Nephrotoxicity of acyclovir and cis-diamminedichloroplatinum(II) – effect of co-administration in rats

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Summary. The effect of co-administration of acyclovir and cis-diamminedichloroplatinum(II) (cisplatin) on nephrotoxicity in male Wistar rats was investigated. Animals received acyclovir (15 mg/kg body weight, s.c., three times per day for 5 days) or cisplatin (5 mg/kg body weight, i.p., one single injection) or a combination of both drugs. Acyclovir plasma levels were determined after one single acyclovir s.c. injection. Urines were monitored for volume, pH, osmolality and excretion of N-acetyl-β-D-glucosaminidase (NAG), lysozyme and total protein. Concentrations of blood urea nitrogen and plasma creatinine were determined on day 6. Renal cortical slices were monitored to assess the accumulation of weak organic bases (tetraethylammonium) and acids (p-aminohippurate). Cisplatin induced a marked increase in the excretion of NAG, lysozyme and total protein and an increase in urine volume, plasma creatinine and blood urea nitrogen. Urine osmolality and accumulation of p-aminohippurate were depressed by cisplatin. Acyclovir treatment alone caused no significant symptoms of nephrotoxicity. Co-administration did not impair renal function more than cisplatin treatment alone, excepting a slight rise in lysozyme excretion on day 6. Short-term antiviral therapy with acyclovir, concomitant to cisplatin treatment, may bring, if at all, a slightly increased nephrotoxic risk.

Key words: Acyclovir – cis-diamminedichloroplatinum(II) – Co-administration – Nephrotoxicity – Rat kidney

Introduction

Acyclovir is an antiviral drug with significant therapeutic benefit in several viral infections e.g. in primary genital herpes infections and shingles (Richards et al. 1983; O'Brien and Campoli-Richards 1989). Previous studies (Chapman and Brigden 1981; Brigden et al. 1982; Tucker et al. 1983; Peterslund et al. 1988; Sawyer et al. 1988; Kimes et al. 1989) have shown that acyclovir treatment might be associated with the impairment of renal function in humans and animals. The administration of acyclovir may be necessary for the treatment of viral infections in patients undergoing an antitumour therapy with cisplatin. Cis-diamminedichloroplatinum(II) (cisplatin) is widely used as a cytostatic drug (Daugaard 1990). Its clinical use can often be limited by several toxic side-effects. Nephrotoxicity is the most common dose-limiting factor (Ward and Fauvie 1976; Goldstein et al. 1981; Hacke et al. 1983; Safirstein et al. 1987; Hannemann and Baumann 1990, 1991). Cisplatin-induced toxicity to the bone marrow may also occur (Bodenner et al. 1986) leading to alterations in the immune response. In addition, the immune system in cancer patients is often suppressed. The concomitant use of acyclovir and cisplatin might potentiate the drug-induced impairment of renal function. Any assessment of an increased nephrotoxic risk through the simultaneous administration of both drugs in humans is problematic, because the first symptoms of nephrotoxicity usually lead to a reduction or cessation of the therapy (Sawyer et al. 1988; Kimes et al. 1989). Therefore the present study reports on the short-term effect of the co-administration of acyclovir and cisplatin on nephrotoxicity using an animal model.

Materials and methods

Drugs. Acyclovir (Zovirax) was kindly provided by Deutsche Wellcome GmbH (Burgwedel, FRG). Cisplatin, chicken egg-white lysozyme (muramidase, mucopeptide N-acetylmuramoylhydrolase, EC 3.2.1.17, grade I) and Micrococcus lysodeikticus were purchased from Sigma (St. Louis, Mo., USA).
Animals and experimental design. Male Wistar rats weighing 270–320 g (Winkelmann, Borchen, FRG) were held in ventilated rooms with controlled temperature (21 °C) and humidity as well as with regular light cycles. The animals were allowed free access to chow (standard diet, Altromin, Lage, FRG) and water, except during periods of urine collection. Animals were divided into seven groups. Group 1 was treated subcutaneously with acyclovir three times a day at equal 6-h doses (10 a.m., 4 p.m., 10 p.m.; 45 mg/kg body weight daily) for 5 days. Group 2 received one single injection of cisplatin (5 mg/kg body weight) intraperitoneally. Group 3 received the combination of cisplatin and acyclovir using the same regime of treatment as used for groups 1 and 2. Cisplatin was administered on day 1. Groups 4, 5 and 6 were treated with the equivalent dose of vehicles. Cisplatin was dissolved in a NaCl (9 g/l) solution and injected at a concentration of 1 mg/ml. Acyclovir was dissolved in NaCl (9 g/l) solution and administered s.c. (skin at the back) at a concentration of 1.5 mg/ml. The subcutaneous injection of acyclovir was tolerated well by the animals. Diluted as above, acyclovir (pH 11 before dilution) did not cause any local irritation or skin reaction. Group 7 was examined to determine acyclovir plasma levels. Animals received one single acyclovir injection (15 mg/kg body weight, s.c.), and blood samples were taken at different times after injection.

Urine collection and analysis. In order to collect urine, rats (groups 1–6) were housed individually in metabolic cages on days 1, 3 and 5 for a period of 16 h after the second acyclovir or control injection. Rats received water ad libitum, but were starved during the urine collection period in order to avoid any food contamination of their urine. The urine was collected in glass beakers surrounded by ice. Urine samples were monitored for volume, pH, osmolality (semimicro osmometer type M, Knauer, Berlin, FRG), N-acetyl-β-D-glucosaminidase (NAG), lysozyme and total protein. Urinary concentrations of NAG were determined using a Test-Combination (Boehringer Mannheim, Mannheim, FRG). Lysozyme concentration was determined according to the method described by Litwack (1955). The decrease in turbidity of a suspension of M. lysodeikticus with aliquots of urine samples was measured spectrophotometrically (645 nm, 25 °C). Quantification of lysozyme concentration was obtained by comparison with a standard curve obtained from adding chicken egg-white lysozyme at various concentrations to the suspensions of M. lysodeikticus (Cojocel et al. 1984). Total urinary protein was determined by using a biuret reagent after precipitation by trichloroacetic acid (Test-Combination Merckotest, Merck, Darmstadt, FRG).

Blood collection and plasma determinations. After weighing, animals (groups 1–6) were sacrificed on day 6 by cervical dislocation and blood was taken for quantification of blood urea nitrogen (BUN) and plasma creatinine concentrations. Determinations were done by using Test-Combinations (Boehringer Mannheim, Mannheim, FRG). Blood samples for determination of plasma levels of acyclovir were taken in the time period 0–12 h after a single acyclovir injection (group 7). Acyclovir levels were determined by high-performance liquid chromatography (HPLC) in the laboratories of Prof. G. Gries (München, FRG). An isocratic solvent (10 mM sodium acetate, pH 4.0) was used. The HPLC system consisted of a Waters pump, equipped with a Waters integrating data module, and a Knauer variable-wavelength module. A RP-18 column (25 cm) was used, the flow rate was 1.5 ml/min and the detection wavelength was 254 nm. A standard of 8.55 mg/ml was used.

Tetraethylammonium (TEA) and p-aminophippurate (PAH) accumulation experiments. Kidneys were removed, decapsulated and weighed (groups 1–6) directly after blood collection on day 6. Renal cortical slices were prepared using a razor blade and a kidney holder device (Hannemann and Baumann 1990). Slices were incubated for 90 min (25 °C, 100% O₂ atmosphere) in a modified Cross and Taggart phosphate buffer (Cross and Taggart 1950) containing either TEA (0.01 mM) and ¹³C]TEA (0.02 μCi) or PAH (0.074 mM). TEA or PAH in renal cortical slices and media were determined as

Statistics. Student's t-test was used to evaluate the significance of differences between experimental groups. Differences were considered significant at the level of P < 0.05.

Results

No significant differences were observed in the results between control groups 4–6. The results were therefore pooled.

Fig. 1. Effect of acyclovir and/or cisplatin treatment on rat body weight. Initial weights of rats on day 1 were set as 100%. Alterations were calculated as percentages. Each point represents the mean value ± SEM of at least eight rats per group. For clarity's sake, SEM is only indicated at significantly different values. SEM did not exceed 4%. *, P < 0.05 compared to the corresponding control value. ○, Control; ▲, acyclovir (15 mg/kg body weight, s.c., three times per day for 5 days); ●, cisplatin (5 mg/kg body weight, i.p., one single injection); ○, acyclovir and cisplatin (dose as described above, cisplatin treatment on day 1)

Fig. 2. Effect of acyclovir and/or cisplatin treatment on rat kidney weight and on the kidney weight/body weight ratio. Kidney weights on day 6 were expressed in grams after percentage adjustment related to initial body weights. Kidney weight/body weight ratios were calculated in relation to body weights on day 6. Results are expressed as the mean value ± SEM of at least eight rats per group. *, P < 0.05 compared to the control group. White columns, control; hatched columns, acyclovir (15 mg/kg body weight, s.c., three times per day for 5 days); grey columns, cisplatin (5 mg/kg body weight, i.p., one single injection); black columns, acyclovir and cisplatin (dose as described above, cisplatin treatment on day 1)