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Review Article

Antineoplastic alkaloids from *Peganum harmala*

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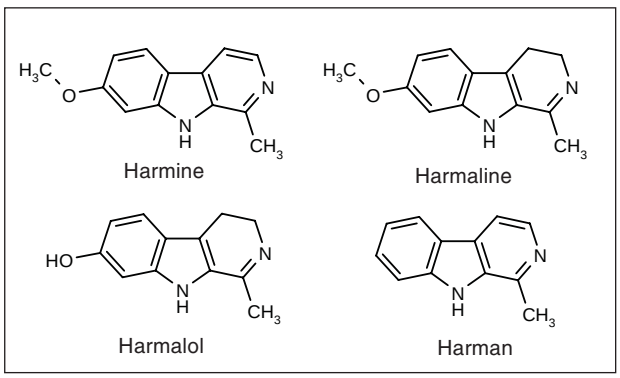

Fig. 1. Major alkaloids of *Peganum harmala*.

Introduction

The seed of *Peganum harmala* (Zygophyllaceae family) has been used as a spice and an intoxicant. Some harmala bases have also been shown to elicit hallucinogenic effects in humans when administered orally (1). 6-Methoxytetrahydroharman, closely related to the harmala bases, was found to be a natural hormone of the pineal gland and also an hallucinogen (1). Further studies demonstrated that some of the harmala alkaloids are inhibitors of monoamine oxidase and *N*-acetyltransferase (2). Some of them induce other biological effects including tremor, immunosuppression, *etc.* (3-8). More than 20 years ago, harmala alkaloids were found to possess antitumor activity. This discovery encouraged scientists in China to investigate the alkaloids in the treatment of various tumors (9). Several characteristics of harmala bases indicate that they have the potential to be developed as new antitumor drugs.

This article describes the isolation and chemistry of harmala alkaloids, their antitumor activity and mechanisms of action, as well as pharmacokinetic, toxicological and clinical studies of the compounds.

Chemistry and isolation

The chemistry of *P. harmala* has been extensively investigated (10). As early as 1841, Goebel reported the presence of the alkaloids in the seed and the root of *P. harmala*. Among the alkaloids from the seed of the plant identified to date are harmine 7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*] indole), harmaline (7-methoxy-3,4-dihydro-β-carboline), harmalol (7-hydroxy-3,4-dihydro-β-carboline) and harman (7-hydroxy-β-carboline (Fig. 1). (4, 8). These alkaloids were also recently isolated from the seed of *Peganum multisectum Maxim* (Zygophyllaceae family), another Chinese species (11).

Harmala alkaloids were isolated using several methods, including chloroform extraction, positive ion exchange resin assay and chloroform:ammonia water (6:4) extraction, *etc*. (12, 13). The chloroform:ammonia water extraction method was very practical and had several advantages including simple technological process, short production period, small amounts of solvents and high yield (13).

Antitumor activity

Experimental investigations have shown that harmala bases possess obvious antitumor activity *in vitro* and *in vivo*.

In vitro *studies*

The total alkaloids of *P. harmala* were shown to inhibit the growth of several types of tumor cell lines *in vitro* as shown in Table I (14-16). In addition, the 5L mixed alkaloids at concentrations of 47 and 94 mg/l were also found

Table I: Inhibitory effect of total alkaloids of P. harmala *on the growth of human cancer cell lines* in vitro.

Cell line	IC_{50} (mg/l)
Retinoblastoma SO-Rb50	1.4
Hepatoma BEL-7402	4.0
Gastric carcinoma MGC-803	17.3
Nasopharyngeal carcinoma CNE-2	24.3
Uterocervical carcinoma HeLa	12.3
Breast cancer MA782'5S	18.3

Table II: Cytotoxic effect of harmaline in vitro.

Cell line	Conc. (mg/l)	Growth inhibition (%)
HeLa	20	21
	40	59
	80	79
	160	92
	320	100
S-180	20	24
	40	60
	80	83
	160	94
	320	100
EAC	80	25
	160	54
	320	86

HeLa = human uterocervical carcinoma cell line; S-180 = mouse sarcoma cell line; EAC = mouse Ehrlich ascites carcinoma cell line.

to suppress proliferation of human uterocervical carcinoma HeLa cells by about 63 and 75%, respectively (17). The cytotoxicity of harmaline for HeLa cells and mouse sarcoma S-180 cells was also significant (IC_{50} = 37.8 and 34.7 mg/l, respectively). However, harmaline's cytotoxic effect on mouse Ehrlich ascites carcinoma cells was weak with an IC_{50} of 139.9 mg/l (18) (Table II). Harmine at concentrations of 47 and 94 mg/l also inhibited growth of HeLa cells *in vitro* by 59 and 72%, respectively (19).

In vivo *studies*

The 5n mixed alkaloids and the total alkaloids of *P. harmala* exerted marked inhibitory effects on the growth of several transplanted tumors in mice (Table III), although no significant influence on mouse Ehrlich ascites carcinoma growth was observed with doses of 15-45 mg/kg i.p. for 8-10 days (12, 16, 17, 20); similar effects were observed for pure harmaline (12, 18) (Table III). Furthermore, when the total harmala bases were used in combination with cisplatin and adriamycin, synergistic inhibition on tumor growth was found (16) (Table IV).

In order to test the cytotoxic effects of the total harmala alkaloids on human tumors *in vivo*, several human cancer cell lines derived from liver (BEL-7402), gastric (MGC-803) and nasopharyngeal cancer (CNE-2) were xenografted subcutaneously into nude mice. As shown in Table V, the total alkaloids of *P. harmala* at tolerable dose levels exhibited significant antitumor activity and a synergistic cytotoxic effect was also observed when the total alkaloids were combined with cisplatin (15, 21).

Table III: Antitumor activity of harmala alkaloids administered i.p. for 8 days in mice.

Cell line	Growth inhibition (%) 5n	Total alkaloids	Harmaline
Sarcoma-180			
	48.5 (11.25)	22.2 (20)	38.8 (22.5)
	51.4 (22.5)	36.9 (40)	47.7 (45)
Reticulosarcoma-L2			
	44.0 (22.5)	25.0 (20)	35.7 (22.5)
	61.0 (45)	51.2 (60)	49.6 (45)
Hepatoma			
	54.4 (22.5)	28.9 (20)	35.6 (22.5)
	66.7 (45)	31.3 (40)	58.2 (45)

Doses (mg/kg) are shown in parentheses.

Table IV: Inhibitory effect of total harmala alkaloids in combination with other antitumor drugs on the growth of mouse S-180 cell line in vivo.

Treatment	Dose (mg/kg)	Growth inhibition (%)
Total alkaloids	40	22
Cisplatin	0.8	32
Adriamycin	1.0	27
Total alkaloids + cisplatin	40 + 0.8	61
Total alkaloids + adriamycin	40 + 1.0	64

Table V: The cytotoxic effect of total harmala alkaloids (i.p.) on human cancer cells in nude mice.

Treatment	Cell line	Dose (mg/kg x d)	Growth inhibition (%)
Total alkaloids	MGC-803	60 x 9	48.4
Total alkaloids	CNE-2	60 x 9	60.0
Total alkaloids	BEL-7402	60 x 9	60.0
Total alkaloids	BEL-7402	40 x 8	30.7
Cisplatin	BEL-7402	0.8 x 8	23.0
Total alkaloids + cisplatin	BEL-7402	40 x 8 + 0.8 x 8	54.7

MGC-803 = human gastric cancer cell line; CNE-2 = human nasopharyngeal cancer cell line; BEL-7402 = human hepatoma cell line.

Mechanisms of antitumor action

Ultrastructural changes of various cancer cells after treatment with the total harmala alkaloids were studied in tumor-bearing mice using electron microscopy. Results

Table VI: Effect[a] of total harmala alkaloids (i.p.) on cell cycle progression of hepatoma cells in mice.

Dose (mg/kg x d)	Time (h)[b]	G_1	S	G_2M
0	0	46.4	36.6	17.0
60	3	40.8	30.2	29.0
60	9	37.0	47.2	21.8
60	24	39.3	33.9	26.8
60	48	51.4	29.8	18.8

[a]Expressed as percent. [b]Hours after administration.

showed that the major effects caused by the total alkaloids included: (i) decreased microvilli of the surface of tumor cells; (ii) enlarged rough endoplasmic reticulum and perinuclear interspace; (iii) local discontinuation of the cell membrane, rough endoplasmic reticulum, mitochondrion membrane and nuclear membrane; (iv) the presence of many cytoplasmic vacuoles; (v) chromatin gathering; and (vi) nucleolus separation, karyopyknosis and nuclear dissolution (16, 22). These results indicate that total alkaloids exert cytotoxic effects mainly through injury of cancer cell biomembranes and DNA.

The total alkaloids suppressed DNA, RNA and protein syntheses in retinoblastoma SO-Rb50 cells *in vitro* with IC_{50} values of 5.9, 7.5 and 8.6 mg/l, respectively (16). Harmine also inhibited DNA synthesis in HeLa cells by 58.0% (47 mg/l) and 66.5% (94 mg/l) *in vitro* (16, 19). The effect of total harmala alkaloids on mouse hepatoma cell cytokinetics was further investigated using a microfluorometric assay and stathmokinetic method. Results showed that the total alkaloids inhibited the transition of cells from G_2 to M phase resulting in accumulation of cells in the G_2 phase (23) (Table VI). The mitotic index of hepatoma cells was also significantly decreased after treatment with 60 mg/kg of the total alkaloids (23). These results indicate that harmala bases could potentially be used in combination chemotherapy with other drugs such as bleomycin.

Chemoprevention, based on the concept that synthetic or naturally occurring chemicals can inhibit the process of carcinogenesis, has played an increasingly significant role in the control of carcinogenesis. There is evidence suggesting a close relationship between the injury to peptic mucous membrane and the presence of tumors in the digestive tract. The total alkaloids of *P. harmala* (20 mg/kg p.o.) were shown to have a protective effect on experimentally induced gastric mucous membrane injury in mice (24). Preliminary clinical studies demonstrated that the total alkaloids had a therapeutic effect in patients with peptic ulcers. In a study in 35 patients, 30 had a good response after oral administration of the total alkaloids, with an efficacy rate of 85.7% (Table VII) (25). It was suggested that the protective effect of harmala bases on peptic mucous membrane may also contribute to their antitumor effect in the digestive tract due to a potential chemopreventive action.

Table VII: Therapeutic effect of total harmala alkaloids in peptic ulcer patients.

	Response			
Ulcer	Good	Partial	No.	Total
Duodenal ulcer	10	16	5	31
Gastric ulcer	3	1	0	4
Total	13	17	5	35

Pharmacokinetics

According to the principle that plasma concentration, effect and toxicity of most drugs are correlated, the measurement of plasma concentration was combined with the calculation of acute mortality of mice by means of drug-accumulation method; acute mortality of mice was used as the index of pharmacological effect and the concentration-effect curve was converted into the corresponding levels in the body. Based on these studies, the pharmacokinetic parameters of the total alkaloids of *P. harmala* were: $t_{1/2}$ = 1.2 h, K_e = 0.5773 h^{-1}, V_d = 2.288 l/kg and CL = 1.321 l/(kg·h) (26). The short $t_{1/2}$ of total alkaloids suggests that elimination from the body is rapid. Data from HPLC analysis of total alkaloids in rabbits (16, 27, 28) were fitted to a one-compartment, open model with a $t_{1/2\beta}$ of 2.3 h. The data from tumor-bearing mice also indicate that trace amounts of the total alkaloids were accumulated in the body.

The pharmacokinetics of harmaline were also examined in rabbits and rats using HPLC and tritium-labeled compound (8, 29). Data from 4/5 tested rabbits fit a one-compartment, open model while data from the other rabbit fit a two-compartment, open model. The main pharmacokinetic parameters calculated were: α = 0.170 min^{-1}, β = 0.037 min^{-1}, MRT = 33.2 min, CL = 508.6 ml/(kg·min), $t_{1/2}\ \alpha$ = 4.08 min and $t_{1/2}\beta$ = 26.5 min. Thirty min after s.c. injection, high radioactivity was found in the small intestine, liver, adrenals, kidneys and lungs of rats. About 40% of harmaline was bound to human serum or rat serum proteins *in vitro*. Blood levels, however, were low at all times in rats, suggesting a rapid turnover and elimination rate. The major route of excretion of harmaline and its metabolites in rats was through the kidneys and the compound was found to undergo demethylation to form harmalol. During the first 8 h, unchanged harmaline in the urine amounted to about 25% of the dose; however, this decreased to only 7% during the 8-24 h period.

Toxicity

The toxicity of harmala alkaloids has been evaluated (16, 26, 30). In acute toxicity tests in mice, central nervous system symptoms such as convulsions and tremor were observed, with LD_{50} values for total alkaloids of 289 mg/kg i.g., 56 mg/kg i.v. and 144 mg/kg i.p. Toxicity tests in rats showed that the total alkaloids given at a dose of

Table VIII: Therapeutic effect of total harmala alkaloids in patients with various tumors.

Tumor	Response Good	Partial	No.	Total
Esophagus carcinoma	2	10	1	13
Gastric carcinoma	2	2	0	4
Colon carcinoma	0	1	0	1
Lymphoma	0	1	2	3
Total	4	14	3	21

300 mg/kg once daily for 28 days induced nephrotoxicity, which was reversible upon drug discontinuation. In rats, the total alkaloids given at lower doses (60 and 134 mg/kg) produced no apparent changes in body weight, organ weight or laboratory tests for hematology, serum chemistry, urinalysis and ECG. No toxic effects on the hemopoietic system were found.

Clinical studies

Based on the results of toxicity studies in animals, the total harmala alkaloids were recommended for clinical trials. Of 21 patients with various tumors, 4 had a good response and 14 had a partial response, with an overall efficacy rate of 85.7% (12) (Table VIII). Symptoms in patients also improved after treatment, and no significant toxic effects were reported.

Conclusions

The antineoplastic activity of harmala alkaloids has been extensively investigated in China. The results of experimental and clinical studies suggest that harmala bases have a wide spectrum of antitumor action although the sensitivity of various tumors to the alkaloids may differ. Several points should be considered regarding harmala alkaloids. First, although harmala alkaloids produce low toxicity, high doses should be carefully administered since the range between therapeutic and toxic doses is relatively small. Second, toxicity tests showing that long-term treatment with high doses of total alkaloids can produce reversible nephrotoxicity indicate that the kidney may be the toxic target organ of these alkaloids. This may be of practical clinical significance. During administration, renal function should be periodically monitored and those patients with poor renal function should be more administered the harmala alkaloids with caution. Third, the protective effect of total alkaloids on peptic mucosa membrane suggests that they are of particular value in the control of cancers of the digestive tract. Finally, since the inhibitory effect of total alkaloids on tumor growth is related to cell cycle accumulation at G_2 phase, harmala alkaloids may be cell cycle-specific. Taken together, these data provide a useful reference for clinical combination chemotherapy of various tumors.

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