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SHORT COMMUNICATION

Experimental Evaluation of Protective Activity of *Echinacea pallida* against Cisplatin Toxicity

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The hydroalcohol standardized extract of *E. pallida* Nutt. given to mice p.o. in association with the i.p. administration of cisplatin exhibited protective effects expressed by a diminished loss and a faster recovery of the animal's body weight. Pretreatment with *E. pallida* also decreased cisplatin nephrotoxicity estimated from the level of kidney homogenate oxygen consumption. © 1997 by John Wiley & Sons, Ltd. Phytother. Res. **11**, 263–265, 1997

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INTRODUCTION

Previous studies have reported the immunostimulatory effects of extracts from many *Echinacea* species. Various polysaccharides (Wagner *et al.*, 1985; Bauer and Wagner, 1990) and more specifically an arabinogalactan (75 kDa) able to activate tumoricidal and antimicrobial capacities of macrophages (Lueting *et al.*, 1989), have been considered as the active principles of these extracts.

The selective stimulation of macrophages might be a profitable approach for diminishing the cytostatic-induced undesirable side effects such as severe toxicity and immunosuppression.

With a view to the prospective use of *Echinacea* extracts to reconstitute the immune response following treatment with cytostatics, we attempted to elucidate the effect of a standardized extract of *Echinacea pallida* on the toxicity of a widely used antineoplastic agent, cisplatin (cis-diamine-dichloroplatinum).

MATERIALS AND METHODS

Plant material. *Echinacea pallida* plants cultivated in the Experimental Garden of the University of Agricultural Sciences, Cluj-Napoca were authenticated both morphologically (Bauer and Wagner, 1990) and by karyotype analysis ($2n = 44$) (Muntean *et al.*, 1991). A voucher specimen is deposited in the Herbarium of Pharmaceutical Botany Department.

Extract preparation. Whole plants harvested in the third year were dried at room temperature and finely powdered. The alcohol extract (1:1) was obtained by repercolation with 60% ethanol. The resulting preparation standardized to 1% immunostimulatory polysaccharides (Ps) (Tamas *et al.*, 1991) contained 2.5% total solid and 52% ethanol. The extract was evaporated to dryness, the residue redissolved in distilled water at a concentration of 10% (0.1% Ps) and administered to animals.

Animals. Swiss mice of either sex weighing 22 ± 2 g, kept on a normocaloric standard diet and water *ad libitum* were used.

Test drug. Cisplatin was from 'Biofarm', Bucharest, each vial containing 100 mg lyophylized power with 5 mg cis-diaminedichloroplatinum, sodium chloride and mannite. Doses administered are reported as mg of pure product.

Acute toxicity assay of cisplatin. The acute toxicity was determined in groups of 10 mice (5 male + 5 female, each) injected i.p. at doses ranging from 2 to 20 mg/kg b.w. of cisplatin. Animals were kept under observation and weighed daily for 15 days. The LD_{50} was calculated using probit analysis.

Protective effect of E.p. extract vs. cisplatin

Effect on body weight. Four groups of 10 mice each were treated as follows: Group 1, with saline solution, p.o., 0.2 mL/mouse on days 0–10 (control); group 2, with *E.p.* extract, p.o., 0.2 mL/mouse (0.2 mg Ps) on days 0–10; group 3, with cisplatin i.p., 2 mg/kg b.w. on days 0–4; group 4, with 0.2 mL *E.p.* extract p.o., 15 min before each cisplatin treatment, on days 0–4 and *E.p.* administration was further continued on days 5–10. The animals were followed-up for 2 weeks, weighed daily and body weight variations expressed, in each group, as percentage of the initial (day 0) values.

Oxygen consumption in kidney homogenates. Kidneys were taken from four groups (5 female mice each), treated with a single dose model: group 1, with saline solution, p.o.,

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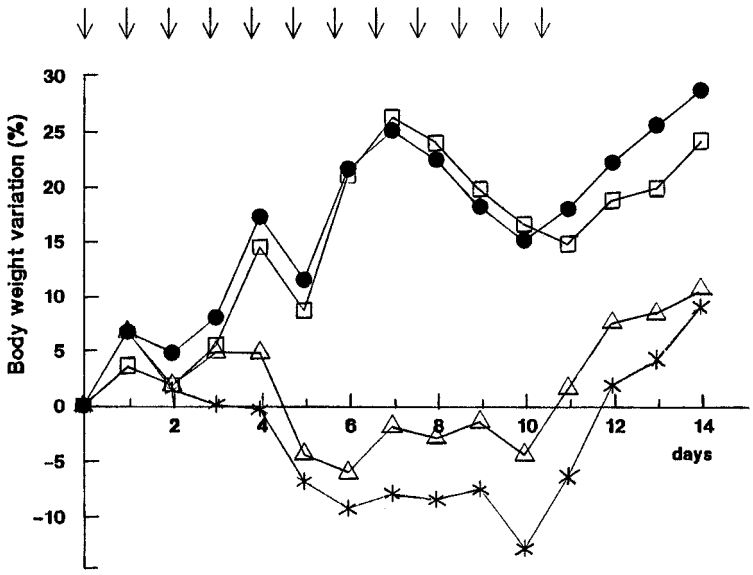

Figure 1. Body weight variation in mice during treatment with fractionated doses (days of treatment with *E.p.* extract are indicated by vertical arrows); ● control; □ *E.p.* extract; ∗ cisplatin; △ *E.p.* extract + cisplatin.

0.1 mL/mouse (control); group 2, with *E.p.* extract, p.o. 0.1 mL/mouse (0.1 mg Ps); group 3, with cisplatin, i.p., 3 mg/kg b.w. in 0.5 mL saline; group 4, with *E.p.* extract, p.o., 0.1 mL/mouse (0.1 mg Ps) followed at 15 min of cisplatin, i.p., 3 mg/kg b.w. in 0.5 mL saline. The mice were killed by cervical dislocation, on day 4. Organs were washed with isotonic saline solution, then pressed between filter paper and weighed. Five mL aliquots of Krebs-Ringer phosphate buffer, pH 7.5, were added to 1 g of wet tissue, and the mixture was homogenized with a Potter teflon pestle homogenizer (A. H. Thomas Co. Philadelphia). The contents were transferred into glass dishes on ice. The oxygen consumption was measured polarographically with a Clark oxygen electrode (Yellow-Springs Instruments Co.) fitted with a 1.35 mL sealed reaction vessel. Working conditions were: oxygen electrode potential -0.8 V, temperature 24°C, homogenate volume 0.2 mL. The results, expressed in 10 nAtoms O_2/min g wet tissue, were mean values ± SE in five replicate determinations.

Statistical analysis. One-way analysis of variance (Anova) was performed with a commercially available computer package, Statistics version 3.5, using the individual body weights of the animals in the four groups observed. The significance versus control was calculated by the multiple comparison Dunnett test, also applied to the oxygen consumption experiments.

RESULTS

Preliminary assays proved that i.p. administration of cisplatin to mice caused body weight loss and mortality depending on dose (data not shown). Under our experimental conditions, the LD_{50} of the drug was 9.3 mg/kg b.w.

When the drug was given alone, in five i.p. fractionated doses (2 mg/kg b.w. each) a marked body weight loss was observed at the end of treatment (days 5 and 6). The decrease was maintained at a quasistationary level in the following 5 days, whereupon recovery began on day 11 (Fig. 1). When the cytostatic agent was associated with *E. pallida*. extract, p.o., body weight variation followed a similar pattern but a diminished loss was evident ($p < 0.01$ on days 4–10, by Anova; $0.01 < p < 0.05$, on days 6 and 7, by Dunnett).

The oxygen consumption of kidney homogenates measured on day 4 after i.p. administration of cisplatin (Fig. 2) indicated a decrease from 53.25 ± 3.66 in controls to 41.25 ± 3.30 (22.53% inhibition, $0.01 < p < 0.05$). When animals were treated, at 15 min before cisplatin inoculation, with *E. pallida* standardized extract the oxygen uptake increased to 49.75 ± 4.76 (6.57% inhibition only, $p > 0.05$).

DISCUSSION

The search for pharmaceutical strategies to modulate the severe toxicity of cisplatin includes, among others, the use of chemoprotectors (Pinzano *et al.*, 1994).

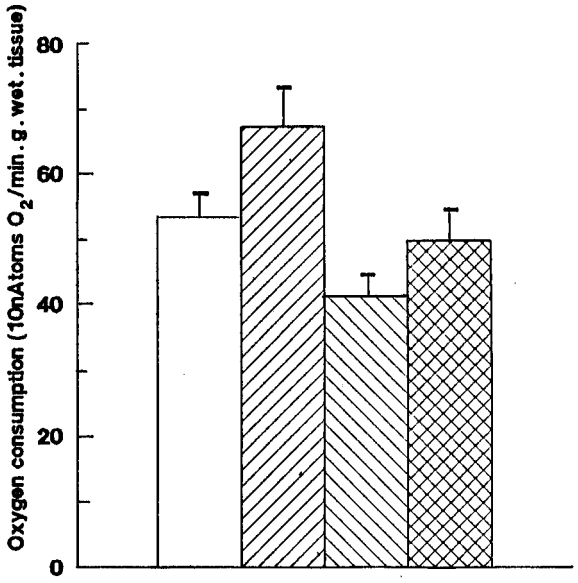

Figure 2. Oxygen consumption in mouse kidney homogenates on day 4 following *E.p.* extract and cisplatin administration; □ control; ▨ *E.p.* extract; ▧ cisplatin; ▩ *E.p.* extract + cisplatin.

We evaluated cisplatin toxicity in mice by a reduction of the body mass following i.p. administration of sublethal doses of cisplatin. The *E. pallida* standardized extract given to mice before and during cisplatin administration showed the protective effect of the extract evidenced by a reduced loss as well as a faster recovery of the animal body weight. According to Singh (1989), this drug acts preponderantly at the kidney level, inducing alterations of some renal functions and biochemical parameters (oxygen consumption, cytochrome C oxidase content). Therefore, we evaluated cisplatin nephrotoxicity and the protective effect of the *E. pallida* extract by measuring the oxygen consumption of kidney homogenates on day 4 after i.p. administration of cisplatin, which has been reported as the time at which the most significant alterations occur (Singh, 1989).

The oxygen consumption, which showed a statistically significant inhibition when cisplatin was given alone, was nearly restored to normal values if cytostatic treatment was associated with administration of *E. pallida* extract.

Our data regarding the protective effect of *E.p.* extract emphasize its possible use as an adjuvant to treatment with cisplatin.

A clinical trial with Novastim®, a solid dosage containing *E. pallida* extract, in patients subjected to chemotherapy with cisplatin alone or in combination with other antineoplastic drugs now in progress in our institute, showed encouraging results.

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