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SHORT COMMUNICATION

METABOLISM OF E1 AND E2 IN ISHIKAWA ENDOMETRIUM CARCINOMA CELLS: INFLUENCE OF TNFα

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Summary—The metabolism of estrone (E1) and estradiol (E2) by Ishikawa endometrial carcinoma cells and its alteration by TNFα treatment was studied. Whereas this cell line practically does not respond to estrogens, it is very sensitive to TNF with respect to growth inhibition and other parameters. E2 and E1 were found to be metabolized by this cell line whereby the main metabolite for both estrogens was estriol. TNF significantly increased the rate of E1 and E2 conversion.

INTRODUCTION

Ishikawa cells were established from a well differentiated human endometrial adenocarcinoma. They were reported to be sensitive to estradiol (E2) and progesterone [1]. The cell line we used in this study had obviously lost hormone sensitivity as cells did not respond, like other estrogen sensitive cells, to 4-OH-tamoxifen in FBS containing medium and to E2 in medium containing stripped FBS. Interferon γ and retinoic acid also had no effect with regard to proliferation. Interestingly cells were very sensitive to TNF [2], however resistance occurred after 24 h [2]. Metabolism of E2 to estrone (E1) and estriol (E3)-sulfate was found by Hata *et al.* [3] in the originally described hormone responsive Ishikawa cells. In this study we investigated E1 and E2 metabolism in the estrogen resistant variant in order to demonstrate an eventual alteration of the E2 metabolism in comparison to the original, estrogen sensitive cells. In addition we were interested in finding out if the enormous TNF sensitivity led to a change in the extent and distribution of metabolites during TNF treatment.

EXPERIMENTAL

Radioactive steroids

[6,7-^{3}H(N)]E1 (sp. act. 54.6 Ci/mmol) and [6,7-^{3}H(N)]E2 (sp. act. 48.3 Ci/mmol) were purchased from New England Nuclear Corp., Boston, MA).

Cell-culture

Ishikawa cells were a gift of Dr Gurpide (Mt Sinai Hospital, New York). Cells were cultured and treated exactly as described previously [2]. Treatment of cells with TNFα occurred for 1 day. Tritiated E1 (20 nM) was added to the culture medium for 24 h, tritiated E2 (20 nM) only for the last 4 h in order to get a reasonable percentage of metabolism. Free steroids and their conjugates were extracted from 2 ml of medium using SEP-PAK-C18-cartridges (Millipore, Bedford, MA). Separation of metabolites was performed by HPLC on ODS-120T, 5 μm, 4.6 × 250 mm columns (LKB, Bromma, Sweden) using a 40–60% gradient of acetonitrile in water (flow-rate 0.3 ml/min, temp. 52°C). Radioactivity was detected in a flow-through scintillation detector (Berthold LD 506 D) with a counting efficiency of 35%. Two separate experiments were performed each in duplicate. Results are indicated as means ± SEM. Statistical evaluation of results was done using Wilcoxon's U Test and Spearman's rank correlation coefficient.

RESULTS

The rate of E1 and E2 metabolism increased with increasing number of cells but not in a linear manner (Fig. 1). Therefore cell numbers in experiments were kept in a narrow range in order to allow acceptable correction of results for cell number. E1 was mainly metabolized to E3 but not to E2, and E2 was also converted into E3 but not into E1. Conjugated estrogens represented about 15% of the total radioactivity and were not further characterized. TNF augmented the rate of overall metabolism (Fig. 2). The increase of E1 and E2 metabolism under TNF was due to an increase in E3 formation over the whole range of TNF whereas the formation of conjugated estrogens from E1 as well as from

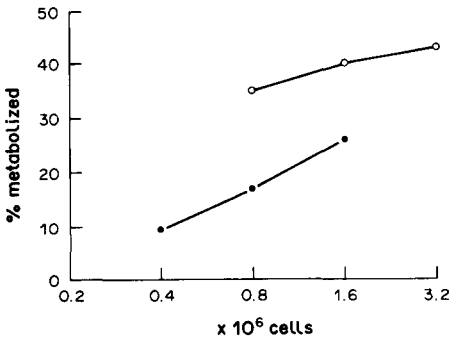

Fig. 1. Dependence of the rate of metabolism on cell number. Cells were incubated for 24 h with [^{3}H]E1 (●) or for 4 h with [^{3}H]E2 (○).

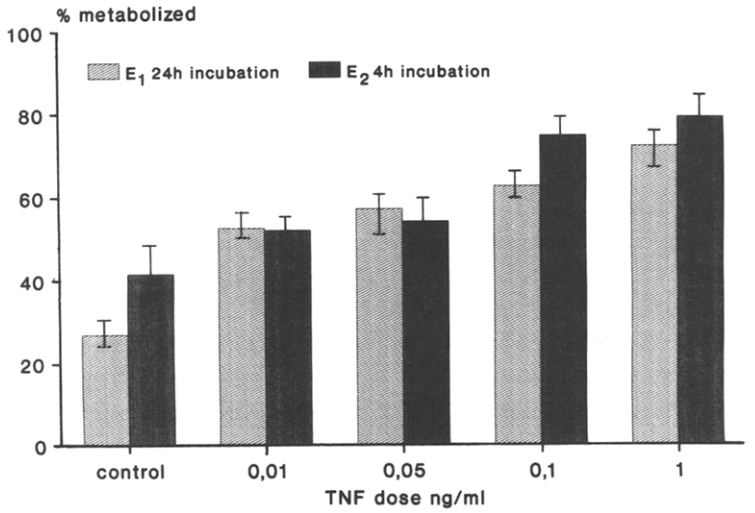

Fig. 2. Metabolism of [^{3}H]E2 and [^{3}H]E1 by 1.5×10^6 cells under TNF treatment in comparison to the control. The treatment group differed significantly ($P < 0.01$) from the control group. Dose dependence was also significant ($P < 0.01$).

E2 was elevated by 0.01 ng/ml TNF and remained at the same level at higher TNF concentrations. The growth of cells was influenced by TNF almost in the same manner as the estrogen metabolism, maximal growth inhibition occurred at 1 ng/ml TNF [2].

DISCUSSION

In contrast to the results of Hata *et al.* [3] conversion of E2 to E3 was predominant whereas no E1 formation occurred. Thus together with the loss of hormone sensitivity the variant of Ishikawa cells investigated in this study also changed the metabolic pathway of E2. As both E1 and E2 are converted practically exclusively to E3, one may assume that estrogen potency is irreversibly reduced to a minimum, whereas metabolism of E2 to E1 and E1-sulfate, as found in the E2 sensitive cells, leads to a state of at least potential estrogen activity, as 17βol oxidation and sulfation are reversible processes. This would explain why our clone of Ishikawa cells does not respond to 4-OH-tamoxifen in the presence of E2 containing fetal calf serum, as the antiestrogen probably only influences E2 induced growth as in mammary carcinoma cells [4].The drastic action on proliferation of TNF is accompanied by an increase in E1 and E2 metabolism rate but there is no evidence that the latter phenomenon has any relevance for the mechanism of growth reduction by TNF.

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