Hyaluronidase enhances the activity of Adriamycin in breast cancer models in vitro and in vivo*

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Summary. The effect of hyaluronidase and a combination of hyaluronidase with Adriamycin was investigated on several breast cancer models in vitro and in vivo. In vitro enzyme treatment (using concentrations up to 80,000 IU/l) of routine (MXT-, MXT-, and MXT+) and human (MCF-7, ZR-75-1 and T-47-D) breast cancer cell lines did not inhibit tumour cell proliferation (measured by a kinetic crystal violet assay) in either case. Although high-dose hyaluronidase (1.2 x 10^6 IU/kg) was ineffective, when administered peritumourally to the MXT M3.2 mammary carcinoma of the B6D2F1 mouse, it is remarkable that five "megadoses" were excellently tolerated. However, the antineoplastic activity of Adriamycin against the oestrogen-receptor-positive variant of the MXT tumour was significantly enhanced by combination with concentrations of hyaluronidase that were inactive per se, both in vitro and in vivo. Interestingly, the enhancement of the in vivo antitumour activity was not compromised by toxic side-effects.

Key words: Hyaluronidase – Adriamycin – Breast cancer models – Enhancement of antitumour activity – No toxic side-effects

Introduction

Whereas numerous studies have confirmed the diagnostic value of elevated serum levels of hyaluronic acid associated with malignant mesothelioma (Frebourg et al. 1987; Pettersson et al. 1988; Dahl et al. 1989) and Wilms' nephroblastoma (Wu et al. 1984; Stern et al. 1991), there have been few reports of raised serum levels in other types of advanced cancer (Depech et al. 1985; Dahl and Laurent 1988). Cooper and Forbes (1988) presented data on the distribution of serum hyaluronic acid in patients with metastatic cancer or large local tumours (myeloma, melanoma, sarcoma, cancer of the pancreas, stomach, colon, lung – small cell and non-small cell, prostate, ovary and breast). Within each type of cancer individual patients showed strongly elevated levels of hyaluronic acid, but statistically significant, overall increased levels were only present in pancreatic cancer, small-cell lung cancer and carcinoma of the prostate.

Although increased synthesis of glycosaminoglycans is not a universal characteristic of tumours, it seems likely that hyaluronic acid production plays an important role in tumour cell proliferation, differentiation, invasion and metastasis (Knudson et al. 1989). In this context, hyaluronan has been implicated in the pathology of experimental and human breast cancer (Angel0 et al. 1982; Kimata et al. 1983; Marotta et al. 1985; Knudson et al. 1989; Decker et al. 1989; Prehm 1990).

Therefore, it is conceivable that breast cancer might be an indication for the therapeutic application of hyaluronidase, an enzyme cleaving hyaluronic acid. Hyaluronidase has for some time been used as an additive to chemotherapy in several studies (Baumgartner 1988) including a phase I trial (Baumgartner et al. 1988).

The pharmacology of hyaluronidase and its potential role in the treatment of malignant disease has been reviewed recently (Baumgartner 1987; Baumgartner and Moritz 1988). Although the clinical trials have shown encouraging but not definite indications of possible augmentation of the antitumour activity of the chemotherapeutics used after preinfusion of hyaluronidase, to date most of the evidence is "pilot" in nature.

In addition, preclinical data obtained in cell culture (Liu et al. 1987; Kohno et al. 1988; Scheithauer et al. 1988; Lehner et al. 1989) and experimental rodent tumour models (Seipel and Kohlheb 1967; Pawlowski et al. 1979; Sargent et al. 1983) are scanty and inconsistent.

As a first approach to characterize the potential efficacy of hyaluronidase in the therapy of breast cancer, we systematically assayed the in vitro chemosensitivity of various murine (MXT-, MXT+, MXT+) and human...
(MCF-7, T-47-D, ZR-75-1) breast cancer cell lines against increasing concentrations of hyaluronidase. As an extension of these experiments, the antitumour activity of hyaluronidase was tested in vivo, using the syngeneic MXT M3.2 mammary carcinoma of the B6D2F1 mouse. Further studies using the same model clearly demonstrated that hyaluronidase combined with Adriamycin significantly enhanced the antineoplastic effect of the antitumour agents, both in vitro and in vivo.

Materials and methods

Chemicals. Reagents (A-grade purity) were obtained from Merck (Darmstadt, FRG). N-Hexamethylypararosaniline (crystal violet) was purchased from Serva (Heidelberg, FRG). All culture media were from Sigma (München, FRG), and fetal calf serum (FCS) was from Gibco (Eggenheim, FRG). Millipore-filtered water was used throughout.

Cell lines and routine culture conditions. The murine cell lines used in the in vitro studies were established from different variants of the MXT mammary carcinoma of the B6D2F1 mouse (Beckenlehner 1991). The human breast cancer cell lines (MCF-7, T-47-D and ZR-75-1) were obtained from the American Type Culture Collection (Rockville, Md., USA). Cell line banking and quality control were performed according to the "seed stock concept" reviewed by Hay (1988).

Proliferation kinetics, karyology, and hormone receptor content of the human breast cancer cell lines used in our laboratory have been described elsewhere (Bernhardt et al. 1992).

MCF-7 cells were grown in Eagle's minimum essential medium containing 1-glutamine, 2.2 g/l NaHCO3, 110 mg/l sodium pyruvate (Sigma, München, FRG), and 10% FCS. T-47-D was cultivated in RPMI-1640 medium containing 1-glutamine, 2.0 g/l NaHCO3 and 10% FCS. The culture medium was supplemented with 10 mg/l bovine insulin (Sigma, München, FRG). The ZR-75-1 cell line was maintained in RPMI-1640 medium with 1-glutamine, 2.0 g/l NaHCO3, 10% FCS and 11.6 mg/l tylosin.

The hormone-independent MXT- and MXT+ sublines were oestrogen-receptor-negative. Cells were maintained in RPMI-1640 medium containing an additional 0.6 g/l 1-glutamine (i.e. 0.9 g/l), 2.0 g/l NaHCO3 and 10% FCS.

The hormone-sensitive and oestrogen-receptor-positive MXT+ variant required 100 ng/l oestradiol (Sigma, München, FRG) supplementation of the same basic medium formulation. All culture media contained 50 mg/l gentamycin (Serva, Darmstadt, FRG) and water was provided ad libitum. The ovary-dependent MXT M3.2 mammary carcinoma was propagated by s.c. implantation of about 2-mm3 tumour pieces into the right thoracic mammary fat pad of intact 8-week-old female B6D2F1 mice (Charles River Wiga, Sulzfeld, FRG). The detailed testing procedure and the characteristics of this tumour model have been described elsewhere (Spruβ et al. 1991, 1992). Briefly, tumours were implanted subcutaneously into the right flank of intact females on day 0, and the animals were randomly assigned to groups of ten.

In the monotherapy experiment treatment was started on day 5 by injecting 1.2 x 106 IU/kg hyaluronidase and vehicle (gelatin carrier) s.c. into the right flank, near to the site of transplantation. In the combination therapy experiment hyaluronidase (1.2 x 106 IU/kg, weekly) was administered peritumourally, 4 h prior to the i.p. injection of Adriamycin (0.2 mg/kg, three times a week). For positive control, one group was ovariec tomised 1 day after tumour transplantation (day 1).

Tumour diameters were measured with a caliper. Tumour areas were calculated as the product of two perpendicular diameters, one measured across the greatest width of the tumour.

Statistics. Significance levels of the in vitro data were calculated according to the t-test (Sokal and Rohlf 1987a). The significance levels of the median tumour areas after combination therapy (in vivo experiment) were determined according to the Mann-Whitney U-test (Sokal and Rohlf 1987b).

Results

Effect of hyaluronidase on the cell proliferation of breast cancer cells in vitro

Increasing concentrations of hyaluronidase were tested on various murine and human breast cancer cell lines. Monotherapy with 2500, 5000, and 10 000 IU/l hyaluronidase had no effect on the growth kinetics of the murine MXT+ mammary carcinoma. In accordance with this observation, 10 000 IU/l enzyme did not affect cell proliferation of the MXT+ variant. Increasing doses of hyaluronidase (10 000, 20 000, 30 000, 40 000, and 50 000 IU/l) were not inhibitory to the oestrogen-receptor-positive MXT+ subline. Further dosage escalation up to 80 000 IU/l was ineffective against the human hormone-sensitive MCF-7, ZR-75-1, and T-47-D cell lines.