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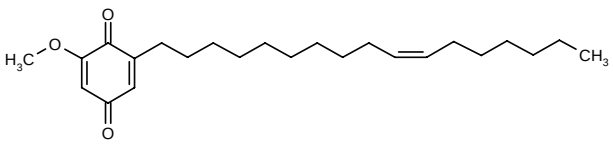
Irisquinone

Antineoplastic
Radiosensitizer

Irisquinone A
Iq-7611

2-[10(*Z*)-Heptadecenyl]-6-methoxy-1,4-benzoquinone



$C_{24}H_{38}O_3$ Mol wt: 374.5612

CAS: 056495-82-0

EN: 229421

Introduction

Treatment of tumors with either radiotherapy or chemotherapy alone has generally given poor results. Combination treatment using both strategies has been evaluated and some investigators have reported a 5-year increase in survival rates compared to radiation or chemotherapy alone (1). Irisquinone is a quinone derivative which was isolated from the seed coating of *Iris pallasii Fisch. var. chinensis Fisch.* and the seed oil of *Iris pseudacorus L*. (Iridaceae) (2, 3). The compound has also been synthesized from orthe-vanillin via a seven-step process (4). Irisquinone has been shown to have significant inhibitory effects on tumor growth in experimental animals. Furthermore, data have inidicated that a combination of irisquinone and radiation may be more effective in the treatment of cancer than either of the modalties alone.

Antineoplastic Effects

The seeds of *Iris pallasii Fisch.* have been used for cancer therapy in Chinese folk remedies. Studies in mice have shown that intraperitoneal irisquinone was effective against uterocervical carcinoma U14, hepatoma and Ehrlich ascites carcinoma (EAC), and both intraperitoneal and oral irisquinone was effective against lymphosarcoma (5) (Table I).

Table I: Effects of irisquinone on mouse tumor growth.

Tumor	Dose (mg/kg)	Route	Treatment schedule	% Growth inhibition
U14	3	i.p.	d1-d14*	40.5
U14	7	i.p.	d1-d14	55.5
Lymphosarcoma	3	i.p.	d1-d10	33.3
Lymphosarcoma	200	p.o.	d1-d10	41.8
Hepatoma (solid)	3	i.p.	d1-d10	29.0
Hepatoma (ascites)	5	i.p.	d1-d7	158.0**
EAC	5	i.p.	d1-d7	83.3**

U14 = mouse uterocervical carcinoma. EAC = Ehrlich ascites carcinoma. *Day after tumor implantation. **Increase of life span (%).

Radiosensitizing Effects

In addition to its antitumor activity, irisquinone also acts as a radiosensitizer. The cytotoxic effects of local radiotherapy against mouse uterocervical carcinoma U14 were found to be potentiated by *in vivo* treatment with irisquinone (5) (Table II). Similar results were obtained in mouse breast cancer cell line Ma737. No effects on tumor growth were observed in animals treated with irisquinone (200 mg/kg p.o.) alone. Treatment with irisquinone in combination with radiation (1500 rad) was more effective than radiation alone, with a sensitive enhancement ratio (SER) of 1.25 (6).

The combined effects of multiple doses of irisquinone and radiation on mouse breast cancer Ma737 growth were also investigated. Irisquinone was given orally at 250 mg/kg on days 10, 12, 14 and 16 after tumor implantation; 18 h after treatment with irisquinone, radiation was administered at 500 rad/mouse for 4 times. As shown in Table III, the radiosensitizing effects of irisquinone were significant. The tumor growth delay (TGD) in the combination treatment group was 16 days compared to 11 days in the radiation alone group. These results indicated that irisquinone given at multiple doses in combination with fractionated radiation enhanced the radiosensitivity of tumors in mice (7).

A dose-effect relationship study of mouse breast cancer cell growth showed that the 500 mg/kg p.o. dose of irisquinone in combination with radiation (1500 rad) had

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Table II: The combined effects of irisquinone with radiation on the growth of mouse uterocervical carcinoma U14 in vivo.

Group	Day 13		Day 17		Day 20	
	Weight (g)	% Inhibition	Weight (g)	% Inhibition	Weight (g)	% Inhibition
Control	1.55	–	1.67	–	2.51	–
Radiation	1.19	23.2	1.08	35.3	1.40	44.2
Irisquinone	0.95	38.7	1.15	31.1	1.87	25.5
Combined	0.76	50.9	0.64	61.7	1.00	60.2

Table III: The combined effects of irisquinone and radiation on mouse breast carcinoma Ma737 growth in vivo.

Group	Treatment schedule (dose x time)	Tumor growth (mm^3)			
		Day 0*	Day 10	Day 20	Day 25
Control	–	152	1239	2260	3129
Radiation	500 rad x 4**	143	883	1913	2617
Irisquinone	250 mg/kg x 4	135	1069	2238	2765
Combined	500 rad + 250 mg/kg x 4	137	851	1720	2278

*Day after therapy. **Irisquinone or radiation was given on days 10, 12, 14 and 16 after tumor implantation. Radiation was given 18 h after oral administration of irisquinone.

Table IV: Effects of radiation in combination with various doses of irisquinone on mouse breast carcinoma Ma737 growth in vivo.

Group	Dose	Tumor growth (mm^3)			
		Day 0*	Day 10	Day 15	Day 20
Control	–	128	1301	2087	3154
Radiation	1500 rad	140	861	1353	2286
Irisquinone	250 mg/kg	122	1490	2373	4429
Irisquinone	500 mg/kg	100	1529	2554	3474
Irisquinone	1000 mg/kg	116	1236	2455	3636
Combined	250 mg/kg + 1500 rad	150	1002	1585	2272
Combined	500 mg/kg + 1500 rad	146	812	1191	1582
Combined	1000 mg/kg + 1500 rad	138	739	1168	1921

*Day after therapy. Radiation was given 18 h after oral administration of irisquinone.

Table V: The combined effects of irisquinone and radiation on hypoxic tumor cells.

	HeLa cells *in vitro*		Ma737 tumor *in vivo*	
	SER	SER/OER	SER	SER/OER
Oxic	1.17	0.39	1.10	0.65
Hypoxic	1.68 (1.73)	0.57 (0.55)	1.30	0.76

SER = sensitizing enhancement ratio. OER = oxygen enhancement ratio. Values for reference compound misonidazole are given in parentheses.

greater inhibitory effects than the other combinations studied (8) (Table IV).

The radiosensitizing effects of irisquinone on hypoxic tumor cells *in vivo* have been investigated. Mouse breast carcinoma cells were made hypoxic by clamping the tumor-bearing leg for 5-8 min. The SER of irisquinone was larger on the hypoxic tumor than on the oxic tumor (1.3 *vs.* 1.1). No radiosensitizing effects on foot skin were observed (9) (Table V). Irisquinone was also shown to increase the radiosensitivity of hypoxic human uterocervical carcinoma HeLa cells. Hypoxia was induced *in vitro* by inflating nitrogen into HeLa cell suspensions. The SER values of irisquinone (0.1 mM) under hypoxic and oxic conditions were 1.68 and 1.17, respectively, indicating that the compound has selective radiosensitizing effects. As a positive control, the SER of misonidazole (0.1 mM) was 1.73. The ratios of SER to oxygen enhancement ratio (OER) were 0.57 and 0.55 for irisquinone and misonidazole, respectively, demonstrating that the radiosensitizing effects of the two drugs were similar(10) (Table V).

In a study in nude mice, the growth inhibitory rate and tumor growth delay of combined irisquinone (500 mg/kg p.o.) and radiation (1000 rad) against hypoxic human intestinal mucoadenocarcinoma were significantly higher than those of radiation or irisquinone alone (11) (Table VI).

Mechanism of Action

Irisquinone has demonstrated direct cytotoxic effects against EAC cells, as seen by damage to the nuclei of cancer cells after drug treatment. Moreover, the mitosis rate of cancer cells was also inhibited (5). Irisquinone (20

Table VI: Radiosensitizing effects of irisquinone against hypoxic human intestinal mucoadenocarcinoma in nude mice.

Tumor	Dose	% Growth inhibition	TGD (day)
Radiation	1000 rad	34.1	1
Irisquinone	500 mg/kg	19.9	11
Combined	1000 rad + 500 mg/kg	68.5	19

TGD = tumor growth delay. Irisquinone was given orally 24 h after radiation.

and 40 μg/ml) also induced DNA single-strand break in L1210 leukemia cells *in vitro*. These actions may be responsible for the compound's antitumor and radiosensitizing effects.

The effects of irisquinone on oxygen consumption of murine leukemia P388 cells were investigated *in vitro* by using oxygen cathod electrode. The results showed that irisquinone (100 μg/ml) significantly inhibited the respiration of P388 cells and decreased the rate of oxygen consumption (Table VII). These findings were supported by ultrastructure observation of EAC cells, in which damage to the mitochondria was observed (12).

Glutathione in tumor cells has been shown to decrease radiation killing capacity by capturing free radicals. The effects of irisquinone on glutathione content in HeLa cells *in vitro* were investigated. Results showed that irisquinone decreased glutathione content more significantly under hypoxic conditions than under oxic conditions (Table VIII), which may also contribute to the drug's radiosensitizing effect (13).

The effects of irisquinone on the cell cycle of EAC cells were examined by microspectrophotometry. Results showed that irisquinone arrested EAC cells in the G1-phase, indicating that the compound is cell-cycle specific (14).

The immunostimulatory effects of irisquinone have been investigated in mice and humans. The delayed-type hypersensitivity reaction induced by dinitrochlorobenzene in normal mice and mice bearing uterocervical carcinoma U14 was found to be increased after oral and i.p. treatment with irisquinone (15) (Table IX). In clinical trials, irisquinone was shown to enhance the depressed immune functions of patients with various tumors (15). The immunostimulatory effects of irisquinone, therefore, may be associated with its antitumor and radiosensitizing effects.

Toxicology

In studies in mice, the LD_{50} values of i.p. and p.o. irisquinone were 5.4 and 2800 mg/kg, respectively. LD_{50}/ED_{50} values were 5.0 mg/kg (i.p.) for mouse uterocervical carcinoma U14 and 13.9 mg/kg (p.o.) for mouse lymphosarcoma. In dogs, oral administration of irisquinone at 4-16 mg/kg/day for 14 days caused no significant changes in body weight, hematology, serum chemistry, urinalysis or ECG tests. There were also no histopathological changes in vital organs including the liver, heart, kidney, lung, spleen, thymus and brain. Similar results were obtained in rats. No bone marrow toxicity was observed and allergic test in guinea pigs was negative (5).

Hereditary tests showed no mutagenic effects of the drug (16).

Clinical Studies

Preliminary results from clinical trials have shown that irisquinone alleviates the symptoms of some patients suffering from acute leukemia, as well as lung, liver, gastric and esophageal carcinomas (5).

The radiosensitizing effects of irisquinone in the treatment of various tumors have been evaluated in multicenter phase III clinical trials (Table X).

Table VIII: Effects of irisquinone on glutathione content in HeLa cancer cells in vitro.

Group	Concentration (mM)	Glutathione (μg/10^6 cells)	
		Oxic	Hypoxic
Control	–	1.94	1.19
Irisquinone	0.1	1.43	0.62

Table IX: Effects of irisquinone on delayed-type hypersensitivity reaction in mice.

Group	Dose (mg/kg)	Route	% DTH enhancement	
			Normal	U14-bearing
Irisquinone	5	i.p.	159.6	114.5
Irisquinone	300	p.o.	ND	350.3
Levamisole*	0.5	i.p.	109.2	301.4
Levamisole	100	p.o.	ND	85.7

*Reference compound. ND = not determined.

Table VII: Effects of irisquinone on oxygen consumption of murine leukemia P388 cells in vitro.

Group	Oxygen consumption rate (%)							
	2 min	4 min	6 min	8 min	10 min	12 min	14 min	16 min
Control	2.2	14.1	37.9	62.3	78.3	88.6	95.7	100.0
Irisquinone	1.6	4.3	6.2	10.3	24.9	37.4	47.7	56.9

Table X: Therapeutic effects of irisquinone combined with radiation in patients with tumors.

	Total response rate (%)		
Tumor	Combined	Radiation	SER
Lung carcinoma	70	41	2.10
Esophageal carcinoma	81	53	1.74
Superficial metastatic cancer	52	35	1.70
Bone metastatic cancer	93	72	?

In patients with lung cancer, combination treatment with irisquinone and radiation resulted in significant increases in survival, with 1-, 2-, 3- and 5-year survival rates of 59, 27, 25 and 24%, respectively, compared to 30, 8.2, 0 and 0%, respectively, for radiation alone. The recurrence rate at 5 years was also decreased by irisquinone (17, 18).

The radiosensitizing effects of irisquinone on esophageal, superficial metastatic, breast and bone metastatic cancers have also been evaluated in clinical trials. Bone marrow suppression caused by radiation in some patients was alleviated by combination treatment with irisquinone. Anorexia and nausea were observed in some patients but did not require interruption of treatment (19, 20).

Conclusions

Since the 1970s, radiosensitizers have been synthesized and introduced into clinical trials for the treatment of cancer. However, serious toxicity with these agents limits their practical application. For example, misonidazole has been associated with CNS toxicity. Development of novel radiosensitizers with better therapeutic effects and fewer side effects, therefore, is still necessary. In animal experiments and clinical trials, irisquinone administered by different routes and treatment schedules exhibited broad-spectrum antitumor and radiosensitizing effects with low toxicity. In particular, no mutagenic or myelosuppressive effects were found with the agent. Since the radiosensitizing effects of irisquinone are enhanced under hypoxic conditions, the compound may be developed as a new antitumor drug and radiosensitizer of hypoxic tumor cells.

Studies using the modified alkaline elution method have shown that irisquinone inhibited the rejoining of DNA single-strand breaks induced by bleomycin in L1210 leukemia cells (21), indicating that the compound may have chemotherapeutic sensitizing effects. The thermosensitizing effects of irisquinone have been demonstrated *in vitro* in HeLa cells and *in vivo* in mouse U14 carcinoma (22), which may be useful in clinical practice with irisquinone.

Considerable interest in finding new radiosensitizers of natural origin has been stimulated in China ever since the isolation and structural elucidation of irisquinone were reported in the 1970s. Several quinone compounds, including Iq-7612 and Iq-7613, have been isolated from *Iris pallasii Fisch.* (Iridaceae) and have shown significant antitumor and radiosensitizing effects (23, 24). Further pharmacological and toxicological studies on these compounds are expected to be performed in the near future.

Sources

Tianjin Inst. of Materia Medica (CN), Shandong Xinghua Pharmaceutical Factory (CN) and Shanghai Inst. of Pharmaceutical Industry, Shanghai (CN).

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