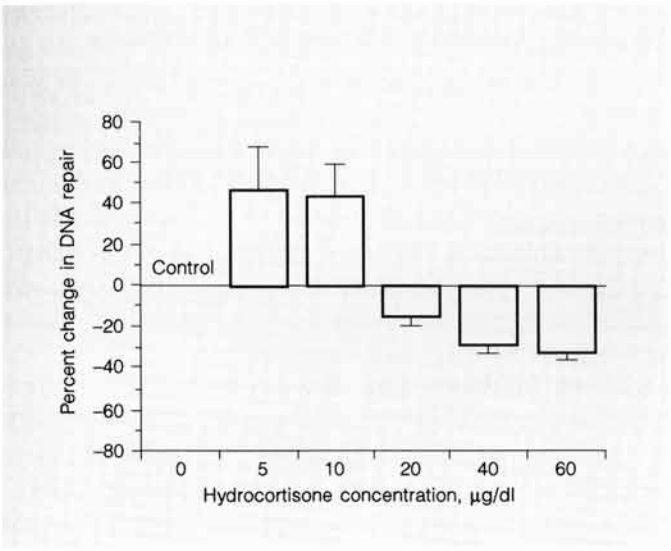

Fig. 1. Dose response of exogenously added hydrocortisone on DNA repair capacity (Cmax) in human peripheral lymphocytes. Each value represents the mean percentage change ± SEM of repair capacity relative to the control in 3–5 donors.

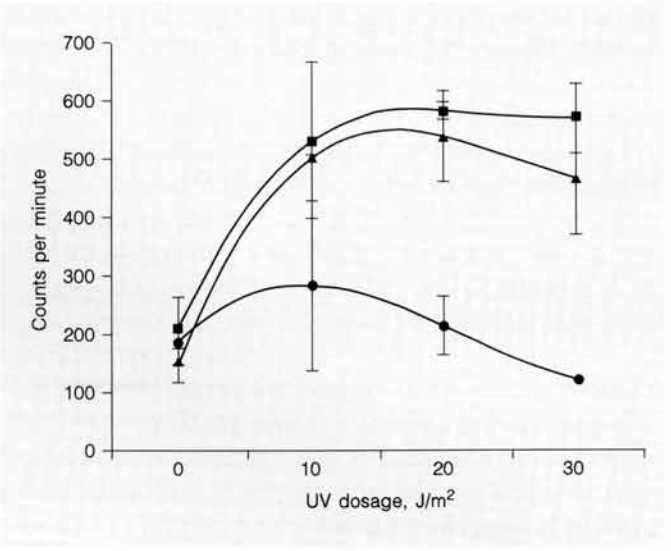

Fig. 2. RU38486 antagonism of hydrocortisone (●) during DNA repair of pyrimidine dimers in human peripheral lymphocytes. UV-damaged lymphocytes were allowed to perform repair in medium with no hydrocortisone (■), in medium supplemented with 40 µg/dl HC, and in medium supplemented with 40 µg/dl HC and 400 µg/dl RU38486 (▲).

The cortisol antagonist RU38486 abrogated the inhibitory effect of cortisol. The results of a typical experiment are given in figure 2. In this experiment, a 74.8% decrease in DNA repair capacity was measured for UV-irradiated mononuclear cells cultured in 40 µg/dl hydrocortisone. Addition of 400 µg/dl RU38486 almost completely restored repair capacity. RU38486 alone had no effect on DNA repair.

Plasma Cortisol Concentration and DNA Repair Capacity in Females and Males

We measured total plasma cortisol in healthy male and female donors by radioimmunoassay. Although free plasma cortisol is the biologically active form, total plasma cortisol (free and bound) is reported more often clinically [11]. To minimize the variability caused by the circadian and episodic secretory patterns of cortisol [12], we confined the time of sampling to a 2-hour period in the late morning [10:00 a.m. to 12:00 noon).

Females not on estrogen therapy had a mean total plasma cortisol concentration similar to that of males. The values ranged from a minimum of 4.4 µg/dl to a maximum of 10.5 µg/dl (table 1). In addition, the DNA repair capacity values observed for males and for females not on estrogen were not significantly different. In contrast, females on therapeutic estrogen (7 on oral contraceptive pills, 2 on estrogen replacement therapy) had a significantly elevated plasma cortisol concentration (range 13.1–28.9 µg/dl) and a significantly decreased DNA repair capacity when compared with females not on estrogen therapy.

Regression analyses between DNA repair capacity and total plasma cortisol concentrations were performed for groups categorized according to age and gender (table 2). For this comparison, females on estrogen therapy were not included. When data for females were combined, the correlation between DNA repair capacity and total plasma cortisol concentration was positive. Young females (premenopausal) demonstrated an even stronger correlation. In older females (perimenopausal and postmenopausal), this relationship is not exhibited. No significant correlation was observed when data for all males or for only young males were analyzed. However, older males demonstrated a weakly significant ($p < 0.10$) negative correlation.

Table 1. Total plasma cortisol concentration and DNA repair capacity in males, females not on estrogen, and females on oral contraceptive or estrogen replacement therapy

	Males	Females not on estrogen	Females on OCP or ERT
Donors	22	21	9
Mean total plasma cortisol concentration µg/dl	7.8 (0.51)	7.5 (0.43)	20.9 (2.01)*
Mean DNA repair capacity, cpm	353.7 (43.7)	351.1 (35.6)	242.1 (39.5)**

OCP = Oral contraceptive pill; ERT = estrogen replacement therapy.
* $p < 0.0001$ and ** $p < 0.054$ vs. females not on estrogen.

Table 2. Correlation coefficients for DNA repair capacity versus total plasma cortisol concentration in females and males of different age groups

Donors	Samples	Correlation between DNA repair capacity and total plasma cortisol, r	Significance p
Females[1]			
Total	21	0.47	0.03
Age 19–39	12	0.62	0.03
Age 40–65	9	0.17	0.67
Males			
Total	22	−0.22	0.33
Age 19–39	10	0.06	0.86
Age 40–63	12	−0.52	0.08

[1] Females on estrogen therapy were not included.

Discussion

Our results provide evidence that cortisol affects DNA repair in human peripheral lymphocytes cultured in vitro in a dose-dependent fashion. Low concentrations were associated with increased DNA repair capacity. Concentrations of cortisol exceeding 20 µg/dl resulted in significant inhibition of DNA repair.

Glucocorticoids have been reported to have dual effects on other biological phenomena. Lymphocyte proliferation is enhanced at low glucocorticoid concentrations and inhibited at high concentrations in vitro and in vivo [13, 14]. Low concentrations of glucocorticoids enhance Il-2 production by activated human peripheral blood leukocytes; high concentrations inhibit Il-2 production [1].

Abrogations of the inhibitory effect by RU38486 indicates that the modulation involved is possibly mediated via the glucocorticoid receptor. The binding of cortisol and its receptor to gluococorticoid-responsive elements enhances or suppresses transcription [15]. RU38486 is thought to prevent the translocation of the activated cortisol-receptor complex into the nucleus [16].

Several feedback mechanisms insure that elevated plasma cortisol levels sustained during secretory bursts or stress responses are transient. During pregnancy, increased estrogen levels cause an increased production of

CBG. However, cortisol concentrations exceed the binding capacity of CBG [11]. In addition, decreased clearance of cortisol results in elevated levels of free plasma cortisol throughout pregnancy [17, 18]. Yet, pregnant women do not manifest overt symptoms of hypercortisolemia. It is postulated that maternal tissues become insensitive to cortisol [18]. But despite maternal tissue refractoriness to the influence of cortisol, there is a glucocorticoid-associated lymphocytopenia which is restored at approximately 20 weeks [19]. In addition, certain decreases in cell-mediated immunity suggest that the hypercortisolemia may have an effect in some pregnant women [19]. Suppression of DNA repair in pregnant women has been reported [Skoner, J.S., Larcom L.L., unpubl. data].

High estrogen states are artificially induced in nonpregnant women with oral contraceptive or estrogen replacement therapy. Mean cortisol levels are greatly increased in women on oral contraceptive therapy [4]. Plasma cortisol levels of 40–60 μg/dl have been reported [20]. Our in vitro studies indicate that these concentrations significantly inhibit DNA repair, indicating that patients on oral contraceptive therapy may be more likely to experience the inhibitory effects of elevated cortisol on DNA repair processes.

Inhibition of DNA repair processes can have deleterious effects. DNA lesions induced by physical and chemical agents are the initiating events for carcinogenesis [6, 7, 21]. Enzymatic repair of these lesions is essential to protecting the organism from the somatic mutations that produce cancer. Suppression of DNA repair capacity in combination with the promoting effects of high estrogen levels may place these individuals at risk for the development of neoplasia.

Birth control pills have been associated with increased risk of developing endometrial, hepatic, and breast cancer [22, 23]. Recent epidemiological studies have indicated a significant rise in breast cancer incidence in women [24, 25]. Although several studies have refuted an association between oral contraceptives and the development of breast cancer [26], other more current studies indicate a significant relationship [24, 27, 28].

We found a significant positive correlation between in vitro DNA repair capacity and cortisol concentration in premenopausal women. This effect may be indirectly related to estrogen levels since we did not find this relationship in perimenopausal and postmenopausal women, or men.

In conclusion, our data provide evidence that cortisol affects DNA repair capacity in human peripheral lymphocytes in vitro. Cortisol concentrations of 20 μg/dl or greater appear to inhibit DNA repair. In addition, women on therapeutic estrogen have significantly elevated plasma cortisol concentration and suppressed ability to repair genetic lesions.

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References

1 Munck A, Guyre PM, Holbrook NJ: Physiological function of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev 1984;5:25–44.

2 Feldman D: Mechanisms of action of cortisol; in DeGroot LJ, Martini L (eds): Endocrinology. Philadelphia, Saunders, 1989, pp 1557–1571.

3 Jaffe RB: Integrative maternal-fetal endocrine control systems; in Yen S, Jaffe RB (eds): Reproductive endocrinology. Philadelphia, Saunders, 1986, pp 770–778.

4 Van Cauten E, Golstein J, Vanhaelst L, Leclerq R: Effects of oral contraceptive therapy on the circadian patterns of cortisol and thyrotropin (TSH). Eur J Clin Invest 1975;5:115–121.

5 Friedman EC: DNA Repair. New York, Freeman, 1986.

6 Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, Vogelstein B, Kinzler KW: APC mutations occur early during tumorigenesis. Nature 1992;359:235–237.

7 Bishop JM: Molecular themes in oncogenesis. Cell 1991;64:235–248.

8 Larcom LL, Smith ME: Quantitative assay for evaluation immunological competence and DNA repair capacity. J Natl Cancer Inst 1988; 80:1112–1118.

9 Pero RW, Ostlund C: Direct comparison in human lymphocytes of the interindividual variations in unscheduled DNA synthesis induced by acetoxy-2-acetyl-aminofluorene and UV radiation. Mutat Res 1980;73:349–357.

10 Henricks DM, Cooper JW, Spitzer JC, Grimes LW: Sex differences in plasma cortisol and growth in the bovine. J Animal Sci 1984;59: 376–383.

11 Meikle AW: Secretion and metabolism of the corticosteroids and adrenal function and testing; in Degroot LJ (ed): Endocrinology. Philadelphia, Saunders, 1989, vol 3, pp 1610–1632.

12 Weitzman ED, Fukushima D, Nogeire C: Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. J Clin Endocrinol Metab 1971;33:14–22.

13 Cupps TP, Fauci AS: Corticosteroid-mediated immunoregulation in man. Immunol Rev 1982;65:133–155.

14 Ritter MA: Embryonic mouse thymocyte development: Enhancing effect of corticosterone at physiological levels. Immunology 1977;33: 241–246.

15 Beato M: Gene regulation by steroid hormones. Cell 1989;56:335–344.

16 Moguilewsky M, Philibert D: RU38486: Potent antagonistic activity correlated with strong binding to the cytosolic receptor followed by an impaired activation. J Steroid Biochem 1984; 20:271–276.
17 Rosenthal HE, Slaunwhite WR, Sandbert AA: Transcortin: A corticosteroid-binding protein of plasma: Cortisol and progesterone interplay and unbound levels of these steroids in pregnancy. J Clin Endocrinol Metab 1969;29:352–355.
18 Nolten WE, Linheimer MD, Rueckert PA, Oparil S, Ehrlich EN: Diurnal patterns and regulation of cortisol secretion in pregnancy. J Clin Endocrinol Metab 1980;51:466–472.
19 Mitchell MS, Capizzi RL: Neoplastic diseases; in Burrow GN, Ferris TF (eds): Medical complications during pregnancy. Philadelphia, Saunders, 1982.
20 Felig P, Baxter JD, Broadus AE, Frohman LA (eds): The adrenal cortex; in Endocrinology and Metabolism. New York, McGraw-Hill, 1987, chapter 12.
21 Lehmann AR: Ageing, DNA repair of radiation damage and carcinogenesis; in Likhachev V, Anisimov V, Montesano R (eds): Age-Related Factors in Carcinogenesis. New York, Oxford University Press, 1985, pp 203–213.
22 Henzl MR: Contraceptive hormones and their clinical use; in Yen SC, Jaffe RR (eds): Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management. Philadelphia, Saunders, 1986, pp 643–682.
23 Henderson BE, Ross RK, Pike MC: Hormonal chemoprevention of cancer in women. Science 1993;259:633–638.
24 Norman C: Search for a killer: Focus shifts from fat to hormones. Science 1993;259:618–621.
25 Devesa SS, Silverman DT, Young JL, Pollack ES, Brown CC, Horm JW, Percy CL, Myers MH, McKay FW, Fraumeni JF: Cancer incidence and mortality trends among whites in the United States, 1947–84. J Natl Cancer Inst 1987;79:701–770.
26 Prentice RL, Thomas DB: On the epidemiology of oral contraceptives and disease. Adv Cancer Res 1987;49:285–401.
27 Miller DR, Rosenberg L, Kaufman DW, Warshauer ME, Shapiro S: Breast cancer before age 45 and oral contraceptive use: New findings. Am J Epidemiol 1989;129:269–280.
28 Olsson H, Ranstam MA, Baldetorp B, Ewers SB, Ferno M, Killander D, Sigurdsson H: Proliferation and DNA ploidy in malignant breast tumors in relation to early oral contraceptive use and early abortions. Cancer 1991;67:1285–1290.