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Reduction of cisplatin-induced nephrotoxicity by cystone, a polyherbal ayurvedic preparation, in C57BL/6J mice bearing B16F1 melanoma without reducing its antitumor activity

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Abstract

The effect of cystone, a polyherbal ayurvedic preparation, on the nephrotoxicity and antitumor activity of cisplatin is studied in C57BL/6J mice bearing B16F1 melanoma. Intraperitoneal administration of cisplatin 6 mg/kg, resulted in significant reduction of body weight, elevation of blood urea nitrogen (BUN) and serum creatinine levels on day 5. Cystone was found to protect tumor-bearing mice from cisplatin-induced nephrotoxicity, when given i.p. 1 h before cisplatin. At 1000 mg/kg, it showed 46, 57 and 66% protection on body weight, BUN and serum creatinine levels, respectively. Treatment of cisplatin alone to tumor bearing mice resulted in significant antitumor activity as measured by tumor appearance, tumor volume and tumor weight. Pre-treatment with cystone (1000 mg/kg) did not reduce the antitumor activity of cisplatin. These results suggested that cystone protects against cisplatin-induced nephrotoxicity without interfering with its antitumor activity. The present study has many clinical implications in cisplatin chemotherapy.

Keywords: Cisplatin; Nephrotoxicity; Cystone; Free radicals; Antitumor activity

1. Introduction

Cisplatin is a potent antineoplastic agent against several types of solid tumors (Rozeneweig et al., 1977). However, its clinical use is limited by its renal toxicity (Madias and Harrington, 1978; Goldstein and Mayor, 1983). Although the mechanism of action of cisplatin renal toxicity is still not clear, it has been suggested that oxygen free radicals play an important role both in vitro and in vivo (Bull et al., 1988; Nakano and Gemba, 1989; Zhang and Lindup, 1993; Inselmann et al., 1995; Rao and Rao, 1998a,b). Naturally occurring antioxidants such as sodium malate, an active constituent from *Angelicae radix* (Sugiyama et al.,

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1994) and silibinin (Bokemeyer et al., 1996) are known to reduce the nephrotoxicity of cisplatin without reducing its antitumor effect.

Cystone is an ayurvedic polyherbal preparation and extensively used in many urinary tract complications (Nadkarni, 1992). Our earlier study showed that cystone protects against cisplatin-induced increased lipid peroxidation in rat renal cortical slices and nephrotoxicity in rats (Rao and Rao, 1998a). Hence, in the present study, we have investigated the effect of cystone on the cisplatin-induced nephrotoxicity and antitumor activity in C57BL/6J mice bearing B16F1 melanoma.

2. Materials and methods

2.1. Drug solutions

To 1000 mg cystone powder (Himalaya Drug, Bangalore. India), 10 ml distilled water was added and kept overnight at room temperature (25 ± 2°C) followed by boiling for 5 min. After cooling, the extract was filtered and the volume was made up to 10 ml. A separate experiment showed that this treatment yielded 218 mg of water soluble extract. The filtrate (equivalent 100 mg/ml of cystone powder) was used for the study The composition of cystone powder is given in Table 1.

Cisplatin was purchased from Sigma, St. Louis, MO. BUN (diacetyl monoxime method) and serum creatinine (alkaline picrate method) kits were obtained from Ranbaxy diagnostics, New Delhi, India.

2.2. Animals and tumor model

C57BL/6J mice (originally procured from National Institute of Nutrition, Hyderabad, India), 6–8 weeks age, weighing 18–20 g of either sex were used. They were maintained under controlled temperature and humidity with sterile bedding and food and water ad libitum. B16F1 melanoma cells (obtained from Department of Radiobiology, Kasturba Medical College, Manipal, India) were maintained and propagated intradermally by serial transplantation in adult female mice. Solid tumors were obtained by intradermal inoculation of 5×10^5 viable tumor cells on the dorsal side of mice (Uma and Rao, 1993). Tumor diameter was measured in three planes with a plastic vernier caliper. Tumor volume (V) was calculated using the formula, $V = \pi/6\ (D_1 \times D_2 \times D_3)$, where D_1, D_2, D_3 are the three diameters (Uma and Rao, 1993).

Table 1
Composition of cystone[a,b]

Plant name	Family	Part used	Quantity (mg)
Didymocarpus pedicellata R.Br.	Gesneriaceae	Flower	65
Saxifraga ligulata Walld.	Saxifragaceae	Stem	49
Ruhia cordifolia L.	Rubiaceae	Stem	16
Cyperus scariosus R.Br.	Cyperaceae	Root	16
Achyranthes aspera L.	Amaranthaceae	Whole plant	16
Onosmabracteatum Walld.	Boraginaceae	Whole plant	16
Vernonia cinerea L.	Compositae	Whole plant	16
Shilajeet (purified)	Bituminous material oozing from rock in summer		13
Hajrul Yahood Bhasma	Fossil stone occurring as a petrified oblong pointed fruit		16

[a] Processed with *Ocimum basilicum* L. (Labiatae), *Tribulus terrestris* L. (Zygophyllaceae), *Mimosa pudica* L. (Mimosaceae), *Dolichos biflorus* L. (Papilionaceae), *Pavonia odorata* Willd. (Malvaceae), *Equisetum arvense* L. (Equisetaceae) and *Tectona grandis* L.f. (Verbenaceae).

[b] Cystone is a ayurvedic formulation prepared and marketed in India.

Table 2
Effect of cystone against cisplatin-induced nephrotoxicity in C57BL/6J mice bearing B16F1 melanoma

Treatment[a]	*n*	Change in body weight (g)	BUN (mg/dl)	Serum creatinine (mg/dl)
Control	10	3.3 ± 0.5	23.9 ± 1.9	0.71 ± 0.04
Cisplatin 6 mg/kg	10	-3.2 ± 0.8*	91.0 ± 12 7*	2.41 ± 0.19*
Cystone 1000 mg/kg	5	3.4 ± 0.6	24.3 ± 3.2	0.74 ± 0.10
Cisplatin 6 mg/kg plus Cystone 1000 mg/kg	10	-0.2 ± 0.2*	$52.9 \pm 6.3^{*,**}$	$1.32 \pm 0.11^{*,**}$

[a] Cisplatin was administered i.p. Cystone was given i.p. 1 h prior to cisplatin. On day 5, blood was collected to measure the BUN and serum creatinine level. Body weight was recorded daily. Results are expressed as mean ± S.E.M.

* $P < 0.05$ compared to control.

** $P < 0.05$ compared to cisplatin.

2.3. Treatment

Animals were implanted with 5×10^5 viable tumor cells, intradermally, 24 h later, cisplatin 6 mg/kg i.p. was administered. Cystone 1000 mg/kg, was given i.p. 1 h prior to cisplatin. The control animals received vehicle. On day 5, blood was collected to measure the BUN and serum creatinine. Body weight was recorded once a day. Tumor appearance and tumor volume was recorded on day 7 and 15. On day 15, all the animals were sacrificed and tumor weight was recorded.

2.4. Statistical analysis

Results are expressed as mean ± S.E.M. For comparison, one-way ANOVA followed by Student's Newman–Keuls test was used. Statistical significance was set at $P < 0.05$. All the statistical analysis was done using SPSS-PC version 3.1 computer package.

3. Results

3.1. Effect on body weight

Animals which received cisplatin 6 mg/kg, i.p. showed decrease in their body weight by 3.2 ± 0.8 g on day 5 compared to control animals which gained 3.3 ± 0.5 g weight during the same period. Cystone 1000 mg/kg, i.p. 1 h prior to cisplatin, protected against decrease in the body weight, which accounted to 46% protection. Treatment of cystone alone had no effect on body weight (Table 2).

3.2. Effect on BUN and serum creatinine

The level of BUN increased to 91.0 ± 12.7 mg/dl when the animals were treated cisplatin (6 mg/kg, i.p.) compared to 23.9 ± 1.9 mg/dl in control animals (Table 2). When cystone was administered at 1000 mg/kg, i.p. given 1 h before cisplatin, the BUN levels were 52.9 ± 6.3 mg/dl which accounted 57% protection. Similar results were obtained with serum creatinine. The serum creatinine level was increased to 2.41 ± 0.19 mg/dl when the animals were treated with cisplatin (control = 0.71 ± 0.04 mg/dl). After pre-treatment with cystone, the serum creatinine levels were increased only to 1.32 ± 0.11 mg/dl, which accounted to 66% protection. Treatment of cystone alone had no effect on BUN and serum creatinine.

3.3. Effect on tumor appearance, tumor volume and tumor weight

Table 3 gives the effect of cystone on antitumor activity of cisplatin as measured by tumor appearance, tumor volume and tumor weight.

Administration of cisplatin 6 mg/kg, i.p. resulted in 60%, tumor appearance on day 7 compared to 100% for control animals. On day 15, both control and cisplatin treated animals showed 100% tumor appearance. On day 7, tumor volume

Table 3
Effect of cystone on antitumor activity of cisplatin in C57BL/6J mice bearing B16F1 melanoma

Treatment[a]	Tumor appearance (%)		Tumor volume (mm^3)		Tumor weight (g)
	Day 7	Day 15	Day 7	Day 15	Day 15
Control	100	100	63 ± 8	701 ± 105	1.65 ± 0.24
Cisplatin 6 mg/kg	60	100	12 ± 4*	192 ± 37*	0.79 ± 0.23*
Cystone 1000 mg/kg	100	100	76 ± 11	679 ± 90	1.58 ± 0.17
Cisplatin 6 mg/kg plus Cystone 1000 mg/kg	65	100	17 ± 5*	211 ± 36*	0.89 ± 0.19*

[a] Cisplatin was administered i.p. Cystone was given i.p. 1 h prior to cisplatin. Results are expressed as mean $\pm$ S.E.M.
* $P < 0.05$ compared to control.

decreased significantly when cisplatin was administered i.p. to mice (12 ± 4 mm^3). The control value was 63 ± 8 mm^3 on day 7. On day 15, there was 11- and 16-fold increase in the tumor volume in control and cisplatin treated animals, respectively. Treatment of cisplatin significantly decreased tumor weight on day 15. The value was found to be 0.79 ± 0.23 g as compared to control (1.65 ± 0.24 g). Treatment with cystone 1000 mg/kg, 1 h before cisplatin i.p. did not alter cisplatin-induced decreased tumor appearance, tumor volume and tumor weight (Table 3).

4. Discussion

Cisplatin is an important antitumor agent useful in treating many types of solid tumors. However, its use is limited by its nephrotoxicity. Free radicals are known to play an important role in cisplatin nephrotoxicity. Our earlier study showed that cystone, a polyherbal ayurvedic preparation partially but significantly protected cisplatin-induced nephrotoxicity in rats (Rao and Rao, 1998b). In the present study, we have investigated the effect of cystone on cisplatin-induced nephrotoxicity and antitumor activity in C57BL/6J mice bearing B16F1 melanoma. Treatment of cystone 1000 mg/kg, i.p. to tumor bearing animals 1 h before cisplatin administration, resulted in lesser nephrotoxicity as characterized by BUN and serum creatinine levels. Also pre-treatment with cystone did not interfere with the antitumor activity of cisplatin in tumor bearing mice as measured by tumor appearance, tumor volume and tumor weight. Naturally occurring antioxidants such as sodium malate, an active constituent from *A. radix* (Sugiyama et al., 1994) and silibinin (Bokemeyer et al., 1996) also reduced the nephrotoxicity of cisplatin without reducing its antitumor effect.

In conclusion, our findings show that cystone, a polyherbal ayurvedic preparation, provides significant protection against cisplatin-induced nephrotoxicity in C57BL/6J mice bearing B16FI melanoma without interfering with its antitumor effect. Hence, the present study has many clinical implications in cisplatin chemotherapy.

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