A COMPARISON OF THE EFFECTS OF OESTROGEN AND TAMOXIFEN ON THE SYNTHESIS OF UTERINE HIGH MOLECULAR MASS RNA

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1. Introduction

The anti-oestrogen, tamoxifen, is an effective chemotherapeutic agent in the treatment of metastatic mammary carcinoma yet its mode of action is not understood. We have shown that the inhibitor, at an optimal dose of 1–2 mg/kg exerts a profound effect on the synthesis of uterine RNA measured as incorporation of precursor into acid insoluble material [1]. This effect occurred over a totally different time course to that induced by oestrogens. Thus, a single administration of oestradiol-17β caused a stimulation of uterine RNA synthesis which peaked at 7–12-times control levels, 2–4 h after hormone injection [1–3]. Tamoxifen had no effect at 2 h but induced a broad peak of stimulated RNA synthesis which lasted from 12–32 h after administration and peaked at 7-fold control levels 24 h after administration [1]. As part of our study on the mode of action of anti-oestrogens we here describe a comparison of the effects of oestrogen and tamoxifen on the incorporation of precursor into purified high M_r uterine RNA.

2. Materials and methods

The administration of tamoxifen and oestradiol-17β by subcutaneous injection in corn oil has been described [1]. The purification of high M_r uterine RNA and its fractionation and analysis on 2.7% polyacrylamide gels has been described [2].

Abbreviations: hnRNA, heterogeneous nuclear RNA, pre-rRNA, precursors to ribosomal RNA; Oestradiol-17β, 1,3,5(10)-estratriene-3,17β-diol, Tamoxifen, [1-(4-β-dimethylaminoethoxyphenyl) 1,2-diphenylbut-1-ene]

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3. Results and discussion

Fig.1 shows the incorporation of precursor into the high M_r uterine RNA of untreated (received carrier only), 3-week-old 30–35 g rats. The result reveals very little incorporation and confirms our findings that the uterus of the immature rat is a very quiescent tissue. It synthesizes little RNA [2], little protein [4] and most of its ribosomes are in the form of monosomes [4]. Fig.1 illustrates the results obtained when carrier was administered 2 h before death but essentially identical results were obtained when it was given 12 h and 24 h before death or if no carrier was administered.

Fig.2 shows the effect of oestrogen, tamoxifen and a combination of oestrogen and tamoxifen on the incorporation of precursor into high M_r, uterine RNA which has been fractionated on 2.7% polyacrylamide gels. It can be seen that, 2 h after the administration of 1 μg oestradiol-17β/30–35 g rat, there was a marked stimulation in the incorporation of precursor into 28 S and 18 S RNA. This increased synthesis could be followed through the 45 S and 32 S pre-rRNA species by varying the time of precursor incorporation and by cofractionating with ^{14}C-labelled 45 S and 32 S RNA isolated from HeLa cell nucleoli [2]. The 32 S species is detectable in the 2 h oestrogen treatment panel of fig.2 as a peak of radioactivity migrating slightly slower than the 28 S species. The 45 S species was most clearly defined in experiments where the radioactive precursor was administered 15 min before death (not shown). Clearly, the synthesis of uterine rRNA is strongly stimulated at 2 h after oestrogen administration and the response appears to result from increased transcription of the 45 S rRNA precursor. These results confirm [2]. It is notable that the peaks of radioactive precursor incorporation into rRNA and

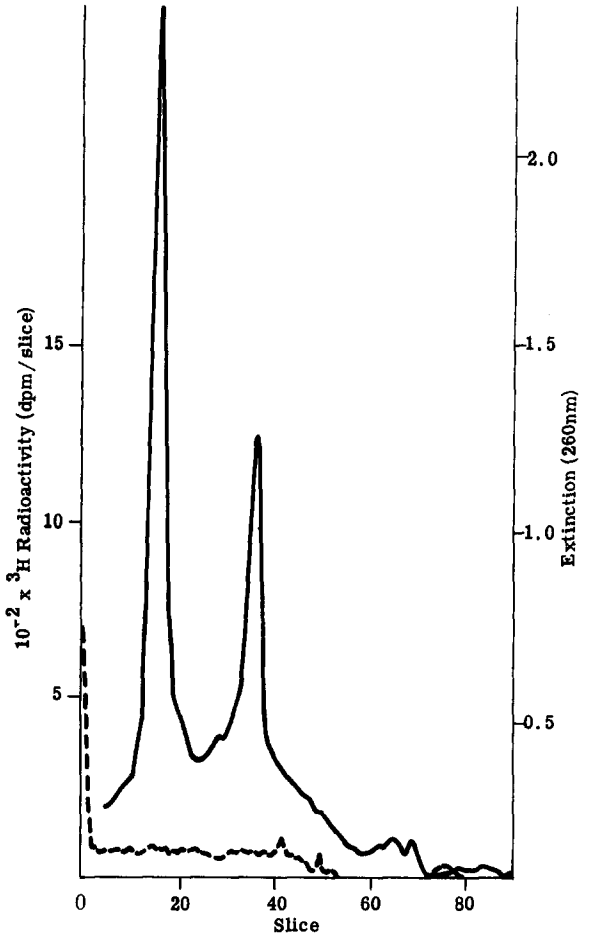

Fig.1. The incorporation of precursor into the high M_r RNA of immature rat uterus. Eight 19–21-day-old rats, (30–35 g) received a subcutaneous injection of 0.1 ml corn oil 2 h before death. They subsequently received 100 μCi each of [5-^{3}H]-uridine and [8-^{3}H]guanosine (each at 5 Ci/mmol) in 0.2 ml 0.9% NaCl by lateral tail-vein injection 30 min before death. Uterine high M_r RNA was then purified and 80 μg was fractionated on 2.7% polyacrylamide gels, scanned at an extinction of 260 nm, the gel sliced into 1 mm sections and the radioactivity in each slice determined as in section 2. (——) E_{260}; (– – –) radioactivity in dpm/slice. For reasons of clarity, the E_{260} trace is omitted for the first few slices of the gels.

pre-rRNA are superimposed on a background of incorporation into other high M_r RNA species. Extensive characterization and fractionation studies conducted by this laboratory in the past have shown that these species are of nuclear location, have a rapid turnover, lack methylation, contain poly (A) sequences and a base composition that identifies them as hnRNA [5,6]. We have further shown that oestrogen-induced hnRNA synthesis precedes and is a pre-requisite for the subsequent stimulation of rRNA synthesis [5–7].

The marked stimulation of RNA synthesis in immature rat uterus peaks at 2–4 h after a single injection of oestradiol-17β and then rapidly falls off, presumably due to hormone metabolism [1,3]. Fig.2 shows the incorporation of precursor into high M_r uterine RNA at 12 and 24 h after oestradiol-17β administration and it is seen that synthesis is much reduced by 12 h and, by 24 h, rRNA synthesis has returned almost to control levels although some stimulation of hnRNA synthesis is still observed (fig.2).

Tamoxifen alone had no significant effect on in vivo uterine RNA synthesis when administered 2 h before death (fig.2). This finding contrasted with in vitro analyses which have examined RNA polymerase activity in isolated uterine nuclei [8,9]. In these analyses some effects of tamoxifen on enzyme activity were observed though the stimulation was less marked than that induced by oestrogen. Considerable stimulation of the incorporation of precursor into high M_r uterine RNA was observed at 12 and 24 h after the administration of tamoxifen (fig.2). This confirmed our previous findings on the incorporation of precursor into acid-insoluble material [1]. As in the earlier stimulation of RNA synthesis by oestrogen, rRNA, pre-rRNA and hnRNA appear to be involved in the response (fig.2).

The slower response in the rat uterus responding to tamoxifen may reflect its low rate of metabolism compared with that of oestradiol. The half-life of tamoxifen in human blood is 4 days compared with

Fig.2. The incorporation of precursor into the high M_r uterine RNA of immature rats responding to oestrogen and tamoxifen. Groups of eight 19–21-day-old rats (30–35 g) received subcutaneous injections of 0.1 ml corn oil in which was dissolved 1.0 μg oestradiol-17β or 30 μg tamoxifen or a combination of both at 2, 12 or 24 h before death. They subsequently received radioactive RNA precursor 30 min before death and high M_r uterine RNA was prepared and analyzed as in fig.1. (——) E_{260}; (– – –) radioactivity in dpm/slice. For reasons of clarity, the E_{260} trace is omitted for the first few slices of the gels.

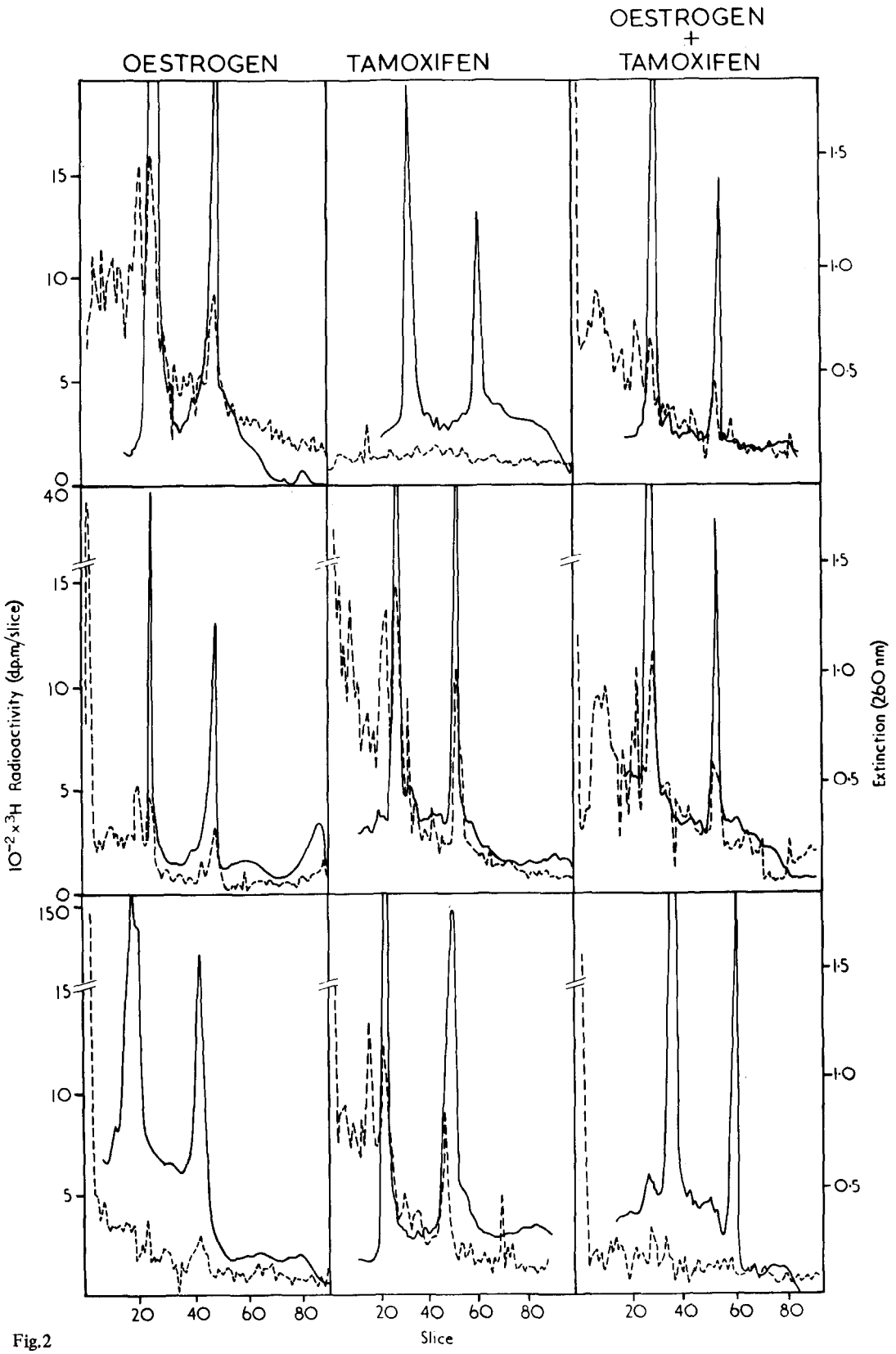
OESTROGEN
TAMOXIFEN
OESTROGEN + TAMOXIFEN
2 HOURS
12 HOURS
24 HOURS
$10^{-2} \times {}^3H$ Radioactivity (dpm/slice)
Extinction (260 nm)
Slice

Fig.2

<6 h for oestradiol [10]. The accumulation of tamoxifen in target cell nuclei is also much slower than that of oestrogen, though its nuclear retention is much increased. Thus, oestradiol nuclear concentration is maximal 2 h after its injection while tamoxifen concentration peaks at 24 h after administration [12]. Nuclear oestradiol levels have returned to those of untreated controls by 24 h after injection whereas tamoxifen is still present in nuclei 4 days after administration [12]. It is not clear, however, whether the prolonged nuclear retention is merely a reflection of the long half-life in blood.

Tamoxifen is an anti-oestrogen which competes with the hormone for its receptor [12], is carried as a receptor complex to the nucleus [11,13] and is assumed to competitively inhibit oestrogen-induced nuclear events. Fig.2 shows the results obtained when tamoxifen was administered together with oestradiol-17β 2, 12 and 24 h prior to death. It can be seen that the anti-oestrogen was unable to totally inhibit oestrogen-induced nuclear events. Thus it was only partially able to inhibit oestrogen-induced RNA synthesis 2 h after administration. Stimulated synthesis of both rRNA and hnRNA was again seen 12 h after the simultaneous administration of oestrogen and tamoxifen. In view of the effects of the individually administered compounds, this was assumed to be caused by tamoxifen rather than oestradiol-17β. At 24 h after the joint administration, very little RNA synthesis was observed. This again confirms our observations of precursor incorporation into acid-insoluble material [1] and suggests that, at this time, oestrogen may be acting as a tamoxifen inhibitor rather than the reverse.

The results presented reveal that the mode of action of tamoxifen is complex and that it certainly does not totally inhibit oestrogen-induced RNA synthesis. It is hoped that further studies on the effect of the inhibitor on oestrogen-induced changes in mRNA populations and in oestrogen-induced ribosome accumulation will clarify the site of action of the compound.

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