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Albumin and the Unique Pattern of Inhibitors of Cortisol Catabolism by Lymphocytes in Serum of Cancer Patients

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Abstract. We have shown previously that sera of cancer patients (CPS) possess ethanol-extractable substances which can inhibit the catabolism of cortisol by lymphocytes (CCL). In the present study an attempt was made to purify the inhibitory material by gel filtration. Chromatography of normal serum and CPS on a Sephadex G-10 column showed one peak of CCL inhibition with control serum and two peaks with CPS. The one peak which was common to both sera appeared with the void volume and was identified as albumin. The second peak which was present with CPS only, appeared at a molecular weight range of 300–350 daltons. We postulate that CPS may contain a relatively high concentration of small molecules which are not bound to proteins and which might modulate the normal function of the immune system.

Introduction

The ability of lymphocytes to effect alterations in the molecular structure of cortisol has been demonstrated by a number of workers, including Dougherty et al. [1], Jenkins and Kemp [2] and Klein et al. [3]. Jenkins and Kemp [2] and Klein et al. [3] demonstrated that human lymphocytes are capable of metabolizing cortisol to tetrahydrocortisol (3α,11β,17α,21-tetrahydroxy-5-pregnan-20-one) and the rest to 20α-dihydroxycortisol and 20β-dihydroxycortisol with no formation of 11-keto derivatives of cortisol. Recently, we showed that when cortisol catabolism by lymphocytes (CCL) is minimal either due to the nature of the cells under investigation (e.g. thymocytes) [4] or inhibition [4–7] the cells become more vulnerable to the effects of cortisol.

Since the sera of cancer patients (CPS) was shown to possess CCL inhibitors [8], an attempt was made to isolate and purify the inhibitory material in CPS by gel filtration. Our previous studies indicated that the inhibitors have a relatively small molecular weight [5-8] and are not digested by protease [6]. Accordingly, a Sephadex G-10 column was used as an initial step towards isolation and purification of the inhibitory material.

Material and Methods

Sera from patients (n = 9) with colorectal carcinoma or breast cancer were used in the study. The patients were diagnosed recently and were untreated. 0.5 ml of serum from either patients or normal subjects was loaded on a Sephadex G-10 column (height 50 cm; diameter 0.9 cm). Chromatography was carried out with 0.01 *M* phosphate buffer as a running solution. Allowing the void volume to pass through the column 1-ml fractions were collected, lyophilized and extracted with ethanol. The ethanol phase was evaporated to dryness.

Since the region where proteins come off the column showed an inhibition of CCL, and we suspected albumin to be responsible for this phenomenon, 20 mg globulin-free human albumin (Sigma, St. Louis) was run on the column and fractions collected and treated as described above.

Lymphocytes, for the bioassay, were isolated from buffy coast of blood bank material as described in our previous studies [4, 5]. The capacity of these cells to catabolize cortisol and the effect of the chromatography fractions on this catabolism was then measured.

Assay of Cortisol Catabolism

The lymphocytes were divided into flasks so that each flask contained 2.5×10^7 lymphocytes in 1-ml suspension as described previously [4–8]. The incubation medium consisted of glucose-containing PBS (5.5 mmol/l), which was found to be the most suitable for assessing rates of cortisol metabolism [9]. Each flask contained 1 ml of medium, 1.0 μCi 1,2-^{3}H-cortisol (specific activity 51.9 Ci/mmol) plus nonradioactive cortisol 10 times the cortisol level in 0.5 ml serum (final concentration = 1.4×10^{-6} *M*), and one of the above-mentioned dry ethanol extractions; i.e., chromatographed fractions of either serum or albumin.

The sealed flasks were incubated in a shaking bath at 37 °C for 17 h as described in our previous work [4, 8] (viability after incubation was no less than 95%). At the end of the incubation period, the contents of each flask were extracted with chloroform. After the chloroform was evaporated, the residues were applied on silica gel HF-254 thin-layer plates. The plates were developed in chloroform:methanol (90:10 vol/vol). All cortisol metabolites; i.e., tetrahydrocortisol, 20α, and 20β-dihydrocortisol were located in *one* spot on the plate (3) and ultraviolet light (254 nm) was used for detecting the spots of cortisol and its metabolites. The product and substrate spots were scraped off and transferred into scintillation vials, and the radioactivity counted. The counts corresponding to the spot of 'metabolites' in the blank flask (which contained no lymphocytes) were substracted from the spot count of the metabolites of all samples.

Results

Chromatography of the sera on a Sephadex G-10 column elicited two peaks of CCL inhibitory activity with CPS and one peak of activity with the control serum (fig. 1). In both sera a peak was observed corresponding to the protein peak, between fractions 1 and 5 (fig. 2). The second CCL inhibitory peak noted only with CPS, was obtained between fractions 10 and 12. This region corresponds to a molecular weight range of 300–350 daltons. Comparing the CCL inhibitory effects obtained with the fractions of CPS and control serum by using a one-way analysis of variance, showed a significant overall group effect ($p < 0.005$). Duncan's multiple range test showed that the only significant differences were found between fractions 10 ($p < 0.05$) and 11 ($p < 0.01$) of both sera.

Figure 2 shows the inhibitory effect of albumin chromatographed under the same conditions as the sera. The inhibitory peak corresponds to the inhibitory peak of control serum and to the first inhibitory peak of CPS, at fractions 1–5.

Discussion

In an attempt to establish whether there is a correlation between CCL and resistance of lymphocytes to cortisol, we showed that the CCL of thymocytes, that are cortisol-sensi-

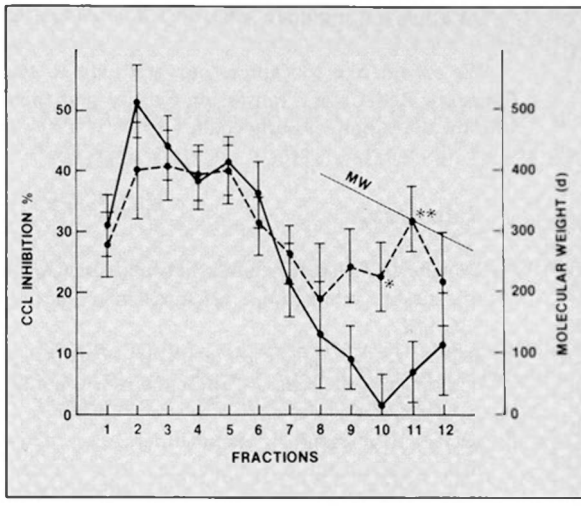

Fig. 1. Effect of ethanol-extracted fractions, obtained by Sephadex G-10 gel filtration, of sera of either cancer patients (– – –) or control (——) on CCL. MW = Molecular weight. * $p < 0.05$; ** $p < 0.01$.

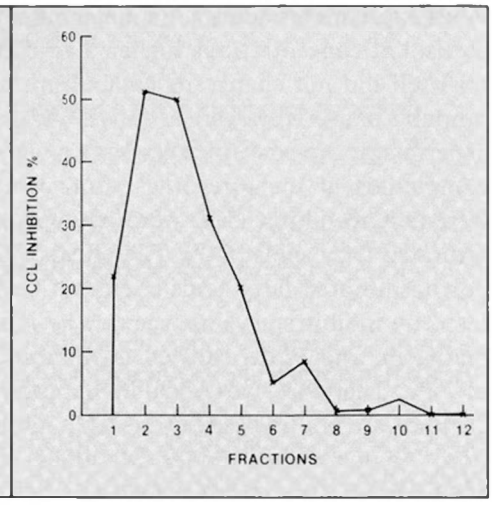

Fig. 2. Effect of ethanol-extracted fractions, obtained by Sephadex G-10 gel filtration, of human albumin on CCL.

tive in vitro, was 11 times lower than that obtained by peripheral lymphocytes [4]. These results raised the question as to whether inhibition of CCL might make peripheral lymphocytes vulnerable to the influence of cortisol. Looking for CCL inhibitors, we found that ethanol extracts of plasma of patients with either AIDS or cancer and unsaturated fatty acids are capable of inhibiting CCL [4–8]. As expected, this inhibition of CCL was also associated with an increase in the vulnerability of the cells to cortisol [4–7].

As mentioned, we have shown that CPS contains ethanol extractable components that are able to inhibit CCL [6, 8]. Such an inhibition could be obtained also with serum of healthy subjects when relatively large volumes of the serum were used [8]. Thus, it seems that the difference obtained between CPS and control serum was due to quantitative rather than qualitative differences.

Chromatography of the sera revealed a major qualitative difference; that is, two inhibition peaks with CPS and one peak with the control serum. The common peak for the two sera appeared in the protein region (fig. 1). Since polyunsaturated fatty acids were found to be CCL inhibitors [5–7] and since albumin is the main carrier of free fatty acids, we suspected that FFA carried by albumin are the inhibitors of CCL. Running albumin on the column on one hand (fig. 2) and lack of defatted albumin to inhibit CCL on the other hand [6] proved this assumption to be correct.

Of particular interest is the additional CCL inhibition peak found in CPS, with a molecular weight between 300 and 350 daltons. A variety of molecules such as fatty acids, steroids, prostaglandins etc., which are known to be immunomodulators, have a molecular weight within this range. In pre-

vious experiments adding the metabolites of cortisol at concentrations higher than cortisol itself did not change its catabolism rate [unpubl. data]. However, as far as our own experiments are concerned the only group of compounds of the foregoing nature which were able to inhibit CCL were free polyunsaturated fatty acids [5–7]. The effect of the polyunsaturated fatty acids could not be altered by inhibiting cyclooxygenase by indomethacin, and PGE_2 showed no inhibition of CCL. Therefore, we conclude that prostaglandins are not involved in the CCL inhibition. As mentioned under Materials and Methods, the free cortisol found in serum could also not change the rate of CCL. The question then arises as to whether cancer patients have a higher concentration of free polyunsaturated fatty acids than normal, and whether other compounds with similar molecular weights appear in CPS and exhibit CCL inhibitory capability. In addition, since the activity of small molecules, like steroids and fatty acids, is expressed when they are free of their protein carrier, attempts to study their relationship to disease necessitates measurement of their protein-free concentration as well as their total concentration. Although the mean ages of the normal and cancer groups were not matched (control 35 ± 9 vs. cancer 49 ± 13 years), those individuals whose ages were matched showed a CCL inhibition pattern which was typical of the group to which they belonged. Moreover, whether the phenomenon is more widespread than cancer was not addressed in this study.

Finally, the source of these compounds and the overall effect they exert by virtue of their potential for influencing the process of immune surveillance merits further investigation.

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