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Cortisol catabolism by lymphocytes of patients with chronic lymphocytic leukemia

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A low rate of catabolism of cortisol by lymphocytes correlates with high sensitivity of the cells to the steroid and causes them to die at a greater rate than control samples. Since lymphocytes of patients with chronic lymphocytic leukemia respond to treatment with glucocorticosteroids and are cortisol sensitive, we attempted to see whether their capability to catabolize cortisol differs from that of normal lymphocytes. No difference was found between the two groups of cells with regard to the pattern of cortisol metabolites. However, the lymphocytes of the chronic lymphocytic leukemia groups showed a total cortisol catabolism per cell that was significantly lower than that of the control group. Patients with low lymphocyte count in peripheral blood showed a relatively higher cortisol metabolism by lymphocytes per cell than those with high counts.

Key words: lymphocytes, cortisol, catabolism, chronic lymphocytic leukemia, morbidity.

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Le catabolisme réduit du cortisol dans les lymphocytes correspond à la sensibilité élevée des cellules à ce stéroïde et cause leur mort à un taux supérieur à celui des échantillons contrôles. Comme les lymphocytes des patients atteints de leucémie lymphoïde chronique répondent au traitement avec les glucocorticostéroïdes et sont sensibles au cortisol, nous avons tenté de voir si leur pouvoir de cataboliser le cortisol diffère de celui des lymphocytes normaux. Il n'existe aucune différence entre les deux groupes de cellules pour ce qui est du profil des métabolites du cortisol. Cependant, dans les lymphocytes des patients souffrant de leucémie lymphoïde chronique, le catabolisme du cortisol total par cellule est significativement plus faible que celui du groupe contrôle. Chez les patients avec un faible compte lymphocytaire dans le sang périphérique, le métabolisme du cortisol par les lymphocytes par cellule est relativement plus élevé que chez les patients avec des comptes élevés.

Mots clés : lymphocytes, cortisol, catabolisme, leucémie lymphoïde chronique, morbidité.

[Traduit par la revue]

ABBREVIATIONS: tetrahydrocortisol, 3α,11β,17α,21-tetrahydroxy-5-pregnan-20-one; AIDS, acquired immune deficiency syndrome; ARC, AIDS-related complex; CLL, chronic lymphocytic leukemia.

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Introduction

The ability of lymphocytes to metabolize cortisol has been demonstrated by a number of workers, including Dougherty *et al.* (1961), Jenkins and Kemp (1969), and Klein *et al.* (1978). Jenkins and Kemp (1969) and Klein *et al.* (1978) demonstrated that human lymphocytes are capable of metabolizing cortisol mainly to tetrahydrocortisol and the remainder to 20α-dihydrocortisol and 20β-dihydrocortisol. Recently, we attempted to ascertain whether thymocytes, which are cortisol-sensitive lymphocytes (in terms of viability), metabolize cortisol at a different rate than cortisol-resistant lymphocytes and whether lymphocytes in which cortisol catabolism is inhibited become sensitive to cortisol. The capability of thymocytes to catabolize cortisol was found to be one eleventh that of peripheral lymphocytes (Klein *et al.* 1987*a*). Inhibition of cortisol catabolism by lymphocytes with either an ethanol extract of sera from AIDS/ARC and cancer patients or the unsaturated fatty acids, linoleic, arachidonic, and eicosapentaenoic acids (Klein *et al.* 1987*a*, 1987*b*, 1988, 1989) made the cells vulnerable to cortisol, causing them to die at a rate significantly greater than that of control samples.

Since lymphocytes of patients with chronic lymphocytic leukemia (CLL) usually respond to treatment with cortisol derivatives, we tried to see whether their capability to catabolize cortisol differs from that of control lymphocytes. Three parameters have been examined: (*i*) total cortisol catabolism, (*ii*) the spectrum of metabolites, and (*iii*) correlation of catabolism with morbidity, i.e., lymphocyte count in peripheral blood.

Materials and methods

Twenty CLL patients, followed in the Princess Margaret and Mount Sinai Hospitals, were included in the study. The criterion for diagnosis of CLL was absolute lymphocytosis of $>10 \times 10^9$ cells/L in the peripheral blood. (However, when the experiments were carried out, some of the patients had normal peripheral blood count.) Bone marrow aspiration was done in most patients and showed diffuse infiltration of small lymphocytes, but was not a prerequisite for the diagnosis. All patients evaluated were of B type, as confirmed by the presence of low intensity immunoglobulin staining on the cell surface membrane. Monoclonality was established by the presence of a single light chain of either κ or λ type on the cell membrane. No patient was treated with corticosteroids within 2 months of evaluation. The control group was composed of 10 healthy blood donors.

The lymphocytes were isolated by centrifugation at 400 × *g* with Ficoll-Isopaque (Boyum 1968), washed twice, and suspended in RPMI-1640 (Gibco Laboratories, New York). Monocytes were separated by their adherence to the plastic walls of tissue culture flasks after incubation of the cells at 37°C in a medium made up of RPMI-1640 plus 10% fetal calf serum (Flow Laboratories, McLean, VA).

Assay of cortisol catabolism

The incubation of the lymphocytes was done in triplicate in a 96-well plate. Each well contained 5×10^6 lymphocytes and 1.0 Ci [1,2-^{3}H]cortisol (specific activity = 51.9 Ci/mmol; 1 Ci = 37 GBq) plus nonradioactive cortisol (final concentration = 1.4×10^{-6} M). RPMI-1640 (0.2 mL) was used as incubation medium. Incubation was carried out in a humidified atmosphere of 5% CO_2 in air at 37°C for 17 h (Klein *et al.* 1986*a*, 1987*a*). At the end of the incubation period, the contents of each well were extracted with chloroform. After the chloroform was removed by evaporation, the residues were applied to silica gel HF-254 thin-layer plates. The plates were developed in chloroform–methanol (90:10 v/v). Ultraviolet light (254 nm) was used for detecting the spots of cortisol and its metabolites. All cortisol metabolites, (i.e., tetrahydrocortisol, 20α-dihydrocortisol, and 20β-dihydrocortisol) were run together and located in one spot on the plate (Klein *et al.* 1978). The product and substrate spots were scraped off and the steroids were extracted with ethanol.

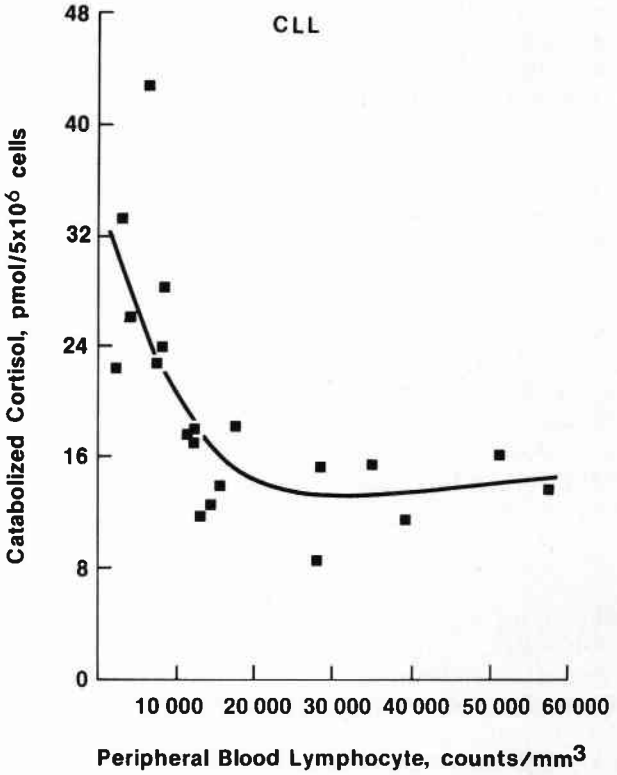

FIG. 1. The relationship between peripheral blood lymphocyte count and the metabolism of cortisol obtained by 5×10^6 cells. Third-order polynomial regression was used to fit the curve to the experimental points. ANOVA was used to calculate the significance of regression ($F_{3,16} = 6.8$, $p = 0.0036$).

An aliquot of each extract was transferred into scintillation vials (Hydroflour scintilation liquid, National Diagnostic, Manville, NJ) and the radioactivity was measured. The remainder of each extract was transferred to the starting line of Whatman No. 1 chromatography paper. Chromatography was carried out in a Bush B-5 system (benzene–methanol–water, 100:50:50 by volume) for 16 h (Klein *et al.* 1978; Jenkins and Kemp 1969). A control strip contained 20α-dihydrocortisol, 20β-dihydrocortisol, and tetrahydrocortisol as standards. Following chromatography, the paper was examined under ultraviolet light and using a radioactivity scanner. The control, in addition, was sprayed with a blue tetrazolium solution to detect those steroids with a reduced A ring and the presence of 17-hydroxy-20-keto groups (tetrahydrocortisol). The radioactive spots were cut out and extracted with ethanol, and their radioactivities were counted.

Results

Measuring the total catabolism of cortisol by using thin-layer chromatography showed that all the cortisol metabolites were located in one spot on the plate. Out of 280 pmol cortisol added to each well, 32.2 ± 2.12 pmol was catabolized by the lymphocytes of the control group ($n = 10$), while 18.7 ± 1.7 pmol was catabolized by those of the CLL group ($n = 20$). A Student's *t*-test showed a significant difference between the two groups ($p < 0.001$).

Separating the metabolites by paper chromatography showed that with regard to the control group the pattern of the metabolites was as follows: tetrahydrocortisol, 60 ± 0.7%; 20β-dihydrocortisol, 32 ± 2%; 20α-dihydrocortisol, 7.8 ± 1%. The pattern obtained with the CLL group was 60 ± 6, 34 ± 5, and 6 ± 0.7%, respectively.

Thus, these results indicated that the low catabolic activity found with CLL group was due to diminished activity of all three enzymes involved in reduction of the cortisol.

Figure 1 shows the relationship between the peripheral lymphocyte count and cortisol catabolism by lymphocytes obtained from the patients with CLL. There is a significant regression of the cortisol catabolism rate as related to the lymphocyte count ($p = 0.0036$). Between 0 and 20 000 cells/mm^3, a significant linear relationship was found ($r = 0.69, p = 0.0067$). A similar experiment with lymphocytes of the control group (1700–4800 cells/mm^3) showed no correlation between cell count and metabolism of cortisol.

Discussion

The major organ for catabolism of cortisol is the liver (Liddle 1981). It can catabolize half of the plasma content of cortisol in 100 min (Cope 1972). Therefore, according to our present and previous results (Klein *et al.* 1978, 1980, 1986*a*, 1986*b*), the cortisol catabolism obtained by lymphocytes is not high enough to make the lymphocytes play a role in the regulation of plasma cortisol levels. However, this does not mean that the cortisol catabolism capability of lymphocytes does not play a role in their own protection against cortisol.

In an attempt to establish whether there is a correlation between cortisol catabolism by lymphocytes and their resistance to cortisol, we showed that the catabolism obtained by thymocytes, which are cortisol sensitive *in vitro*, was one eleventh that obtained by peripheral lymphocytes (Klein *et al.* 1987*a*). These results raised the question of whether inhibition of cortisol catabolism by lymphocytes might make peripheral lymphocytes vulnerable to the influence of cortisol. As expected, inhibition of cortisol catabolism in lymphocytes was found to be associated with an increase in the vulnerability of the cells to cortisol (Klein *et al.* 1987*a*, 1987*b*, 1988, 1989).

The fact that only 12% of the added cortisol was metabolized by the lymphocyte preparation does not support the hypothesis that this metabolism acts to protect the cells against cortisol. However, the capacity of the cells to metabolize cortisol depends on the concentration of added cortisol and might rise linearily as much as 500 times (A. Klein, unpublished data). Since activation of intracellular cortisol depends on binding to cortisol receptors, it is possible that the cortisol-metabolizing enzymes are located in a position which allows them to regulate the concentration of receptor-bound cortisol. Furthermore, the hypothesis that the metabolism of cortisol plays a role in protecting lymphocytes against cortisol is supported by our work with the cortisol analogue prednisolone (containing an additional double bond at position 1–2), which is a more potent immunosuppressor and is metabolized much less effectively than cortisol (A. Klein and A.W.-L. Chan, unpublished data). Thus, we still do not know whether inhibition of the cortisol-metabolizing enzymes *per se* or their inherent low capability to metabolize the steroid modulates the sensitivity of lymphocytes to cortisol or is only indicative of other changes.

Since lymphocytes of CLL patients are vulnerable to cortisol and respond to treatment with cortisol derivatives, we expected that their ability to catabolize the steroid would be lower than that of normal lymphocytes. A lower rate of catabolism obtained with the CLL group was shown. This low catabolic activity was not accompanied by a change in the spectrum of the three metabolites, namely tetrahydrocortisol and the 20-hydroxy epimers. Since all three metabolites of cortisol are in a reduced form of the steroid and since NADPH and NADH are the hydrogen suppliers of the enzymatic reactions (Klein *et al.* 1986*b*), it is possible that changes in the availability of the coenzymes is responsible for the low catabolism of cortisol obtained by lymphocytes of the CLL group.

Figure 1 describes the relationship between the catabolism of cortisol by lymphocytes and the peripheral lymphocyte count in the CLL patients. The curve starts with a cortisol catabolism rate equal to that obtained with the control group (32 pmol), followed by a regression of cortisol catabolism with increasing lymphocyte count and ending with a plateau at a level of 14 pmol correlating to peripheral lymphocyte count of 20 000 cells/mm^3 and greater. This could be due to a difference in cortisol catabolism rate of two populations of lymphocytes, namely malignant and nonmalignant cells. We propose that the malignant cells are characterized by a low basic cortisol catabolism rate (14 pmol, Fig. 1), whereas the nonmalignant cells are characterized by high metabolic rate. Therefore, at high cell count, when the majority of the cells are malignant, relative low metabolism per cell becomes characteristic.

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Glutaraldehyde effect on hemoglobin: evidence for an ion environment modification based on electron paramagnetic resonance and Mossbauer spectroscopies

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Glutaraldehyde is a widely used reagent for hemoglobin cross-linking in blood substitutes research. However, hemoglobin polymerization by glutaraldehyde involves modifications of its functional properties, such as oxygen affinity, redox potentials, and autoxidation kinetics. The aim of this article is to investigate, by electron paramagnetic resonance and Mossbauer spectroscopies, the changes that occur in the iron environment after glutaraldehyde cross-linking. Spectrometric studies were performed with native hemoglobin and hemoglobin cross-linked as soluble and insoluble polymers. Spectrometry data comparison with glutaraldehyde-modified hemoglobin functional properties allows to interpret from a structural point of view that glutaraldehyde action occurs as a decrease of the O—N(F8His) distance, an increase of the Fe—N(F8His) bond length, and the decrease of the distal-side steric hindrance.

Key words: hemoglobin, glutaraldehyde, Mossbauer spectroscopy, electron paramagnetic resonance, blood substitute.

CHEVALIER, A., GUILLOCHON, D., NEDJAR, N., PIOT, J. M., VIJAYALAKSHMI, M. W., et THOMAS, D. 1990. Glutaraldehyde effect on hemoglobin: evidence for an ion environment modification based on electron paramagnetic resonance and Mossbauer spectroscopies. Biochem. Cell Biol. **68** : 813–818.

Le glutaraldéhyde est un réactif largement utilisé pour la réticulation de l'hémoglobine dans la recherche de substituts sanguins. Cependant, la polymérisation de l'hémoglobine par le glutaraldéhyde implique des modifications de ses propriétés fonctionnelles comme l'affinité pour l'oxygène, les potentiels rédox et la cinétique d'autooxydation. Utilisant la résonance paramagnétique électronique (EPR) et la spectroscopie Mossbauer, nous avons recherché les changements qui se produisent dans l'environnement du fer après la réticulation par le glutaraldéhyde. Nous avons effectué les études spectrométriques avec l'hémoglobine native et l'hémoglobine réticulée sous forme de polymères solubles et insolubles. La comparaison des données spectrométriques avec les propriétés fonctionnelles de l'hémoglobine modifiée par le glutaraldéhyde permet d'interpréter d'un point de vue structural l'action du glutaraldéhyde comme une diminution de la distance O—N(F8His), une augmentation de la longueur du lien Fe—N(F8His) et une diminution de l'empêchement stérique du côté distal.

Mots clés : hémoglobine, glutaraldéhyde, spectroscopie Mossbauer, résonance paramagnétique électronique, substituts sanguins.

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ABBREVIATION: EPR, electron paramagnetic resonance.

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