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Inhibition of chemical carcinogenesis by berberine in rats and mice

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Abstract

Berberine, an alkaloid isolated from the plant *Berberis aristata,* has been found to inhibit significantly the carcinogenesis induced by 20-methylcholanthrene (200 μg/0.1 mL/mouse) or *N*-nitrosodiethylamine (NDEA; 0.02 % NDEA in distilled water, 2.5 mL/animal by gavage, five days a week for 20 weeks) in a dose-dependent manner in small animals. Administration of berberine (0.5, 2.5 or 5.0 mg kg^{-1}) could reduce significantly the incidence of tumour in animals after an injection of 20-methylcholanthrene and increased their life span compared with the control. When berberine (10, 25 or 50 mg kg^{-1}) was administered simultaneously with NDEA, the markers of liver injury (liver weight, γ-glutamyl transpeptidase activity and glutathione S-transferase level) were reduced significantly compared with animals treated with NDEA only, which resulted in all the values being elevated. A similar decrease was noted in the serum levels of lipid peroxide, bilirubin and glutamate pyruvate transaminase. Morphology of liver tissue and levels of marker enzymes indicated that berberine offered protection against chemical carcinogenesis.

Introduction

Hepatocellular carcinoma is widely prevalent in some areas of the Far East and in Africa. The causative agents are reported to be hepatitis B virus and environmental pollutants, of which nitrosoamines have a major role. *N*-Nitrosodiethylamine (NDEA), a potent hepatocarcinogen, is a byproduct of the nitrosation of primary amines in the acidic condition of the stomach, and is present in many food products e.g. meat, beer etc. NDEA has been shown to be metabolized to its active ethyl radical metabolite ($CH_3CH_2^+$) and the reactive product interacts with DNA producing mutation and further oncogenesis. Similar activation is also involved in the carcinogenic action of polyaromatic hydrocarbons.

Berberine, an isoquinoline alkaloid, has been reported to have multiple pharmacological actions (Ckless et al 1995). Antitumour activity of berberine against human and rat malignant brain tumour cells has been reported (Zhank et al 1990). Recently, we reported that berberine could potentiate the antitumour activity of cyclophosphamide and radiation in animals (Anis et al 1999). Chang et al (1990) showed that berberine was able to down-regulate c-ki-ras 2 oncogene expression in human teratocarcinoma cells, indicating that berberine may be able to inhibit the proliferation of cancer cells produced by chemical carcinogens. In this study we have investigated the chemopreventive activity of berberine against 20-methylcholanthrene-induced sarcoma in mice and NDEA-induced hepatocarcinogenesis in rats.

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Materials and Methods

Animals

Fifty female Wistar rats (120–150 g) were obtained from the Veterinary College, Mannuthy, India. They were housed in ventilated cages and fed with a pelleted diet (Lipton, India Ltd) with water freely available. The male Swiss albino mice (20–25 g) used were reared in our facility.

Materials

Berberine hydrochloride was a gift from Dr Rajpal, Kisalaya Herbals Ltd. (Indore, India). 20-Methylcholanthrene was purchased from ICN-Pharmaceuticals (New York, NY). *N*-Nitrosodiethylamine (NDEA) was obtained from Sigma Chemicals (St Louis, MO). 1-Chloro-2, 4-dinitrobenzene, glutathione, and 5,5-dithiobis(2-nitrobenzoic acid) were purchased from Sisco Research Laboratory (Bombay, India). Thiobarbituric acid was obtained from E-Merck (Germany). All other chemicals used were of analytical reagent grade.

Drug preparation

Desired concentrations of berberine hydrochloride were dissolved in hot water and administered orally to the animals.

Determination of the effect of berberine hydrochloride on 20-methylcholanthrene-induced sarcoma

Swiss albino mice (20–25 g, 15/group) were used for the study. Hair was removed from the dorsal side of all animals and a single dose of 20-methylcholanthrene (200 μg/0.1 mL/mouse) was injected subcutaneously on the dorsal side. The animals were divided into four groups. Group 1, which received 20-methylcholanthrene only, was kept as a positive control. Groups 2, 3 and 4 received 20-methylcholanthrene and different concentrations (5, 2.5 and 0.5 mg kg^{-1}) of berberine hydrochloride, respectively. This dosage, which was non-toxic to the animals, was selected after determining the LD50 of berberine in the animals (results not shown). Administration of the drug started five days before 20-methylcholanthrene injection and continued for eight weeks, thrice a week orally. The control group was kept without any treatment. The animals were observed for the onset of sarcoma as well as for their survival up to 180 days.

Determination of the effect of berberine hydrochloride on NDEA-induced hepatocarcinogenesis

Liver tumours were induced by the administration of NDEA as described by Narurkar & Narurkar (1989) with slight modifications. Six-week-old female Wistar rats (120–125 g) were divided into five groups (10 animals/group). Group 1 served as untreated normal animals. Group 2 received 0.02% NDEA in distilled water, 2.5 mL/animal by gavage, five days a week for 20 weeks. This dosage was found to induce liver tumours in the rats (Jose Jeena et al 1999) and animals died by 29–31 weeks. Groups 3, 4 and 5 were administered 50, 25 and 10 mg kg^{-1} berberine hydrochloride, respectively, along with NDEA. This dosage was found to be non-toxic to rats. Berberine hydrochloride was administered 24 h before the first dose of NDEA and was continued for 20 weeks. Animals were kept without any treatment for a further 10 weeks before being killed. At the end of the 30th week animals were administered diethylether anaesthesia, and blood and liver tissues were collected immediately. Change in liver weight and tumour incidence produced by NDEA treatment was noted. Serum was separated and part of the tissue was fixed in formalin and the rest were kept frozen until analysed.

The following serum and liver parameters were measured to assess the effect of berberine hydrochloride on hepatocarcinogenesis. Serum levels of γ-glutamyl transpeptidase activity were measured by Szasz's method using an AVT diagnostics kit (Szaz 1976) and tissue γ-glutamyl transpeptidase was measured using γ-glutamyl *p*-nitroanilide as substrate (Tate & Meister 1974). Cytosolic glutathione S-transferase activity was determined by its ability to conjugate glutathione with 1-chloro-2,4-dinitrobenzene (Habig et al 1974). Tissue levels of reduced glutathione were determined by its reaction with 5,5-dithiobis(2-nitrobenzoic acid) (Moron et al 1979). Lipid peroxidation in serum was estimated by the thiobarbituric acid method (Ohkawa 1979). Protein was analysed by the method of Lowry et al (1951). Glutamate pyruvate transaminase activity was measured in serum (Bergmeyer & Bernt 1980). Serum total bilirubin level was estimated by the method of Jendrassic & Grof (1938).

Statistical analysis

Results were expressed as mean $\pm$ s.d. and evaluated by Student's *t*-test.

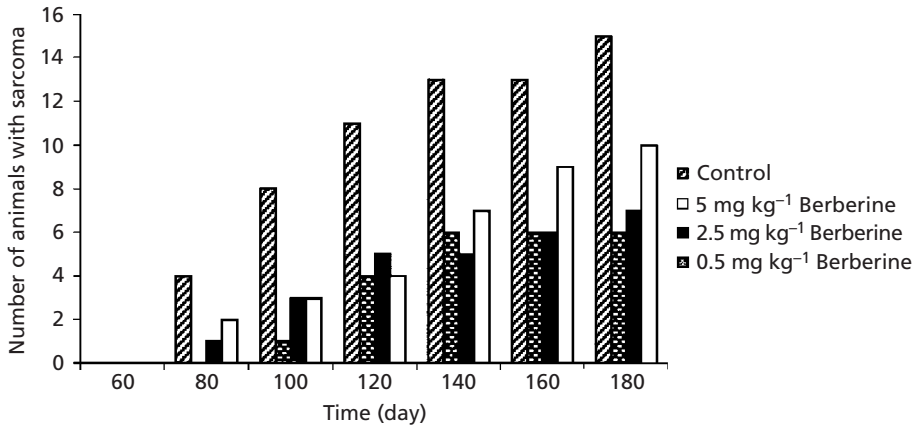

Figure 1 Effect of berberine on sarcoma development induced by the administration of 20-methylcholanthrene (200 μg/0.1 mL/mouse).

Results

Berberine hydrochloride could inhibit sarcoma development induced by 20-methylcholanthrene in a dose-dependent manner. Oral administration of the drug (5, 2.5 or 0.5 mg kg^{-1}) was found to inhibit sarcoma development by 60 %, 53 % and 33 %, respectively (Figure 1). Animals treated with berberine and 20-methylcholanthrene were shown to have an increased life span (Table 1). Control animals began dying of tumour burden after 80 days of carcinogen treatment, with all the animals dying by 180 days, whereas in the treated groups only 6, 7 and 10 animals, respectively, had died by 180 days.

The effect of berberine hydrochloride on NDEA-induced liver tumour incidence is shown in Table 2. The NDEA-treated group showed 100 % tumour incidence by the end of the 30th week, whereas the berberine-treated groups showed a dose-dependent reduction of tumour incidence. Inhibition of tumour incidence was also reflected in changes in liver weights. Liver weight of normal animals was shown to be 2.3 ± 0.2 g/100 g, which was significantly increased by NDEA treatment to 8.9 ± 2.1 g/100 g ($P < 0.001$). Liver weight of the berberine-treated groups was found to be reduced significantly in a dose-dependent manner.

γ-Glutamyl transpeptidase enzyme activity, a non-specific marker of liver neoplasm, was significantly elevated from 30.4 ± 5.8 to 152 ± 37 U L^{-1} in serum and from 0.01 ± 0.002 to 0.15 ± 0.02 nmol min^{-1} (mg protein)$^{-1}$ in liver by the administration of NDEA. Administration of berberine (10, 25 or 50 mg kg^{-1}) reduced the serum γ-glutamyl transpeptidase levels to 119, 93 and 82 U L^{-1}, respectively. Liver γ-glutamyl transpeptidase levels were also significantly ($P < 0.001$) reduced by the administration of berberine hydrochloride (Table 3).

NDEA-treated groups showed significantly elevated liver glutathione S-transferase activity (1769 ± 898) compared with the normal group (377 ± 13; Table 4), which may be due to induced detoxification mechanisms for elimination of NDEA and its metabolites. A dose-dependent decrease in glutathione S-transferase level (837 ± 605 to 549 ± 128 nmol min^{-1} (mg protein)$^{-1}$) was observed in the berberine-treated group (10–50 mg

Table 1 Effect of berberine on the survival of animals after induction of sarcoma with 20-methylcholanthrene (200 μg/0.1 mL/mouse).

Treatment	Number of animals that survived (days)						
	60	80	100	120	140	160	180
Control	15/15	14/15	12/15	4/15	3/15	3/15	0/15
5 mg kg^{-1}	15/15	15/15	13/15	12/15	12/15	9/15	9/15
2.5 mg kg^{-1}	15/15	14/15	12/15	11/15	11/15	10/15	8/15
0.5 mg kg^{-1}	15/15	14/15	11/15	8/15	7/15	6/15	5/15

Table 2 Effect of berberine on NDEA-induced hepatocarcinogenesis in rats.

Group	Treatment	Number of animals	% of tumour incidence	Liver weight/100 g body weight
1	Normal	10	0	2.3 ± 0.2
2	Control (NDEA alone)	10	100 %	8.9 ± 2.1^{b}
3	NDEA + berberine (10 mg kg^{-1})	10	100 %	5.2 ± 1.2^{a}
4	NDEA + berberine (25 mg kg^{-1})	10	50 %	3.7 ± 1.5^{b}
5	NDEA + berberine (50 mg kg^{-1})	10	50 %	3.5 ± 0.4^{b}

$^{a}P < 0.01$, $^{b}P < 0.001$.

Table 3 Effect of berberine on γ-glutamyl transpeptidase activity in rats treated with *N*-nitrosodiethylamine (NDEA).

Group	Treatment	γ-Glutamyl transpeptidase activity	
		Serum (U L^{-1})	Tissue (nmol min^{-1} (mg protein)$^{-1}$)
1	Normal	30 ± 5.8	0.01 ± 0.002
2	Control (NDEA alone)	152 ± 37^{d}	0.15 ± 0.02e
3	NDEA + berberine (10 mg kg^{-1})	119 ± 34^{a}	0.09 ± 0.02^{e}
4	NDEA + berberine (25 mg kg^{-1})	93 ± 39^{b}	0.04 ± 0.02^{e}
5	NDEA + berberine (50 mg kg^{-1})	82 ± 35^{c}	0.04 ± 0.03^{e}

$^{a}P < 0.2$, $^{b}P < 0.05$, $^{c}P < 0.02$, $^{d}P < 0.005$ and $^{e}P < 0.001$.

Table 4 Effect of berberine on liver glutathione S-transferase and glutathione levels in rats treated with *N*-nitrosodiethylamine (NDEA).

Group	Treatment	Glutathione S-transferase (nmol min^{-1} (mg protein)$^{-1}$)	Glutathione (nmol (mg protein)$^{-1}$)
1	Normal	377 ± 13	3 ± 0.2
2	NDEA alone	1769 ± 898^{a}	5.3 ± 0.8^{d}
3	NDEA + berberine (10 mg kg^{-1})	837 ± 605^{b}	5 ± 1.8
4	NDEA + berberine (25 mg kg^{-1})	674 ± 370^{c}	4.6 ± 1.6
5	NDEA + berberine (50 mg kg^{-1})	549 ± 128^{c}	4.1 ± 0.8

$^{a}P < 0.02$, $^{b}P < 0.05$, $^{c}P < 0.005$ and $^{d}P < 0.001$.

kg^{-1}). Reduced glutathione activity was also elevated by the administration of NDEA. However, berberine treatment had only a marginal effect on glutathione levels (Table 4).

Lipid peroxide and glutamate–pyruvate transaminase levels in normal rat serum were found to be 1.3 ± 0.25 nmol mL^{-1} and 170 ± 52 U mL^{-1}, respectively. These were elevated to 3.0 ± 0.67 nmol mL^{-1} and 589 ± 168 U mL^{-1}, respectively, after the administration of NDEA. Simultaneous administration of berberine significantly lowered the lipid peroxide and glutamate–pyruvate transaminase level in a dose-dependent manner (Table 5). Similarly, serum bilirubin, which is a biomarker for liver damage, was significantly ($P < 0.01$)

Table 5 Effect of berberine on lipid peroxide, glutamate–pyruvate transaminase and bilirubin in serum of rats treated with *N*-nitrosodiethylamine (NDEA).

Group	Treatment	Glutamate pyruvate transaminase (U mL^{-1})	Lipid peroxide (nmol mL^{-1})	Bilirubin (mg/100 mL)
1	Normal	170 ± 52	1.3 ± 0.25	0.21 ± 0.05
2	NDEA alone	589 ± 168^{c}	3.0 ± 0.67^{c}	0.60 ± 0.19^{b}
3	NDEA + berberine (10 mg kg^{-1})	542 ± 100	2.0 ± 0.6^{a}	0.31 ± 0.09^{b}
4	NDEA + berberine (25 mg kg^{-1})	315 ± 190^{a}	1.9 ± 0.1^{b}	0.29 ± 0.07^{c}
5	NDEA + berberine (50 mg kg^{-1})	295 ± 129^{c}	1.7 ± 0.3^{d}	0.24 ± 0.03^{d}

$^{a}P < 0.02$, $^{b}P < 0.01$, $^{c}P < 0.005$ and $^{d}P < 0.001$.

increased by the NDEA treatment. In the animals treated with berberine, bilirubin levels were almost equal to normal values (Table 5).

Discussion

Berberine is an alkaloid widely distributed in nature. *Berberis aristata* and *Berberis asiatica* are plants rich in this alkaloid. *B. aristata* is used in Chinese medicine and is much used in Indian medicinal preparations, for instance as an hepatoprotector (Antarkar et al 1980). Berberine is an antibacterial agent, active against Gram-positive and Gram-negative bacteria. Zhou et al (1995) demonstrated that berberine acted as a calcium channel agonist, and Matsumato et al (1994) reported that it induced platelet aggregation. Kuo et al (1995) showed berberine to produce aggregation of DNA and induction of apoptosis. Moreover, it was found to produce anti-tumour activity by down regulation of c-ki-ras2 oncogenes (Chang et al 1990). Also, berberine was found to inhibit the crypt formation in the rat colon during azoxymethane-induced carcinogenesis (Fukutake et al 1998).

The results of this study indicated a chemopreventive action of berberine in the two animal models studied. Berberine was found to reduce liver weight, γ-glutamyl transpeptidase, glutamate–pyruvate transaminase and bilirubin, all markers of liver injury, induced by NDEA. Berberine treatment was found to reduce the glutathione S-transferase levels in the liver, but had only a marginal effect on glutathione. Berberine reduced 20-methylcholanthrene-induced sarcoma in mice, as seen from the increased life span compared with the control animals and the reduction in the number of tumour-bearing animals. However, at present, the mechanisms of action of berberine are not known and require further investigation.

References

Anis, K. V., Kuttan, G., Kuttan, R. (1999) Role of berberine as an adjuvant response modifier during tumour therapy in mice. *Pharm. Pharmacol. Commun.* **5**: 697–700

Antarkar, D. S., Ashok, B. V., Doshi, J. C., Athavale, A. V., Vinchoo, K. S., Natekar, M. R., Thathed, P. S., Ramesh, V., Kale, N. (1980) A double-blind clinical trial of *Arogya-Wardhani* an ayurredic drug in acute viral hepatitis. *Indian J. Med. Res.* **72**: 588–593

Bergmeyer, H. U., Bernt, E. (1980) Calorimetric method for aspartate and alanine aminotransferases. In: Varley, H., Gowenlock, A. G., Bell, M. (eds) *Practical Clinical Biochemistry*, vol. 1. 5th edn, William Heinemann, Medical Books Ltd, London, pp 741–742

Chang, K. S., Gao, C., Wang, L. C (1990) Berberine induced morphologic differentiation and down regulation of C-Ki-ras 2 proto-oncogene expression in human teratocarcinoma cells. *Cancer Lett.* **55**: 103–108

Ckless, K., Schlottfeldt, J. L., Pascal, M., Moyana, P., Henriques, J. A., Wajner, M. (1995) Inhibition of in vitro lymphocyte transformation by the isoquinoline alkaloid berberine. *J. Pharm. Pharmacol.* **47**: 1029–1031

Fukutake, M., Yokota, S., Kawamura, A., Lizuka, A., Amagaya, S., Fukuda, K., Komatsu, Y. (1998) Inhibitory effect of *Coptidis rhizoma* and *Scutellaria radix* on azoxymethane induced aberrant crypt foci formation in rat colon. *Biol. Pharm. Bull.* **21**: 814–817

Habig, W. H., Pabst, M. J., Jakobsky, W. R. (1974) Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249**: 7130–7139

Jendrassic, L., Grof, P. (1938) Simplified photometric method for the determination of the blood bilirubin. *Biochem. Z.* **297**: 81–89

Jose Jeena, K., Joy, K. L., Kuttan, R. (1999) Effect of *Emblica officinalis*, *Phyllanthus amarus* and *Picrorrhiza kurroa* on N-nitrosodiethylamine induced hepatocarcinogenesis. *Cancer Lett.* **136**: 11–16

Kuo, C. L., Chou, C. C., Yung, B. Y. (1995) Berberine complexes with DNA in the berberine induced apoptosis in human leukemic HL-60 cells. *Cancer Lett.* **93**: 193–200

Lowry, O. H., Rosebrough, N. J., Farr, L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275

Matsumato, M., Ischida, S., Ichikawa, T., Sakiya, Y. (1994) Aggregation of DNA enhanced by the protoberberine

alkaloids, coralyne and berberine. *Chem. Pharm. Bull.* (*Tokyo*) **42**: 1556–1561

Moron, M. A., Depierre, J. W., Mannervick, B. (1979) Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat liver. *Biochim. Biophys. Acta* **582**: 67–68

Narurkar, L. M., Narurkar, M. V. (1989) Role of nicotinamide in suppression of diethylnitrosamine hepatocarcinogenesis in rats. In: Bhide, S. V., Maru, G. B. (eds) *Chemoprevention of Cancer*. Omega Scientific Publishers, New Delhi, pp 162–177

Ohkawa, H., Ohishi, N., Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**: 351–358

Szaz, G. (1976) Reaction rate method for γ-glutamyl transpeptidase activity in serum. *Clin. Chem.* **22**: 2031–2055

Tate, S. S., Meister, A. (1974) Interaction of γ-glutamyl transpeptidase with amino acid peptides and derivatives and analogues of glutathione. *J. Biol. Chem.* **23**: 7593–7602

Zhank, R. X., Dougherty, D. V., Rosenblum, M. L (1990) Laboratory studies of berberine used alone and in combination with 1,3-bis(2-chloroethyl)-1-nitrosourea to treat malignant brain tumours. *Chin. Med. J.* (*Engl.*) **103**: 658–665

Zhou, Z., Lan, T., Li, H., Zhang, Y., Wang, Y. (1995) Effects of berberine on single Ca^{2+} channel current in cultured embryonic chick ventricular myocytes. *Hua His I Ko Ta Hsueh Hsueh Pao* **26**: 287–290