Oestrogen as an inhibitor of human NK cell cytolysis

M.M. Ferguson and F.G. McDonald

Department of Oral Medicine and Pathology, Glasgow Dental Hospital and School, 378 Sauchiehall Street, Glasgow G2 3JZ, Scotland

Received 22 August 1985

Natural killer (NK) cells are large granular lymphocytes attributed with the ability to lyse certain tumour cells. Previous studies on NK cells have demonstrated only an in vivo suppression of NK cell activity by 17β-oestradiol. The suppressive action of oestrogen on other peroxidase-containing leukocytes by virtue of its redox potential has already been documented. In the present study oestrogen suppressed NK cell cytolysis in vitro (determined by the release of [^{51}Cr]chromate from radiolabelled cells) in a dose-dependent manner ($p < 0.01$). Parallel experiments demonstrated a similar reduction in NK cell luminol chemiluminescence during activation by K562 tumour cells. Therefore, it would appear that there may be an association between NK cell lysis and their peroxidase/oxygenase activity.

Estrogen NK cell Peroxidase Cytolysis inhibition

1. INTRODUCTION

Natural killer (NK) cells constitute an important component of the immune surveillance system against malignant neoplasms, restraining both their growth and dissemination. These large, granular lymphocytes are present in particularly high numbers in the spleen and lymph nodes where they exhibit target specificity for a number of different tumours. The NK cells are widely distributed throughout the body where, in addition to neoplastic cell surveillance, they have been associated with a number of other functions including apoptosis of effete cells, graft rejection, and activation in viral and bacterial infections. They have also been implicated in certain autoimmune disorders and connective tissue diseases.

It is well established that oestrogens can influence many different parameters of the immune response although there does not appear to be a specific leukocyte receptor for this group of steroid sex hormones. Hence an alternative mode of action upon leukocytes must be postulated. Oestrogens, by virtue of their phenolic A ring, are capable of undergoing oxidation. Their oxidation potential is of the order of +500 mV [1] and as such can be oxidised by peroxidase; this results in the formation of catecholic oestrogens [2]. The synthetic oestrogen, fosfestrol, has been shown to be a substrate for the peroxidase/oxygenase systems in both polymorphoneutrophils [1] and monocytes [3].

Treatment of mice with high concentrations of 17β-oestradiol depletes the NK cell population [4–6]. Initially this was attributed to a decreased marrow space although subsequent work by the same group [7] suggested a direct effect upon the NK cells. Diethylstilboestrol similarly reduced murine NK cell activity when given neonatally and caused the animals to have an increased tumour susceptibility [8].

The mechanism by which NK cells lyse target cells is unclear. Proteases, esterases and phospholipases have all been implicated, perhaps by injecting these lytic enzymes directly through their microvilli into the target cell. Peroxidase, or possibly oxygenase activity, has been identified by chemiluminescence studies in the NK cells [9] and it is possible that such enzyme systems may play a direct role in killing by generating active free radicals of oxygen.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

2. MATERIALS AND METHODS

Venous blood was removed at the same time each day (8–9 a.m.) from 8 healthy volunteers, aged 22–39 years, and mixed immediately with preservative-free heparin – 200 U heparin in 5 ml Dulbecco's phosphate-buffered saline (PBS) per 50 ml blood. Mononuclear leukocytes were removed by density centrifugation at 400 × *g* for 30 min on Ficoll-Hypaque (Pharmacia). The cells were washed twice with medium RPMI 1634 containing 5% foetal calf serum (Gibco Biocult), buffered to pH 7.4 with 0.02 M Hepes, and then incubated at 37°C for 1 h in plastic flasks which had been pre-incubated for 15 min with autologous serum to remove monocytes by adherence. The B-lymphocytes were then depleted by passage through a 10 cm nylon wool column. The resultant cells were found to be 95% viable using trypan blue exclusion and also after 5 h incubation with fosfestrol there was no decrease in cell viability.

K562 cells, an erythroid leukaemic line, were cultured in medium RPMI 1634 containing 5% foetal calf serum (Gibco Biocult), penicillin (100 units · ml^{-1}) and streptomycin (100 ur · ml^{-1}); 0.02% glutamine was added every second day. These target cells were used for both luminometry and cytolysis resuspended in PBS at a ratio of 10 lymphocytes to 1 K562 cell.

The lymphocytes, at 1×10^7 cells in 250 μl, were suspended in Gey's solution and allowed to dark adapt in borax glass tubes for 30 min at 36°C in a Packard Picolite luminometer. Luminol (59 μl) was added, to give a final concentration of 10^{-4} M, and 50 μl K562 cells were injected. Chemiluminescence was measured by 30 s sequential counts and peak chemiluminescence was reached from 30 to 40 min. For each individual's cells, counting was commenced 5 min before the peak was reached and then for a further 10 min. All experiments were carried out in triplicate. Controls containing either lymphocytes alone or K562 cells alone produced no chemiluminescence. When K562 cells were added to monocytes there was negligible chemiluminescence although the separated monocyte fraction did produce chemiluminescence on addition of opsonised zymosan. Likewise when zymosan was added to the lymphocytes a negligible response was seen.

The effect of including fosfestrol (disodium salt of diethylstilboestrol phosphate) was examined over the concentration range 10^{-6}–10^{-3} M. This synthetic oestrogen is readily water soluble and permitted a wide range of concentrations in aqueous media. Further, hydrolysis of the phosphate group is unnecessary to permit oxidation of the phenolic ring which is in contrast to the interaction of this hormone with specific oestrogen nuclear receptors.

To measure cytolysis of target cells, the K562 cells were first incubated at 37°C for 1 h with a solution of [^{51}Cr]chromate (sodium chromate in aqueous solution containing 0.9% NaCl: specific activity of ^{51}Cr, 350–600 mCi/mg chromium); 100 Ci [^{51}Cr]chromate in 1 ml medium RPMI 1634, containing 5×10^6 cells. The cells were then washed 3 times with culture medium and again suspended in medium RPMI 1634 containing 5% foetal calf serum.

Cytolysis was performed by incubating 200 μl lymphocytes with 200 μl K562 cells together with either 100 μl PBS or fosfestrol in PBS, at 37°C in a humidified atmosphere of 5% CO_2:95% air for 5 h. All incubations were performed in triplicate. At the end of this time, 500 μl medium RPMI 1634 was added and the tubes then centrifuged at 400 × *g* for 10 min. Using a gamma spectrometer, the radioactivity was counted in both a 500 ml aliquot of supernatant and the remaining 500 μl of medium plus cells.

Spontaneous release of [^{51}Cr]chromate from K562 cells into the supernatant was measured when these cells were incubated in the absence of lymphocytes. To determine maximal release of [^{51}Cr]chromate, 3 detergents were evaluated: Triton X-100, Lissapol NX and sodium deoxycholate. Of these detergents, sodium deoxycholate was found to give optimal release and therefore used for all subsequent experiments.

It has been conventional to calculate cytolysis by the formula:

$$\% \text{ cytolysis} = \frac{(R_{exp} - R_{sp})}{(R_{max} - R_{sp})} \times 100$$

where R_{exp} is the radioactivity in the supernatant of experimental tubes, R_{sp} the spontaneously released radioactivity and R_{max} the maximally released radioactivity.

However, such an equation does not take

cognisence of the fact that lymphocytes may take up [^{51}Cr]chromate from the medium once this has been released from the K562 cells. This would give an underestimate of cytolysis in the experimental tubes and it is therefore desirable to introduce a further factor, the uptake constant (K_u), giving the corrected formula.

$$\% \text{ cytolysis} = \frac{(R_{exp} \times K_u) - R_{sp}}{R_{max} - R_{sp}} \times 100$$

The magnitude of this correction factor was found to be 1.04 for the experimental conditions described, where supernatant counts exceeded 1×10^5 in 100 s for a 500 μl volume of lymphocytes, at 1×10^7 cells·ml^{-1}.

The effect of including fosfestrol, at 10^{-6}–10^{-3} M, was investigated by pre-incubating the mononuclear cells for 10 min with the oestrogen prior to addition of the K562 target cells. Controls were examined containing no fosfestrol.

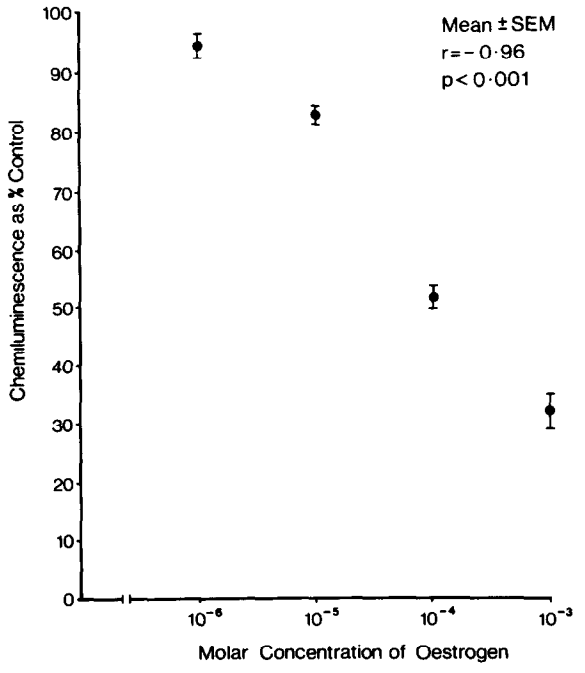

Fig.1. Effect of increasing concentrations of fosfestrol on the luminol chemiluminescence of NK cells. Values are expressed as a percentage of control containing no oestrogen.

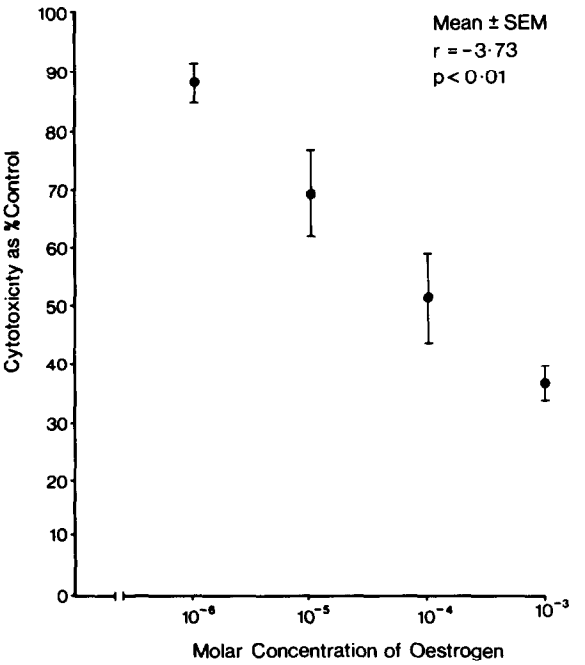

Fig.2. Effect of increasing concentrations of fosfestrol measured as ^{51}Cr release from K562 target cells. Values for NK cell cytotoxicity are expressed as a percentage of controls.

3. RESULTS

Over the concentrations of fosfestrol examined there was a progressive decrease in chemiluminescence indicating that the oestrogen could interact with the peroxidase system of the NK-cell (fig.1). The correlation coefficient showed this to be highly significant $p < 0.01$.

The results of these cytolysis experiments show a significant reduction in cell killing ($p < 0.01$) with increasing concentrations of fosfestrol (fig.2) and it is apparent that the magnitude of this decrease is comparable to the diminution in peroxidase/oxygenase based chemiluminescence seen in fig.1.

4. DISCUSSION

Our data support the previous in vivo findings that oestrogen suppresses NK cell activity. By adopting an in vitro system it would seem that this is accomplished by a direct action upon the NK cells rather than indirectly, as was postulated either

by decreasing bone marrow space or by altering the NK cell/interferon pathway [7]. Depression of NK cell activity by oestrogen may partly account for sex differences in a variety of disorders but the actions of progestogens and androgens must also be taken into account.

Although the precise role of the peroxidase/oxygenase systems in the NK cell still requires elucidation the data indicate that this activity is obligatory for killing, either directly or indirectly. Under these circumstances the possibility is raised that the NK cell generates free oxygen radicals as part of its killing mechanism.

The interacting of oestrogens with peroxidase/oxygenase enzyme systems represents a mechanism whereby these sex hormones can influence leukocytes in the absence of specific receptors.

ACKNOWLEDGEMENT

Supported by a grant from the Scottish Home and Health Department (F.G.McD.).

REFERENCES

[1] Ferguson, M.M., Alexander, W.D., Connell, J.M., Lappin, A.E., McCruden, D.C., MacLure, R., Mairs, R.J. and Younger, A. (1984) Biochem. Pharmacol. 33, 757–762.

[2] Ball, P. and Knuppen, R. (1980) Acta Endocrinol. Suppl.232.

[3] Ferguson, M.M., Mairs, J.R., McDonald, F.G. and McGroarty, J.M. (1984) Interaction of Oestrogens with Human Leucocyte Sub-populations, 3rd Joint Meeting of the British Endocrine Society.

[4] Seaman, W.E., Blackman, M.A., Gindhart, T.D., Roubinian, J.R., Loeb, J.M. and Talal, N. (1978) J. Immunol. 121, 2193–2198.

[5] Seaman, W.E. and Gindhart, T.D. (1979) Arthritis Rheum. 22, 1234–1240.

[6] Seaman, W.E., Gindhart, T.D., Greenspan, J.S., Blackman, M.A. and Talal, N. (1979) J. Immunol. 122, 2541–2547.

[7] Seaman, W.E., Merigan, T.C. and Talal, N. (1979) J. Immunol. 123, 2903–2905.

[8] Kalland, T. and Forsberg, J.G. (1981) Cancer Res. 41, 5134–5140.

[9] Roder, J.C., Helfand, S.L., Werkmeister, J., McGarry, R., Beaumont, T.J. and Dawe, A. (1982) Nature 289, 569–572.