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KINETICS AND MECHANISM OF UPTAKE OF PLATINUM-BASED PHARMACEUTICALS BY THE RAT SMALL INTESTINE

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Abstract—The absorption of two platinum-based pharmaceuticals, cisplatin and carboplatin, was studied using *in vitro* and *in situ* models. By utilizing everted rat small intestine, it was found that absorption of both drugs was linear with time up to 60 min and was not saturable up to a concentration of 1.0 mM. Moreover, uptake against a concentration gradient could not be demonstrated and absorption was not reduced by metabolic inhibition or anoxic conditions. These results indicate the lack of involvement of an active transport mechanism for cisplatin and carboplatin and imply that absorption across the gastrointestinal tract is by passive diffusion. Cisplatin was absorbed more readily than carboplatin, both *in vitro* and *in situ*. *In situ* both drugs were found to disappear from the intestinal lumen following first-order kinetics. The results of *in situ* studies indicate that a decrease in pH of the perfusion medium leads to an increase in absorption of carboplatin into the systemic blood. This report establishes the fact that both cisplatin and carboplatin are absorbed across the gastro-intestinal tract and indicates that pre-clinical trials involving oral administration of platinum-based pharmaceuticals could be justified.

The anti-tumour activities of cisplatin [cis-diammine dichloroplatinum (II)] and its congener carboplatin [cis-diammine-1,1-cyclobutane dicarboxylate platinum (II)], have usually been evaluated following their parenteral administration [1–3]. However, since oral application of anti-tumour drugs is of importance in the clinical management of patients, investigations involving this route of administration have been initiated. Recently, Siddik and colleagues reported anti-tumour, pharmacokinetic and toxicity studies with orally administered cisplatin and its congeners [4]. This study and our earlier work [5] clearly demonstrate that platinum does enter the blood after oral administration of cisplatin or carboplatin. Moreover, it has been reported that orally administered cisplatin is effective against several murine tumours and has suggested that clinical trials involving oral administration of platinum-based compounds would be justified [6]. The present study was undertaken to determine the kinetics of intestinal transport of cisplatin and carboplatin by measuring the *in vitro* uptake of the compounds into everted intestinal tissue of the rat jejunum and *in situ* disappearance from perfused whole small intestine. The aims of this investigation were: (a) to determine the extent to which cisplatin and carboplatin are absorbed from the small intestine, and (b) to clarify the characteristics of the transport processes involved.

MATERIALS AND METHODS

Chemicals. All reagents and chemicals used were of analytical grade except where stated otherwise. The 95% oxygen, 5% carbon dioxide gas mixture and oxygen free nitrogen were supplied by the medical gases division of the British Oxygen Company Ltd (Wolverhampton, West Midlands, U.K.). Sodium pentobarbitone (Sagatal) was obtained from May and Baker Ltd (Dagenham, Essex, U.K.). Platinum standard stock solution for atomic absorption spectrophotometry was atomic absorption grade purchased from BDH Chemicals Ltd (Poole, Dorset, U.K.). Cisplatin and carboplatin were gifts from the Johnson Matthey Research Centre (Sonning Common, U.K.).

Animals. Male albino Wistar rats (University of Surrey strain), kept on standard laboratory pellet diet, and allowed water *ad libitum*, were kept in a controlled environment. Temperature was 21° ± 2° with a 50% humidity and lighting controlled to give 12 hr light and 12 hr dark per 24 hr. Twenty hr prior to the experiment animals were rehoused in plastic cages with wire mesh floors which prevented them from coprophagy and eating the wood shavings. Food was withheld, but water was available at all times.

In vitro *absorption technique*. The technique used was based on that of a previously published method [7]. After a 20-hr fast, the animals were anaesthetised with an i.p. injection of sodium pentobarbitone (45 mg/kg bodyweight) and placed on a heated blanket to maintain body temperature of 36° ± 1°. The abdomen was opened with a midline incision and L-shaped glass cannulae were inserted into the small intestine at the upper duodenum and the ileal caecal junction. Ice-cold Krebs–Henseleit buffer, pH 7.4, was then perfused through the intestine using a Gilson Minipuls 2 perfusion pump (Anachem, Luton, Beds, U.K.) at a flow rate of 2 mL/min. When all solid remnants had been flushed out, a reproducible

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region of the small intestine (25–50 cm from the stomach) was rapidly excised. This was then everted over a smooth glass rod and replaced into ice-cold Krebs–Henseleit medium, pH 7.4, that was continuously gassed with 95% oxygen, 5% carbon dioxide. Four 5-cm lengths of everted intestine were cut and one end of each segment was tied off with a thread ligature. With minimum handling the open end of each everted intestinal segment was placed over the end of a Venisystems Abbocath T short 18G cannula (Abbott Ireland Ltd, Sligo, Republic of Ireland), which was attached to a 1 mL syringe. A second ligature was tied around the cannula and 0.5 mL of pregassed, 37° Krebs–Henseleit medium containing 10 mM D-glucose (serosal fluid) was introduced. Using the second ligature the sacs were carefully withdrawn from the cannula and tied off. The four sacs were then placed into a 100 mL volumetric flask containing 75 mL of test mixture (mucosal fluid). This test mixture consisted of cisplatin or carboplatin at the appropriate concentration, and 10 mM D-glucose in Krebs–Henseleit medium, pH 7.4, at 37° and gassed with 95% oxygen, 5% carbon dioxide. Samples of mucosal and serosal fluids were retained and stored at −18° until they could be analysed for glucose and platinum. During the incubation period, flasks were shaken reciprocally at a rate of 35 times/min and purged with 95% oxygen, 5% carbon dioxide at a constant rate. The incubation period was generally 60 min. In the study of the effect of metabolic inhibition, 5 mM potassium cyanide was included in both mucosal and serosal fluids. To study the effect of anoxia on transport, incubations were carried out under oxygen-free nitrogen. Upon completion of the incubations, further samples of mucosal fluid were taken and intestinal sacs were removed from the flasks, washed in ice-cold isotonic saline for exactly 5 sec, blotted on hardened filter paper and weighted. The contents of each sac was then removed using a hypodermic syringe, placed in a polypropylene tube and stored at −18°. Empty sacs were then weighed (to give by difference the volume of serosal fluid at the end of the incubation period), dried overnight at 105°, reweighed and stored until analysed for platinum by electrothermal atomic absorption spectroscopy. Both the incubation medium (mucosal fluid) and the solution collected from inside the sacs (serosal fluid) were analysed for glucose by a method based on the Somogyi–Nelson procedure [8]. Since only those preparations in which glucose accumulates against the concentration gradient maintain functional tissue, only those were subjected to platinum analysis.

In situ *absorption technique*. The technique is described in detail elsewhere [9, 10] and therefore it will only be briefly outlined here. After a 20-hr fast, the animals were anaesthetised with an i.p. injection of sodium pentobarbitone and the entire small intestine from the top of the duodenum to the ilealcaecal junction was cannulated. The intestine was then cleaned by perfusion of 25 mL of warm (37°) perfusion solution [11] at a rate of 4.3 mL/min. During this period the test compound (cisplatin or carboplatin) was dissolved in buffered medium to a concentration of 2.8 mM. The buffer used was Tris hydroxymethylaminomethane (20 mM), containing 130 mM sodium chloride, adjusted to pH 7.0, 7.5 or 8.0 with 6 M HCl and made isotonic prior to perfusion. Once the test compound had completely dissolved, it was perfused through the intestine at a rate of 4.3 mL/min. The temperature of the test compound in buffer was maintained at 37° throughout the course of the perfusion. At regular time intervals during the next 90 min, a 100 μL sample was collected from the perfusate, diluted in distilled water and stored at −18° until analysis for platinum within 1 week. At the same time as samples of perfusate were taken, 25 μL of systemic blood was taken from the tail vein. Preliminary data concerning the appearance of platinum in portal blood was obtained from two animals by cannulation of the portal vein as described by Van der Voet and de Wolff [9]. Whole blood was diluted with 100 μL of 0.05 M sodium EDTA and stored at 4° prior to platinum analysis.

Platinum analysis. Platinum concentrations in solutions collected from *in vitro* or *in situ* experiments were determined by electrothermal atomic absorption spectrophotometry on a Pye Unicam SP9090 spectrophotometer fitted with deuterium background correction. The conditions used were based on published methods [12, 13] and optimized for the spectrophotometer employed. In all assays quantification of platinum levels in samples was achieved by comparison with suitably matrix matched platinum standards. Intestinal tissue samples were "wet" ashed in concentrated nitric acid at 170° in a DG-1 block digester (Techne Limited, Cambridge, U.K.) and resuspended in 1% hydrochloric acid prior to platinum analysis.

Calculation of results. For *in vitro* studies, transport of platinum from mucosal to serosal sides of the everted intestinal sacs was expressed as μg platinum/min/g dry tissue weight. The SE of the rate of uptake was usually below 15%. Uptake into intestinal tissue was expressed as μg platinum/g dry tissue weight after a 60-min incubation period. In the case of *in situ* experiments, the amount of platinum present in the perfusate was corrected for the reduction of the total volume of the perfusate during the perfusion period by a mathematical method described previously [9]. Semilogarithmic plots of platinum concentration in the intestinal fluid against time were constructed using ordinary least squares regression of the log data. The first-order rate constants (K_a) and half-life of the absorption process ($T_{1/2}$) were then calculated. All estimations of transport were made from the mean values obtained from four preparations.

RESULTS

In vitro *absorption studies*

Time course of drug uptake. The time course of uptake from mucosal to serosal sides of everted sacs of rat small intestine was examined for cisplatin and carboplatin. Figure 1 shows that the uptake of these compounds increased linearly with time up to at least 60 min. As can be seen from Fig. 1, cisplatin with an uptake rate of 2.75 μg platinum/min/g dry weight was absorbed more rapidly than carboplatin; which had an uptake rate of 1.66 μg platinum/min/g dry weight. Incubation of sacs in cisplatin for 60 min resulted in a serosal platinum concentration that was

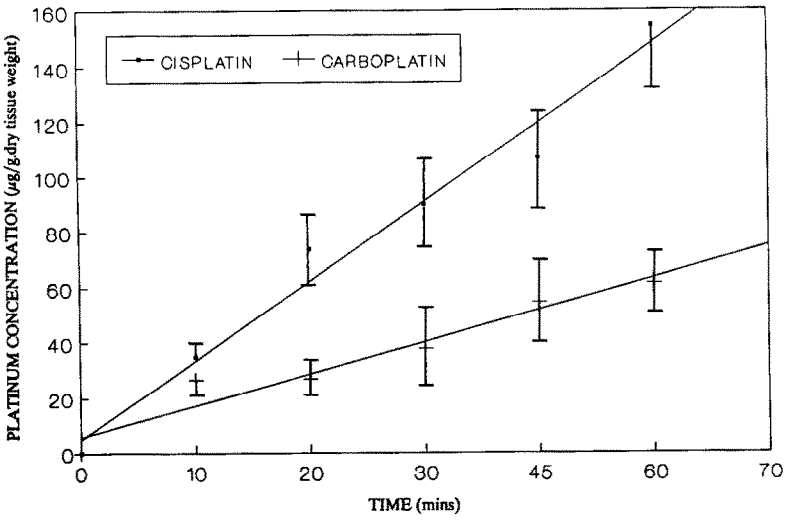

Fig. 1. Time course of the uptake of cisplatin and carboplatin by everted sacs of rat small intestine. The concentration of compound used was 0.25 mM. Each point is the mean ± SE of four determinations.

12.9 ± 0.73% (N = 16) of that found in the incubation medium. In comparison, serosal concentrations of platinum reached only 8.26 ± 0.66% (N = 16) of that in an incubation medium after an equivalent time in carboplatin. The better absorption of cisplatin was also illustrated by the higher platinum tissue levels that were attained after incubation of everted sacs in medium containing this compound. Whereas incubation in cisplatin (0.25 mM) for 60 min resulted in intestinal tissue platinum levels of 184 ± 16 μg/g dry weight, incubation in an equivalent dose of carboplatin resulted in tissue platinum levels of only 35 ± 15 μg/g dry weight.

Concentration dependency of initial uptake. Uptake rates of cisplatin and carboplatin were measured over a concentration range up to 1.0 mM. Figure 2 shows the relationship between the uptake rate and concentration of substrate in the incubation medium. A linear dependence of the uptake rate on mucosal drug concentration was observed for both compounds up to at least 1 mM. When a double reciprocal (Lineweaver–Burk) plot was constructed using this data, the lines drawn for both compounds converged at the origin, indicating that uptake is not governed by Michaelis–Menten kinetics.

Effects of metabolic inhibitors and anoxic conditions on drug uptake. The effect of metabolic inhibitors and anoxic conditions on the uptake of cisplatin and carboplatin were examined with an initial concentration of 0.25 mM compound in the incubation medium. A

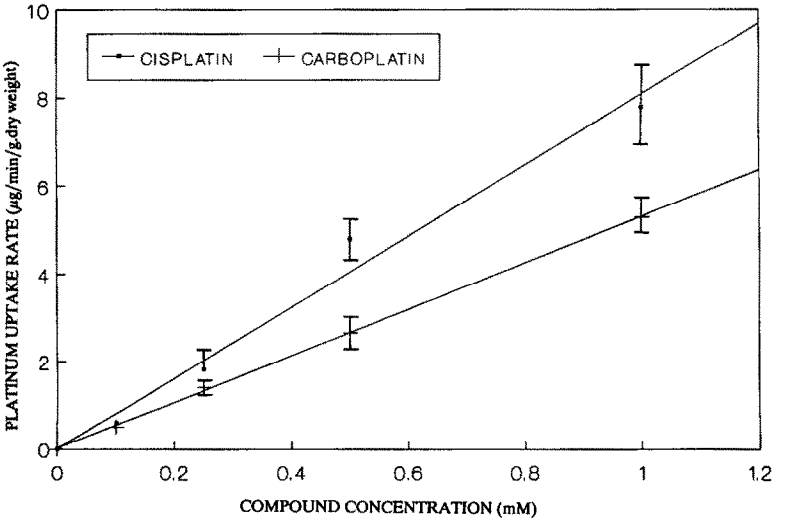

Fig. 2. Influence of cisplatin and carboplatin concentration in the incubation medium on the uptake by isolated everted sacs of rat small intestine. Sacs were incubated for a period of 60 min. Each point is the mean ± SE of four experiments.

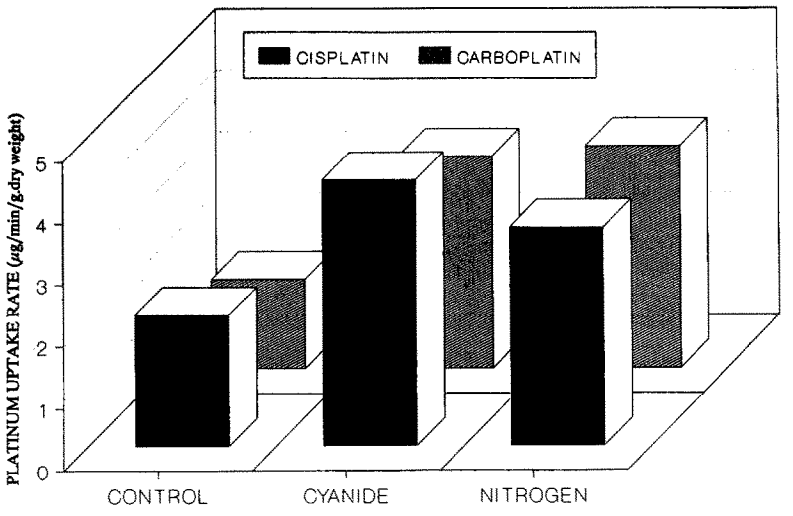

Fig. 3. The effect of metabolic inhibition and anoxia on the uptake of cisplatin and carboplatin by sacs of everted rat small intestine. Under control conditions sacs were incubated in Krebs–Henseleit medium at 37° gassed with 95% oxygen/5% carbon dioxide for a period of 60 min. Metabolic inhibition was achieved by addition of 5 mM potassium cyanide to the incubation medium, whereas anoxia was maintained by incubation under oxygen-free nitrogen. The concentrations of cisplatin and carboplatin were 0.25 mM. Results are presented as means of at least three experiments.

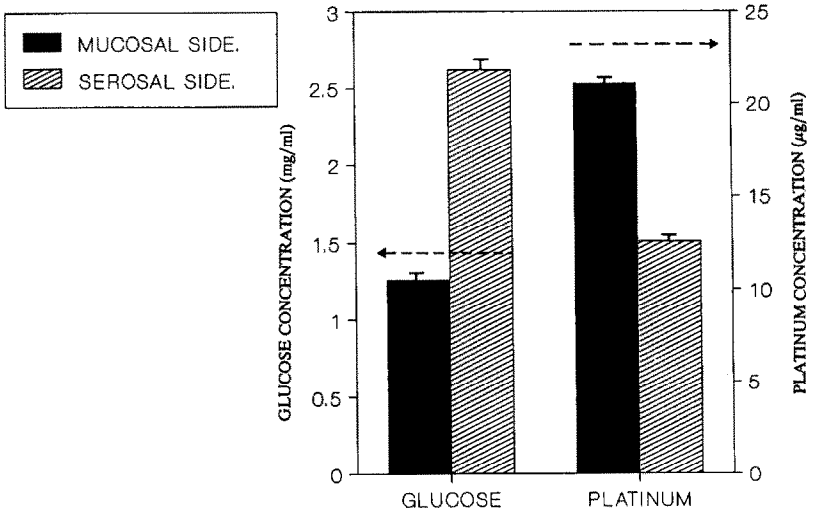

Fig. 4. Concentration gradients developed by everted intestinal sacs after a 60-min incubation in Krebs–Henseleit medium containing cisplatin and glucose. ←--- Initial concentration. Results are means ± SE.

60-min incubation of intestinal sacs in medium containing 5 mM potassium cyanide or under anoxic conditions prevented the absorption of glucose against a concentration gradient (indicating that active transport processes had been eliminted). However, these conditions failed to decrease the uptake of either cisplatin or carboplatin (Fig. 3). Conversely, both sets of conditions were seen to significantly increase the uptake of the platinum-based compounds into the serosal fluid and intestinal tissue.

Transport of cisplatin against a concentration gradient. Sacs of everted rat small intestine were incubated in Krebs–Henseleit medium gassed with 95% oxygen, 5% carbon dioxide for 60 min. The initial concentration of test compound and glucose was the same on the two sides of the intestinal tissue. After the 60-min incubation period, glucose concentrations on the serosal side of the intestinal sac were always found to be higher than those on the mucosal side (i.e. the S/M ratio was always greater than unity). This indicated

Table 1. Half-lives ($T_{1/2}$) and first-order rate constants (K_a) for the disappearance of cisplatin and carboplatin from the rat small intestine after *in situ* perfusion

Compound*	pH†	$T_{1/2}$‡ (min)	$K_a \times 10^{-3}$‡ (/min)
Cisplatin	7.0	75 ± 15.7	9.20 ± 1.1
Carboplatin	7.0	139 ± 14.1	4.98 ± 0.52
Carboplatin	7.5	164 ± 5.1	4.21 ± 0.80
Carboplatin	8.0	180 ± 4.6	3.83 ± 0.21

* The concentration of cisplatin and carboplatin in the perfusate was 2.8 mM.

† The buffer used was Tris hydroxymethyl-aminomethane 20 mM and 130 mM NaCl adjusted to the required pH with aristar HCl or NaOH.

‡ First-order rate constants and half-lives were the mean (±SE) of four experiments.

that glucose was being transported against a concentration gradient. Conversely, cisplatin was never transported against the concentration gradient (Fig. 4).

In situ absorption studies

Drug disappearance from the intestinal lumen. Cisplatin and carboplatin disappearance from the intestinal lumen was found to follow first-order kinetics for the entire experimental period (90 min). At pH 7.0, the absorption rate (K_a) of cisplatin (9.2×10^{-3}/min) was found to be significantly greater ($P < 0.05$) than carboplatin (4.98×10^{-3}/min). Absorption half-lives ($T_{1/2}$) were estimated to be 75 min and 139 min for cisplatin and carboplatin respectively. The absorption of carboplatin from the intestinal lumen was pH dependent; with the half life of absorption at pH 7.0 being significantly lower ($P < 0.05$) than that at pH 8.0 (Table 1).

Drug appearance in the peripheral blood. The time-dependent increase in platinum in the systemic blood after intestinal perfusion with 2.8 mM cisplatin or carboplatin is shown in Fig. 5. As was illustrated with the disappearance from the intestinal lumen, perfusion with cisplatin resulted in a greater increase in systemic blood platinum levels. The pH of the perfusate profoundly affected the time-dependent increase in platinum in the systemic blood. Figure 6 clearly demonstrates the effect of pH on systemic blood platinum levels after a 70-min perfusion with carboplatin. Preliminary data, obtained from two animals, indicates that platinum levels in blood collected directly from the hepatic portal vein are significantly higher than that originating from the systemic circulation (Fig. 7).

DISCUSSION

The mechanism by which platinum-based anti-tumour drugs enter cells has been the subject of some debate. Although some evidence exists for carrier-mediated uptake of cisplatin by an amino acid-mediated transport system [14] most evidence suggests that platinum-based drugs passively diffuse through the plasma membrane of the cell [15–17]. Clearly, to aid the development of platinum anti-tumour drugs capable of oral delivery, the mechanism by which such drugs cross the cells of the gastro-intestinal tract should be elucidated.

The uptake of cisplatin and carboplatin by intestinal sacs was concentration dependent and increased with increasing concentrations of the two compounds. A saturation of platinum uptake by everted sacs was not observed after incubation for 60 min. Although both *in vitro* and *in situ* experiments demonstrated that cisplatin is more readily absorbed by the rat small intestine than carboplatin, both compounds were shown to follow similar first-order kinetics. When the results of *in vitro* absorption studies were drawn as a double reciprocal plot of uptake of platinum by the intestine against external drug concentration, the lines drawn for both compounds converged at the origin. This suggests that the Michaelis constant for the formation of a hypothetical carrier–substrate intermediate complex is approximately infinite. Hence the rate of formation of a complex is essentially zero, or the rate of dissociation is infinitely slow. The results indicate no rate-limiting factor other than drug concentration and strongly mitigate against carrier transport and for passive diffusion. Further evidence supporting this conclusion emerged from the effects of potassium cyanide, a chemical capable of metabolic inhibition, and anoxia on the rate of uptake. Although both conditions prevented the absorption of glucose against a concentration gradient indicating that active transport processes had been eliminated, neither condition slowed down the absorption of the two platinum-based pharmaceuticals. Conversely these conditions stimulated the uptake rate possibly by compromising the integrity of cellular membranes. Such a response has also been reported by Casey and colleagues [17] in a study of cisplatin uptake into a renal proximal tubular cell line (LLCPK cells). Further evidence that the platinum-based pharmaceuticals cisplatin and carboplatin are transported across the small intestine by a mechanism of passive diffusion was obtained with the finding that cisplatin could not generate serosal to mucosal concentrations (S/M ratios) greater than unity.

Apart from providing data that may facilitate the characterization of the mechanism of intestinal uptake of platinum-based pharmaceuticals, it is hoped that this methodology can be applied to the questions concerning intestinal metabolism of this group of compounds as was done with the orally administered gold-based drug auranofin [18]. Information concerning metabolism specific to the oral route is indispensable as intestinal-induced modifications in structure could potentially alter the toxicity and anti-tumour activity associated with the compounds.

In offering more physiological conditions, the *in situ* studies were able to shed some light on the relationship between absorption of the platinum-based drugs from the gastro-intestinal tract and their appearance in the blood stream. One important finding was that both the disappearance of carboplatin from the intestinal lumen and its appearance in the

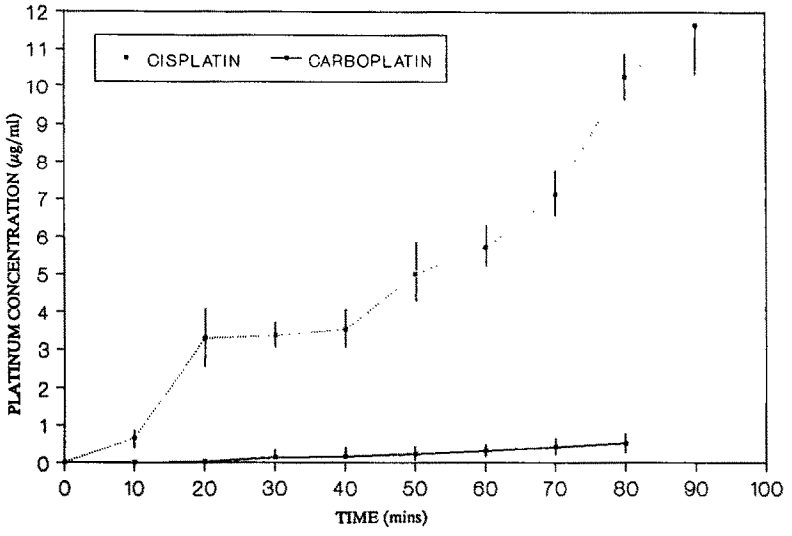

Fig. 5. Absorption of platinum into systemic blood after 60-min perfusion with cisplatin (2.8 mM) or carboplatin (2.8 mM). Compounds were dissolved in TRIS–HCl-saline at pH 7.0. Each point is the mean ± SE of at least four experiments.

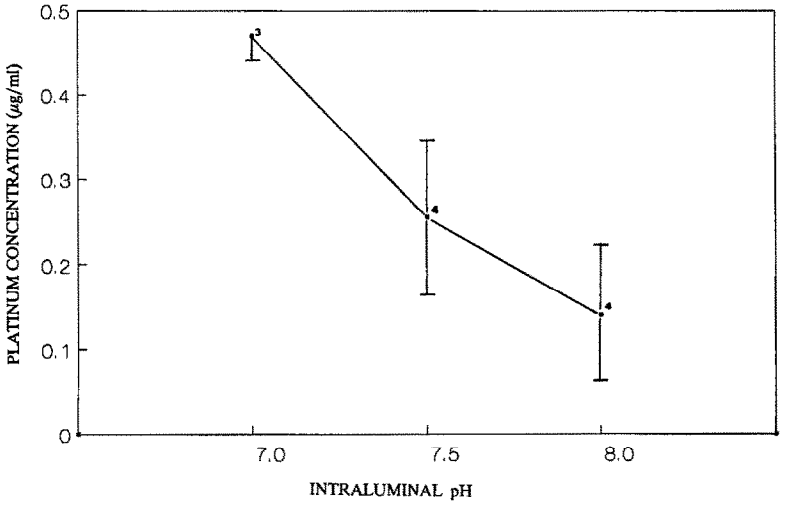

Fig. 6. Relationship between appearance of platinum in systemic blood and intraluminal pH after a 70-min perfusion of carboplatin in medium of either pH 7.0, 7.5 or 8.0. Each symbol represents the mean platinum concentration found in the systemic blood of a certain number of rats indicated next to the symbol. The standard errors of the mean values (SE) are indicated by vertical bars.

peripheral circulation was dependent on pH; absorption being potentiated at a lower pH. This pH dependency of carboplatin absorption is probably not surprising since the platinum species available from absorption are expected to differ in chemical form and quantity from one pH to another. For example, studies by Roos and Stokes [19] have illustrated that although carboplatin does not react with chloride ions at neutral pH, it readily does so under acidic conditions forming cisplatin. Since cisplatin has been shown to be absorbed across the small intestine more readily than carboplatin, this chemical reaction in the high chloride concentrations present in the perfusion medium could explain the higher absorption rate at lower pH. This could have important consequences on the potential clinical efficacy of orally administered carboplatin since if when given by mouth it is converted into cisplatin in the acidic environment of the stomach, then its non-nephrotoxic properties would be lost. Fortunately, this does not appear to be the case since *in vivo* studies carried out in the rat have demonstrated that although cisplatin retains

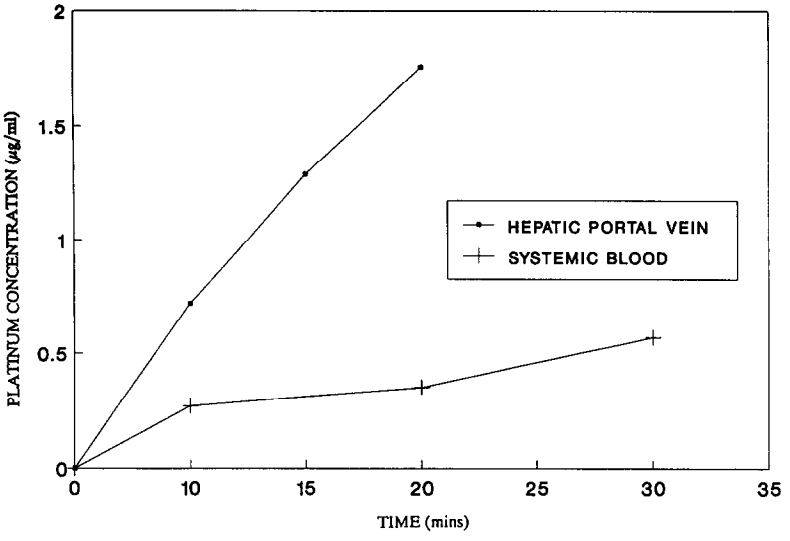

Fig. 7. Platinum levels in whole blood collected from portal and systemic veins after intestinal perfusion with carboplatin (2.8 mM). Carboplatin was dissolved in Sorensen buffer, pH 6, prior to *in situ* perfusion. Each point is the mean of two experiments.

its nephrotoxic side effect when administered orally, carboplatin when given by mouth is essentially not nephrotoxic [20].

The preliminary findings that platinum concentrations in systemic blood were lower than in portal blood is another significant finding from the *in situ* studies. This may suggest a possible (liver) tissue trapping of platinum species before entering the peripheral circulation and may have a profound effect on the toxicity associated with the oral administration of platinum-based pharmaceuticals. In support of this hypothesis, our earlier *in vivo* studies [5] have indicated that oral administration may potentiate any hepatotoxicity associated with treatment with platinum-based pharmaceuticals. Sampling of both portal and systemic blood and measurement of platinum in liver tissue will be of value to elucidate the process of liver retention in further studies.

To conclude, this study confirms our previous *in vivo* observation that the platinum-based pharmaceuticals cisplatin and carboplatin are absorbed from the rat intestine. Transport appears to be by a simple diffusion mechanism as no evidence for active transport was found. Further studies to characterize the intestinal transport system and the toxicity resulting from oral administration of platinum-based anti-tumour agents are currently being undertaken in our laboratory.

REFERENCES

1. Rosenberg B, Fundamental studies with cisplatin. *Cancer* **55**: 2303–2316, 1983.
2. Barnard C, Cleare and Hydes P, Second generation anticancer platinum compounds. *Chem Brit* **22**: 1001–1004, 1986.
3. Calvert AH, Harland SJ, Newell DR, Siddik ZH, Jones AC, McElwain TJ, Raju S, Wiltshaw E, Smith IE, Baker JM, Peckham MJ and Harrap KR, Early clinical studies with cis-diammine-1,1-cyclobutane dicarboxylate platinum II. *Cancer Chemother Pharmacol* **9**: 140–147, 1982.
4. Siddik ZH, Boxall F, Goddard P, Barnard C and Harrap K, Antitumour, pharmacokinetic and toxicity studies with orally administered cisplatin, CBDCA and CHIP. In: *Abstracts of Papers, Seventy-fifth Annual Meeting of American Association for Cancer Research, Toronto, March* 1984, p. 369.
5. Binks S and Dobrota M, A comparative study of cisplatin toxicity and route of administration. *Biochem Soc Trans* **14**: 694–695, 1986.
6. Hasegawa Y and Morita M, Antitumour effect of oral cisplatin on certain murine tumours. *Chem Pharm Bull* **33**: 5511–5514, 1985.
7. Wilson TH and Wiseman G, Method for studying intestinal metabolism and absorption. *J Physiol* **121**: 45P, 1953.
8. Somogyi M, A new reagent for the determination of sugars. *J Biol Chem* **160**: 61–68, 1945.
9. Van der Voet G and de Wolff F, A method of studying the intestinal absorption of aluminium in the rat. *Arch Toxicol* **55**: 168–172, 1984.
10. Van de Voet G and de Wolff F, The effect of Di-and Trivalent iron on the intestinal absorption of aluminium in rats. *Toxicol Appl Pharmacol* **90**: 190–197, 1987.
11. Doluisio J, Billups N, Dittert L, Sugita E and Swintosky J, Drug absorption 1: An *in situ* rat gut technique yielding realistic absorption rates. *J Pharm Sci* **58**: 1196–1202, 1969.
12. Delves HT and Shutter IL, Interferences in the measurement of platinum in body tissues and fluids using electrothermal atomization and atomic absorption spectrophotometry. In: *Biochemical Mechanisms of Platinum Antitumour Drugs* (Eds. McBrien DCH and Slater TF), pp. 329–346. IRL Press, Oxford, 1986.
13. Farago ME and Parsons PJ, Determination of platinum, palladium and rhodium by atomic absorption spectroscopy with electrothermal atomisation. *Analyst* **107**: 1218–1220, 1982.
14. Byfield J and Calabro-Jones P, Further evidence for

carrier-mediated cell uptake of cis-dichlorodiammine platinum (CDDP). In: *Abstracts of Papers, Seventy-third Annual Meeting of American Association for Cancer Research, March* 1982, p. 167.
15. Gale G, Morris C, Atkins L and Smith A, Binding of an antitumour platinum compound to cells as influenced by physical factors and pharmacologically active agents. *Cancer Res* **33**: 813–818, 1973.
16. Andrews P, Mann S, Velvry S and Howell S, Cisplatin uptake mediated cisplatin-resistance in human ovarian carcinoma cells. In: *Abstracts of Papers, Fifth International Symposium on Platinum and other Metal Coordination Compounds in Cancer Chemotherapy, Albano Terme, Italy, July* 1987, p. 195.
17. Casey B, McGuiness S, Pratt I, Ryan MP, McAuliffe C, Sharma HL and Tinker N, Uptake of cisplatin (^{195}Pt) into LLCPK cells in the presence of DDTC, MESNA and Amiloride. In: *In Vitro to In Vivo and from Animals to Man. Proceedings of the Third International Symposium on Nephrotoxicity. Guildford, U.K. 3–7 August* 1987 (Eds. Bach PH and Lock EA), pp. 353–356. Plenum Press, 1989.
18. Tepperman K, Finer R, Donervan S, Elder R, Doi J, Ratliff D and Ng K, Intestinal uptake and metabolism of auranofin, a new oral gold-based antiarthritis drug. *Science* **225**: 430–431, 1984.
19. Roos I and Stokes K, Kinetic studies of the stability of platinum drugs. *Med Ped Oncol* **15**: 125, 1987.
20. Binks S, Absorption, toxicity and deposition of transition metal based pharmaceuticals following oral administration. Ph.D. Thesis, University of Surrey, U.K., 1988.