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Difference in uptake of ^{3}H-estramustine in two human prostatic carcinoma cell lines, LNCaP and LNCaP-r

M. Hasenson

Department of Clinical Chemistry I, Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden

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Summary. Estramustine, estradiol-3-N-bis(2-chloroethyl)carbamate (EM), has been shown to inhibit growth of the human prostatic carcinoma cell line LNCaP as well as its subline LNCaP-r. The hormone sensitive LNCaP showed greater sensitivity to the drug than the hormoneresistant LNCaP-r. LNCaP has also shown an increasing sensitivity to 10^{-7} M EM, when incubated with different concentrations of steroid hormones. We studied whether the increasing sensitivity was caused by increased uptake of EM. ^{3}H-estramustine was added to medium and the cells were incubated 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, and 4 h, or 2 h, 4 h, 6 h, and 8 h. No effect of steoids on the uptake of EM was noted, but LNCaP showed a higher uptake of EM compared to LNCaP-r. The uptake of EM in LNCaP increased for eight hours, whereas LNCaP-r reached its maximum uptake after six hours of incubation.

Key words: Carcinoma of the prostate – Cell line – Estramustine – LNCaP

Introduction

As estramustine phosphate (Estracyt®) (EM) is often used for treatment of hormone resistant prostatic carcinomas [1, 2], we wanted to study the effect of EM, on the growth rate of two cell lines, LNCaP and LNCaP-r [3]. In these studies EM was found to inhibit the growth of both cell lines, but LNCaP was more sensitive. We also found that addition of estradiol-17β (E_2) or 5α-dihydrotestosterone (DHT) significantly altered the growth rate of EM treated cells. The change in growth rate was found to be dependent on the concentration of EM but not on the type or concentration of steroid, i.e. E_2 had roughtly the same effect as DHT. These studies have been expanded here and the uptake of EM in the two cell lines in the presence or absence of steoid hormones was investigated.

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Materials and methods

Cell lines

The LNCaP cell line at passage 60 was a gift from Dr. Horoszewicz, Roswell Park Memorial Institute, Buffalo, NY, USA. The present studies were carried out with cells between 60th and 70th passages. The LNCaP-r subline was derived from the LNCaP cell line and characterized at our laboratory [4]. This subline is hormone resistant and has a different chromosomal distribution pattern and morphology, compared to that of the LNCaP cell line.

Cell culture

The cells were cultured in a constant environment (37°C, 5.0% CO_2) using RPMI1640 medium (Flow Lab, Scotland) supplemented with 10% (v/v) inactivated fetal calf serum (Gibco, Scotland), 50 μg/ml penicillin, 50 μg/ml streptomycin, and 2.0 μmol/ml L-glutamine. Final concentrations of endogenous hormones were as previously reported [4]. Culture medium was changed every second day, and the confluent cells subcultured 1:5 in 80 cm^2 or 25 cm^2 flasks (A/S Nunc, Roskilde, Denmark).

Substances

Estramustine, estradiol-3-N-bis((2-chloroethyl)carbamate, and ^{3}H-estramustine were a gift from AB Leo, Helsingborg, Sweden. Estradiol-17β (E_2), and testosterone (T) were purchased from Sigma (St. Louis).

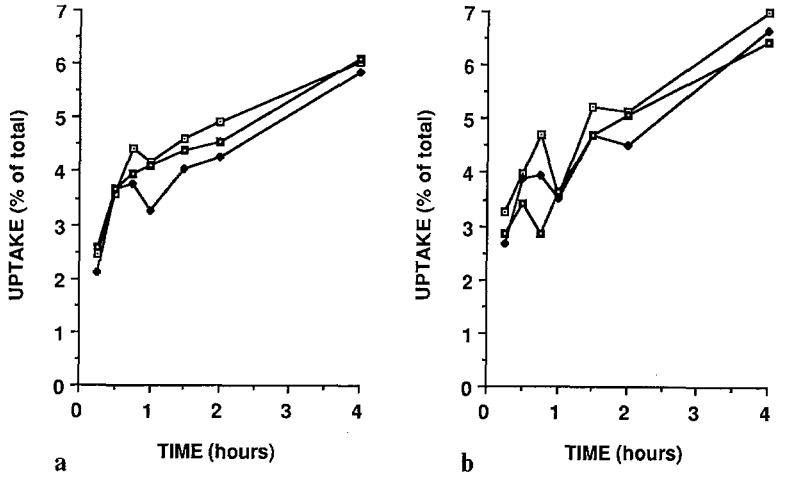

Fig. 1. a LNCaP cells incubated with (▣) 10^{-8} M ^{3}H-estramustine, (◆) 10^{-8} M ^{3}H-estramustine and 10^{-7} M estradiol, (◘) 10^{-8} M ^{3}H-estramustine and 10^{-7} M testosterone. b LNCaP cells incubated with (▣) 10^{-8} M ^{3}H-estramustine, (◆) 10^{-8} M ^{3}H-estramustine and 10^{-5} M estradiol, (◘) 10^{-8} M ^{3}H-estramustine and 10^{-5} M testosterone

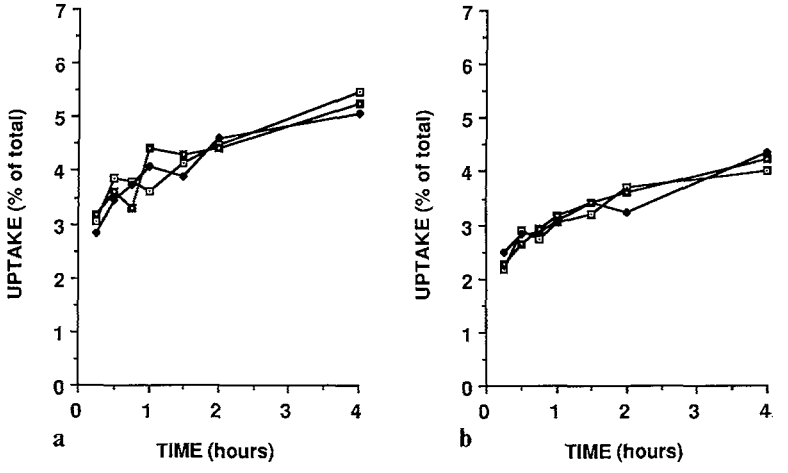

Fig. 2. a LNCaP-r cells incubated with (▣) 10^{-8} M ^{3}H-estramustine, (◆) 10^{-8} M ^{3}H-estramustine and 10^{-7} M estradiol, (◘) 10^{-8} M ^{3}H-estramustine and 10^{-7} M testosterone. b LNCaP-r cells incubated with (▣) 10^{-8} M ^{3}H-estramustine, (◆) 10^{-8} M ^{3}H-estramustine and 10^{-5} M estradiol, (◘) 10^{-8} M ^{3}H-estramustine and 10^{-5} M testosterone

Studies on estramustine uptake

The LNCaP cells were stored in liquid nitrogen in our laboratory since their arrival from Buffalo 1982. The LNCaP-r cells were stored in liquid nitrogen since the analysis that proved their difference from the original cell line [4]. The cells were subcultured 1:5 in 25 cm^2 tissue culture flasks, two days before incubation. On the day of incubation, new media, containing estramustine and the steroids in different concentrations (see figure legends) were added.

Incubation was carried out for 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, and 4 h, or 2 h, 4 h, 6 h, and 8 h. Each concentration had triplicate flasks and all experiments were repeated. After incubation the medium was removed, the cells washed with 1 ml PBS (pH 7.4) twice and 1 ml of trypsin-EDTA (Gibco, Scotland) was added to remove the cells from the flasks. The cells were then filtered on Whatman GF/C filters in a Millipore multihole filter holder, washed twice with PBS and the radioactivity on the filters measured in a scintillation counter, using LKB Optiphase scintillation cocktail. Two flasks with each type of medium (see figure legends), but without cells, were also incubated to determine the non-specific binding of radioactivity to the vessels.

Statistical analysis

Statistical analysis was carried out using Students *t*-test.

Results

Short time incubation (4 h) of LNCaP cells in ^{3}H-EM-containing medium, with or without addition of E_2 or T at different concentrations, did not reveal any measurable differences in ^{3}H-EM uptake ($P>0.05$) (Fig. 1a and b). Similarly, incubation of LNCaP-r cells with or without addition of steroids to ^{3}H-EM-medium did not reveal any differences in uptake of radioactivity to the cells (Fig. 2a and b).

However comparison of uptake of ^{3}H-EM did reveal differences between the cell lines. Incubation of the LNCaP cell line in ^{3}H-EM-containing medium

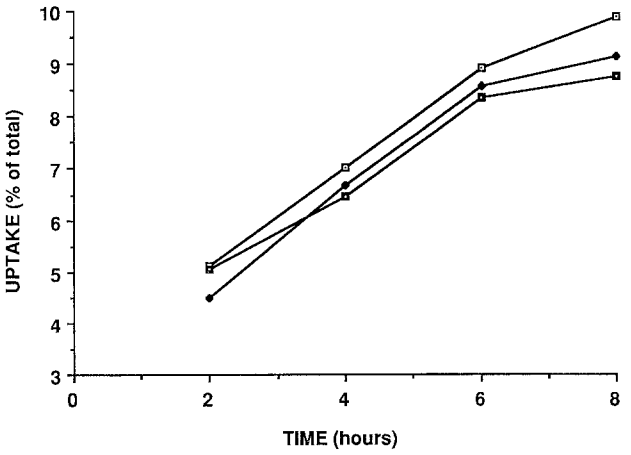

Fig. 3. LNCaP cells incubated with (▣) 10^{-8} M ^{3}H-estramustine, (◆) 10^{-8} M ^{3}H-estramustine and 10^{-7} M estradiol, (◘) 10^{-8} M ^{3}H-estramustine and 10^{-7} M testosterone

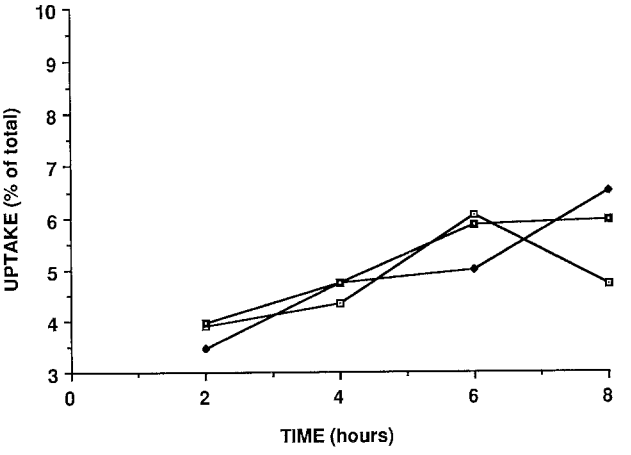

Fig. 4. LNCaP cells incubated with (▣) 10^{-8} M ^{3}H-estramustine, (◆) 10^{-8} M ^{3}H-estramustine and 10^{-7} M estradiol, (◘) 10^{-8} M ^{3}H-estramustine and 10^{-7} M testosterone

Table 1. Uptake of ^{3}H-EM in LNCaP and LNCaP-r cell lines after incubation in medium without added steroids. Incubation in medium with steroid addition gave similar results

	LNCaP	LNCaP-r
4 h (% uptake of total)	7.01	4.35
8 h (% uptake of total)	9.88	4.70

showed that the increase in uptake of the radioactive drug continued even after eight hours, while the LNCaP-r showed clear saturation after six hours (Figs. 3 and 4). Also the uptake in percentage of total added, after four and eight hours was lower for the LNCaP-r cells than for the LNCaP cells (Table 1).

Discussion

The LNCaP cell line from Roswell Park Memorial Institute was originally shown to be hormone sensitive [5, 6]. The LNCaP cell line and its subline LNCaP-r have previously also been reported to have differing sensitivities to hormonal stimulation, when compared by ATP measurement and cell enumeration [3, 4, 7]. Furthermore the effect of estramustine on growth was found to differ between the cell lines, as LNCaP was shown to be more sensitive to EM treatment than was LNCaP [3].

In view of these findings and the fact that steroids had been shown to modulate the inhibitory effect of EM on LNCaP cells [3], we studied whether these effects could be explained by differences in the cellular uptake of ^{3}H-EM. The present studies did not reveal any differences in the amount of uptake of ^{3}H-EM in the two cell lines in the presence of low or high concentrations of steroid hormones, compared to incubation with ^{3}H-EM only. Thus the original object of this study was not achieved.

However clear differences between the cell lines were found, when the time required for maximum uptake and the total percentage of uptake was compared. The steroid sensitive LNCaP clearly showed a higher uptake of ^{3}H-EM at all times during the four hour incubation.

This did not show any sign of declining even after eight hours of extended incubation. Conversely the steroid resistant cell line did not reach the same high uptake as LNCaP, during a 4 h incubation and between four and eight hours no further increase was found.

The higher sensitivity of LNCaP to EM [5] may be due to the higher uptake of the drug shown in the present study. In LNCaP-r the EM uptake mechanism may be disturbed, as was found for other systems in the cell line, such as production of PAP or functionally active 5α-reductase [4]. Whether the lack of sensitivity to EM and to steroid hormones was due to the general deterioration of enzyme systems in LNCaP-r or was caused by malfunction of one mechanism for uptake of both EM and hormones could not be determined.

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Dr. med. sc. Michael Hasenson
Department of Clinical Chemistry I
Karolinska Institutet
Huddinge University Hospital
S-14186 Huddinge
Sweden