Cancer Chemother Pharmacol (2003) 51: 525–529
DOI 10.1007/s00280-003-0578-z

CLINICAL TRIAL REPORT

Miranda Verschraagen · Epie Boven · Ivon Zegers
Frederick H. Hausheer · Wim J. F. Van der Vijgh

Pharmacokinetics of BNP7787 and its metabolite mesna in plasma and ascites: a case report

Received: 21 June 2002 / Accepted: 17 January 2003 / Published online: 15 May 2003

Abstract *Purpose*: BNP7787 (2′,2′-dithio-bis-ethane sulfonate sodium) is a novel protector against cisplatin-induced toxicities. The pharmacokinetics of BNP7787 and its metabolite mesna were investigated in plasma and ascites of a cancer patient. We also evaluated potential pharmacokinetic interactions between BNP7787 and cisplatin. *Methods*: BNP7787 and mesna were measured as mesna in deproteinized plasma and ascites using high-performance liquid chromatography with an electrochemical detector provided with a wall-jet gold electrode. *Results*: After the i.v. administration of 41 g/m^2 BNP7787, BNP7787 and mesna had a half-life of 1.5 and 3.4 h, respectively. The AUC_∞ of mesna was approximately 8% of the AUC_∞ of BNP7787. Coadministration of cisplatin did not appear to influence the plasma concentration-time curves of BNP7787 and mesna. In ascites, approximately 0.02% of the BNP7787 dose was present as mesna, whereas approximately 4% of the dose was present as BNP7787 at the time of the maximum concentration. *Conclusions*: It can be concluded that the presence of ascites did not have a major impact on the pharmacokinetics of BNP7787 and coadministration of cisplatin did not influence the pharmacokinetics of BNP7787 and mesna.

Keywords BNP7787 · Mesna · Pharmacokinetics · Plasma · Ascites

M. Verschraagen (✉) · E. Boven · I. Zegers
W. J. F. Van der Vijgh
Department of Medical Oncology,
Vrije Universiteit Medical Center,
De Boelelaan 1117, 1007 MB
Amsterdam, The Netherlands
E-mail: M.Verschraagen@vumc.nl
Tel.: +31-20-4443846
Fax: +31-20-4443844

F. H. Hausheer
BioNumerik Pharmaceuticals Inc.,
8122 Datapoint Drive,
San Antonio, TX 78229, USA

Introduction

The disulfide BNP7787 (2′,2′-dithio-bis-ethane sulfonate sodium) is under clinical investigation as a novel agent to protect against platinum- and taxane-induced toxicities. BNP7787 is selectively converted into mesna in the kidney, intestine and liver by enzymatic reduction (Fig. 1) [6, 10, 11, 12]. Mesna can locally react with (monohydrated) cisplatin species in the kidneys and thereby prevent cisplatin-induced nephrotoxicity.

In preclinical studies in rats and beagle dogs [7], BNP7787 has been shown to protect against cisplatin-induced toxicities such as vomiting, nephrotoxicity and myelosuppression, without reducing antitumor activity in vitro or in vivo as shown in tumor-bearing rats and mice [2, 7]. Furthermore, intravenously administered BNP7787 has been observed to be non-toxic at doses that greatly exceed the LD_{50} for oral table salt in rats [7]. In rats, it has been observed that BNP7787 pretreatment greatly enhances the antitumor activity of cisplatin [7]. Based on these results, a clinical phase I trial was started in our hospital to identify a safe and effective dose of BNP7787 and to investigate whether saline hydration could be substantially reduced when cisplatin was combined with BNP7787 pretreatment. During this phase I trial the pharmacokinetics of BNP7787 and cisplatin were studied. Investigation of the pharmacokinetic profile in one patient with tumor-associated ascites was possible, and this represented an important opportunity to characterize the pharmacokinetic behavior of BNP7787 in a cancer patient with a common clinical problem that can affect the distribution and elimination of drugs: the third space accumulation of fluid (ascites). Investigation of the pharmacokinetic behavior of BNP7787 including the (mixed) disulfides and mesna in plasma and ascites allowed us to determine whether BNP7787 or mesna are distributed into the ascites. We were also able to gain a first impression of the possible influence of cisplatin on the distribution of BNP7787 as BNP7787 and mesna.

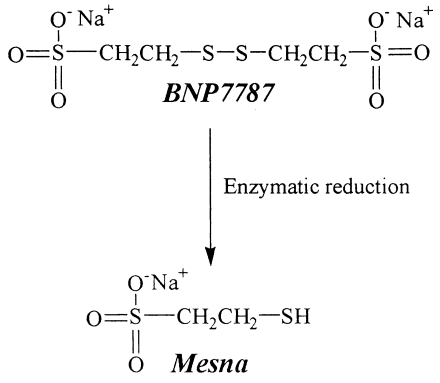

Fig. 1 Enzymatic reduction of BNP7787 to mesna in the kidneys, intestine and liver

Materials and methods

Drugs

BNP7787 (2′,2′-dithio-bis-ethane sulfonate sodium) was provided in sterile single-use vials of 10 g by BioNumerik Pharmaceuticals (San Antonio, Tx.). For each gram of BNP7787, 0.4 ml 8.4% (w/v) sodium bicarbonate (Hospital Pharmacy, Vrije Universiteit Medical Center, Amsterdam, The Netherlands) was added to the formulation to obtain a pH of about 7. BNP7787 was given in isotonic saline (0.9% NaCl) (Baxter, München, Germany) in a total volume of 500 ml. Cisplatin (Platosin, 1 mg/ml) was provided by Pharmachemie (Haarlem, The Netherlands). Cisplatin was administered in 0.9% NaCl in a total volume of 180 ml.

Patient characteristics

A 47-year-old male patient (body surface area 1.88 m^2, weight 75 kg) presented with gastric cancer, concurrent ascites due to peritoneal metastases and obstruction of the right ureter in the pelvic area. Physical examination upon admission revealed an ECOG performance status of 2, mainly due to massive ascites. He had normal blood values, normal liver function tests, normal creatinine and a creatinine clearance of 83 ml/min. After having given written informed consent, he was enrolled in the phase I trial of BNP7787 preceding cisplatin.

Drug administration

Hydration and drug administration were given i.v. in the following sequence. Over 90 min 1000 ml 0.9% NaCl including 20 mmol KCl and 2 g $MgSO_4$ were given, followed by 100 ml 20% mannitol over 10 min. BNP7787 was then administered in 500 ml 0.9% NaCl over 15 min and cisplatin in 180 ml 0.9% NaCl over 60 min, again followed by 1000 ml 0.9% NaCl including 20 mmol KCl and 2 g $MgSO_4$ over 90 min and 100 ml 20% mannitol over 10 min. The patient received 75 mg/m^2 cisplatin, and 3 weeks later he received cisplatin immediately preceded by 41 g/m^2 BNP7787. The same dose of BNP7787 was also given alone 1 week prior to the cycle with cisplatin. In all instances the total infusion volume as well as the KCl, $MgSO_4$ and mannitol supplements were the same. Except for when BNP7787 was given alone, the patient received i.v. antiemetic premedication, i.e. ondansetron 8 mg and dexamethasone 8 mg. BNP7787 and cisplatin were administered by syringe infusion pumps (Ivac P6000, Alaris Medical Systems, Amersfoort, The Netherlands). On a continuous daily basis the patient received omeprazole 40 mg, paracetamol 500 mg, tramadol 100 mg, spironolactone 4×25 mg and occasionally metoclopramide 10 or 20 mg during the treatment period.

Sample collection

In the phase I trial of BNP7787 preceding cisplatin, the pharmacokinetics of both cisplatin and BNP7787 were determined. This report is limited to the pharmacokinetics of BNP7787 and mesna, which were determined when BNP7787 was given alone and immediately before cisplatin.

During and after the administration of BNP7787, 3 ml blood and ascites samples were collected in cooled glass tubes containing EDTA (K3 EDTA, Becton Dickinson Vacutainer Systems, Plymouth, UK) at the following time-points: just before treatment (0 min), 8, 15, 25, 35 and 45 min and 1, 1.25, 1.75, 2.25, 4.25 and 6.25 h after starting the BNP7787 infusion. During administration of the combination of BNP7787 and cisplatin, blood and ascites samples were obtained at the same time-points. Ascites samples were taken from the peritoneal cavity using a temporary indwelling catheter commonly used for drainage of ascites. The catheter was flushed with heparin after each sample collection to prevent blockage upon lock. Unfortunately, because the catheter was temporarily clogged, no ascites samples could be taken at 25, 35 and 45 min after starting the BNP7787 infusion, when BNP7787 was given immediately before cisplatin. After sample collection drainage of ascites was carried out resulting in 4.8 l after the BNP7787 infusion alone and 1.7 l after administration of the combination with cisplatin.

Blood and ascites samples were centrifuged at 4°C for 15 min at 3000 *g* and deproteinized immediately by adding one volume of 0.33 *M* sulfuric acid and one volume of sodium hexametaphosphate (5 g/100 ml) to one volume of plasma or ascites sample. After centrifugation for 2 min at 12,000 *g*, the supernatant was transferred to polypropylene micro test tubes and stored at −20°C until analysis.

Analysis of plasma and ascites samples

A high-performance liquid chromatography (HPLC) method with electrochemical detection was developed to measure mesna and BNP7787 [14]. Mesna could be determined immediately in the deproteinized plasma and ascites samples after adding citrate buffer to increase the pH to about 3.5. Mesna was analyzed by HPLC using a Phenomenex Customsil 5 ODS-4 column (100×4.6 mm; Bester, Amstelveen, The Netherlands) preceded by a refillable guard column (20×2 mm) with pellicular C18 (35–50 μm) fill (Alltech, Deerfield, Ill.). The mobile phase was an aqueous solution of trisodium citrate dihydrate (0.1 *M*), tetrabutyl ammonium dihydrogenphosphate (1.0 m*M*) and cysteamine (0.1 μ*M*), adjusted to pH 3.5 with 85% *o*-phosphoric acid. The flow rate used was 1 ml/min and the column was kept at a temperature of 36°C. A sensitive electrochemical detector (Antec Leyden, Leiden, The Netherlands) set at an operating potential of +1.00 V relative to an Ag/AgCl reference electrode and provided with a wall-jet Au electrode was used for the detection of mesna. After deproteinization of plasma and ascites, BNP7787 was analyzed after reduction with sodium borohydride to free mesna.

Our analytical method could not discriminate between BNP7787 itself and possible mixed mesna disulfides. The BNP7787 concentration was calculated as $([\text{mesna}]_{\text{after reduction}}-[\text{mesna}]_{\text{before reduction}})/2$. Thus, for the practical purpose of assessing the pharmacokinetic profile of BNP7787 and mesna, we assumed that all mesna disulfides were present as BNP7787. However, it should be kept in mind that part of the BNP7787 might have been present as mixed mesna disulfides originating from mesna reacted with endogenous thiols including glutathione and (homo)cysteine or proteins containing a sulfhydryl group. This means that the actual concentration of BNP7787 could have been lower than the presently reported concentration, depending upon the extent of mixed mesna disulfide formation. Because BNP7787 represents all the mesna disulfides present, this assumption will not change the conclusion on the distribution of BNP7787 into ascites. In addition, as reported in the literature [3, 8], the mesna concentration in

plasma represents the total extractable mesna, i.e. the free mesna plasma concentration plus the non-covalently protein-bound fraction of mesna.

Drug-free EDTA-plasma from healthy volunteers was used as a medium for the calibration and quality control samples for the analysis of mesna and BNP7787 in plasma. Blank ascites, which was obtained from the patient studied in this report just before BNP7787 was administered, was used as the medium for the ascites analysis. The assays for BNP7787 and extractable mesna were linear over the ranges 1.6–500 μ*M* and 0.63–320 μ*M*, respectively. For BNP7787 the within- and between-day accuracy was within 2% and 4% of the nominal value, respectively. The within- and between-day accuracy of mesna was better than 12%. The precision for the within-day analysis of mesna was better than 5% and of BNP7787 better than 3%. Between-day precisions of the mesna and BNP7787 assays were better than 5% and 6%, respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters, i.e. peak concentration (C_{max}), area under the concentration-time curve over the time interval 0–6.25 h ($AUC_{0–6.25}$), area under the curve to infinity (AUC_{∞}), final half-life ($t_{1/2}$), mean residence time (MRT) and steady-state volume of distribution (V_{ss}), were determined by non-compartmental analysis using the pharmacokinetic data analysis program WinNonLin Standard Edition version 1.5 (Pharsight Corporation, Mountain View, Calif.). For the calculation of the final half-life the four final data points were used, i.e. for BNP7787 and for mesna the concentrations at 1.75, 2.25, 4.25 and 6.25 h.

Results

Pharmacokinetics

The plasma concentration-time curves for BNP7787 and mesna are shown in Fig. 2. The peak plasma concentration of BNP7787 was reached at the end of the 15-min BNP7787 infusion, whereas the peak value for mesna was reached not earlier than 75 min after starting the infusion. Without and with cisplatin administration, the final half-lives of BNP7787 (Table 1) were 1.5 and 1.6 h, respectively, and of mesna (Table 1) were 3.4 and 2.8 h, respectively. The MRT of mesna (i.e. 5.4 and 4.7 h) was more than two times higher than that of BNP7787 (i.e. 2.2 and 2.1 h; Table 1). Following both administrations, the AUC_{∞} of mesna was approximately 8% of the AUC_{∞} of BNP7787 (Table 1). Coadministration of cisplatin did not appear to influence the plasma concentration-time curves of BNP7787 or mesna

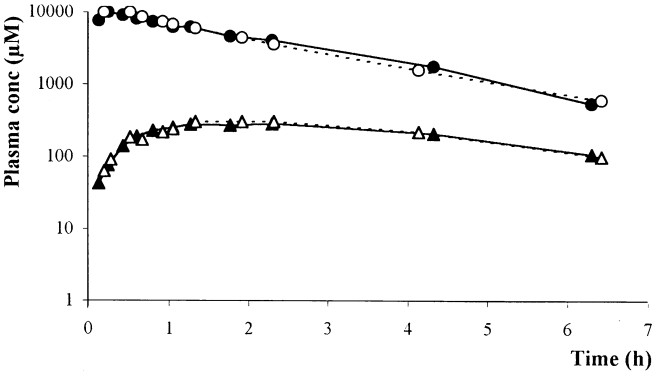

Fig. 2 Semilogarithmic concentration-time curves of BNP7787 (●, ○) and mesna (▲, △) in plasma of a patient who received a 15-min i.v. infusion of 41 g/m^2 BNP7787 alone (*closed symbols* and *solid line*) and in combination with a 1-h i.v. infusion of 75 mg/m^2 cisplatin (*open symbols* and *dotted line*)

(Fig. 2) nor the values of the pharmacokinetic parameters of BNP7787 and mesna (Table 1).

The concentration-time curves of BNP7787 and mesna in ascites are shown in Fig. 3. The peak concentration of BNP7787 in ascites was reached not earlier than 2 h after the end of the BNP7787 infusion. This peak concentration was approximately 17% of the BNP7787 peak plasma concentration (Table 1). The total amount of BNP7787 present in the ascites at that time was about 4% of the administered dose of BNP7787, when based on the ascites volume recovered on the same day after the pharmacokinetic study. The concentration of BNP7787 in ascites 4 h after the end of the BNP7787 infusion was similar to the BNP7787 concentration in plasma. The peak concentration of mesna in ascites was reached 4 h after the end of the BNP7787 infusion, which was 2 h after the peak concentration in plasma. Over the whole concentration-time curve, the concentrations of mesna in ascites were 5–47 times lower than the concentrations in plasma as reflected by the $AUC_{0–6.25}$ of mesna in ascites which was approximately 4.5% of the $AUC_{0–6.25}$ of mesna in plasma when BNP7787 was administered alone. The BNP7787/mesna concentration ratio decreased with time and was higher in ascites (range 38–409) than in plasma (range 5–181). This was also reflected by the BNP7787/mesna $AUC_{0–6.25}$ ratio, which was approximately 141 in ascites

Table 1 Pharmacokinetic data of BNP7787 and mesna in plasma and ascites of a patient who received a 15-min i.v. infusion of 41 g/m^2 BNP7787 alone and in combination with a 1-h infusion of 75 mg/m^2 cisplatin

Analyte	Drug infusions	Parameters in plasma						Parameters in ascites	
		C_{max}(μM)	Final $t_{1,2}$(h)	$AUC_{0–6.25}$ (μ*M*·h)[a]	AUC_{∞} (μ*M*·h)	MRT (h)	V_{ss} (l)	C_{max} (μ*M*)	$AUC_{0–6.25}$ (μ*M*·h)[a]
BNP7787	BNP7787 alone	10,044	1.5	21,864	23,169	2.2	22	1939	8178
	BNP7787 + cisplatin	11,015	1.6	21,653	23,146	2.1	21	1712	8891
Mesna	BNP7787 alone	282	3.4	1,289	1,881	5.4	–	16	58
	BNP7787 + cisplatin	304	2.8	1,329	1,792	4.7	–	45	188

[a]$AUC_{0–6.25}$ represents the AUC determined over a time interval of 0–6.25 h

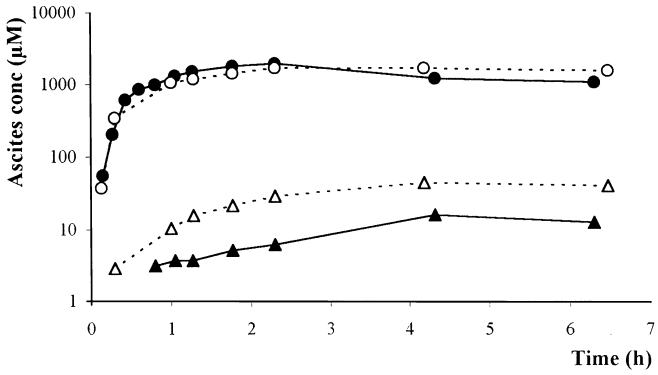

Fig. 3 Semilogarithmic concentration-time curves of BNP7787 (●, ○) and mesna (▲, △) in ascites of a patient who received a 15-min i.v. infusion of 41 g/m^2 BNP7787 alone (*closed symbols* and *solid line*) and in combination with a 1-h i.v. infusion of 75 mg/m^2 cisplatin (*open symbols* and *dotted line*)

and 17 in plasma when BNP7787 was administered alone. The concentration of mesna in ascites was lower when BNP7787 was administered alone than when it was given in combination with cisplatin, but at that time the volume of ascites was larger than when BNP7787 was given in combination with cisplatin. When BNP7787 was administered alone, the total amount of mesna (in micromoles) in the recovered ascites was similar to the total amount of mesna in the recovered ascites at the same time-points when given in combination with cisplatin.

Discussion

The pharmacokinetics of mesna in plasma have previously been described in cancer patients receiving different i.v. dosages of mesna in combination with ifosfamide [4] or cyclophosphamide treatment [3] and in healthy volunteers receiving mesna orally and intravenously [5, 8]. The reported final half-lives of mesna determined after i.v. mesna administration range from 0.36 to 2.12 h [3, 4, 5, 8], which are shorter than the final half-life we found for mesna after BNP7787 administration (2.8–3.4 h). This, as well as the longer MRT of mesna in comparison to that of BNP7787, indicates that the formation of mesna is not very rapid, which is also reflected by the time at which the peak value of mesna was reached, i.e. not earlier than 1 h after the end of the BNP7787 infusion. BNP7787 was more rapidly eliminated than mesna. The half-life of BNP7787 (1.5–1.6 h) was only slightly higher than the reported half-life of BNP7787 after mesna infusion (1.17–1.29 h) [3, 5, 8]. The volume of distribution of BNP7787 was 21–22 l, which corresponds to a distribution of BNP7787 to the extracellular volume and to a minor extent to the ascites.

Pharmacokinetic studies in patients and healthy volunteers have shown that after mesna administration, the concentration of mesna in plasma is similar to or higher than the BNP7787 concentration [3, 5, 8]. After the administration of BNP7787, however, the AUC_∞ of mesna in plasma was only 8% of the AUC_∞ of BNP7787, which is in agreement with earlier findings [11]. Thus, after BNP7787 administration the concentration in the circulation of the thiol mesna, which is more reactive with (hydrated) cisplatin than its disulfide [9], is much lower than that observed after an equivalent dose of mesna. This large difference in mesna plasma concentrations explains in part why BNP7787 administration does not inactivate cisplatin in the circulation and therefore does not interfere with the antitumor activity of cisplatin [2, 7], whereas mesna administration has been shown to interfere with the antitumor activity of cisplatin [2, 7]. Coadministration of cisplatin with BNP7787 did not influence the plasma concentrations, AUC_∞ and final half-life of mesna or BNP7787 in plasma. This is in agreement with the results obtained in 11 patients during the same phase I trial receiving BNP7787 with and without cisplatin [15].

No data were previously available on the distribution of mesna and BNP7787 into ascites. Our results show that approximately 4% of the BNP7787 dose was present in the recovered ascites as BNP7787 at the time of its maximum concentration in ascites, whereas only 0.02% of the BNP7787 dose was present as mesna. Thus, because of this very low distribution of BNP7787 and mesna into the ascites, the presence of ascites does not have an impact on the plasma pharmacokinetics of BNP7787 and mesna. This means that no dose adjustments of BNP7787 have to be made when patients with ascites are being treated with BNP7787. The fact that the uptake of BNP7787 and mesna was only very limited in ascites on two occasions in the same subject indicates that further investigation in an extensive study in more patients is unnecessary. Furthermore, the low distribution of mesna into the ascites and the expectedly high local mesna concentrations in the kidneys [10] makes BNP7787 a suitable candidate for nephroprotection, e.g. for the two-route application in patients with residual ovarian cancer, i.e. intraperitoneal cisplatin plus i.v. BNP7787 [1, 13].

The BNP7787/mesna concentration ratio was higher in ascites (range 38–409) than in plasma (range 5–181) over the whole concentration-time curve, indicating that it is not very likely that BNP7787 is reduced to mesna in ascites. Apparently, ascites does not contain the combination of disulfide-reducing enzymes and thiol transferase, glutathione and NADPH, which have been reported to be responsible for the reduction of BNP7787 to mesna in the kidneys of rats [10, 11].

In conclusion, in plasma BNP7787 was present at much higher concentrations than mesna. The amounts of BNP7787 and mesna in ascites were so low that the presence of ascites did not have an impact on the plasma pharmacokinetics of BNP7787 and mesna. Coadministration of cisplatin did not influence the plasma pharmacokinetics of BNP7787 and mesna in this patient. Neither BNP7787 or its metabolite, mesna, appears to accumulate in ascites.

References

1. Abe R, Akiyoshi T, Baba T (1990) 'Two-route chemotherapy' using cisplatin and its neutralizing agent, sodium thiosulfate, for intraperitoneal cancer. Oncology 47:422
2. Boven E, Verschraagen M, Hulscher TM, Erkelens CAM, Hausheer FH, Pinedo HM, Van der Vijgh WJF (2002) BNP7787, a novel protector against platinum-related toxicities, does not affect the efficacy of cisplatin or carboplatin in human tumour xenografts. Eur J Cancer 38:1148
3. El-Yazigi A, Ernst P, Al-Rawithi S, Legayada E, Raines DA (1997) Pharmacokinetics of mesna and dimesna after simultaneous intravenous bolus and infusion administration in patients undergoing bone marrow transplantation. J Clin Pharmacol 37:618
4. Goren MP, Anthony LB, Hande KR, Johnson DH, Brade WP, Frazier MW, Bush DA, Li JT (1998) Pharmacokinetics of an intravenous-oral versus intravenous-mesna regimen in lung cancer patients receiving ifosfamide. J Clin Oncol 16:616
5. Goren MP, Houle J-M, Bush DA, Li JT, Newman CE, Brade WP (1998) Similar bioavailability of single-dose oral and intravenous mesna in the blood and urine of healthy human subjects. Clin Cancer Res 4:2313
6. Goren MP, Hsu LC, Li JT (1998) Reduction of dimesna to mesna by the isolated perfused rat liver. Cancer Res 58:4358
7. Hausheer FH, Kanter P, Cao S, Haridas K, Seetharamulu P, Reddy D, Petluru P, Zhao M, Murali D, Saxe JD, Yao S, Martinez N, Zukowski A, Rustum YM (1998) Modulation of platinum-induced toxicities and therapeutic index: mechanistic insights and first- and second-generation protecting agents. Semin Oncol 25:584
8. James CA, Mant TGK, Rogers HJ (1987) Pharmacokinetics of intravenous and oral sodium 2-mercaptoethane sulphonate (mesna) in normal subjects. Br J Clin Pharmacol 23:561
9. Leeuwenkamp OR, Neijt JP, Van der Vijgh WJ, Pinedo HM (1991) Reaction kinetics of cisplatin and its monoaquated species with the modulating agents (di)mesna and thiosulphate. Eur J Cancer 27:1243
10. Ormstad K, Uehara N (1982) Renal transport and disposition of Na-2-mercaptoethane sulfonate disulfide (dimesna) in the rat. FEBS Lett 150:354
11. Ormstad K, Orrenius S, Låstbom T, Uehara N, Pohl J, Stekar J, Brock N (1983) Pharmacokinetics and metabolism of sodium 2-mercaptoethanesulfonate in the rat. Cancer Res 43:333
12. Shaw IC, Graham MI (1987) Mesna—a short review. Cancer Treat Rev 14:67
13. Van Rijswijk RE, Hoekman K, Burger CW, Verheijen RH, Vermorken JB (1997) Experience with intraperitoneal cisplatin and etoposide and i.v. sodium thiosulphate protection in ovarian cancer patients with either pathologically response or minimal residual disease. Ann Oncol 8:1235
14. Verschraagen M, Zwiers THU, De Koning P, Welink J, Van der Vijgh WJF (2001) Quantification of BNP7787 (dimesna) and its metabolite mesna in human plasma and urine by high-performance liquid chromatography with electrochemical detection. J Chromatogr B 753:293
15. Verschraagen M, Baen E, Ruijtes R, Van de Ban K, Berkhof J, Hausheer FH, Van der Vijgh WJF (2003) Pharmacokinetics and preliminary clinical data of the novel chemoprotectant BNP7787 and cisplatin and their metabolites. Clin Pharmacol Ther (in press)