Transport and biotransformation of the new cytostatic complex *cis*-diammineplatinum(II)-chlorocholylglycinate (Bamet-R2) by the rat liver[1]

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Abstract **Rat liver uptake and bile output of the cytostatic complex *cis*-diammineplatinum(II)-chlorocholylglycinate (Bamet-R2) were studied. Up to 100 μm, Bamet-R2 uptake by rat hepatocytes in primary culture followed saturation kinetics (V_{max} = 0.65 ± 0.12 nmol/5 min per mg protein; K_M = 45.2 ± 10.7 μm). Bamet-R2 uptake was lower than that of cholylglycinate (CG) but higher than that of cisplatin. Replacement of 116 mm NaCl by 116 mm choline chloride did not significantly reduce Bamet-R2 uptake. Addition of 500 μm CG, cholic acid, estrone sulfate, or ouabain to 50 μm Bamet-R2-containing incubation media inhibited Bamet-R2 uptake. No liver biotransformation of Bamet-R2 occurred, as indicated by HPLC analysis of bile collected from anesthetized rats after intravenous administration of the drug. Bamet-R2 uptake and secretion into bile by isolated rat livers exceeded those of cisplatin but were lower than those of CG. Differences between Bamet-R2 and CG were more marked for bile output than for liver uptake. Thus, higher Bamet-R2 than CG or cisplatin liver content was found. Coadministration of Bamet-R2 and CG revealed that CG induced a slight reduction in Bamet-R2 uptake and a marked inhibition in Bamet-R2 bile output. By contrast, Bamet-R2 had no effect on CG on either liver uptake or bile output. In sum, the present data indicate that Bamet-R2 is efficiently taken up and secreted into bile by the rat liver by mechanisms shared in part by natural bile acids.—**Macias, R. I. R., M. J. Monte, M. Y. El-Mir, G. R. Villanueva, and J. J. G. Marin. **Transport and biotransformation of the new cytostatic complex *cis*-diammineplatinum(II)-chlorocholylglycinate (Bamet-R2) by the rat liver. *J. Lipid Res.* 1998.** 39: **1792–1798.**

Supplementary key words bile acid • cancer • chemotherapy • cisplatin • glycocholate • metal • platinum (Pt)

Bile acids have been used in several attempts to shuttle drugs to the liver (1–4). In the case of cytostatic compounds, this possibility has been questioned (5) because cell lines derived from liver tumor cells have been reported to fail to efficiently take up bile acids (6–9). Despite this controversy, which should be elucidated in further investigations, another interesting potential use of bile acid derivatives with cytostatic activity is their ability to enhance biliary excretion of the drug after exposure of extrahepatic tumors to the cytostatic agent during regional chemotherapy. In this sense, our group has synthesized several antitumoral compounds by binding derivatives of transition metals to bile acids. The resulting complexes are called Bamet, from Ba- (for bile acid) and -met (for metal) (10). One of these compounds is Bamet-R2, which was obtained by binding cisplatin to the carboxylate group of cholylglycinate (CG) (11).

Cisplatin was selected because it is a small and efficient drug used to treat a variety of solid tumors (12). However, its dose-limiting toxicity has encouraged the search for new cisplatin derivatives. Although many different compounds with cytostatic activity have been obtained, few of them are currently used in clinical practice (13). Among the derivatives, *cis*-diammine(1,1-cyclobutanedicarboxylate)-platinum(II) (carboplatin) and 1,2-cyclohexane diammineplatinum(II) (DACH-Pt) and their derivatives are particularly interesting. One beneficial property of these cisplatin analogues is that although they are not organotropic, their physical–chemical characteristics do facilitate their excretion, and hence they are usually less nephrotoxic than cisplatin itself. However, other side effects such as myelotoxicity and neurotoxicity have been reported to be similar or even greater than those of the parent drug (14). Other authors have previously complexed DACH-Pt with bile acids to increase both the lipophilicity and water miscibility of the compounds (15, 16). Although promising results have been obtained as regards the antitumoral activity of the resulting drug, no further investigation has been conducted in this direction.

Abbreviations: CA, cholic acid; CG, cholylglycinate; K_M, affinity constant; HPLC, high performance liquid chromatography; V_{max}, maximal velocity of transport.

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Previous investigations by our group have revealed that Bamet-H2, a negatively charged compound that contains two CG moieties directly bound to a platinum(II) atom, is a cytostatic complex with hepatobiliary organotropism (10). Efficient blood-to-bile Bamet-H2 transfer is due to both bile acid and non-bile acid transport pathways (17). Bamet-R2 is a simpler compound with marked cytostatic ability, which is probably due to the presence of the cisplatin moiety in the complex. The aim of the present work was to further explore the ability of the liver to carry out Bamet-R2 uptake from plasma and to secrete the compound into the bile.

METHODS

Chemicals

Sodium cholylglycinate (CG), cholic acid (CA), ouabain, estrone sulfate, cisplatin, and chemicals used for isolation and culture of rat hepatocytes were purchased from Sigma Chemical Co. (St. Louis, MO). *Cis*-diammineplatinum(II)-chlorocholylglycinate (Bamet-R2, see the inset of Fig. 1) was synthesized and chemically characterized as previously reported (11). [^{14}C]-CG and [^{3}H]-inulin were from New England Nuclear (Itisa, Madrid, Spain). Labeled and non-labeled CG were more than 95% pure by thin-layer chromatography. All other reagents were from Merck (Darmstadt, Germany).

Rat hepatocytes in primary culture

Fasting male Wistar rats (180 g) from the Animal House, University of Salamanca, Salamanca, Spain, were used as donors to obtain isolated hepatocytes. They were fed commercial rat pelleted food from Panlab (Madrid, Spain). They had free access to food and water. Temperature (20°C) and the light/dark cycle (12 h:12 h) were controlled. All animals received humane care as outlined in Guide for the Care and Use of Laboratory Animals (NHI Publication No. 80-23, revised 1985). Hepatocytes were isolated by a modified procedure of Berry and Friend (18), suspended in William's medium E supplemented with 26.2 mm $NaHCO_3$, 10 mm HEPES, 100 nm Na_2SeO_3, 30 nm dexamethasone, 100 nm insulin, 11.1 mm galactose and antibiotics (streptomycin 0.02 mg/mL, penicillin 20 U/mL, and amphotericin B 0.05 μg/mL), pH 7.40, and cultured as previously reported (17).

Measurements of cisplatin, radiolabeled CG, and Bamet-R2 uptake by hepatocytes were carried out at 24 h of culture by an adaptation of the method described by Liang et al. (19). In brief, culture medium was replaced by incubation medium containing the following: 116 mm NaCl, 5.3 mm KCl, 1.8 mm $CaCl_2$, 0.8 mm $MgSO_4$, 1.1 mm KH_2PO_4, 11 mm d-glucose, 10 mm HEPES, pH 7.40. Hepatocytes were then incubated at 37°C in a prewarmed cabinet for 1 h. After this time, the medium was removed from culture dishes and drug uptake was initiated by adding 0.75 ml of prewarmed (37°C) fresh incubation medium containing the desired drug (cisplatin, [^{14}C]CG, or Bamet-R2) concentration. Hepatocytes were incubated under these conditions for 5 min. This incubation time was chosen on the basis of preliminary experiments on the time course of drug uptake, which revealed that this was linear for approximately 20 min and that the amount of compound retained by the cultures remained relatively constant at longer incubation times (data not shown). Uptake was terminated by removing the incubation medium. This was followed by four washes with ice-cold drug-free incubation medium. The amount of drug taken up by the hepatocytes was recovered by digesting them in 1 mL Lowry solution (100 mm NaOH, 189 mm Na_2CO_3) at room temperature for 2 h. In some experiments, 500 μm CG, CA, ouabain, or estrone sulfate was added to incubation medium containing 50 μm Bamet-R2. To check the sodium dependency of Bamet-R2 uptake by rat hepatocytes in a separate set of experiments, 116 mm NaCl in the incubation medium was replaced by 116 mm choline chloride.

In order to measure the amount of drug present in the sample due to contamination from extracellular water, [^{3}H]inulin (5×10^6 dpm/dish) was added to separate dishes, which were processed as above for drug-containing dishes. Radioactivity for [^{3}H]inulin and [^{14}C]CG, and platinum for cisplatin and Bamet-R2 were measured. Contamination from extracellular water (55.3 ± 2.1 nL/cm^2) was measured in each experiment to determine the amount of extracellular drug (6.3 ± 0.8% of that retained in the dishes). This value was used to calculate uptake, which was corrected by the amount of protein in each culture dish.

Characterization of biliary Bamet-R2

Hepatic Bamet-R2 biotransformation was studied in the following way. Surgery and bile collection were performed in vivo in Wistar rats (approximately 250 g) under sodium pentobarbital (Nembutal N.R., Abbott, Madrid, Spain) anesthesia (5 mg/100 g body weight, i.p.). After a body temperature equilibration period of 20 min and after surgical implantation of a catheter into the common bile duct, bile was collected in preweighed vials at 15-min intervals. After a first 15-min basal period, a single bolus of 500 μl Bamet-R2 solution (1 mm) in 150 mm NaCl was injected through the penial vein and bile samples were collected for an additional 2-h period, after which the animals were killed by exsanguination.

Rat liver perfusions

Fasting male Wistar rats (200 g) were anesthetized by i.p. administration (5 mg/100 g body weight) of sodium pentobarbital (Nembutal N.R., Abbott, Madrid, Spain). The liver was then in situ perfused by a modification (20) of the method of Miller et al. (21) in a prewarmed (38°C) cabinet. The recirculating perfusion medium was 150 mL of a modified Krebs-Henseleit solution containing the following in mm: 120 NaCl, 5 KCl, 1.29 $CaCl_2$, 0.65 $MgSO_4$, 25 $NaHCO_3$, 1.17 KH_2PO_4, 5.0 d-glucose, and gentamicin sulfate (100 mg/L) plus Bamet-R2, cisplatin or GC at the desired initial concentrations. The medium was oxygenated with a mixture of 5% CO_2–95% O_2. A similar solution without any of the three drugs under investigation was used during liver isolation as pre-perfusion medium. Bile flow, oxygen uptake, perfusion pressure, perfusion flow, potassium and lactate dehydrogenase activity released into the perfusate were determined during the experiments to assess the viability of the preparations, which was similar to that usually found in our laboratory for this experimental model (20). Perfusion medium and bile samples were collected during the experiments to measure radioactivity due to [^{14}C]CG or the platinum content when cisplatin and Bamet-R2 were used.

Analytical methods

Bile flow was determined gravimetrically assuming 1 mg = 1 μL. [^{3}H]inulin and [^{14}C]CG were measured on a liquid scintillation counter (LS-1800-Beckman, Beckman Instruments, Madrid, Spain). Ready-Safe Scintillation Cocktail, also from Beckman, was used as scintillant. After digesting the samples in nitric acid, platinum contents in perfusate, bile, cells, and liver homogenates were measured by flameless atomic absorption spectrophotometry (Z-8100 Polarized Zeeman apparatus with a graphite furnace, Hitachi, Pacisa, Madrid, Spain). The presence of Bamet-R2 and its related compounds, if any, in bile was investigated by high-performance liquid chromatography (HPLC) using a System Gold (Beckman Instruments, Madrid, Spain) with a pro-

grammable solvent module of two pumps connected to a gradient controller and a manual injection loop of 100 μL. HPLC was performed on a reverse-phase octadecylsilane (C_{18}) column (Spherisorb ODS, 250 × 4.6 mm, Symta, Madrid) equilibrated with solvent A (10 mm KH_2PO_4–methanol 25:75 (v/v) pH 7.02), which after 5 min was changed from 100% A to 20% A and 80% methanol over 15 min at 1 mL/min flow rate. HPLC fractions (0.5 min) were analyzed for platinum. Protein was determined by a modification of the classical method of Lowry et al. (22) with bovine serum albumin as standard.

Statistical analysis

Results are expressed as means ± SE. The statistical significance of differences among groups was calculated using paired and unpaired *t*-tests and the Bonferroni method of multiple-range testing, as appropriate. Linear and non-linear regressions were obtained using Ultrafit Software (Biosoft, Cambridge, U.K.). Statistical analyses were performed on a Macintosh LC-III computer (Apple Computer, Inc., Cupertino, CA).

RESULTS

The kinetics of CG uptake by rat hepatocytes in primary culture were studied in the 0 to 800 μm concentration range (data not shown). Results from CG uptake were fitted to a Michaelis-Menten equation (V_{max} = 9.0 ± 0.6 nmol/5 min per mg protein, K_M = 165 ± 35 μm) plus a diffusional term with a negligible (less than 0.001 μL/5 min per mg protein) diffusional constant (K_d). Owing to the low solubility and high toxicity of cisplatin, the uptake of this compound by rat hepatocytes was studied using a narrower concentration range (0–400 μm). In this range, no saturation was observed (data not shown), in agreement with a diffusional process. However, the existence of a very high capacity carrier-mediated process cannot be ruled out. The best fit for cisplatin uptake was a linear equation, whose slope was assumed to be the diffusional constant (K_d = 1.8 ± 0.1 μL/5 min per mg protein). Results on the kinetics of CG and cisplatin are in agreement with previous studies by our group (17). A dual behavior for Bamet-R2 uptake by rat hepatocytes was observed. Up to approximately 100 μm substrate concentrations (**Fig. 1**), Bamet-R2 uptake followed Michaelis-Menten kinetics (V_{max} = 0.65 ± 0.12 nmol/5 min per mg protein; K_M = 45.2 ± 10.7 μm). In this range, Bamet-R2 uptake was lower than that of CG, but higher than that of cisplatin. At substrate concentrations higher than 200 μm a non-saturable linear increase in Bamet-R2 uptake was found (data not shown). The slope of this curve (19.8 ± 6.2 μL/5 min per mg protein) was 10-fold steeper than that of cisplatin. Bamet-R2 uptake was not significantly modified by sodium replacement at 50 μm substrate (**Fig. 2**). Absence of significant sodium dependency in Bamet-R2 uptake was also found at substrate concentrations higher than 200 μm (data not shown). Bamet-R2 uptake was significantly reduced in the presence of CG, CA, ouabain, or estrone sulfate (Fig. 2).

To check whether Bamet-R2 undergoes liver biotransformation, HPLC analysis was carried out on bile samples. After intravenous administration of 0.5 μmol Bamet-R2 to anesthetized rats, approximately 90% of the Pt given was recovered in the bile within the ensuing 2 h. Approximately 35% of this amount was collected during the first 15 min (data not shown). This investigation revealed that

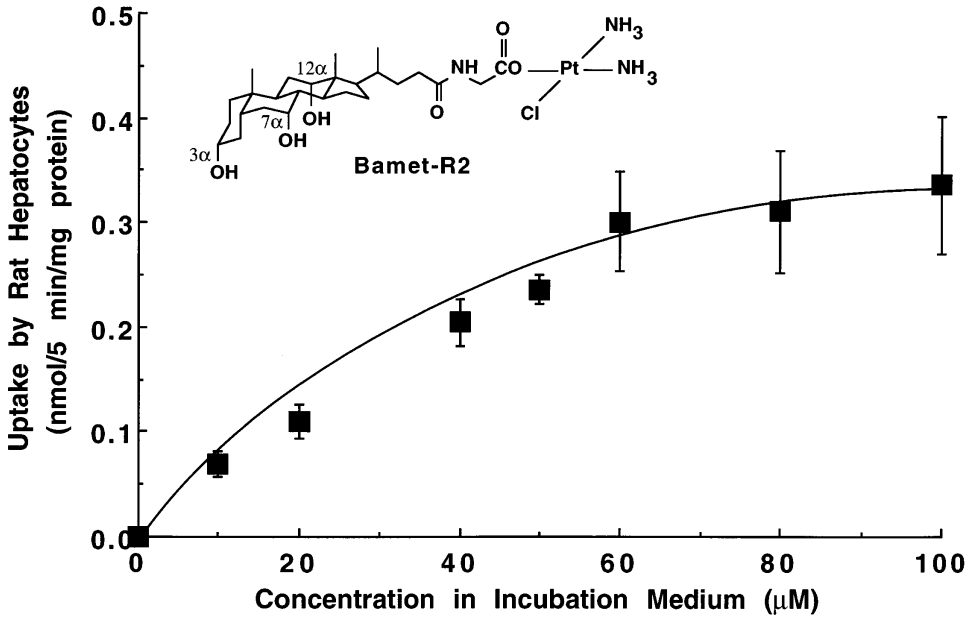

Fig. 1. Uptake of Bamet-R2 by rat hepatocytes in primary culture for 24 h. Uptake was initiated by adding 0.75 ml of prewarmed (37°C) fresh incubation medium containing the desired Bamet-R2 concentration. After incubation for 5 min, the amount of platinum retained by the cells was measured and corrected by the amount of protein in each culture dish and the amount of extracellular drug calculated from the volume of [^{3}H]inulin-containing extracellular water measured in separate dishes. Values are means ± SE from measurements carried out in triplicate on four different cultures. Inset: Bamet-R2 molecular structure.

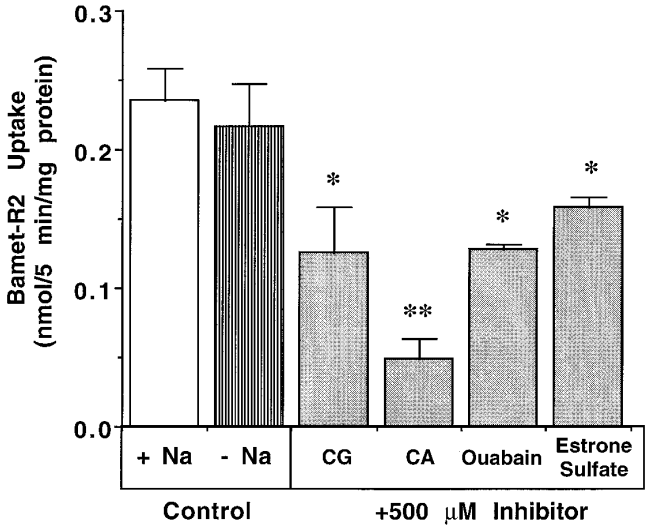

Fig. 2. Effect of NaCl replacement in culture medium by choline chloride and of the presence of 500 μm cholylglycinate (CG), cholic acid (CA), ouabain, or estrone sulfate on Bamet-R2 uptake by rat hepatocytes in primary culture for 24 h. Uptake was initiated by adding 0.75 ml of prewarmed (37°C) fresh incubation medium containing 50 μm Bamet-R2 together with none or one of the inhibitors mentioned above. Values are means ± SE from measurements carried out in triplicate on four different cultures. *$P < 0.05$; **$P < 0.01$ as compared to Control in the presence of sodium by the Bonferroni method of multiple range testing.

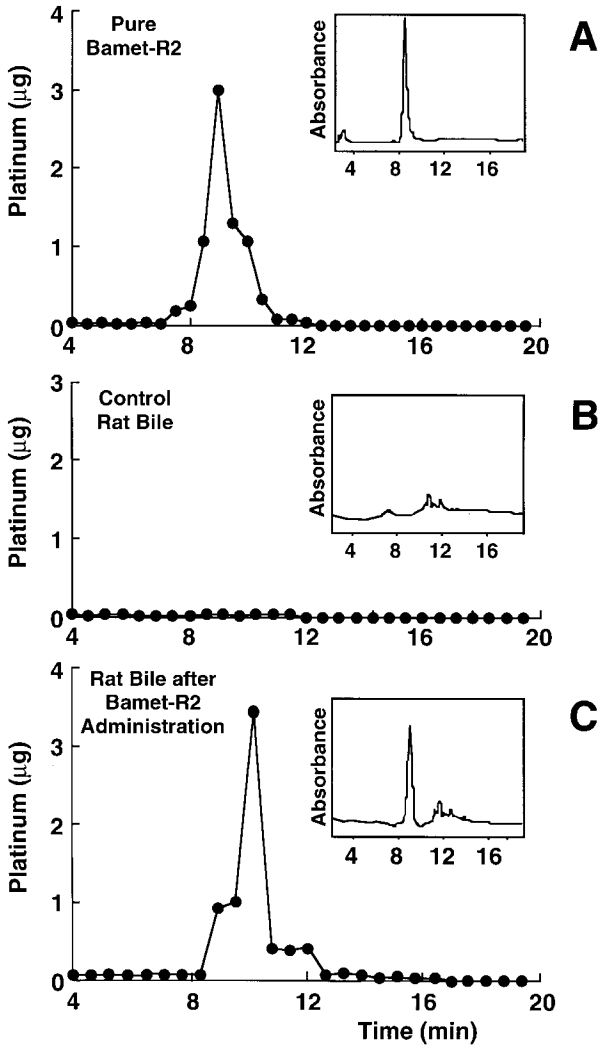

Fig. 3. Typical profile obtained during reverse phase HPLC by platinum determination in 0.5 min collected fractions and continuous recording of the absorbance at 250 nm wavelength (insets) after injection into the chromatograph of pure Bamet-R2 solution (A) or bile samples that were obtained before (B) and after (C) intravenous 0.5 μmol Bamet-R2 administration to anesthetized rats (n = 3).

both the continuous UV-absorbance and the Pt content profiles for pure Bamet-R2 and bile collected after Bamet-R2 administration were very similar (**Fig. 3**). Approximately 96% of the Pt measured in the HPLC fractions was found at the elution time corresponding to the pure Bamet-R2 standard. This suggests that the compound was mainly excreted into bile without undergoing biotransformation.

Experiments carried out using isolated perfused rat livers revealed that this organ was able to efficiently take up Bamet-R2. Comparison of liver uptakes over 40 min revealed a significant difference among CG, Bamet-R2, and cisplatin (**Fig. 4A**). Bamet-R2 uptake by the liver was lower than that of the natural bile acid, i.e., CG, but much higher than that of cisplatin. As compared with CG, Bamet-R2 bile output was approximately 50% lower; however this was several-fold higher than that of cisplatin (Fig. 4B). Measurement of drug liver contents revealed that the amount of Bamet-R2 remaining in the liver at the end of the perfusion period was significantly higher than that of both cisplatin and CG (Fig. 4C). Calculation of fractional first pass hepatic clearance by isolated rat liver revealed that this for Bamet-R2 (35.9% ± 3.9) was lower than for CG (52.2% ± 4.6; $P < 0.05$) but higher than for cisplatin (4.5% ± 1.5; $P < 0.05$). Addition of CG to the perfusion medium (50 μm CG versus 0.1 μm Bamet-R2) reduced both Bamet-R2 fractional first pass hepatic clearance (to 14.2% ± 4.6; $P < 0.05$) and total 40 min uptake (−20%; **Fig. 5**, left panel). A marked reduction (−72%) in Bamet-R2 bile output (Fig. 5, right panel) was also observed. Conversely, addition of 50 μm Bamet-R2 to the medium containing 0.1 μm [^{14}C]CG did not significantly affect either [^{14}C]CG fractional first pass hepatic clearance (49.1% ± 5.3; $P > 0.05$), total 40 min liver uptake (**Fig. 6**, left panel) or bile output (Fig. 6, right panel). The changes in radioactivity or platinum content in liver at the end of these two sets of experiments were consistent with the changes in the overall uptake–secretion process (data not shown).

DISCUSSION

The development of new cytostatic drugs with reduced effects on extratumor tissues is of enormous importance. Side effects are one of the major problems related to the clinical usefulness of a variety of powerful antiproliferative agents, such as cisplatin. This work shows that Bamet-R2, a

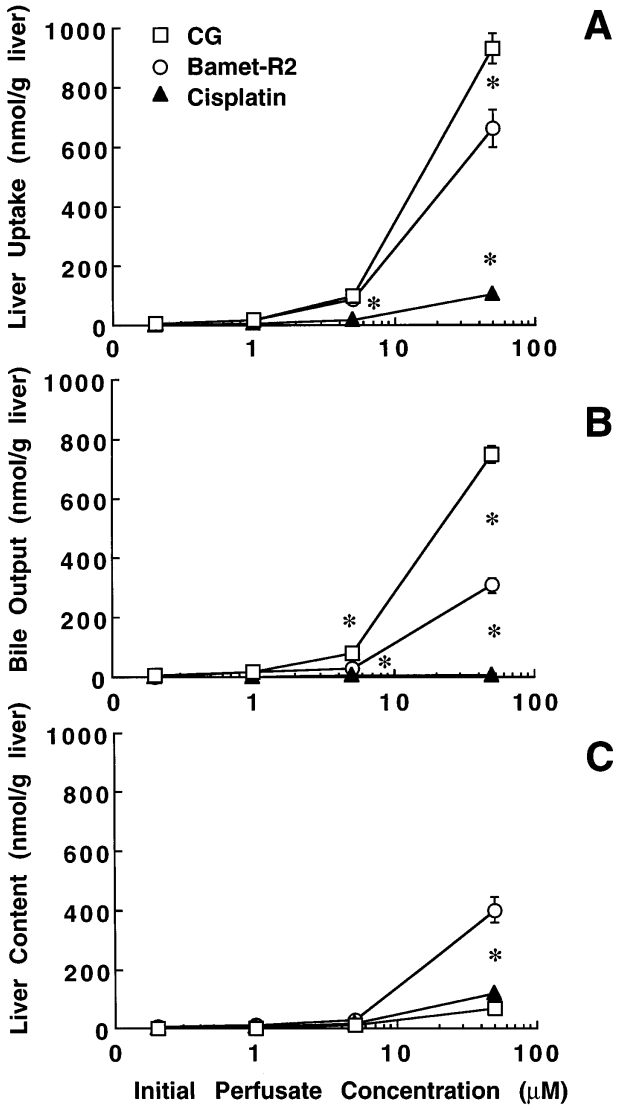

Fig. 4. Liver uptake (A), bile output (B) and liver content (C) of cholylglycinate (CG), Bamet-R2, and cisplatin over 40 min perfusion of isolated rat livers. The erythrocyte- and albumin-free Krebs-Henseleit perfusion medium contained CG, Bamet-R2, or cisplatin at the logarithmically indicated initial concentrations. Values are expressed as means ± SE; n = 4 for each group. *$P < 0.05$ as compared to Bamet-R2 group by the Bonferroni method of multiple range testing.

new cisplatin derivative obtained by binding cisplatin to the carboxylate group of cholylglycinate, is efficiently taken up by the rat liver and subsequently excreted into bile. The cytostatic and antitumoral activities of this compound have been reported elsewhere (11).

The facts that the Bamet-R2 molecule is not much larger than that of CG and that its formation does not substantially affect the main part of the bile acid moiety hint that it may be able to interact with bile acid carrier systems located at the plasma membrane of the hepatocyte and that it may endow this complex with the hepatobiliary organotropism that has been observed in vivo (23). The findings reported here partly support these hypotheses. Absence of toxic effect of up to 200 μm Bamet-R2 on rat hepatocytes has been observed (unpublished data). However, disruption of the plasma membrane permeability barrier at higher concentrations cannot be ruled out. This may account for the sharp increase in the amount of platinum found in rat hepatocytes after incubation for 5 min with Bamet-R2 concentrations above 200 μm (data not shown). At lower Bamet-R2 concentrations, a carrier-mediated process with high affinity and low capacity seems to account for Bamet-R2 transport across the sinusoidal membrane. This has been found to be sensitive to the inhibition by CG. In contrast to previously reported data for Bamet-H2 (17), another platinum-containing bile acid derivative with a negative charge, this process is not affected by sodium removal. This and the absence of a negative charge in the Bamet-R2 molecule together with the lack of an inhibitory effect of Bamet-R2 on CG uptake suggest that this carrier system is not the major sodium-dependent pathway known to be responsible for bile acid transport across the sinusoidal membrane of rat hepatocytes, the Ntcp (24). Carriers of the oatp family are involved in sodium-independent transport of a variety of amphipathic organic compounds including bile acids, neutral steroids, and the cardiac glycoside ouabain across the basolateral membrane of rat hepatocytes (24). Inhibition of Bamet-R2 uptake by estrone sulfate, ouabain, CG, and more markedly by the less polar bile acid CA suggests that these transporters may be involved in the uptake of this neutral bile acid derivative by rat hepatocytes.

Cisplatin uptake by the liver cell is not well understood, although it has been proposed to be mediated by mechanisms similar to those responsible for small neutral aminoacid uptake (for a review, see ref. 25). The marked difference with Bamet-R2 in uptake kinetics at non-toxic substrate concentrations suggests that cisplatin does not share the saturable transport process found in rat hepatocytes for Bamet-R2.

When cisplatin, and presumably Bamet-R2, are dissolved in the perfusate, where the chloride ion concentration is approximately 100 mm, the chloride of the compound's structure remains bound to them. Once in the intracellular space, where the chloride concentration drops to approximately 3 mm, these compounds are activated by chloride displacement to allow the formation of aquo species, which are the reactive forms of this type of drug due to their ability to act as potent electrophiles. These react with any intracellular nucleophile, including the sulphydryl groups of proteins and nucleophilic groups of nucleic acids, leading to the formation of DNA adducts. This is believed to account for their cytotoxicity. If this was also true for Bamet-R2, two interesting hypotheses could be advanced from the present results. Aquo species only result in the loss of one chloride ion, leaving the diammine-aquo-platinum(II) bound to CG, because after crossing the intracellular space of the hepatocyte most of the platinum(II) is recovered in bile still forming part of the Bamet-R2 molecule. This suggests that DNA–Bamet-R2 adducts, if formed as reported (26), should be monoadducts but not interstrand or intrastrand DNA cross-links. The other consequence of this assumption is that the cat-

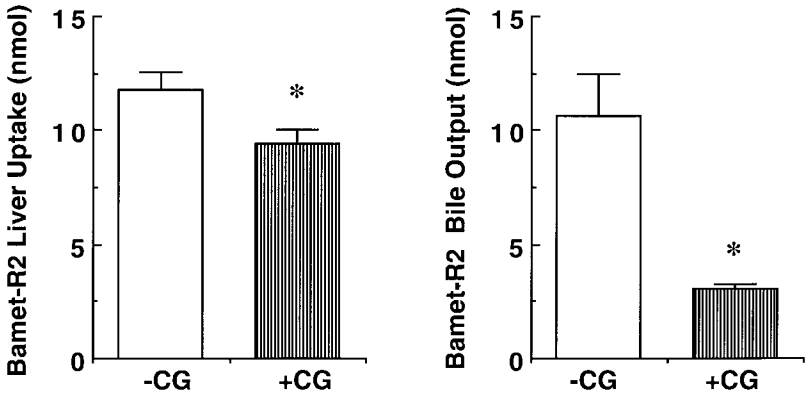

Fig. 5. Effect of cholylglycinate (CG) on Bamet-R2 uptake and bile output by isolated rat livers perfused in a recirculating system with an erythrocyte- and albumin-free Krebs-Henseleit solution for 40 min. The initial perfusate contained 0.1 μm Bamet-R2 without (open bars) or with (striped bars) 50 μm CG. Values are means ± SE (n = 4). *$P < 0.05$ as compared by the Student's *t*-test.

ionic form of Bamet-R2, where Cl^- is replaced by H_2O, forms part of the pool of intracellular molecular species. Whether this is the major form transported across the canalicular membrane is not known.

The formation of glutathione–cisplatin complexes is considered to be a significant part of the cellular metabolism of this drug and the ATP-dependent glutathione S–conjugate export pump has been suggested to play a role in the elimination of the glutathione–cisplatin complex from tumor cells (27). Recently, the human gene homologous to the rat canalicular multispecific organic anion transporter (cMOAT) or multidrug resistance protein MRP2 gene has been found to be overexpressed in cisplatin-resistant human cancer cell lines, and this is accompanied by a decreased cisplatin accumulation in these cells (28). This protein mediates the excretion of multivalent anionic conjugates (29). The absence of Bamet-R2 conjugation suggests that this pathway does not play any role in Bamet-R2 bile output.

Another canalicular ABC transporter protein, the multidrug resistance protein (mdr1), mediates the excretion of cytotoxic compounds. Additionally, surveys for similarities among the substances transported by this P-glycoprotein indicate that most are amphiphilic, have a polar planar structure, and are 400- to 1,400-KDa cations (for a review see ref. 30). Intracellular Bamet-R2 fits these characteristics perfectly, suggesting that this compound is a possible candidate for a mdr1 substrate.

In the present work, strong inhibition of CG on Bamet-R2 bile output was observed, but no reciprocal inhibition of Bamet-R2 on CG bile output was found. This is consistent with the idea of Bamet-R2 being transported across the canalicular membrane by mechanisms other than that mainly responsible for natural bile acid secretion into bile, such as the canalicular bile acid transporter cBAT, which mediates the secretion of monovalent bile salts (29).

Bile acids are not substrates for P-glycoproteins but are able to inhibit P-glycoprotein-mediated drug transport (31). Therefore, alternative to the possibility of their sharing canalicular transport systems, which is unlikely, CG may affect Bamet-R2 bile output by inhibiting mdr1 and hence reducing its efflux across the hepatocyte canalicular membrane.

In sum, these results reveal that Bamet-R2 is efficiently and rapidly cleared from plasma by the liver and is subsequently excreted into bile. These together with previously reported cytostatic ability are promising properties in the development of new cytostatic compounds with enhanced hepatobiliary organotropism.

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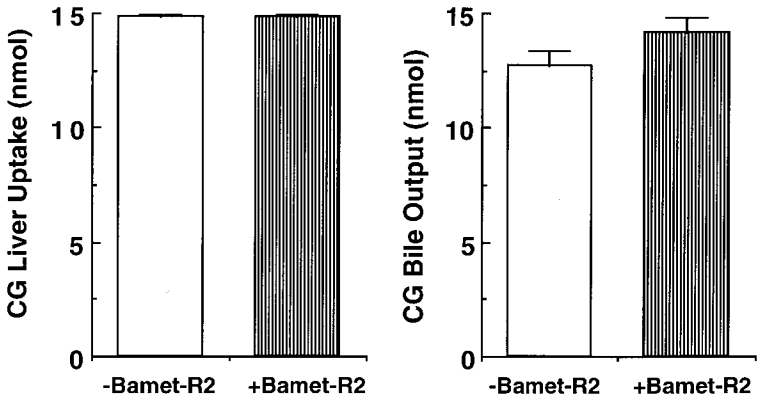

Fig. 6. Effect of Bamet-R2 on cholylglycinate (CG) uptake and bile output by isolated rat livers perfused in a recirculating system with an erythrocyte- and albumin-free Krebs-Henseleit solution for 40 min. The initial perfusate contained 0.1 μm CG without (open bars) or with (striped bars) 50 μm Bamet-R2. Values are means ± SE (n = 4). *$P < 0.05$ as compared by the Student's *t*-test.

REFERENCES

1. Ho, N. F. H. 1987. Utilizing bile acid carrier mechanisms to enhance liver and small intestine absorption. *Ann. N.Y. Acad. Sci.* **907:** 315–329.
2. Betebenner, D. A., P. L. Carney, A. M. Zimmer, and J. M. Kaziewicz. 1991. Hepatobiliary delivery of polyaminopolycarboxylate chelates: synthesis and characterization of a cholic acid conjugate of EDTA and biodistribution and imaging studies with its indium-111 chelate. *Bioconjugate Chem.* **2:** 117–123.
3. Stephan, Z. F., E. C. Yurachek, R. Sharif, J. M. Wasvary, R. E. Steele, and C. Howes. 1992. Reduction of cardiovascular and thyroxine-suppressing activities of L-T3 by liver targeting with cholic acid. *Biochem. Pharmacol.* **43:** 1969–1974.
4. Kramer, W. and G. Wess. 1996. Bile acid transport systems as pharmaceutical targets. *Eur. J. Clin. Invest.* **26:** 715–732.
5. Meijer, D. K. F. 1993. Drug targeting to the liver with bile acids—The Trojan Horse resurrected. *Hepatology.* **17:** 945–948.
6. Kroker, R., M. S. Anwer, and D. Hegner. 1978. The lack of active bile acid transport in AS-30D ascites hepatoma cells. *Naunyn Schmiedeberg's Arch. Pharmacol.* **303:** 299–301.
7. Von Dippe, P., and D. Levy. 1990. Expression of the bile acid transport protein during liver development and in hepatoma cells. *J. Biol. Chem.* **265:** 5942–5945.
8. Marchegiano, P., F. Carubbi, C. Tiribelli, S. Amarri, M. Stebel, G. C. Lunazzi, D. Levy, and S. Bellentani. 1992. Transport of sulfobromophthalein and taurocholate in the HepG2 cell line in relation to the expression of membrane carrier proteins. *Biochem. Biophys. Res. Commun.* **183:** 1203–1208.
9. Kullak-Ublick, G. A., U. Beuers, and G. Paumgartner. 1996. Molecular and functional characterization of bile acid transport in human hepatoblastoma HepG2 cells. *Hepatology.* **23:** 1053–1060.
10. Criado, J. J., M. C. Herrera, M. F. Palomero, M. Medarde, E. Rodriguez, and J. J. G. Marin. 1997. Synthesis and characterization of a new bile acid and platinum(II) complex with cytostatic activity. *J. Lipid Res.* **38:** 1022–1032.
11. Criado, J. J., R. I. R. Macias, M. Medarde, M. J. Monte, M. A. Serrano, and J. J. G. Marin. 1997. Synthesis and characterization of the new cytostatic complex *cis*-diammineplatinum(II) chlorocholylglycinate. *Bioconjugate Chem.* **8:** 453–458.
12. Loeher, P. J., and L. H. Einhorn. 1984. Cisplatin. *Ann. Int. Med.* **100:** 704–713.
13. Bradner, W. T., W. C. Rose, and J. B. Huftalen. 1980. Antitumor activity of platinum analogs. *In* Cisplatin: Current Status and New Developments. A.W. Prestayko, S.T. Crooke, and S.K. Carter, editors. Academic Press, New York. 171–182.
14. Christian, M. C. 1992. The current status of new platinum analogs. *Sem. Oncol.* **19:** 720–733.
15. Maeda, M., N. Takasuka, T. Suga, and T. Sasaki. 1990. New antitumor platinum(II) complexes with both lipophilicity and water miscibility. *Jpn. J. Cancer Res.* **81:** 567–569.
16. Maeda, M., T. Suga, N. Takasuka, A. Hoshi, and T. Sasaki. 1990. Effect of bis(bilato)-1,2-cyclohexanediammineplatinum(II) complexes on lung metastasis of B16-F10 melanoma cells in mice. *Cancer Lett.* **55:** 143–147.
17. Marin, J. J. G., M. C. Herrera, M. F. Palomero, R. I. R. Macias, M. J. Monte, M. Y. El-Mir, and G. R. Villanueva. 1998. Rat liver transport and biotransformation of a cytostatic complex of bis-cholylglycinate and platinum(II). *J. Hepatol.* **28:** 417–425.
18. Berry, M. N., and D. S. Friend. 1969. High-yield preparation of isolated rat liver parenchymal cells. *J. Cell Biol.* **43:** 506–520.
19. Liang D., B. Hagenbuch, B. Stieger, and P. J. Meier. 1993. Parallel decrease of Na^+-taurocholate cotransport and its encoding messenger RNA in primary cultures of rat hepatocytes. *Hepatology.* **18:** 1162–1166.
20. Marin, J. J. G., A. Esteller, and G. R. Villanueva. 1988. Diabetes-induced cholestasis in the rat. Possible role of hyperglycemia and hypoinsulinemia. *Hepatology.* **8:** 332–340.
21. Miller, L. L., C. G. Bly, M. L. Watson, and W. F. Bale. 1951. The dominant role of the liver in plasma protein synthesis. *J. Exp. Med.* **94:** 431–445.
22. Markwell, M. A. K., S. M. Haas, L. L. Bieber, and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87:** 206–210.
23. Marin, J. J. G., M. C. Herrera, M. F. Palomero, R. I. R. Macias, M. J. Monte, G. R. Villanueva, M. Y. El-Mir, M. A Serrano, and J. J. Criado. 1996. In vivo distribution, toxicity and cytostatic capacity of platinated bile acid analogs. [Abstract] *Hepatology.* **24:** 372A.
24. Hagenbuch, B. and P. J. Meier. 1996. Sinusoidal (basolateral) bile salt uptake systems of hepatocytes. *Semin. Liver Dis.* **16:** 129–136.
25. Chu, G. 1994. Cellular responses to cisplatin—the roles of DNA-binding proteins and DNA repair. *J. Biol. Chem.* **269:** 787–790.
26. Marin, J. J. G., J. J. Criado, M. F. Palomero, M. C. Herrera, R. I. R. Macias, M. J. Monte, G. R. Villanueva, M. Y. El-Mir, and M. A. Serrano. 1996. Platinated bile acids as cytostatic drugs. [Abstract] *Hepatology.* **24:** 542A.
27. Ishikawa, T., and F. Ali-Osman. 1993. Glutathione-associated *cis*-diammine dichloroplatinum(II) metabolism and ATP-dependent efflux from leukemia cells. Molecular characterization of glutathione–platinum complex and its biological significance. *J. Biol. Chem.* **268:** 20116–20125.
28. Taniguchi, K., M. Wada, K. Kohno, T. Nakamura, T. Kawabe, M. Kawakami, K. Kagotani, K. Okumura, S. Akiyama, and M. Kuwano. 1996. A human canalicular multispecific organic anion transporter (cMOAT) gene is overexpressed in cisplatin-resistant human cancer cell lines with decreased drug accumulation. *Cancer Res.* **56:** 4124–4129.
29. Muller, M., and P. L. M. Jansen. 1997. Molecular aspects of hepatobiliary transport. *Am. J. Physiol.* **35:** G1285–G1303.
30. Gatmaitan, Z. C., and I. M. Arias. 1995. ATP-dependent transport systems in the canalicular membrane of the hepatocyte. *Physiol. Rev.* **75:** 261–275.
31. Mazzanti, R., O. Fantappie, Y. Kamimoto, Z. C. Gatmaitan, P. Gentilini, and I. M. Arias. 1994. Bile acid inhibition of P-glycoprotein-mediated transport in multidrug-resistant cells and rat liver canalicular membrane vesicles. *Hepatology.* **20:** 170–176.