The Effect of Fatty Acids on the Vulnerability of Lymphocytes to Cortisol

Ami Klein, Barbara Bruser, and Aaron Malkin

We have shown previously that cortisol-sensitive lymphocytes (thymocytes) have a much lower capacity than cortisol-resistant cells to catabolize cortisol and that linoleic acid inhibits the catabolism of cortisol by lymphocytes and modulates the sensitivity of lymphocytes to cortisol. In the present study, we attempted to see whether other fatty acids are inhibitory and if inhibition of cortisol catabolism by lymphocytes indicates a change in resistance of the cells to cortisol. Measuring the effect of fatty acids on cortisol catabolism by lymphocytes indicated that the polyunsaturated fatty acids, linoleate, arachidonate, and eicosapentaenoic, inhibit cortisol catabolism by lymphocytes. Using prostaglandin PGE_2 and indomethacin as a blocker of prostaglandin formation, we observed that the effect of the polyunsaturated fatty acids was not due to the formation of prostaglandins. Examining the effect of fatty acids on the vulnerability of lymphocytes to cortisol, we noted that saturated fatty acids had no significant effect, whereas the aforementioned polyunsaturated fatty acids make lymphocytes more sensitive to cortisol.

THE ABILITY of lymphocytes to effect alterations in the molecular structure of cortisol has been demonstrated by a number of workers, including Dougherty, Berliner, and Berliner,[1] Jenkins and Kemp,[2] and Klein, Kaufmann, Mannheimer, et al.[3] Jenkins et al[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), 20-alpha-dihydrocortisol and 20-beta-dihydrocortisol. Recently, we attempted to ascertain whether cortisol-sensitive lymphocytes, eg, thymocytes, metabolize cortisol at a different rate than cortisol-resistant cells, and whether lymphocytes in which cortisol catabolism was inhibited become cortisol-sensitive. The capacity of thymocytes to catabolize cortisol was found to be 11 times lower than that of peripheral lymphocytes.[4] Inhibition of cortisol catabolism by lymphocytes with either an ethanol extract of plasma from acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) patients or linoleic acid showed that the cells became more vulnerable to cortisol, dying at a rate significantly greater than that of control samples.[4,5] The results obtained with linoleic acid have led us to an investigation of the effect of saturated and unsaturated fatty acids on cortisol catabolism by lymphocytes and its relationship to the sensitivity of lymphocytes to cortisol.

MATERIALS AND METHODS

Lymphocytes were isolated from buffy coats of blood bank material obtained within two hours of the time the blood was donated. The lymphocytes were isolated by centrifugation at 400 × g with Ficoll-Isopaque (Pharmaia, Uppsala, Sweden),[6] washed twice, and resuspended in phosphate-buffered saline containing 100 IU penicillin and streptomycin. 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 Roswell Park Memorial Institute medium-1640 (RPMI-1640) plus 10% heat-inactivated fetal calf serum (FCS) (RPMI-1640 was supplied by Gibco Laboratories, New York, and FCS by Flow Laboratories, McLean, VA). Duplicate aliquots of cells were prepared and divided into groups with the following additions: (1) 66 μmol/L fatty acids (caproic [6:0], heptanoic [7:0], lauric [12:0], myristic [14:0], palmitic [16:0], stearic [18:0], linoleic [18:2], arachidonic [20:4], and eicosapentaenoic [20:5], Sigma, St Louis); (2) PGE_2 (20 μg/mL) and polyunsaturated fatty acids either in the presence or absence of indomethacin (10 μg/mL) (Sigma, St Louis). The capacity of these cell groups to catabolize cortisol and their resistance to cortisol was then measured.

Assay of Cortisol Catabolism

Each preparation of lymphocytes was divided into flasks so that each flask contained 2.5 × 10^7 lymphocytes in 1 mL suspension as described previously.[4,7] The incubation medium consisted of glucose-containing phosphate-buffered saline (5.5 mmol/L), which was found to be the most suitable for assessing cortisol metabolism rates.[8] Each flask contained 1 mL of medium and 1.0 μCi (7 × 10^5 CPM) cortisol (New England Nuclear, Billerica MA), [1, 2, ^{3}H] cortisol plus nonradioactive cortisol, final concentration 1.4 mol/L, and one of the above-mentioned additions, ie, fatty acids (in the presence or absence of indomethacin), prostaglandin PGE_2.

The sealed flasks were incubated in a shaking bath at 37°C for 17 hours as described previously.[4,7] 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). Following chromatography, the plates were viewed under UV light at 254 nm. The product and substrate spots were scraped off and transferred into scintillation vials, and the radioactivity was counted. With the chromatographic procedure, the one product peak provides the sum of radioactivity of all the metabolites.[3] The counts corresponding to the spot of metabolites in the blank flask (which contained no lymphocytes) were subtracted from the spot count of the metabolites of all samples.

Assay of Cortisol Sensitivity

The cells were immersed at a concentration of 2 × 10^6 cells/mL in medium made up of RPMI-1640 plus 10% heat-inactivated FCS and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Using the trypan blue exclusion method, cultures were observed daily for cell viability. Lymphocytes were immersed in 1 mL medium with or without cortisol (2.7 μmol/L) with one of the following fatty acids: caproic, heptanoic, myristic, lauric, palmitic, stearic, linoleic, arachidonic, or eicosapantaenoic, (66 μmol/L).

From the Department of Clinical Biochemistry, Sunnybrook Medical Centre, University of Toronto, Toronto.

Address reprint requests to Ami Klein, PhD, Department of Clinical Biochemistry, Sunnybrook Medical Centre, 2075 Bayview Ave, Toronto, Ontario, M4N 3M5, Canada.

0026-0495/89/3803-0015$03.00/0

RESULTS

Figure 1 shows the effect of nine fatty acids on cortisol catabolism by lymphocytes. The polyunsaturated fatty acids, linoleic (18:2), arachidonic (20:4), and eicosapentaenoic (20:5), were the only ones that had an inhibitory effect on cortisol catabolism ($P < .01$). Figure 2 illustrates the inhibitory effect of arachidonic acid on cortisol catabolism by lymphocytes which could not be changed by the addition of indomethacin. Likewise, cortisol catabolism by lymphocytes is not affected by PGE_2. (Similar results were obtained with eicosapentaenoic acid.) It should be noted that measuring the viability of the cells after the initial 17-hour incubation indicated a death rate in either group of no more than 5%. Figure 3 shows the effect of saturated and unsaturated fatty acids, in the presence or absence of cortisol, on the killing of lymphocytes after three days of incubation. Figure 3A illustrates the results obtained with the control group of cells (no fatty acids added) incubated in medium in the presence or absence of cortisol. A slight rise in death rate of cells was found after the second and third days of incubation. The same results were observed when the cells were incubated with each of the saturated fatty acids. A sharp rise in the death rate of the cells in the presence of cortisol was noted at the second and third days of incubation when the polyunsaturated fatty acids arachidonic and eicosapentaenoic were added ($P < .01$) (Fig 3B and 3C). A significant rise in the death rate of the cells ($P < .05$) was observed with linoleate and cortisol only after three days of incubation (15% ± 4.2% with linoleate and cortisol and 7.33% ± 0.88% with linoleate alone).

DISCUSSION

In 1961, Dougherty and Berliner[1] found that cortisol metabolites have no suppressive effect upon lymphocytes. They even postulated that the ability of lymphocytes to effect changes in cortisol constitutes an important homeostatic mechanism in the regulation of the lymphocyte population. We recently used a lymphocyte subset, namely thymocytes, that are cortisol-sensitive in vitro, in an attempt to establish whether there is a correlation between cortisol catabolism by lymphocytes and resistance of lymphocytes to cortisol. This study showed that the cortisol catabolism of thymocytes was 11 times lower than that obtained by peripheral lymphocytes.[4] Accordingly, these results raised the question as to whether inhibition of cortisol catabolism might correlate with an enhanced vulnerability of peripheral lymphocytes to cortisol. Looking for inhibitors of cortisol catabolism, we

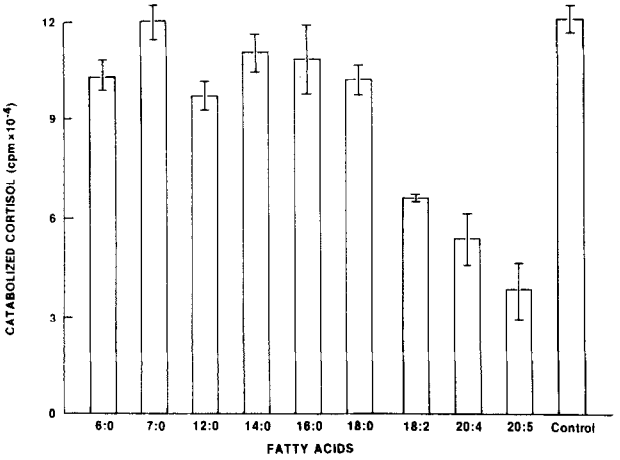

Fig 1. The effect of saturated and polyunsaturated fatty acids on the catabolism of cortisol by lymphocytes. The results represent mean ± SEM. One-way ANOVA indicated that the overall group effect is significant ($F9, 40 = 15.68$; $P < .01$). Using Duncan's multiple-range test showed a significant inhibition of CCL ($P < .01$) obtained only with the polyunsaturated fatty acids linoleic (18:2), arachidonic (20:4), and eicosapentaenoic (20:5).

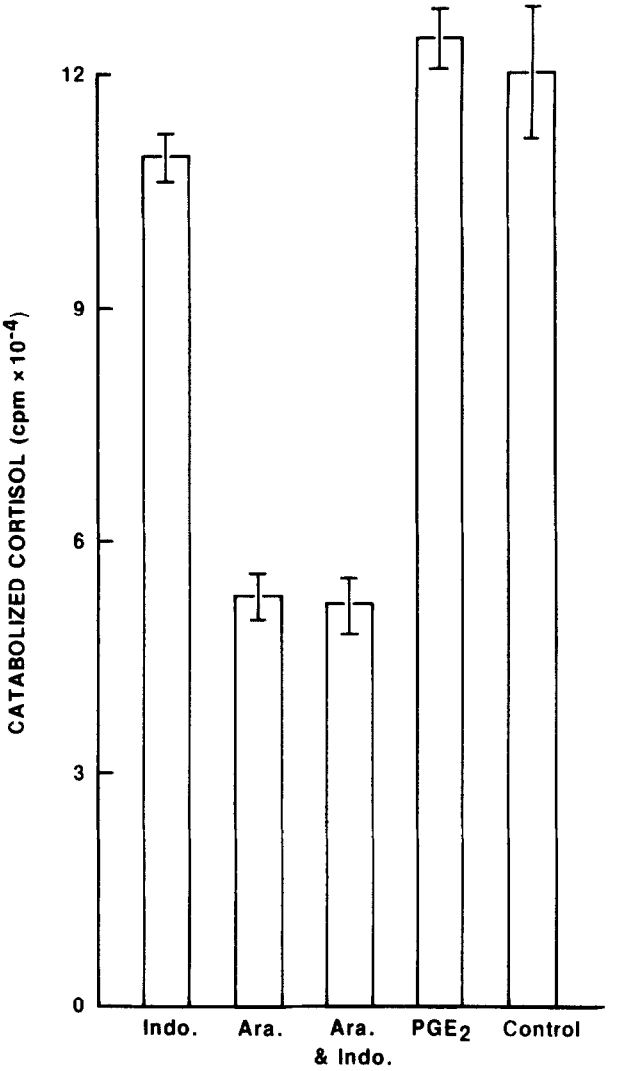

Fig 2. Effect of arachidonic acid, indomethacin, and PGE_2 on catabolism of cortisol by lymphocytes. The results represent mean ± SEM. One-way ANOVA indicated that the overall group effect is significant ($F3, 23 = 115.07$; $P < .01$). Duncan's test showed no significant difference between arachidonic *v* arachidonic + indomethacin and indomethacin *v* PGE_2. A significant difference of $P < .01$ was found between indomethacin and both arachidonic and arachidonic + indomethacin. The same difference was found between PGE_2 and both arachidonic and arachidonic + indomethacin.

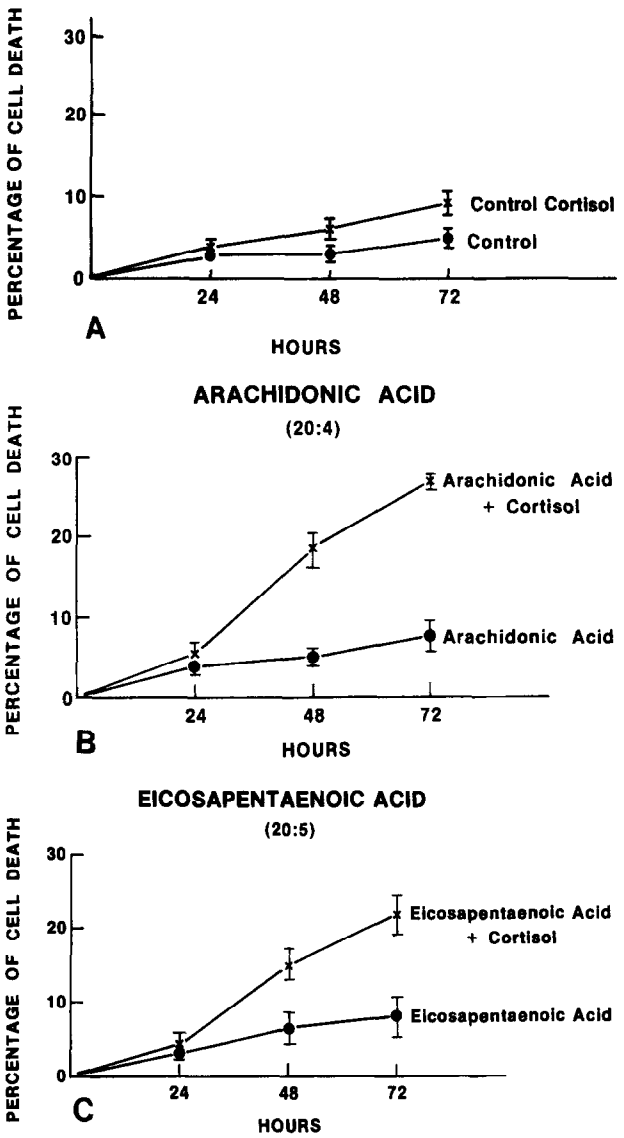

Fig 3. The effect of saturated and unsaturated fatty acids, in the presence or absence of cortisol, on the death rate of lymphocytes after three days of incubation. Values are mean ± SEM. One-way ANOVA and Duncan's test were used for the statistical analysis. (A) Results obtained with the control group of cells (no fatty acid added). Overall group effect was significant ($F5, 68 = 11.81$; $P < .01$). A slight rise in death rate of cells was found after the second and third days of incubation when cortisol was present ($P < .05$). The same results were observed when the cells were incubated with each of the saturated fatty acids. (B) Results obtained with arachidonic acid. ($F5, 24 = 37.7$; $P < .01$). A significant rise in the death rate of the cells was noted at the second and third days of incubation ($P < .01$). (C) Results obtained with eicosapentaenoic acid ($F5, 24 = 22.36$; $P < .01$). A significant rise in the death rate of the cells was noted at the second ($P < .05$) and third ($P < .01$) days of incubation.

found that ethanol extracts of plasma of patients with AIDS and linoleic acid are capable of inhibiting cortisol catabolism by lymphocytes.[4,5] As expected, the inhibition of cortisol catabolism observed with an ethanol extract of AIDS patients' plasma and linoleic acid was also associated with an increase in the vulnerability of the cells to cortisol.[4,5] (Ethanol extract of controls showed no significant inhibition of cortisol catabolism by lymphocytes and no significant increase in the vulnerability of the cells to cortisol.[4]) It should be noted that Christeff et al[8] showed recently a rise in the level of polyunsaturated fatty acid and cortisol in serum of AIDS and ARC patients. We still do not know whether inhibition per se of the cortisol-metabolizing enzymes or their inherent low capability to metabolize the steroid is effective in modulating the sensitivity of lymphocytes to cortisol or is only indicative of other changes.

We do not as yet have any idea as to the mechanism by which the inhibitors affect cortisol catabolism by lymphocytes. It might occur as a consequence of either a direct effect on the enzymes responsible for cortisol catabolism or, alternatively, an indirect effect on the formation of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) and nicotinamide adenine dinucleotide, reduced form (NADH) coenzymes necessary for cortisol reduction by lymphocytes.[9] This last assumption is supported by Arslan et al,[10] who showed that unsaturated fatty acids can act as mitochondria uncouplers. In any event, it appears from our previous work with either plasma of AIDS patients[4] or linoleic acid,[5] and from the present study with polyunsaturated fatty acids, that cortisol catabolism by lymphocytes can be used as a convenient indicator of changes occurring in the cells which make them more vulnerable to the effect of cortisol.

The inhibition of cortisol catabolism by lymphocytes does not seem to have an immediate effect on lymphocytes in the sense of making them sensitive to cortisol. Figure 3(B and C) shows a lag period of at least 24 hours preceding the increment of cell death following the addition of cortisol to polyunsaturated fatty acid-treated lymphocytes. Using thymocytes, we showed that their cortisol sensitivity is characterized also by the same lag period obtained with fatty acid-treated lymphocytes.[4] This lag period is perhaps a result of the time needed for the formation of proteins,[11] as a result of cortisol induction.

Immunosuppression at various levels, such as the response to stimulation by mitogens,[12] complement-dependent rosette formation,[13] or lymphocytotoxicity to malignant cells[14] has been attributed to fatty acids.[12,15,16] Cortisol, meanwhile, has been found to increase free fatty acid concentrations in plasmas.[17-19] Contradictory information is obtained with regard to the effect of cortisol on the concentration of fatty acids in lymphocytes. Turnell et al[20,21] even proposed that a rise in intracellular free fatty acid is responsible for the lymphocytolysis induced by cortisol. However, Jardieu et al[11] showed that lipomodulin, the product of cortisol induction, inhibits phospholipase A_2.

Linoleate (18:2) was found to have the smallest effect on lymphocytes when compared with arachidonate (20:4) and eicosapentaenoic acid (20:5). A significant killing of cells appeared only after three days of incubation in the presence of linoleate and cortisol; this rate was half that obtained with either arachidonate or eicosapentaenoic acid. However, in another study[5] we showed that using twice the concentration of linoleate caused the death rate to double. Therefore, it seems that the effect of polyunsaturated free fatty acids on

the vulnerability of lymphocytes to cortisol depends in part, at least, on the number of double bonds in the free fatty acids.

In our previous[5] and present work, we have demonstrated that PGE_2 does not have any effect on cortisol catabolism by lymphocytes. This implies that the effect of polyunsaturated fatty acids on cortisol catabolism is not a result of prostaglandin production. However, it leaves open the possibility that other prostaglandins or leukotrienes could have caused cortisol catabolism by lymphocytes inhibition. But on using indomethacin as an inhibitor of prostaglandin production, that is, by adding it to either linoleate,[5] arachidonate, or eicosapentaenoic acid, we showed no change in the effect of the fatty acid on cortisol catabolism. Thus, it seems that the effect is due to polyunsaturated fatty acids per se and not to the production of prostaglandins. However, the effect of leukotrienes could not be excluded.

It appears from the present studies that cortisol catabolism by lymphocytes can be used as a convenient indicator of changes occurring in the cell that make them more vulnerable to the effect of cortisol. It is possible that certain fatty acids act synergistically with cortisol in the sense that an increase in their concentration may be a prerequisite for the action of cortisol on lymphocytes.

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