

Reaction Kinetics of Cisplatin and its Monoaquated Species with the Modulating Agents (Di)mesna and Thiosulphate

O.R. Leeuwenkamp, J.P. Neijt, W.J.F. van der Vijgh and H.M. Pinedo

The reactive and rapidly excreted thiol mesna (2-mercaptoethane-sulphonate sodium) has the potential to reduce the dose-limiting nephrotoxicity of cisplatin by chemical neutralisation of the latter in the kidney. The reaction kinetics of cisplatin with mesna and its disulphide, dimesna, was studied at 37°C in unbuffered 0.15 mol/l NaCl (pH 5.3) and in 0.15 mol/l NaCl buffered with 0.02 mol/l Hepes (pH 7.4). The reaction mixtures were analysed for intact cisplatin. In the presence of mesna or dimesna 0.5 mol/l as anticipated in urine for conditions of renal protection, the half-life ($t_{1/2}$) of 0.2 mmol/l cisplatin was less than 6 min. $t_{1/2}$ of 151 and 629 min were found in the presence of mesna and dimesna concentrations of 5 mmol/l and 3 mmol/l, respectively, anticipated in plasma under conditions of renal protection. Cis-diamminemonoaquamono-chloroplatinum(II) 0.2 mmol/l reacted rapidly with 50 mmol thiosulphate and 0.5 mol/l (di)mesna ($t_{1/2} \leq 1$ min). This platinum species also reacted rapidly with 2.6 mmol/l thiosulphate ($t_{1/2} < 1$ min), a concentration reached in plasma for conditions under renal protection. Reaction of the monoaquated form of cisplatin proceeded slowly in the presence of dimesna or mesna concentrations (< 5 mmol/l), as anticipated in plasma under renal protecting conditions. It is hypothesised that renal protection by the strong nucleophiles, thiosulphate, mesna and dimesna occurs rather by neutralisation of the aquated species in the lumen of the renal tubulus than by neutralisation of intact cisplatin, and that neutralisation of these species in plasma contributes significantly to the protecting effect.

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INTRODUCTION

CISPLATIN is a powerful antitumour drug. Its serious side-effects include nephrotoxicity [1]. Howell and Taetle [2] proposed concurrent administration of thiosulphate to protect renal function. According to these investigators, the protecting action of the nucleophile thiosulphate is based on chemical neutralisation of cisplatin by the formation of less (nephro)toxic Pt(II)-thiosulphate complexes, especially in the renal tubuli where rapidly cleared thiosulphate is concentrated. Thiosulphate has proven its renal protecting efficacy permitting escalation of the dose by about a factor of two [3, 4]. The inactivation rate of cisplatin by thiosulphate [5] accounts for the renal protecting effect of thiosulphate and explains why the area under the concentration-time curve (AUC) of intraperitoneally administered cisplatin [4, 6] is decreased under conditions of thiosulphate protection.

Thiols exhibit a high nucleophilic reactivity towards Pt(II) complexes [7]. The thiol mesna (2-mercaptoethanesulphonate sodium) is rapidly cleared into the urine [8, 9], resulting in high urinary concentrations. Mesna is rapidly oxidised in plasma into its presumably less reactive disulphide dimesna (2,2-dithiodiethane sulfonate disodium). In renal tubular cells, dimesna is converted into more reactive mesna. Because of these unique processes, we anticipated that mesna affords protection against cisplatin-related nephrotoxicity, while reducing the AUC of

cisplatin to a lesser extent than thiosulphate. The supposed protecting action of mesna is in line with recent findings of Dorr and Lagel [10], but is contradicted by other animal studies (P. Hilgard, J. Pohl and J. Stekar, Asta-Werke, Frankfurt, and ref 11).

This contradiction and the supposed advantage of mesna over thiosulphate in terms of maintaining cisplatin's antitumour effect prompted us to a detailed study of the reaction kinetics of cisplatin and its monoaquated species with mesna and dimesna. In this paper the kinetic data are presented. Its clinical implications and an alternative mechanism of renal protection are discussed.

MATERIALS AND METHODS

Reagents

Cisplatin and dimesna trihydrate were generous gifts of Bristol Myers (Brussels) and Dr J. Phol (Asta-Werke, Bielefeld, Germany), respectively. Mesna was from Sigma, sodium thiosulphate (Ph. Eur. grade) from Brocacef (Maarsse, The Netherlands), hexadecyltrimethylammonium hydroxide 0.5 mol/l aqueous solution from Eastman Kodak Company (Rochester, New York) and Hepes from BDH Chemicals (Poole, UK). Anhydrous sodium sulphate, sodium chloride, sodium hydroxide and sulphuric acid were all Merck analytical grade. Doubly distilled water was used for the preparation of all solutions, including the mobile phase.

Chromatography

The (dynamic ion-exchange) chromatographic system of Elferink *et al.* [5] was used for the quantitation of intact cisplatin in the reaction mixtures.

Under the chromatographic conditions used the retention

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times of aquated cisplatin, Hepes, dimesna, cisplatin, and mesna were 1.1, 1.5, 1.8, 3.5 and 20 min, respectively. The effluent was monitored at 214 nm by a Kratos Spectroflow 773 variable wavelength detector (Kipp & Zonen, Delft, The Netherlands).

Linear calibration graphs for cisplatin ($r > 0.999$) were obtained in the concentration range of 5 $\mu\text{mol/l}$ –0.2 mmol/l. At a concentration of 0.02 mmol/l the within-day coefficient of variation was 2.9% ($n = 7$).

Reaction kinetics

To prevent (auto)oxidation of mesna to dimesna, the reaction mixtures containing mesna were kept under an atmosphere of nitrogen. To avoid photodegradation of cisplatin, the reaction mixtures were protected from light. Oxygen-free solutions of mesna were prepared by dissolving the appropriate amount of mesna in normal saline (pH 5.3) or 0.15 mol/l NaCl buffered with 0.02 mol/l Hepes (pH 7.4). Solutions were purged with nitrogen for at least 20 min before use. Vials containing the resulting solutions were placed in a waterbath of 37°C, nitrogen was passed over and after 10 min the required volume of a 3.4 mmol/l stock solution of cisplatin in 0.15 mol/l NaCl was added. At $t = 0$ min (zero time control) and preselected time intervals 100 μl samples were taken from the reaction mixtures (initial volume 3.5 ml) and analysed immediately on the chromatographic system. All measurements were performed at least in duplicate. Under the described conditions of incubation approximately 2% of mesna was converted to dimesna.

Additionally, the reaction kinetics of mesna, dimesna and thiosulphate with the monoaquated species of cisplatin was studied. Monoaquated cisplatin was obtained by aging (15 h) of an aqueous solution of cisplatin. Calculations according to Le Roy *et al.* [12] and analysis on a cation chromatographic system (ref 13 and own unpublished results) demonstrated that cisplatin

was converted into cis-diamminemonochloromonoaqua-platinum(II) (monoaquated species) for about 50%. Aliquots of these solutions were diluted with solutions containing the nucleophile of interest to obtain about 0.2 mmol/l of monoaquated species (and 0.2 mmol/l cisplatin) and the desired concentration of the nucleophile. These solutions were used to study the reaction kinetics.

Analysis of reaction kinetics

The initial concentrations of mesna (5 mmol/l–0.5 mol/l) and dimesna (3 mmol/l–0.5 mol/l) were in large excess over the initial concentration of cisplatin (0.2 mmol/l or 0.02 mmol/l). Therefore, disappearance of intact cisplatin obeyed the first-order rate law:

$$-d[\text{cisplatin}]/dt = k_{\text{obs}} \cdot [\text{cisplatin}] \quad \{1\}$$

with k_{obs} representing the observed (pseudo) first-order rate constant for loss of intact cisplatin. Integration of {1} yields

$$\ln[\text{cisplatin}] = \ln[\text{cisplatin}]_{t=0} - k_{\text{obs}} \cdot t \quad \{2\}$$

According to {2}, semilogarithmic plots of the measured concentration of cisplatin vs. time will be linear. The plots were fitted with the least-squares method.

RESULTS

Reaction kinetics of cisplatin and mesna

The reaction kinetics of cisplatin and mesna were studied in unbuffered medium pH 5.3 (0.15 mol/l NaCl) to avoid competitive interaction of buffer components (e.g. phosphate, acetate, citrate) with cisplatin and in particular its aquation products [14, 15]. During incubation, the pH of the reaction mixture decreased to 5.1. Due to its pK of 9.2, mesna remains fully protonated despite the shift in pH from 5.1 to 5.3.

In Fig. 1 typical chromatograms are shown as obtained at the indicated time points for the reaction mixture of 0.2 mmol/l cisplatin and 0.02 mol/l mesna. Linear semilogarithmic plots of the cisplatin concentration vs. time were obtained ($r > 0.999$) for the reaction of 0.2 mmol/l cisplatin with 0.02–0.5 mol/l mesna, indicating monoexponential loss of intact cisplatin. The kinetic parameters are summarised in Table 1.

In 0.02 mol/l Hepes buffer pH 7.4 (0.15 mol/l NaCl), intact cisplatin disappeared monoexponentially (Fig. 2), yielding

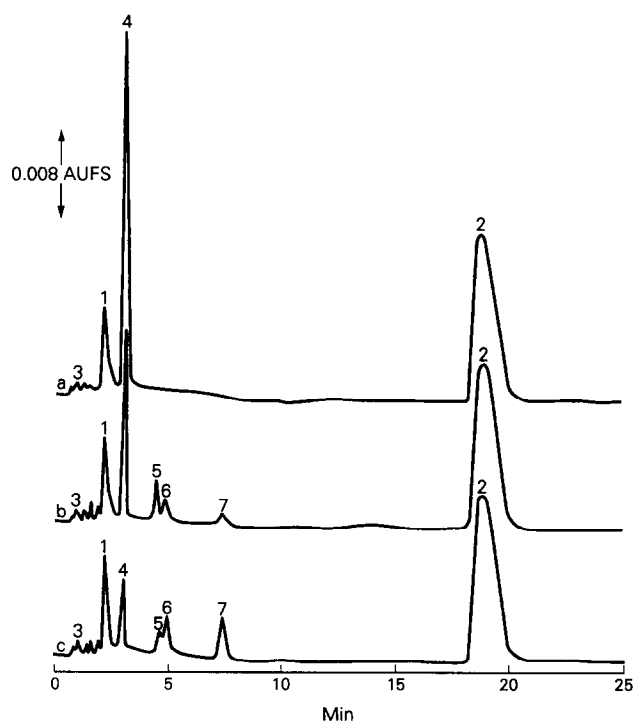


Fig. 1. Typical chromatograms obtained for reaction mixtures of 0.2 mmol/l cisplatin and 0.02 mol/l mesna (pH 5.3, 0.15 mol/l NaCl, 37°C) at (a) $t = 0$ (b) 69 and (c) 125 min. (1) Chloride, (2) mesna, (3) dimesna, (4) cisplatin and 5–7 interaction products formed during reaction.

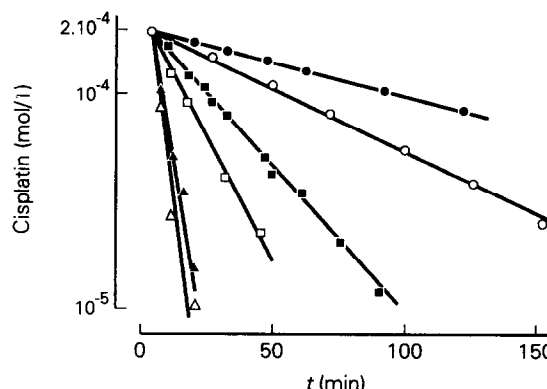


Fig. 2. Semilogarithmic plots of cisplatin concentration vs. time obtained during reaction of 0.2 mmol/l cisplatin with 0.02 mol/l (\circ , \bullet), 0.1 mol/l (\square , \blacksquare) and 0.5 mol/l (\triangle , \blacktriangle) (di)mesna in 0.02 mol/l Hepes buffer pH 7.4 (0.15 mol/l NaCl, 37°C). Mesna = open symbols and dimesna = closed symbols.

Table 1. Half-life times of 0.2 mmol/l cisplatin on incubation with mesna

Mesna concentration (mol/l)	pH 5.3		pH 7.4	
	$t_{1/2, \text{obs}}$ (min)	k_{obs} (per min)	$t_{1/2, \text{obs}}$ (min)	k_{obs} (per min)
0.005*	223.6	$3.1 \cdot 10^{-3}$	150.7	$4.6 \cdot 10^{-3}$
0.01	126.0	$5.5 \cdot 10^{-3}$	94.9	$7.3 \cdot 10^{-3}$
0.02	63.0	$1.1 \cdot 10^{-2}$	53.3	$1.3 \cdot 10^{-2}$
0.1	16.9	$4.1 \cdot 10^{-2}$	13.9	$5.0 \cdot 10^{-2}$
0.5*	5.3	$1.3 \cdot 10^{-1}$	3.3	$2.1 \cdot 10^{-1}$

* Concentrations of mesna as anticipated in plasma (5 mmol/l) and urine (0.5 mol/l) under the supposed conditions of renal protection.

interaction products with retention times identical to those observed in acidic medium. At pH 7.4 somewhat higher rate constants and lower half-lives ($t_{1/2}$) were found (Table 1). Reaction of 0.02 mmol/l cisplatin with 5 mmol/l and 0.02 mmol/l mesna, exceeding the plasma concentration anticipated for renal protection (see Discussion), resulted in half-life times of 151 min ($r = 0.990$) and 95 min ($r = 0.996$), respectively. At pH 8.0 and in presence of 0.2 mol/l mesna, 0.2 mmol/l cisplatin disappeared with a much shorter $t_{1/2}$ of 7.0 min.

Reaction kinetics of cisplatin and dimesna

In unbuffered 0.15 mol/l NaCl of pH 5.3 linear semilogarithmic concentration–time plots ($r > 0.99$) were obtained for the reaction of 0.2 mmol/l cisplatin with dimesna in the concentration range of 0.02–0.5 mol/l. The kinetic data are summarised in Table 2. In 0.02 mol/l Hepes buffer pH 7.4 (0.15 mol/l NaCl), intact cisplatin also disappeared monoexponentially (Fig. 2). $t_{1/2}$ and rate constants are summarised in Table 2 together with the $t_{1/2}$ as observed at pH 7.4 for the reaction of cisplatin with 3 mmol/l dimesna. The kinetic parameters at pH 5.3 and pH 7.4 were very similar.

Reaction kinetics of the monoaquated species of cisplatin with thiosulphate, mesna and dimesna

Reaction of the monoaquated species of cisplatin (0.2 mmol/l) with mesna or dimesna yielded interaction products with retention times identical to those observed during reaction of cisplatin with mesna or dimesna. Linear semilogarithmic concentration–time plots were found. The half-life times are summarised in

Table 2. Half-life times of 0.2 mol/l cisplatin on incubation with dimesna

Dimesna concentration (mol/l)	pH 5.3		pH 7.4	
	$t_{1/2, \text{obs}}$ (min)	k_{obs} (per min)	$t_{1/2, \text{obs}}$ (min)	k_{obs} (per min)
0.003*	—	—	629.0	$1.1 \cdot 10^{-3}$
0.005	216.7	$3.2 \cdot 10^{-3}$	385.0	$1.8 \cdot 10^{-3}$
0.02	110.0	$6.3 \cdot 10^{-3}$	101.4	$6.8 \cdot 10^{-3}$
0.1	22.4	$3.1 \cdot 10^{-2}$	22.4	$3.1 \cdot 10^{-2}$
0.5*	5.8	$1.2 \cdot 10^{-1}$	5.3	$1.3 \cdot 10^{-1}$

* Concentrations of dimesna as anticipated in plasma (3 mmol/l) and urine (5 mmol/l) under the supposed conditions of renal protection.

Table 3. Half-life times of 0.2 mmol/l monoaquated species observed on incubation with (di)mesna and thiosulphate (pH 5.3, 37°C)

Nucleophile	Concentration* (mmol/l)	$t_{1/2, \text{obs}}$ (min)	Concentration† (mmol/l)	$t_{1/2, \text{obs}}$ (min)
Thiosulphate	2.6	< 1	50	≤ 1
Mesna	5.0	18	500	≤ 1
Dimesna	3.0	60	500	≤ 1

Concentrations as anticipated in *plasma and †urine under the supposed conditions of renal protection.

Table 3. The monoaquated species reacted rapidly ($t_{1/2} \leq 1$ min) with 2.6 mmol/l thiosulphate as anticipated in plasma under conditions of renal protection.

DISCUSSION

Substitution reactions involving the labile chloride ligands of cisplatin yield more stable and therefore probably less toxic Pt(II) complexes. Rapid exchange of these ligands at the site of toxicity (e.g. kidney) will result in reduction of cisplatin-induced (nephro)toxicity while the antitumour effect is not impaired. When the nucleophile is strong and concentrated in the kidney due to its rapid renal clearance, high reaction rates may be achieved. Non-toxic thiosulphate appeared to be suited for rescue of cisplatin-induced nephrotoxicity [2–4, 6, 7]. However, intraperitoneally administered cisplatin was neutralised to some extent in plasma [4, 6]. These findings are consistent with the reaction kinetics of cisplatin with thiosulphate [5].

Like thiosulphate, thiols have a high nucleophilic reactivity towards Pt(II) complexes [7]. The thiol mesna and its presumably less reactive disulphide dimesna are rapidly cleared into the urine [8, 9] resulting in high urinary concentrations. The anticipated renal protecting action of mesna is in line with recent findings [10] but contradicted by other animal studies [11]. This contradiction, the expected advantage of mesna as a rescue agent over thiosulphate and a mechanism of renal protection possibly involving ligand exchange by mesna or dimesna prompted us to study the reaction kinetics of cisplatin, mesna and dimesna.

New chromatographic peaks arose during reaction of cisplatin with mesna or dimesna. Most probably these peaks resulted from step-wise substitution of the chloride and ammine ligands by (di)anionic (di)mesna. The retention times of these peaks appeared to be increased compared to cisplatin. Increase of the retention times in the anion-exchange chromatographic system used is in line with ligand exchange by dianionic mesna or dimesna, yielding negatively-charged platinum species. As the

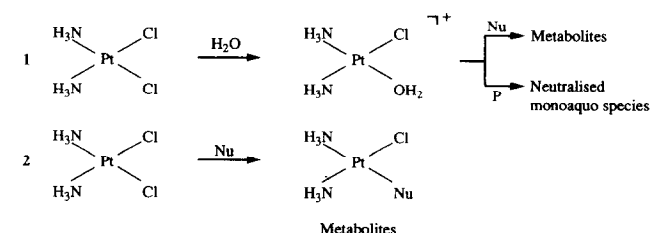


Fig. 3. Reactions of endogenous nucleophiles (Nu) or renal protecting agents (P) with cisplatin along the parallel pathways of (1) the aquation products and (2) direct displacement of the chloride ligands in cisplatin.

primary objective of the present study was the reaction kinetics of cisplatin and its aquated forms, the exact nature, stability and (cyto)toxicity of the interaction products formed were not investigated.

In buffered medium pH 7.4, mesna reacted faster than in acidic medium pH 5.3 (Table 1). At pH 7.4 about 1% of mesna ($pK_{SH} = 9.2$) is deprotonated [11]. These deprotonated SH groups will display a higher reactivity towards Pt(II)-complexes because of the higher nucleophilicity of S^- .

Nearly identical kinetic parameters were found for dimesna in acidic medium (pH 5.3) and buffered medium (pH 7.4). Absence of the thiol group explains why the reaction kinetics was virtually pH-independent.

For high concentrations of dimesna and mesna, the half-lives of cisplatin are very similar (Tables 1 and 2). At low concentrations, the half-lives diverge. This may have important (clinical) consequences for the rate at which cisplatin is neutralised in the kidney (renal protection) and the extent of neutralisation in plasma (antitumour effects).

Under clinical conditions of renal protection [4], the urinary concentration of thiosulphate is about 0.5 mol/l. For reaction of 0.2 mmol/l cisplatin with 0.5 mol/l thiosulphate, a $t_{1/2}$ of 3.4 min was found [5]. Therefore, the mechanism of thiosulphate protection may involve substantial neutralisation of cisplatin itself in the kidney. In the presence of 0.5 mol/l (di)mesna, the half-lives were less than 6 min (Tables 1 and 2). In analogy with thiosulphate protection, urinary (di)mesna concentrations of at least 0.5 mol/l may afford protection against cisplatin-mediated nephrotoxicity. Based upon the pharmacokinetics of (di)mesna [16] it can be calculated that this requires infusion rates exceeding 100 mg/kg.h. The toxicity of mesna is low [8, 9]. Hence, such infusion rates may afford protection against cisplatin-related (nephro)toxicity without introducing serious side-effects. This conclusion is in line with the results of a recent study in rats [10] but conflicting with the outcome of such a study in mice (P. Hilgard, J. Pohl, J. Stekar, Asta-Werke, Frankfurt). Species differences with regard to renal toxicity of cisplatin and/or mesna may explain the conflicting results.

Steady state plasma concentrations of (di)mesna can be estimated on basis of pharmacokinetic data [16] and the following assumptions can be made: the volume of distribution is 12 l because distribution of hydrophilic and ionised (di)mesna is limited to the extracellular fluid; the elimination $t_{1/2}$ are 51 min (mesna) and 65 min (mesna + dimesna) [17]. This means that if mesna is infused at a rate of 100 mg/kg.h, estimated steady state plasma concentrations are 4 mmol/l mesna and 2.5 mmol/l dimesna. As *in vitro* $t_{1/2}$ of 95 min (0.01 mol/l mesna) and 385 min (5 mmol/l dimesna) were observed and the plasma elimination $t_{1/2}$ of cisplatin is 38.5 min [3], intact cisplatin will be slowly neutralised by concurrently infused mesna. Dimesna is poorly transported across the membranes of other than renal tubular cells [8]. Therefore, it is anticipated that under conditions of renal protection the antitumour effect of cisplatin is not significantly reduced by concurrently administered mesna. This conclusion is supported by findings of a recent animal study [10].

There are two arguments against the proposed mechanism of renal protection involving substantial chemical neutralisation of intact cisplatin by thiosulphate in the kidneys. In the first place, the transit time for thiosulphate in the proximal tubule identified as the primary site of renal damage [18–20] is very short (10–20 s) [21] compared to the expected half-life time. Secondly, renal Pt concentrations are not significantly reduced in thiosulphate

protected rats [22] as might be presumed, because neutralisation of intact cisplatin by thiosulphate results in highly charged Pt-thiosulphate complexes which probably do not enter renal cells. Therefore, significant reduction of renal Pt levels would be expected when renal protection is based upon substantial neutralisation of intact cisplatin in the kidneys.

The aquated forms of cisplatin may be involved in the mechanism of renal protection, because these species are highly nephrotoxic [23–28]. Therefore, we also studied the reaction kinetics of the monoaquated form of cisplatin representing the principal aquated form in plasma [12, 13].

Very short half-lives ($<< 1$ min) were found for the monoaquated form of cisplatin under conditions expected in urine during protection by thiosulphate or (di)mesna while (di)mesna slowly neutralised this form of cisplatin under conditions expected in plasma during protection (Table 3). Because the aquated species of cisplatin represent only a minor portion of circulating Pt, it is not likely that neutralisation of the (mono)aquated form of cisplatin results in a reduction of renal Pt levels. This supposition is in line with observations of Uozumi and Litterst [22].

In view of the above, (renal) protection may involve rapid neutralisation of the reactive and highly nephrotoxic (mono)aquated form of cisplatin in urine rather than inactivation of intact cisplatin itself. The aquated forms of cisplatin are highly nephrotoxic [23–28]. Therefore, neutralisation of the (mono)aquated form of cisplatin in plasma may contribute substantially to the renal protection. Involvement of this form of cisplatin may explain why thiosulphate is an effective renal protector and mesna may give contradictory results.

In summary, we postulate that chemical inactivation of aquated forms of cisplatin in the circulation contribute significantly to the renal protecting effect of modulating agents. In view of the lower reactivity of mesna compared to thiosulphate, it is anticipated that the antitumour effect of cisplatin is reduced to a lesser extent by mesna than by thiosulphate and that inactivation of the aquated species is a critical process in explaining the contradictory results reported for mesna.

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Inhibition of the Growth of Transplanted Rat Pancreatic Acinar Carcinoma with Octreotide

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The effects of octreotide on transplanted azaserine-induced pancreatic acinar tumours were investigated in the rat. When tumours became palpable, rats were treated either with octreotide (40 µg/kg per day, by infusion) or NaCl 0.9% (controls) for 14 days. Tumours were then analysed for their size, composition and somatostatin receptors. Octreotide induced a 80% reduction in tumour growth rate during the first 2 days of treatment. This rate was less marked from day 4 to day 15. The tumour weight, protein, DNA, RNA and enzyme content were reduced in parallel by 50 to 60%. A homogeneous distribution density and a high affinity of somatostatin receptors were found by receptor autoradiography and *in vitro* binding assays in tumours of both groups. These findings indicate that octreotide reduces the growth rate of the transplanted pancreatic acinar tumour and may exert its inhibitory effect directly via specific somatostatin receptors on tumour cells.

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INTRODUCTION

It is well recognised that pancreatic growth is affected by a number of gastrointestinal hormones and neuropeptides. Some, including the cholecystokinin family (cholecystokinin, cholecystokinin-8, caerulein), the gastrin family (pentagastrin, tetragastrin) and the bombesin family (bombesin, gastrin-releasing peptide), induce pancreatic growth [1–4]. Others, especially somatostatin and its structural analogues exert antitropic effects

on the exocrine pancreas [5, 6]. The role of these factors in the development and growth of pancreatic cancer is not clearly understood, but it is likely that they may influence the growth of malignant cells of the pancreas [7–10]. As reported by Lhoste and Longnecker [11], bombesin and caerulein are able to stimulate growth of preneoplastic acinar cell lesions induced in the rat by azaserine. In addition, it was shown by Howatson *et al.* [8] that cholecystokinin enhances pancreatic ductal carcino-