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Comparison of intestinal toxic effects of platinum complexes: cisplatin (CDDP), carboplatin (CBDCA), and iproplatin (CHIP)

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Summary. The biochemical background of the intestinal side effects of *cis*-diammine-1,1-cyclobutane dicarboxylate platinum (II) (CBDCA) and *cis*-diisopropylammine-*trans*-dihydroxy-dichloro platinum (IV) (CHIP) was compared with those of *cis*-diamminedichloroplatinum (II) (CDDP). Biochemical investigations were carried out on mucosal cells isolated by a combined chemical-mechanical method from the total length of the small intestine. After treatment with single, equitoxic doses of Pt analogues, the activities of thymidine kinase (TK) EC 2.7.1.21, sucrase (SUC) EC 3.2.1.26, maltase (MAL) EC 3.2.1.20, and protein content showed dose-dependent decreases, whereas the activity of alkaline phosphatase (AP) EC 3.2.1.20 increased slightly. The nadir of enzyme activity changes occurred 24–48 h after treatment. For the regeneration of the mucosa more than 96 h was necessary. Of the platinum analogues studied, CHIP proved to be the most toxic to the small intestine. While the highest doses of CDDP and CBDCA ($0.66 \times LD_{50}$) caused significant but less than 50% decreases in TK, SUC, MAL, and protein content (PROT), the CHIP doses needed for 50% reduction were between $0.44–0.66 \times LD_{50}$.

Introduction

Cisplatin has proved to be a valuable drug in the treatment of different human tumors. The major side effects of the drug have been renal, bone marrow, nervous system, and intestinal damage. The surviving fraction of hemopoietic bone-marrow stem cells (CFU_c) in mice has been reported to decrease from 1 to 0.03 after treatment with the LD_{50} dose of CDDP [18]. A 36%–53% decrease in lymphocyte and granulocyte counts has been observed in mouse bone marrow after treatment with CDDP (5 mg/kg i.p., days 1–4) [4]. The maximal increase – 800% – in blood urea nitrogen (BUN) was observed 4 days after a $0.87 \times LD_{50}$ dose of CDDP [4]. The use of various methods, such as hydration [22], induction of diuresis by mannitol, furosemide, or acetazolamide [12, 19, 20], as well as the inactivation of biologically active platinum by thiol compounds [3, 4, 10, 13, 14], has mitigated both kidney and bone marrow toxicity and inhibited CDDP-induced nausea and vomiting in dogs. In addition to central mechanisms, small intestinal mucosal toxicity may also play an important role in the induction of emesis by CDDP [5]. New analogues have recently been developed to find a derivative with a higher therapeutic index [7, 8]. In this study, the biochemical background of the intestinal side effects of CBDCA and CHIP was compared with that of CDDP in rats. Thymidine kinase (TK), an enzyme of the pyrimidine salvage pathway, was selected as the marker for the proliferative activity of the crypt cells, whereas alkaline phosphatase (AP), sucrase (SUC) and maltase (MAL) activities were measured to characterize the digestive function of the mucosa.

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Materials and methods

The platinum compounds tested (Table 1) were kindly provided by Lachema o.p. (Brno, Czechoslovakia). All drugs were dissolved in physiological saline and were administered i.v. as a single dose to Wistar H-Riop outbred male rats weighing 180–200 g. The applied doses were equitoxic, corresponding to 0.2, 0.30, 0.44, and $0.66 \times LD_{50}$ value (Table 1). No higher doses were used in our experiments because we intended to characterize small intestinal side effects in the therapeutic dose range. The animals were provided with water ad libitum, but were deprived of food 24 h before the start of the biochemical assay. In each group 4–6 rats were treated. The animals were killed 6, 24, 48, 72, and 96 h after drug administration, after which the small intestine was removed and washed with 154 m*M* NaCl containing 1 m*M* mercaptoethanol. Intestinal epithelial cells were isolated according to the method of Weiser [23] modified by Kralovánszky et al. [16].

After centrifugation for 15 min at 1500 rpm at +5° C, the cells were collected and homogenized in isotonic KCl. For the determination of AP, SUC, and MAL activities, the whole homogenate was used, and for that of TK, the 100,000-*g* supernatant. Protein content was determined both from the whole homogenate and from the cytosol.

AP activity was determined with *p*-nitrophenylphosphate as the substrate, and the released *p*-nitrophenol was measured spectrophotometrically at 405 nm [2]. SUC and MAL activities were estimated by measuring the rate of hydrolysis of sucrose and maltose, using the glucose oxidase-peroxidase method to measure the amount of glucose

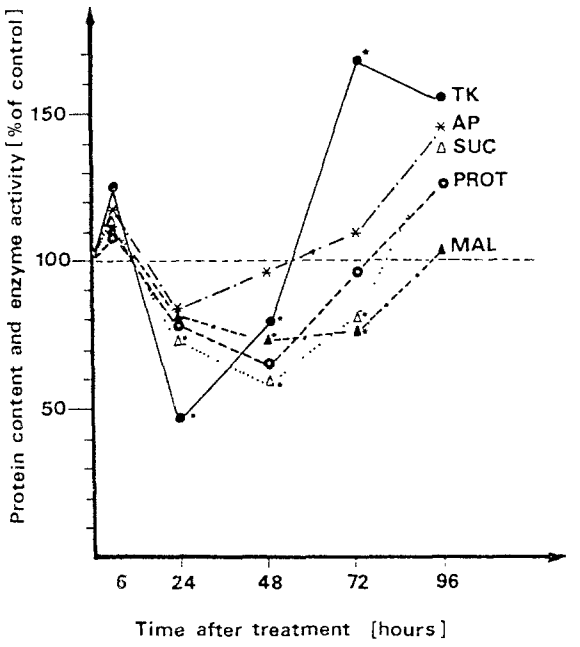

Fig. 1. Time dependence of biochemical changes in rat intestinal cells after CDDP treatment. Animals were treated i.v. with a single dose of 6 mg/kg ($0.66 \times LD_{50}$) cisplatin. Each *point* is the mean of eight determinations from four rats. *x*, significant difference from untreated control ($P < 0.05$)

formed [9]. TK activity was measured by determining the conversion of (2-^{14}C)/Tdr to (2-^{14}C)/dTMP by the DEAE cellulose disk method [15]. Protein was assayed by the method of Hartree [11]. AP, SUC, and MAL activities were calculated as μmol/h per cm of intestine; TK activity is given as nmol/h per cm. Statistical analysis was performed by using *t*-statistics for two means.

Results

Time course of biochemical changes after treatment with CDDP

Six hours after treatment, the level of all enzymes and that of the protein increased slightly. Thereafter, the reduction of all studied parameters began. The decreases in TK and SUC became statistically significant at 24 h as compared to the normal values. The nadir of enzyme activity changes occurred at 24 h after the treatment for TK, and at 48 h for disaccharidases (SUC, MAL) and protein. AP activity was only mildly inhibited. The sudden, significant rise in TK level on day 3 is an indicator of the increased proliferative activity of the crypt cells after CDDP-induced damage. Recovery of the functional enzymes began after day 2; however, the complete regeneration of the mucosa needed more than 4 days (Fig. 1).

Dose dependence of biochemical changes after treatment with various platinum analogues

The changes in enzyme activites and protein content were compared 48 h after treatment with equitoxic doses of the three platinum analogues (Fig. 2 and Tables 1 and 2).

CDDP moderately influenced enzyme activities. Significant decreases could be observed only in TK, SUC, and MAL levels in groups of animals treated with higher doses of the drug. The most marked reduction of enzyme activities was observed in the CHIP-treated groups. TK exhibited the highest sensitivity; its activity decreased proportionally to increasing CHIP concentrations. The inhibition of other enzymes became marked only at higher drug doses. CBDCA treatment caused the least effect on the small intestinal mucosa. Only TK activity was reduced slightly, but no significant difference from control values

Table 1. Characteristics of the studied platinum complexes

Structure	Drug	LD_{50} value (Rat i.v.)	Applied doses (mg/kg i.v.)
	cis-Diamminedichloro platinum (II) NSC-119875 CDDP	9.0 mg/kg	1 × 6.0 1 × 4.0 1 × 2.7 1 × 1.8
	cis-Diammine-1,1-cyclobutane dicarboxylate platinum (II) NSC-241240 CBDCA	80.0 mg/kg	1 × 54.0 1 × 36.0 1 × 24.0 1 × 16.0
	cis-Diisopropylammine-*trans*-dihydroxy-dichloro platinum (IV) NSC-256927 CHIP	50.0 mg/kg	1 × 33.0 1 × 22.0 1 × 15.0 1 × 10.0

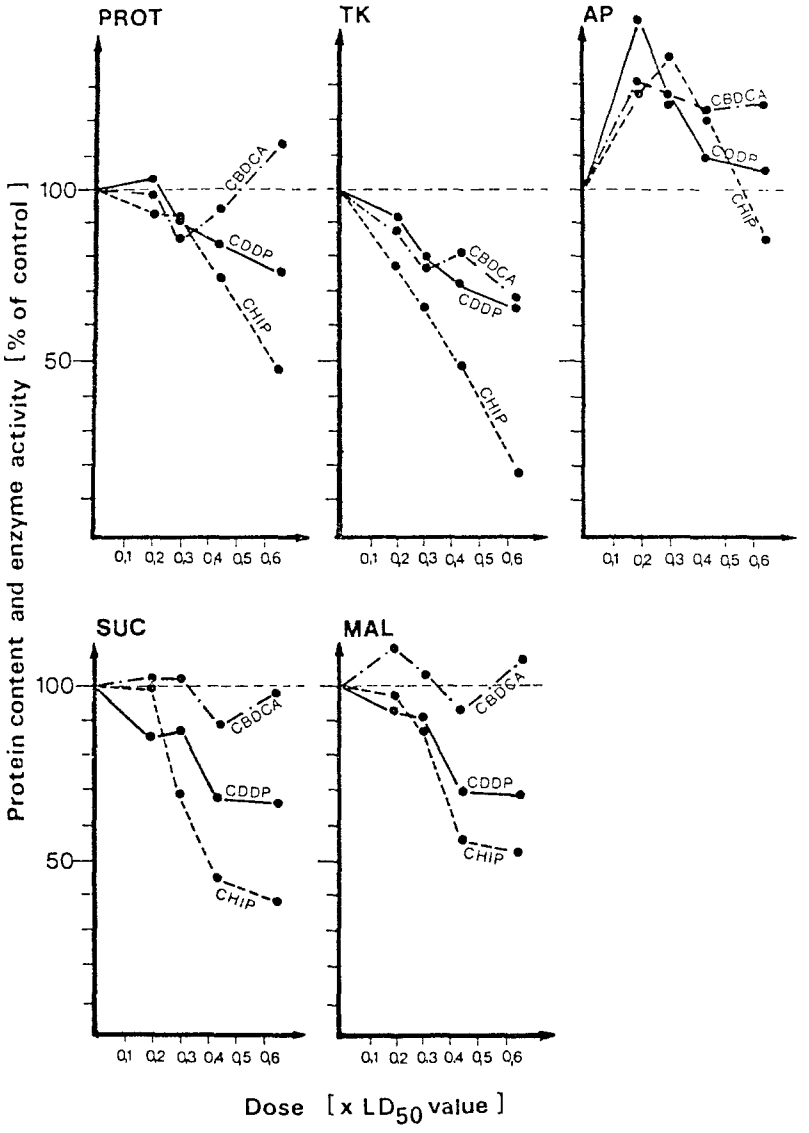

Fig. 2. Dose-response curves of intestinal enzymes and protein after treatment with various platinum analogues. Animals were treated i.v. with single, equitoxic doses of different drugs and killed 48 h after treatment. Applied doses are given in Table 1. Enzyme activities are given as percentages of values in untreated controls

could be demonstrated even at higher drug concentrations. The values of the functional enzymes fluctuated around the level measured in the untreated animals.

The sensitivity of the studied parameters of drug treatment can be evaluated by comparing those drug concentrations that cause 50% enzyme inhibition (I_{50}). This value could be determined only in CHIP-treated animals; the inhibition caused by other analogues remained lower than 50%. The I_{50} values expressed in mg/kg for CHIP were: TK, 21.5; SUC, 20.5; MAL, 32.5; protein, 32.0.

The results were surprising in the case of AP. Instead of a decrease, an increase in enzyme activity was demonstrated, which was the highest at relatively low drug concentrations. To clarify the mechanism of this phenomenon, the possible enzyme activator role of the platinum ion was studied in in vitro experiments. However, no increase in AP activity could be demonstrated by incubating the intestinal cells with the drugs in vitro. Both the prolongation of the preincubation time and use of a noncytostatic platinum compound – K_2PtCl_4 – caused enzyme inhibition instead of activation (Table 3).

Discussion

For the normal function of the intestinal mucosa, a physiological equilibrium between cell proliferation, maturation, fully differentiated function, and cell loss is needed. If this equilibrium is disturbed, e.g., during cytotoxic chemotherapy, mucosal damage will take place. In our earlier studies we have established that the measurement of enzyme activities, which are characteristic for cell proliferation and function, is a sensitive indicator of small intestinal toxicity caused by cytostatic agents [17, 21]. The time course of enzyme activity changes showed that CDDP exerted a reversibly damaging effect on the small intestinal mucosa. The nadir of TK activity, which preceded the nadirs of the other parameters by 24 h, suggested that CDDP acted primarily on the dividing crypt cells and that the decrease of functional enzyme activities, which was concomitant with temporary arrest of mitosis in the crypt cells, was secondary. Due to the inhibition of disaccharide digestion and monosaccharide absorption, osmotically active substances can remain in the intestinal lumen, possi-

Table 2. Statistical evaluation of enzyme activity changes 48 h after treatment with various platinum analogues

Drugs	Dose × LD_{50}	PROT	TK	AP	SUC	MAL
CDDP	0.66	n.s.	$P<0.05$	n.s.	$P<0.05$	$P<0.05$
	0.44	n.s.	n.s.	n.s.	$P<0.05$	$P<0.05$
	0.30	n.s.	n.s.	$P<0.05$[a]	n.s.	n.s.
	0.20	n.s.	n.s.	$P<0.001$[a]	n.s.	n.s.
CHIP	0.66	$P<0.01$	$P<0.001$	n s.	$P<0.001$	$P<0.001$
	0.44	n.s.	$P<0.01$	n.s.	$P<0.001$	$P<0.01$
	0.30	n.s.	$P<0.05$	n.s.	$P<0.05$	n.s.
	0.20	n.s.	n.s.	n.s.	n.s.	n.s.
CBDCA	0.66	n.s.	n.s.	n.s.	n.s.	n.s.
	0.44	n.s.	n.s.	n.s.	n.s.	n.s.
	0.30	n.s.	n.s.	n.s.	n.s.	n.s.
	0.20	n.s.	n.s.	n.s.	n.s.	n.s.

n.s., no significant difference from untreated control
Statistically significant decreases in TK, SUC, and MAL activities was caused only by the highest concentrations used (0.66 LD_{50}) of CDDP and CHIP, respectively, and, in some cases, also by the lower CHIP doses. No significant increase in AP activity could be demonstrated in the case of 0.3 LD_{50} doses of CBDCA and CHIP; however, the AP activity values were higher than the corresponding enzyme activity for *cis*-platin. The following high-standard deviation values are responsible for this discrepancy: CDDP, 129.7 ± 7.6; CHIP, 138.7 ± 31.5; CBDCA, 129.6 ± 34.2
[a] Increase in enzyme activity

Table 3. Activity of the intestinal alkaline phosphatase preincubated with different platinum analogues in vitro

Compound	Concentration (m*M*)	Preincubation time (min)			
		0	30	60	120
CDDP	1.0	102[a] (98–107)	87 (80–94)	72 (65–77)	71 (69–74)
CHIP	1.0	100	88 (80–94)	89 (86–92)	88 (85–90)
	4.3	100	90 (86–94)	70 (67–75)	75 (70–83)
K_2PtCl_4	1.0	93 (89–96)	75 (67–85)	54 (49–58)	32 (28–36)
	4.3	88 (80–94)	64 (63–65)	42 (38–44)	32 (27–35)

Intestinal cell homogenate was preincubated with the platinum compounds for 0–120 min at 37°C. The homogenate was then added to the AP incubation mixture to determine AP activity. The final concentrations of the drugs in the incubation solution are given in the table. In the case of CDDP, no higher concentration could be produced because of the poor solubility of the drug
[a] Alkaline phosphatase activity is given in % of the control, which was preincubated for the same length of time without the drug. Mean of 3 determinations and the range are given

bly resulting in a decrease in water reabsorption, causing diarrhea. Analyzing the jejunal cell-population kinetics – ^{3}H-thymidine incorporation and mitotic index – after CDDP treatment, Burholt et al. have also found reduced proliferative activity during the first 24 h [6]. The rapid increase of TK activity on day 3 is a sign of compensatory cell proliferation, but for the complete regeneration of the mucosa, more than 4 days are needed.

Of the platinum analogues, CHIP proved to be the most toxic agent for the intestinal mucosa, whereas CBDCA was the least toxic. Similar results have been obtained by Allan and Smyth [1], who compared the effect of various platinum complexes on the mouse ileal architecture, villus epithelial cell influx, and disaccharidase activity. According to these authors, CHIP and CDDP were roughly equitoxic to ileal crypt cells, whereas CBDCA showed the least effect. The most serious inhibition of trehalase activity was found in the case of CHIP; the nadir was reached between days 3–7, with recovery by day 10. The differences in time courses of the biochemical changes between their and our results may be due to the different kinetic characteristics of small intestinal mucosal cells in rat and mouse.

On the basis of our model experiments, it can be concluded that the platinum complexes exert moderate toxic effects on the small intestinal mucosa. The finding that CHIP caused the most serious damage to the intestine is in full agreement with the highest frequency of diarrhea in patients treated with CHIP as compared to that observed with other Pt compounds.

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