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OESTRIOL IN HUMAN BREAST TUMOURS

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SUMMARY

The role of oestriol in blocking the accumulation of oestradiol-17β by human breast tumours was investigated in patients with primary breast cancer. It was observed that oestriol is accumulated and retained by these tumours. However, pre-treatment of patients with 0·5 mg of the compound every 4 h for one and 3 days prior to mastectomy failed to reduce the accumulation of oestradiol indicating that, clinically oestriol may not be effective in inhibiting the action of oestradiol. The significance of the finding is discussed.

INTRODUCTION

The role of oestrogens in either the etiology of human breast cancer or the clinical course of the disease remains unsolved despite numerous studies. Some epidemiological evidence supports the concept of oestrogen involvement in that early menarche and late menopause, both related to changes in ovarian function and therefore oestrogen synthesis, are associated with an increased risk of the disease, whereas oophorectomy before the age of 40 for reasons other than cancer leads to diminution in the risk (see MacMahon, Cole and Brown [1]. However, the risk is considerably reduced in women who have their first child at an early age [1] although such women are also exposed early in life to a pronounced oestrogenic stimulus. In order to resolve these paradoxical findings it has been suggested that oestrone and oestradiol-17β are carcinogenic but that oestriol has a protective effect. This hypothesis has been supported by the observations that non-pregnant women of races with a low incidence of breast cancer have a higher ratio of urinary oestriol to oestrone and oestradiol than women from races with a higher incidence of the disease [2, 3]. However, it is argued [4, 5] that the measurement of oestriol excreted as a glucuronide may bear little or no relation to either plasma free oestriol or amounts present in oestrogen sensitive tissues. Very little is known about the physiological function of oestriol in normal women or in patients with breast cancer although it has been shown that oral administration of as little as 1·0 mg per day produces oestrogenic effects such as vaginal cornification and withdrawal bleeding [6].

If oestriol does act in the manner suggested by MacMahon and his collegues [1], it might be potentially useful in the treatment of breast cancer. An attempt was made, therefore, to study the uptake of oestriol by human breast tumours and the effect of pre-treatment with this compound on oestradiol accumulation. This paper reports our findings.

MATERIAL AND METHODS

Solvents were of analytical grade and were redistilled before use.

[6,7-^{3}H]-Oestradiol (S.A. 56 Ci/mmol) [2,4-^{3}H]-oestriol (S.A. 58 Ci/mmol) [4-^{14}C]-oestradiol (S.A. 31·8 mCi/mmol) and [4-^{14}C]-oestriol (S.A. 53 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, England, and were purified by paper chromatography before use. The compounds were dissolved in ethanol to a concentration of 50 μCi/ml. Oestriol tablets (Ovestin 0·25 mg/tablet) were a gift from Organon Laboratories Limited, Crown House, Morden, Surrey.

The patients in this series were women with breast cancer undergoing mastectomy whose informed consent was obtained. The malignancy of the tumour was confirmed by histological examination. Tritiated compounds in ethanol were mixed with 20 ml of 0·9% NaCl and 20 ml of this mixture was injected into an antecubital vein at least 1 h before operation. The tumour specimen was immediately frozen and remained in that state until further processing.

A single 20 ml sample of peripheral blood was taken at the same time as removal of the tumour to determine the peripheral concentrations of the injected compounds. In eight patients the effect of pre-treatment with oestriol on the uptake of oestradiol was studied. The patients were treated with ten tablets per day (i.e. 2·5 mg/24 h) one day prior to mastectomy and radioactive oestradiol was injected at least 1 h before the operation.

The tumour was thawed, the surrounding fat and connective tissue was removed and the tumour was then cut into small pieces. At this stage a small amount of [^{14}C]-labelled oestradiol or oestriol

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Table 1. Concentration of radioactive oestradiol and oestriol in human breast tumours and peripheral blood following a single injection of 50 μCi of these compounds. Sections 3 and 4 indicate the concentration of radioactive oestradiol after one day and three days of pre-treatment with 2·5 mg of oestriol per day, respectively

Section	Hormone administered	Age of patient	Time after injection (min)	Peripheral blood d.p.m./ml.	Tumour d.p.m./g	Tumour: blood
1.	[^{3}H]-Oestradiol	40	115	200	2570	12·8
		45	120	380	1590	4·2
		48	150	470	950	2·0
		38	165	230	2490	10·8
		52	165	300	1660	5·5
		48	170	270	390	1·4
	Mean ± S.D.	45·1	147	310 ± 100	1610 ± 850	6·1
2.	[^{3}H]-Oestriol	53	60	420	1570	3·7
		68	60	490	2020	4·1
		74	120	110	220	2·0
		64	135	380	3120	8·2
		67	205	270	2860	10·5
	Mean ±S.D.	65·1	116	330 ± 150	1960 ± 1160	5·7
3.	[^{3}H]-Oestradiol	53	95	450	2100	4·7
	after pre-treatment	68	120	—	1910	—
	with 0·5 mg cold	53	120	80	1950	24·3
	oestriol 4-hourly	79	120	240	3300	13·7
	for 20 h.	—	130	280	1240	4·4
		54	135	—	2150	—
		62	150	240	1800	7·5
		61	300	280	3480	12·4
	Mean ±S.D.	47·7	142	260 ± 120	2240 ± 760	8·4
4.	[^{3}H]-Oestradiol	50	130	120	1060	8·8
	after pre-treatment	52	135	880	2630	3·0
	with 0·5 mg cold oestriol 4-hourly for 3 days					
	Mean	51	132	500	1840	5·9

(5000 d.p.m.) was added and the radioactivity from the tumour was extracted as described previously [7]. The purified tumour extract was applied to a Whatman No. 1 paper and the chromatogram was developed for 4 h in a Bush B_1 system [8] with radioactive oestradiol or oestriol as reference compounds. The chromatograms were scanned in a Packard radiochromatogram scanner (Model 7200) and radioactivity from the paper strips was eluted with ethanol. The radioactivity was counted as described before [9]. Peripheral blood samples were treated in a similar manner.

RESULTS

The amounts of radioactivity associated with the free unconjugated oestradiol and oestriol in both peripheral blood and in the tumour tissue were measured in eleven patients. The results are shown in Table 1.

In the first investigation (see Section 1, Table 1), the tumour contained six times as many counts derived from oestradiol as the peripheral blood some 2·5 h after injection. Similar results were obtained when oestriol was administered: the tumour–blood ratio for oestriol was 5·7 at just under 2 h. (Section 2, Table 1).

When patients were given four doses each of 0·5 mg of oestriol (p.o.) at four hourly intervals before operation, and when tritiated oestradiol was then administered, the oestriol had no effect on the amount of radioactive oestradiol in peripheral blood at 2 h. (280 d.p.m./ml compared with 308 d.p.m./ml without oestriol treatment). The amount of free oestradiol in tumour tissue at this time was similarly unchanged by the oestriol pre-treatment (2240 d.p.m./g compared with 1610 d.p.m./g).

In the final investigation in which two patients had oestriol p.o. for three days, there was again no evidence of a diminution of plasma or tumour levels of oestradiol.

DISCUSSION

It is widely accepted that oestradiol is accumulated in many human breast tumours by binding to high-affinity low capacity receptor proteins. Endogenous concentrations of oestradiol in tumours appear to be higher than those in peripheral blood [10, 11]. It is a reasonable assumption that the high tumour–blood

ratios of oestradiol that we observed represent specific binding to receptor proteins.

There is no information whether there is a specific oestriol receptor in normal or neoplastic human breast tissue and Millington *et al.* [10] found only very low endogenous concentrations of this steroid in two of six tumours. However, it has been reported that oestriol diminishes the binding of oestradiol to its cytoplasmic receptor in both rat and human breast tumours *in vitro* [12, 13].

The tumour–blood ratios observed after administration of oestriol are of the same order as those found for oestradiol and oestriol may very well have been taken up by binding either to spare oestradiol receptor sites or to a specific oestriol receptor.

Administration of cold oestriol in amounts that are several times as high as the expected normal production had no effect upon oestradiol concentrations in peripheral blood or in the tumour tissue. This result indicates that it is unlikely that the uptake and subsequent biological action of oestradiol in human breast tumours can be blocked by oestriol concentrations within the physiological range. This failure could be due to the existence of a specific site for oestriol; or to a failure to saturate the oestradiol receptor with oestriol at the dose used; or to a displacement of oestriol by oestradiol. Whatever the mechanism, it seems improbable that oestriol would be uniquely useful in the treatment of the disease. Furthermore, if oestriol has a "protective effect" in preventing a carcinogenic action by oestradiol on human breast tissue, as suggested by MacMahon and his associates [1] it seems unlikely that this is effected by blocking oestradiol accumulation.

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