Does this mean that approximately half the patients with DCIS will need treatment by total mastectomy? This would seem to be illogical since it has been proven that patients with invasive cancers up to 4 cm in diameter can be safely treated by breast conservation. Why is it that patients with a condition in which they have a 30% chance of developing an infiltrating cancer are being offered ablative treatment, albeit with immediate or delayed reconstruction? The reason is that there is no body of data which has demonstrated that extensive DCIS can be satisfactorily treated, that is prevented from progressing to invasion by external or interstitial radiotherapy.

It could be claimed that the reverse is true, namely that patients with infiltrating cancer with associated extensive intraductal component are at greater risk of relapse after breast conservation including radiotherapy. However this may represent a different state from DCIS alone or with the earliest signs of micrometastases. No randomised studies are examining the role of either irradiation or tamoxifen for patients with extensive DCIS for which total mastectomy is the standard treatment at present.

1. Betsill WL, Rosen PP, Lieberman PH, Robbins GF. Intraductal carcinoma. Long-term follow up after treatment by biopsy alone. *JAMA* 1978, **239**, 1863–1867.
2. Page DL, Dupont WD, Rogers LW, Laudenberger M. Intraductal carcinoma of the breast: follow-up biopsy alone. *Cancer* 1982, **49**, 751–758.
3. Lagios MD, Margolin FR, Westdahl PR, Rose MR. Mammographically detected duct carcinoma *in situ*. *Cancer* 1989, **63**, 618–624.
4. Fentiman IS. Radiosensitivity of *in situ* carcinoma of the breast. *Lancet* 1985, **ii**, 443–444.
5. van Dongen JA, Fentiman IS, Harris JR. *et al. In situ* breast cancer: report of the EORTC consensus meeting. *Lancet* 1989, **ii**, 25–27.

Eur J Cancer, Vol. 27, No. 4, pp. 452–456, 1991.
Printed in Great Britain

0277-5379/91 $3.00 + 0.00

Tamoxifen Binding Sites Heterogeneity in Breast Cancer: a Comparative Study with Steroid Hormone Receptors

Giuseppe Leo, Gabriella Cappiello, Palmiro Poltronieri, Carmela Giardina, Corrado Manca, Carlo Storelli and Santo Marsigliante

Steroid receptors and tamoxifen binding sites (TBS) were assayed in the soluble fraction of 121 primary breast cancers. Scatchard analysis of TBS in high speed supernatant (100 000 *g*) showed one population of binding sites; however, biphasic plots were obtained in low speed supernatants (40 000 *g*). Isoelectric focussing of supernatants preincubated with radioactive tamoxifen identified two classes of TBS (pI 4.1–4.6) which have different binding affinities and bind neither oestradiol nor diethylstilbestrol. Association between TBS and steroid receptors was: TBS positive/progesterone receptor positive 32.6%, TBS positive/glucocorticoid receptor positive 52.7%, TBS positive/oestrogen receptor positive 60% and TBS positive/androgen receptor positive 72.2%. We conclude that heterogeneous TBS are present in low speed fractions and can be easily separated from the oestrogen receptor by isoelectric focussing. The association between TBS and steroid receptor status could be of clinical value in the management of primary breast cancer.
Eur J Cancer, Vol. 27, No. 4, pp. 452–456, 1991

INTRODUCTION

ALTHOUGH THE antitumoral effect of tamoxifen has been established in the management of advanced and early breast cancer [1], the exact modes of action of antioestrogen on breast cancer cells have not been completely elucidated.

Even though tamoxifen is thought to mediate its effects especially through the oestrogen receptors (ER), several aspects of tamoxifen action such as the response observed in 10% of patients whose tumours do not contain ER [2] suggest that non-oestrogen receptor mediated responses must occur in some tissues.

An alternative signal transmitter that is present in some ER negative tissues is the tamoxifen binding substance (TBS), first described by Sutherland and his colleagues [3]. Though the work of Sudo *et al.* [4] and Sheen *et al.* [5] strongly suggests that binding to the TBS is not an essential feature of the mechanism of action of antioestrogen, recently it has been reported that this binding site may represent a novel histamine receptor [6]. From this viewpoint, in addition to its effects mediated through binding to ER, tamoxifen may also exert antiproliferative effects through blockade of histamine responses.

Correspondence to S. Marsigliante, Department of Biochemistry, Faculty of Basic Medical Sciences, Queen Mary & Westfield College, Mile End Road, London E1 4NF, U.K; reprint requests to G. Leo.
G. Leo and C. Manca are at the Laboratorio di Ricerca Clinica, Ospedale Multizonale "V. Fazzi", 73100 Lecce; G. Cappiello, P. Poltronieri, C. Storelli and S. Marsigliante are at the Laboratorio Fisiologia Generale, Dipartimento di Biologia, Universitá di Lecce, Lecce; and G. Giardina is at the Anatomia Patologica, Universitá di Bari, Italy.
Revised 7 Jan. 1991; accepted 11 Jan. 1991.

The intracellular distribution of TBS is still contradictory. Several studies have indicated their presence in the soluble high speed supernatant, while other studies showed a loss of sites in this fraction and a prevalent distribution in low speed supernatants [4, 7–11]. Moreover, although TBS has been characterised in numerous tissues by radioligand binding assays, and a heterogeneity in sites has been described in the TBS from fetal guinea pig uterus [12], little evidence of the biochemical heterogeneity of the TBS in human breast cancer has been described.

To aid in the resolution of these controversies, we undertook this current study in which we analyse the cellular distribution of TBS in two different cytosol fractions. Moreover, a direct method for partial characterisation of the TBS in human breast cancer tissue is described and, finally, we compare the presence of TBS with the steroid receptor status in all the patients examined.

MATERIALS AND METHODS

Chemicals

All reagents were of analytical grade. [1,2,6,7,-^{3}H]-oestradiol ([^{3}H]-oestradiol), 16-ethyl-21-hydroxy-19-nor[6,7-^{3}H]pregn-4ene-3,20-dione (^{3}H-ORG2058), z-4-hydroxy-[N-methyl-^{3}H]tamoxifen ([^{3}H]-OH-TAM), [1,2,4,6,7,-^{3}H]-dexamethasone, z-4-hydroxy-tamoxifen (OH-TAM), ORG2058, triamcinolone acetonide, were purchased from Amersham. Diethylstilboestrol (DES) and 19-norethindrone were purchased from Sigma, dithiothreitol (DTT) was obtained from BDH and [17-methyl-^{3}H]-R1881 from New England Nuclear.

Tissue handling

Human breast tumour tissue was obtained at operation. The sample was stored in liquid nitrogen until processed. All tissue processing was performed at 4°C. The tissue was homogenised in an Ultraturrax and suspended in a phosphate buffer of low ionic strength (10 mmol/l K_2HPO_4/KH_2PO_4, 1.5 mmol/l EDTA, 10 mmol/l Na_2MoO_4, 3 mmol/l NaN_3, 5 mmol/l DTT, 10 mmol/l $MgCl_2$ and 10% glycerol. The homogenates were centrifuged at 40 000 *g* (low speed supernatant) at 4°C for 1 h; a fraction of this supernatant was retained for analysis. The remaining fraction was then centrifuged at 100 000 *g* (high speed supernatant) at 4°C for 1 h. In order to establish the cellular distribution of the TBS, we assayed both high and low speed fractions. Oestrogen (ER), progesterone (PR), androgen (AR) and glucocorticoid (GR) receptors were assayed in the high speed fraction only.

DCC assay of AR, GR and PR

100 μl of the high speed supernatant (2–3 mg protein/ml) was incubated with a single saturating concentration of [^{3}H]hormone (5 nmol/l, final concentration), in the presence or absence of a 200 fold excess of unlabelled antagonists. Since the steroid receptors produce crossreactions with different steroid radioligands [13, 14] resulting in overestimation of the number of binding sites, we tried to overcome this problem through addition of suitable cold antagonists to the assay. Therefore, we used the following competitors: dexamethasone and ORG2058 (for PR), triamcinolone acetonide and R1881 (for AR), 19-norethindrone and dexamethasone (for GR).

DCC assay of ER

ER were measured in 73 out of the 121 samples by single saturating dose assay using tritiated oestradiol (5 nmol/l, final concentration), or by multipoint analysis followed by Scatchard plot [15] in the 48 remaining tumours (which were large enough to obtain the required volume of cytosol). The multipoint analysis was performed incubating aliquots (100 μl) of tumour supernatant (100 000 *g* fraction) with increasing concentrations of [1,2,6,7,-^{3}H]oestradiol (0.15–10 nmol/l, final concentration) in the presence or absence of 200-fold excess of diethylstilboestrol or tamoxifen.

DCC assay of TBS

TBS were assayed by incubating 100 μl of supernatant (in duplicate) containing 2 μmol/l DES (to eliminate binding of tritiated tamoxifen to oestrogen receptor sites), with seven dilutions (0.15–10 nmol/l, final concentration) of [^{3}H]-OH-TAM, in the presence or absence of a 200-fold excess of unlabelled OH-TAM, made up in 2.5% dimethylformamide and 1% ethanol, to ensure OH-TAM was in solution. We used OH-TAM, a metabolite of tamoxifen, because it is about 20 fold more active as an antioestrogen than tamoxifen.

These incubations were carried out at 4°C for 24 h. Free hormone was separated from bound by incubation with dextran coated charcoal (DCC) (0.5% charcoal and 0.05% dextran T70) at 4°C followed by centrifugation. An aliquot of the supernatant was counted in a liquid scintillation counter.

A further aliquot of these samples was taken for isoelectric focussing (IEF) of TBS and ER.

Isoelectric focussing

The IEF gels were cast in slabs of size 125 × 260 mm and separation was conducted along the long axis. Polyacrylamide gels containing 12% glycerol, 2 mm thick, with high porosity (T = 5%, C = 3%) were used. A pH gradient was achieved using 0.7% (w/v) LKB ampholine 3.5–10 (LKB, Bromma, Sweden) and 0.3% (w/v) LKB ampholine 5–8. Gels were photopolymerised at room temperature by means of a TR 26 polymerisation light (Hoefer S.I., San Francisco), using riboflavin (0.004%) for at least 8 h. IEF was performed in a cold room and the temperature of the cooling water was kept constant at 4°C, using a LKB Multiphor II System. Electrode solutions of 1 mol/l NaOH (cathode) and 1 mol/l H_2SO_4 (anode) were used. After DCC extraction, aliquots of the radioactive supernatant derived from DCC assay, were loaded near the cathode. The run was carried out for 4 h using a 3000 xi CC Power supply (Biorad, Hemel Hempstead, UK) at 20 mA/1200 V/20 W constant power. A mixture of nine natural proteins (Biorad) was used for pH calibration. After the run, the gels were cut into 2.5 mm slices and each slice was incubated with 10 ml scintillation cocktail (Packard) for 24 h at room temperature and radioactivity assayed.

Protein determination

This was carried out using the method of Lowry *et al.* [16] using bovine serum albumin as the standard. Receptors and TBS concentrations were calculated as femtomole of ligand bound per milligram of protein.

RESULTS

121 primary carcinomas of the breast were analysed. Quantitatively, the results of ER assay by DCC method were practically identical using either DES or tamoxifen as antagonists. In fact, comparison of receptor concentration values obtained with DES and with tamoxifen gave an overall correlation coefficient of $r = 0.99$ ($P < 0.001$) and with a slope of 1.02 ($n = 121$). 76

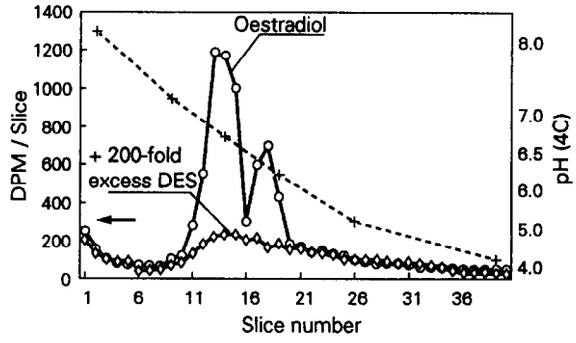

Fig. 1. IEF analysis of oestrogen receptors in a 40 000 *g* supernatant obtained from a primary breast tumour incubated with [^{3}H]-oestradiol. The specific binding components with pI 6.1 and 6.6 represent the ER and no radioactivity is bound to proteins with pI between 6.0 and 3.5. The arrow marks the position of the sample application point. DPM = disintegrations per min.

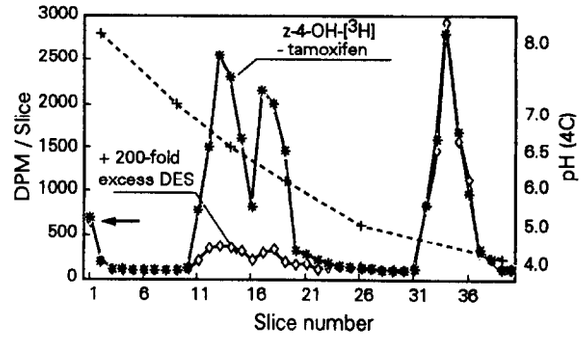

Fig. 3. IEF analysis of oestrogen receptors and tamoxifen binding sites in a 40 000 *g* supernatant from a primary breast tumour incubated with z-4-OH-[N-methyl-^{3}H]-TAM, in the presence or absence of a 200 fold excess of cold DES. The radioactive peak at pI 4.3 shows no appreciable displacement.

out of the 121 specimens (62.8%) were TBS positive (TBS> 10 fmols/mg protein) when tamoxifen was used as an antagonist and in 56 out of the 121 tumours (46.2%) when DES was used in place of tamoxifen, confirming that tamoxifen, besides binding to the ER, also binds to distinct binding sites which do not bind oestrogens.

Analysis of 121 primary tumour cytosols was carried out using IEF in polyacrylamide gel. IEF fractionation of ER positive (ER+) primary tumour cytosols incubated with [^{3}H]-oestradiol were performed in both 40 000 *g* and 100 000 *g* fractions. In both fractions, IEF showed that the specific binding components were present only as two major isoforms with pI 6.1 and 6.6 respectively (Fig. 1). No radioactivity was seen bound to proteins from ER negative (ER−) tumours fractionated by IEF, when incubated with radioactive oestradiol. The IEF using [^{3}H]-hydroxymethyl-TAM instead of labelled oestradiol was performed on the 40 000 *g* fraction only. It showed the presence of two protein species (ER and TBS) which were able to specifically bind TAM. All the possible IEF patterns were presence of activity only at pI 4.1–4.5 (in ER−/TBS+ tumours), presence of activity also in the range of pI 5.9–6.7 (in ER+/TBS+ tumours) (Fig. 2) and, finally, absence of both activities (in ER−/TBS− tumours). The radioactive peak present at pI 4.1–4.5, following IEF fractionation of cytosol previously incubated with labelled tamoxifen, was completely displaced by a 200 fold excess of cold tamoxifen in only 20% of the tumours analysed (in 15 out of the 76 TBS+ samples). The remaining samples showed a different degree of displacement ranging from 80 to 30% of the total binding. This behaviour could suggest the presence of two sites belonging either to the same protein or to different proteins which exhibit approximately the same pI, but with different binding affinities, the lower affinity being non saturable. The IEF using DES as a competitor, while completely displacing the labelled tamoxifen from the ER isoforms, never altered the TBS profile at pI 4.1–4.5 (Fig. 3), showing that TBS do not bind DES.

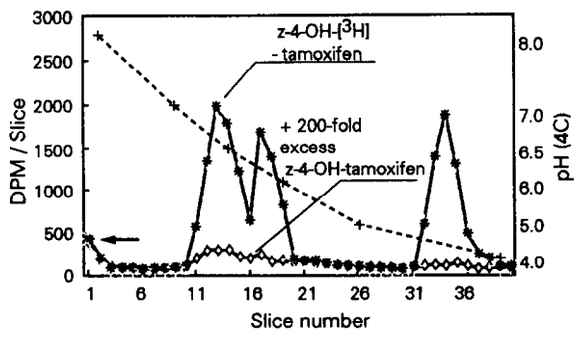

Fig. 2. IEF analysis of oestrogen receptors and tamoxifen binding sites in a 40 000 *g* supernatant from a primary breast tumour incubated with z-4-OH-[N-methyl-^{3}H]-TAM, in the presence or absence of a 200 fold excess of z-4-OH-TAM. The ER is present as two isoforms with pI of 6.1 and 6.6 (cf. Fig. 1). The TBS is present at approximately pI 4.3.

Figure 4 shows the influence of the two centrifugal fields (40 000 *g* and 100 000 *g*) on TBS. In general, the TBS concentration is higher in 40 000 *g* supernatants, producing biphasic Scatchard plots, while only one population of binding sites with a k_d between 1 and 2 nmol/l is present in the high speed fraction. These data demonstrate that heterogeneity of TBS exists mainly in low speed supernatant. The presence of a 200 fold excess of DES in the incubation mixture assures the saturation of the ER isoforms (as shown by IEF) enabling the binding of the labelled tamoxifen to the TBS only.

The steroid receptor content was analysed by DCC (using either SSD or Scatchard assays for ER), with a cut-off value of 10 fmols/mg cytosolic protein. 70 out of the 121 tumours were ER+ (57.8%), 52 were PR+ (42.9%), 36 were GR+ (29.7%) and 35 out of 121 were AR+ (28.9%). TBS (using cold tamoxifen as competitor) were present in 76 out of the 121 samples (62.8%). When the presence of TBS was compared with the presence, in the same samples, of steroid receptors, a good association between TBS and AR was observed. In fact, 26 out of 36 AR+ tumours (72.2%) were also TBS+ ($P < 0.01$). This percentage decreases to 60% for ER (42 of 70 ER+ cytosols were TBS+, $P < 0.001$) to 52.7% for GR (19 of 36 GR+ tumours were also TBS+, N.S.) and to only 32.6% for PR (17 of 52 PR+ tumours were TBS+, N.S.).

DISCUSSION

Laboratory studies during the past decade have shown that tamoxifen shares the same binding site with oestradiol and our correlation between ER concentrations obtained using DES or tamoxifen as competitors for the radioactive oestradiol is in perfect agreement with this concept. In this study we present a method for the identification of TBS in human breast cancer tissue using radiolabelled hormone binding and isoelectric

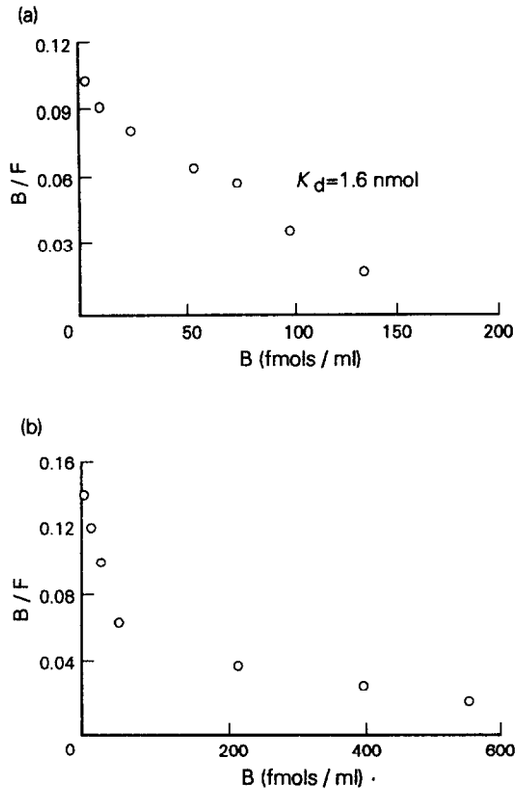

Fig. 4. Scatchard analysis of tamoxifen binding sites in human breast cancer cytosols incubated with seven concentrations of z-4-OH-[N-methyl-^{3}H]-TAM with and without a 200-fold excess of z-4-OH-TAM. (a) High speed supernatant (100 000 *g*), (b) low speed supernatant (40 000 *g*).

focussing. Using this method we have been able to show the effectiveness of tamoxifen as a competitor for the different ER isoforms: in fact both major isoforms of ER present at pI 6.1 and 6.6 disappeared following an incubation with a 200 fold excess of cold tamoxifen. These two isoforms identified by IEF are equivalent to the 8S form (the protein with pI 6.1) and to the 4S form (the isoform with pI 6.6) [17]. It is likely that the protein with pI 6.1 represents a multimeric assembly of at least two receptor molecules and other protein such as the 90 kD heat shock protein, while the 4S form is monomeric [18–20].

The IEF competition profiles show only a partial inhibition of TBS by TAM, suggesting the presence of low affinity sites within the same pI range that cannot be separated effectively from the high affinity ones. In some of the tumours analysed the unsaturable site is in excess with respect to the high affinity sites and it is difficult to visualise them by IEF. In contrast to earlier reports in which TBS detected in human breast cancer specimens were present as one homogeneous population of binding sites, we found non-linear Scatchard plots in the low speed fraction, suggesting either that the tamoxifen binding protein has two classes of binding site or that there are two different receptor molecules. When the sample is subjected to a further centrifugation at 100 000 *g*, only one receptor with high affinity is detectable in the soluble fraction. Sutherland and his associates found one single class of TBS in the high speed supernatant fraction [3, 7, 8], and one single class of TBS was found by Ferno and Borg [9] when analysing TBS in 20 000 *g* supernatant and by Mheta [10] who used the subcellular fraction collected after a 105 000 *g* centrifugation. Thus, this discrepancy could be attributed to the difference in cellular fraction used for the assay. It is interesting to note that two classes of tamoxifen binding sites have been shown already in the soluble high speed fraction from fetal guinea pig uterus which exhibit a significant difference in the binding affinity [12]. It should be pointed out that the physiological role of these two macromolecules in the fetal guinea pig uterus is currently not known.

It has been shown that TBS are present in almost every normal and malignant tissue [21, 22] and in cell lines that are oestrogen responsive or non-responsive; this could lead to the belief that TBS has no direct function in the antitumour action of tamoxifen. Nevertheless, the work of Brandes and Bogdanovic [6] and the association between TBS and steroid receptors (especially with ER and AR) shown here should allow further investigation before excluding any role of TBS in mediating the growth suppressive action of antioestrogens.

It has been shown that TBS bind, with relatively high affinity, to a variety of componds that are classical inhibitors of drug metabolism [23], and an association of the TBS with cytochrome P-450 has been proposed [24, 25]. The finding that low affinity-high capacity TBS exist together with the high affinity species could lead to the speculation of an alternative role of this protein as a means of the retention and/or inactivation of unknown ligands, perhaps compounds bearing aromatic moieties. Whatever the reason for the existence of TBS in the intact cell, the relationship between them and steroid receptor action in normal and disease conditions would seem to be worth further study.

1. Furr BJR, Jordan VC. The pharmacology and clinical uses of tamoxifen. *Pharmacol Ther* 1984, **25**, 126–205.
2. DeSombre ER, Jensen EV. Clinical usefulness of steroid receptor determination in breast cancer. In: Leung BS, ed. *Hormonal Regulation of Mammary Tumours* Montreal, Eden Press, 1982, Vol I, 155–182.
3. Sutherland R, Murphy L, Foo M, Green M, Whybourne A, Krozowski Z. High-affinity antioestrogen binding site different from the oestrogen receptor. *Nature* 1980, **288**, 273–275.
4. Sudo K, Monsma FJ Jr and Katzenellenbogen BS. Antiestrogen binding sites distinct from the estrogen receptor: subcellular localization, ligand specificity and distribution in tissues of the rat. *Endocrinology* 1983, **112**, 425–434.
5. Sheen Y, Simpson D, Katzenellenbogen B. An evaluation of the role of antioestrogen-binding site in mediating the growth modulatory effects of antioestrogens: Studies using t-butil-phenoxy-ethyl diethylamine, a compound lacking affinity for the oestrogen receptor. *Endocrinology* 1985, **117**, 561–569.
6. Brandes LJ, Bogdanovic RP. New evidence that the antioestrogen binding site may be a novel growth-promoting histamine receptor (?H3) which mediates the antioestrogenic and antiproliferative effects of tamoxifen. *Biochem Biophys Res Commun* 1986, **134**, 601–608.
7. Sutherland RL, Green MD, Hall RE, Reddel RR, Taylor IW. Tamoxifen induces accumulation of MCF-7 human mammary carcinoma cells in the Go/G1 phase of the cell cycle. *Eur J Cancer Clin Oncol* 1983, **19**, 615–621.
8. Sutherland RL, Hall RE, Taylor IW. Cell proliferation kinetics of MCF-7 human mammary carcinoma cells in culture and effects of tamoxifen on exponentially growing and plateau-phase cells. *Cancer Res* 1983, **43**, 3998–4006.
9. Ferno M, Borg A. Antiestrogen binding sites in human breast cancer biopsies. *Anticancer Res* 1985, **5**, 307–312.
10. Mehta RR, DasGupta TK. Antiestrogen binding sites in microsomal fractions of malignant and nonmalignant human breast tissues. *Breast Cancer Res Treat* 1987, **9**, 61–67.
11. Watts CKW, Murphy LC, Sutherland RL. Microsomal binding sites for nonsteroidal anti-oestrogens in MCF-7 human mammary carcinoma cells. *J Biol Chem* 1984, **259**, 4223–4229.
12. Gulino A, Pasqualini JR. Specific binding and biological response of antiestrogen in the foetal uterus of the guinea pig. *Cancer Res* 1980, **40**, 3821–3826.

13. Funder JW, *et al.* Mineralcorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 1988, **242**, 583–585.
14. Randall V, Mainwaring W. The assay of androgen receptor proteins. In: *Sex Steroid Receptors* 1985, Acta Medica Press, 37–45.
15. Scatchard GS. The attraction of proteins for small molecules and ions. *Ann N Y Acad Sci* 1949, **51**, 660–672.
16. Lowry OH, Roseburgh NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
17. Wrange O, Nordenskjold B, Gustafsson JA. Cytosol oestradiol receptor in human mammary carcinoma: an assay based on isoelectric focussing in polyacrylamide gel. *Anal Biochem* 1978, **85**, 461–469.
18. Wittliff JL. Molecular basis of endocrine responsive cancers. *Clin Physiol Biochem* 1987, **5**, 160–178.
19. Moncharmont B, Anderson WL, Rosenberg B, Parikh H. Interaction of oestrogen receptor of calf uterus with a monoclonal antibody: probing of various molecular forms. *Biochemistry 1984*, **23**, 3907–3912.
20. Brandt DW, Wittliff JL. Assessment of oestrogen receptor monoclonal antibody interaction by high performance liquid chromatography. *J Chromat* 1987, **397**, 287–297.
21. Lim CL, Walker MJ, Mehta RR, DasGupta TK. Oestrogen and antioestrogen binding sites in desmoid tumors. *Eur J Cancer Clin Oncol* 1986, **22**, 583–587.
22. Mehta RR, Cerny WL, Moon RC. Distribution of antioestrogen specific binding sites in normal and neoplastic mammary gland. *Oncology* 1984, **41**, 387–392.
23. Ruenitz PC, Bagley JR. Affinity of ligands other than triarylethylenes for liver microsomal antioestrogen binding sites. *Biochem Pharmacol* 1985, **34**, 2807–2809.
24. Ruenitz PC, Toledo MM, (1980). Inhibition of rabbit liver microsomal oxidative metabolism and substrate binding by tamoxifen and the geometric isomers of clomiphene. *Biochem Pharmacol* 1980, **29**, 1583–1587.
25. Meltzer NM, Stang P, Stenson LA. Influence of tamoxifen and its N-desmethyl and 4-hydroxy metabolites on rat uterine cytosol. *Biochem Pharmacol* 1984, **33**, 115–124.

Acknowledgements—We are most grateful to the Puglia Region (Italy) for project grant support (L.R. 730 of 27/12/83).

Eur J Cancer, Vol. 27, No. 4, pp. 456–461, 1991.
Printed in Great Britain

0277-5379/91 $3.00 + 0.00

N-nitrosoproline Excretion in the Presence and Absence of Gastric Disease

T.M. Knight, S. Leach, D. Forman, C. Vindigni, P. Packer, S. Venitt, C. Minacci, L. Lorenzini, P. Tosi, G. Frosini, M. Marini and N. Carnicelli

_N_-nitrosoproline (NPRO) excretion, an indicator of endogenous nitrosation, was measured in a group of hospital inpatients who were identified by endoscopy and gastric biopsy as either having gastric lesions or having healthy stomachs. NPRO was assayed in background 24-hour urine samples and samples collected after loading doses of nitrate and L-proline. The presence of gastric lesions was associated with altered gastric pH and concomitant changes in gastric juice nitrate and nitrite concentration. Gastric juice pH increased with increasing severity of gastric disease ($P = 0.031$) and patients with normal stomachs had a lower gastric pH than those with chronic atrophic gastritis (CAG) (3.0 vs. 6.5, $P = 0.017$). The changes in gastric juice nitrate concentration were in the reverse direction ($P = 0.002$ for trend) with normal patients having higher mean levels than CAG patients (12.7 vs. 5.5 μg/ml, $P<0.0001$). Nitrite concentration increased with severity of gastric disease but the results were not significant (normal, 82.9 vs. CAG, 223.4 ng/ml, $P = 0.069$). No association was found between the presence of gastric lesions and increased urinary NPRO excretion. Mutagenic activity was not detected in any of the gastric juice samples.
Eur J Cancer, Vol. 27, No. 4, pp. 456–461, 1991

INTRODUCTION

THIS STUDY was designed to address the question of whether individuals with chronic gastric disease, who are at a higher risk of developing gastric cancer [1, 2] synthesise more *N*-nitrosoproline (NPRO) *in vivo* than individuals without such disease. NPRO is produced as a result of the nitrosation of L-proline by nitrite ingested or generated from bacterial reduction of nitrate *in vivo*. NPRO synthesis has been used as an indicator of nitrosating ability and a marker of endogenous *N*-nitroso compound formation [3]. Most *N*-nitroso compounds, but not NPRO, are proven animal carcinogens [4] and those *N*-nitroso compounds formed intragastrically (especially the *N*-nitrosamides) have been thought to be a possible cause of GC in humans [5–7].

As secondary objectives the study also sought to determine the interrelationships between NPRO synthesis and gastric juice pH, nitrate and nitrite concentration, and smoking and mutagenicity.

MATERIALS AND METHODS

Subjects

The study was conducted amongst inpatients in the Central Hospital, Siena, Italy and was approved by the local research ethical committee. The consent of all patients was obtained. 81