Eur J Cancer, Vol. 27, No. 11, pp. 1445–1448, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Cell Membrane Receptors for Urokinase Plasminogen Activator are Increased in Malignant Ovarian Tumours

Bertil Casslén, Barbro Gustavsson and Birger Åstedt

The binding of ^{125}I-labelled urokinase plasminogen activator (uPA) to cell membranes of ovarian tumours was characterised. Binding was fast, specific to HMW-uPA, and saturated at low concentration [1.5 (range 1.2–1.6) nmol/l]. Scatchard analysis suggested a single class of binding sites with K_d 1.1 (0.9–1.3) nmol/l. These data indicate the presence of a specific cell membrane receptor for uPA in ovarian tumours, whose characteristics are similar to those reported for uPA receptors in other tissues. Endogenously occupied receptors were uncovered by exposing the membranes to acid conditions (pH 2) before assay, thereby allowing quantitation of the total amount of receptor. uPA receptors were assayed in 10 malignant and 6 benign epithelial ovarian tumours. The total number of receptors was higher in the malignant tumours. This was secondary to increases of both free and occupied receptors. We conclude that this reflects the biological function of cell surface bound plasminogen activation in tumour growth and spread.
Eur J Cancer, Vol. 27, No. 11, pp. 1445–1448, 1991.

INTRODUCTION

MALIGNANT TISSUES produce and contain increased amounts of certain proteolytic enzymes, which are instrumental in their growth and spread. The conversion of circulating plasminogen to active plasmin is central in the process of extracellular matrix degradation associated with tissue proliferation [1]. Plasmin acts directly to degrade certain matrix proteins like fibronectin, vitronectin and laminin, and indirectly to activate latent collagenases, which subsequently degrade the collagen matrix [2, 3]. Activation of plasminogen in this context is initiated by urokinase plasminogen activator (uPA), and involves cell-surface binding of both uPA and plasminogen [4–6]. This cell-surface bound interaction seems to also involve the mechanism for activation of the secreted zymogen pro-uPA to active uPA [6]. The receptor binding of uPA serves to focus the proteolytic activity to surfaces in contact with extracellular matrix or other cells [7]. uPA, which is not bound to its receptor, is a less efficient activator, and is more exposed to inactivation by specific inhibitors [3, 8, 9]. Receptor-bound uPA has been demonstrated to mediate tissue invasion and matrix degradation of tumour cells and macrophages [3, 9–11]. Such effect on cell proliferation does, however, require the catalytic site of uPA to be intact [12]. Specific receptors for uPA have now been reported in a variety of neoplastic and normal tissues, all of which produce uPA.

The aim of this study was to identify and characterise the binding of uPA to cell membranes from ovarian carcinomas, since such tumours produce uPA in tissue culture [13]. We also measured the number of free and total binding sites on the membranes of benign and malignant ovarian tumours.

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Revised 8 May 1991; accepted 7 June 1991.

MATERIALS AND METHODS

Material

Bovine serum albumin (BSA), Hepes and *p*-aminobenzamidine was purchased from Sigma (St Louis, Missouri). Hanks' buffered salt solution (HBSS) was obtained from Gibco (Paisley, Scotland), Ukidan was from Serono (Geneva), and recombinant tissue plasminogen activator (tPA) from Boehringer Ingelheim. Sephadex G-100 and CH-Sepharose 4B was obtained from Pharmacia (Uppsala, Sweden).

Clinical material

25 patients with epithelial ovarian tumours were included in the study. Tissue samples, which were not required for the histopathological examination, were obtained immediately at surgery for the study. Approval had been obtained from the Institutional Review Board for studies on human subjects at the University Hospital of Lund. These samples were frozen in HBSS until processed. The remaining tumour was fixed in formalin and routinely processed for histological classification. Each tumour was examined by two independent pathologists before classification was made. The tumours were classified as serous, mucinous or endometroid. Nineteen were malignant and six were benign. In the malignant group, two tumours were highly differentiated, and the others poorly or intermediately differentiated. 2 patients belonged to clinical stage 1, 2 to clinical stage 2, and the rest to clinical stage 3 and 4. Patients with benign tumours were 41–82 (median 60) years old, and those with malignant tumours were 32–65 (median 60) years old.

Preparation of cell membranes

After superficial drying and weighing, the tissue was homogenised in sucrose 0.25 mol/l, (Hepes) 0.01 mol/l buffer pH 7.2, 10 ml/g tissue, for 3×10 seconds, using a homogeniser with rotating knives (Ystral, Germany). The homogenate was first centrifuged at 1700 *g* for 10 min and then at 12 000 *g* for 15 min to remove cell debris and nuclei. The pellet of membranes was collected after centrifugation of the supernatant at 44 000 *g* for 45 min, washed once with the same buffer, and finally redissolved

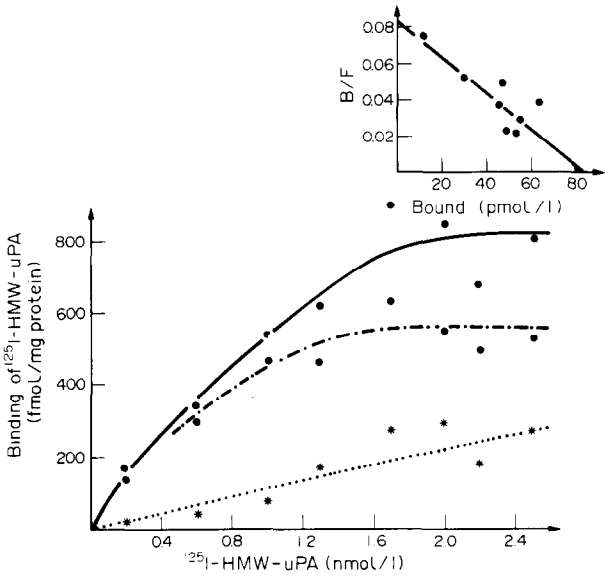

Fig. 1. Binding of ^{125}I-HMW-uPA to cell membranes prepared from a serous ovarian adenocarcinoma. The Scatchard plot (insert) was linear, suggesting a single class of receptors with K_d 1.0 nmol/l.

in Tris 0.05 mol/l NaCl 0.25 mol/l buffer pH 7.4, using a Potter–Elvehjem homogeniser. The suspension of cell membranes was frozen in aliquots until assayed. Protein content was assayed with BSA as a standard [14].

Preparation of radiolabelled ligand

uPA was purified from Ukidan by affinity chromatography on a benzamidine–Sepharose column [15]. The active enzyme fraction was further separated in its high molecular weight (HMW-uPA) and low molecular weight (LMW-uPA) forms by gel filtration on Sephadex G-100. Immunoblotting of the LMW-uPA preparation revealed trace amounts of HMW-uPA. This HMW-uPA contamination was, however, estimated to <1% of the total LMW-uPA. The HMW-uPA preparation was ^{125}I-labelled [16]. Specific radioactivity was in the range 0.4–0.6 MBq/μg protein.

Binding of ^{125}I-HMW-uPA to ovarian tumour membranes

Binding studies were performed in BSA-coated polystyrene tubes. Membrane proteins, 40 μg/tube, were incubated with the radiolabelled ligand in Tris 0.05 mol/l, NaCl 0.25 mol/l buffer pH 7.4, containing BSA 10 g/l. The final volume was 400 μl/tube. The incubation mixture was shaking for 20 min at 20°C. Reaction was stopped by adding 1 ml of ice cold buffer, and the tubes were centrifuged at 9000 *g* for 20 min at +4°C. The pellet was washed twice, and remaining radioactivity was counted in a 1260 Multigamma counter (LKB, Sweden).

Non-specific binding was measured with the same procedure in the presence of 100-fold excess of non-labelled HMW-uPA. Specific binding was calculated as total binding minus non-specific binding.

We examined the efficiency of various pH, in the range between 7 and 2, to remove endogenously bound uPA from the receptors. For this purpose, membranes were incubated in Tris-HCl buffer, 0.05 mol/l, in which pH was adjusted by addition of HCl. After 10 min, the membranes were collected by centrifugation, resuspended in the Tris/NaCl/BSA buffer, and the amount of receptors assayed as described above. Preincubation

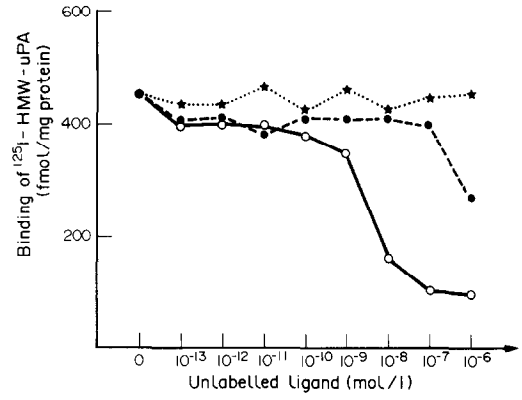

Fig. 2. Membrane binding of ^{125}I-HMW-uPA (2 nmol/l) was assayed in the presence of increasing concentrations of unlabelled ligands. HMW-uPA (○), but not LMW-uPA (●) or tPA (★) competed for the binding sites. Due to trace amounts of HMW-uPA impurity, the highest concentration of LMW-uPA produced a slight inhibition.

of membranes at pH 2 was subsequently used to assay for the total number of binding sites/μg protein.

Statistical methods

Results were given as mean (S.E.), and differences between groups analysed with Student's *t* test for unpaired observations.

RESULTS

Similar binding curves were obtained for four epithelial malignant tumours (one is shown in Fig. 1). Scatchard analysis of the data demonstrated linearity, thus indicating a single class of binding sites. The K_d was 1.1 (range 0.9–1.3) nmol/l, and binding was saturated at 1.5 (range 1.2–1.6) nmol/l. Saturation was rapid, the binding at room temperature being maximal after 5 min and not further increased by prolonged incubation up to 60 min (data not shown).

Binding was specific for HMW-uPA since unlabelled HMW-uPA, but not unlabelled tPA or LMW-uPA, competed for the binding sites. Figure 2 shows the mean of two experiments.

The uPA binding was augmented by acid pretreatment. Figure 3 shows the result of exposure of the membranes to various low pH (mean of three experiments). Pretreatment with pH 2 was the most effective, and it was subsequently used to assay for the total number of binding sites. We examined ten

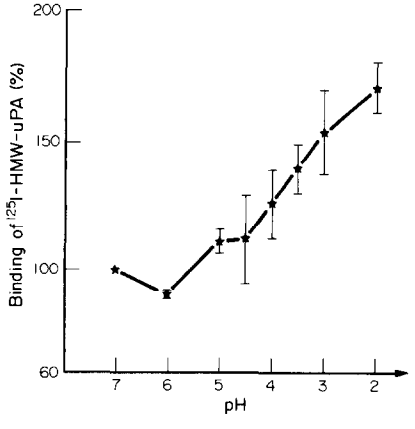

Fig. 3. Ovarian carcinoma membranes were exposed to various pH before assaying the uPA receptors. Binding at individual lower pH was expressed as % binding at pH7.

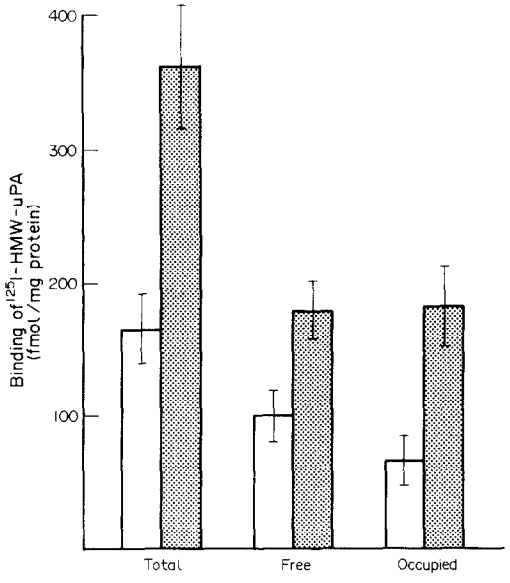

Fig. 4. The amount of uPA receptors was assayed in membranes prepared from malignant (n=10; filled bars) and benign (n=6; open bars) epithelial ovarian tumours.

malignant and six benign tumours for their amount of cell surface receptors (Fig. 4). The total number of binding sites was higher in malignant than in benign tumours (P=0.008). Free (unoccupied) as well as occupied receptors were higher (P=0.03 and P=0.01) in the malignant group. The percentage of occupied receptors was, however, not different between malignant [49(S.E. 3)%] and benign tumours [44(5)%].

DISCUSSION

This study found that ovarian tumour membranes have specific binding sites for HMW-uPA. The receptor-ligand interaction was rapid, the receptor was saturated at low concentrations (1.5 nmol/l), and had high affinity (K_d 1.1 nmol/l) for HMW-uPA. These data suggest the presence of "high specificity, low capacity"-type cell membrane receptors for HMW-uPA in ovarian tumour tissues.

The K_d was calculated in the same range as that reported for the uPA receptor in various cell lines 0.5–2.9 nmol/l [5, 12, 17], and normal granulocytes, 2.6 nmol/l [18]. In contrast, two epidermal carcinoma cell lines expressed uPA receptors with higher affinity, K_d = 0.05 nmol/l [19, 20]. Needham *et al.* reported specific uPA receptors on breast cancer membranes with K_d=0.2 nmol/l [21]. They found the number of binding sites to be 20–250 fmol/mg protein. This number of binding sites is lower than what we found in ovarian cancers, presumably because we measured the total number of receptors after acidification.

Binding was specific for HMW-uPA, since LMW-uPA and tPA did not interfere with the interaction of HMW-uPA with the receptor. LMW-uPA does not interact with the receptor, since the receptor binding sequence is located in the aminoterminal end of the uPA molecule, and is thus included in HMW-uPA but not in LMW-uPA [22]. Furthermore, these data exclude that plasminogen activator inhibitors are involved in the membrane binding of HMW-uPA, since tPA as well as LMW-uPA would interact with these inhibitors [23].

We found the receptors to be partly occupied by endogenous uPA. Saturation was 40–50%, and not different between malignant and benign tumours. Endogenous receptor saturation seems to vary between different cell lines. One carcinoma cell line has, however, 100% saturation of its uPA receptors [24]. Unmasking of receptors, which are endogenously occupied by uPA, has been done at various low pHs [3, 24, 25]. We found pH 2 to be the most effective.

The content as well as the release of uPA is increased in tumour tissue as compared to normal tissue [13, 26, 27]. The serum levels of uPA have been correlated with malignancy and metastasis in genital tract and breast carcinomas, and has been suggested to predict primary disease and relapse of these tumours [28–30]. uPA receptors occur in neoplastic cell lines and in mammary carcinoma tissue [21]. The receptor apparently serves to focus plasminogen activation to cell surfaces in contact with extracellular matrix and adjacent cells [7]. Receptor binding is the structural basis for the crucial function of uPA in extracellular matrix degradation during cell migration and invasion [9–11]. Our finding that malignant ovarian tumours had higher concentration of uPA receptors than benign tumours supports the theory that these receptors are involved in tumour growth and spread. The number of tumours studied needs, however, to be expanded to further validate our observation.

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Acknowledgements—This study was supported by grants from the Swedish Cancer Foundation (2693-B90-02XA) and the Swedish Medical Research Council (04523).

Eur J Cancer, Vol. 27, No. 11, pp. 1448–1453, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Undergraduate Education about Cancer

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The quality, quantity and balance of undergraduate cancer teaching in Australian Medical Schools were investigated by a survey, using a self-administered questionnaire, of recent graduates from all Australian medical schools. Stratified random cluster sampling was used and a response rate of 84% (389 respondents) was achieved. The results revealed substantial differences in knowledge, experience in, and rating of teaching between the medical, surgical, radiotherapeutic and palliative components of cancer management. The proportions of graduates who had never attended radiotherapy and palliative care clinics or units (42.3% and 49.9%, respectively) were more than double the proportion who had never attended medical and surgical cancer clinics or units (17.5% and 10.9%, respectively). More than twice as many graduates rated their instruction in the palliative management of cancer as poor or very poor (29.4%) compared with those rating their instruction as poor or very poor in both cancer prevention (8.4%) and treatment for cure (14.6%). The respondents displayed a considerable lack of knowledge about radiotherapy treatment options, and reported a lack of perceived competence in doing cervical smears. Their answers to questions about 5-year survival of selected cancers, about the existence of screening tests validly shown to reduce mortality, and the ages at which breast and cervical cancers are likely to develop all revealed worrying levels of incorrect knowledge. There was some important disturbing variation in levels of knowledge, experience and rating of cancer instruction between states and between universities.
Eur J Cancer, Vol. 27, No. 11, pp. 1448–1453, 1991.

INTRODUCTION

A SURVEY of cancer education for medical students in 1986 resulted in the Australian Cancer Society (ACS) developing guidelines for an "ideal" cancer curriculum for medical undergraduate teaching. This document has many similarities to guidelines recommended for a European undergraduate oncology curriculum proposed following the consensus workshop on a curriculum for medical students in Europe (jointly organised by the EEC and EORTC) [1, 2]. The present study investigated medical students' knowledge, perceived skills and undergraduate experience in cancer. It was planned to identify any serious deficiencies in undergraduate cancer curricula compared to the ACS guidelines, in order to enable recommendations for curriculum review, and as a baseline against which future curriculum changes could be assessed.

In recent years there have been many publications on what should be included in undergraduate curricula as assessed by teachers, interest groups and institutions [3–7]; how to decide what to include in a curriculum [8–11]; descriptions of the composition of curriculum committees [12] and sources of data and even "how to do it" instructions on the politics and machinery of curriculum change [13].