ACTIVITY OF SAFRACINS A AND B, HETEROCYCLIC QUINONE ANTIBIOTICS, ON EXPERIMENTAL TUMORS IN MICE

TAKEKI OKUMOTO, MARIKO KAWANA, IKURO NAKAMURA,
YOSHIFUMI IKEDA and KATSUYOSHI ISAGAI

Research Laboratories, Yoshitomi Pharmaceutical Industries Ltd.,
3-7-25 Koyata, Iruma-shi, Saitama 358, Japan

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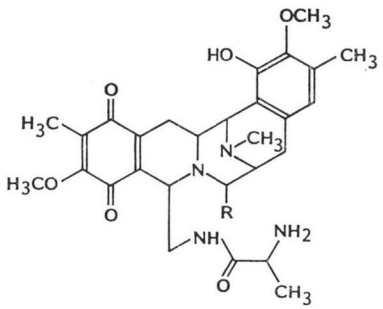
Safracins A and B, new antibiotics produced by *Pseudomonas fluorescens* A2-2, were tested for antitumor activity against mouse tumors. Structurally, these antibiotics belong to the saframycin family of antibiotics, and safracin B is 21-hydroxysafracin A. They showed antitumor activity against L1210 and P388 leukemias and B16 melanoma. The toxic and effective doses of safracin B were much lower than those of safracin A. Safracin B also prolonged the life span of tumor-bearing mice to a greater extent than safracin A. These results indicate that the α-carbinolamine structure plays an important role in the antitumor action of this type of antibiotic. Both safracins were, however, ineffective when their administration route differed from that used for inoculating tumor cells.

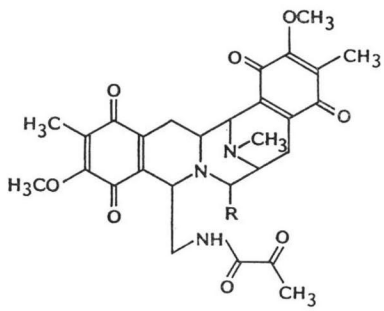
Two new antibiotics, safracins A and B, have recently been isolated from the culture broth of *Pseudomonas fluorescens* A2-2.[1,2] As shown in Fig. 1, these antibiotics have novel structures closely related to saframycins which have been shown to possess antitumor activity on mouse tumors,[3~6] and differ structurally in the following respects from saframycins; the former has only one heterocyclic quinone and the L-alanyl moiety at the 23 position, while the latter has two heterocyclic quinones and the pyruvyl group at the 23 position. Except for the hydroxyl group at the 21 position, safracin B is identical to safracin A.

As with many antitumor drugs,[7,8] safracins A and B have been shown mutagenic in the Ames system (M. TAKEUCHI *et al.*, unpublished data), suggesting that they have an ability to induce cellular DNA damage. They have also been shown to induce morphological changes in *Escherichia coli* cells, and to possess antitumor activity in preliminary studies.[9] In this paper, the antitumor activity of

Fig. 1. Structures of safracins and saframycins.
R=H: Safracin A or saframycin B.
R=OH: Safracin B or saframycin S.



Safracin



Saframycin

safracins A and B against L1210 and P388 leukemias, B16 melanoma and Lewis lung carcinoma in mice is presented.

Materials and Methods

Antibiotics

Safracins A and B isolated from *P. fluorescens* A2-2 cultures were prepared as $2HCl \cdot H_2O$ salts. Their isolation and chemical properties have been reported previously.[1,2] Safracins were dissolved in 0.9% NaCl solution just prior to use. Drug solutions were given to animals at 0.01 ml/g body weight.

Animals and Tumors

Female BALB/c×DBA/2 F_1 (hereafter called $CD2F_1$) mice weighing 19~24 g, male C57BL/6× DBA/2 F_1 (hereafter called $B6D2F_1$) mice weighing 18~23 g and male C57BL/6 mice were obtained from Charles River Japan, Inc., Kanagawa, Japan. Animals were given pelleted feed and water *ad libitum*. Tumors used were maintained by serial transplantation: Leukemias were inoculated ip into $CD2F_1$ mice; B16 melanoma and Lewis lung carcinoma were implanted sc into C57BL/6 mice.

Antitumor Studies

$CD2F_1$ mice were implanted ip, sc or iv with 0.2 ml of diluted ascitic fluid containing 10^5 L1210 or 10^6 P388 cells. $B6D2F_1$ mice received ip or sc implants of B16 melanoma homogenates prepared by the method of GERAN *et al.*[12] or 10^6 Lewis lung carcinoma cells. The iv and sc routes used are as follows: The tail vein for the iv route; sc injection of leukemias and antibiotics in the back, and sc implantation of other tumors in the right axillary region of mice. Each group consisted of 5~11 mice. Control animals received no vehicle. After tumor implantation, survival days of tumor-bearing animals were recorded, and perpendicular diameters of sc-implanted B16 melanoma and Lewis lung carcinoma were measured on day 20. T/C values were calculated for either mean or median of survival time and mean tumor weight estimated from tumor diameters,[12] where T and C are the value of the treated and untreated groups, respectively. Safracins were considered active, if they produced T/C values *of* $\geqq 125\%$ for survival systems, and $\leqq 42\%$ for tumor-weight inhibition.

Acute Toxicity Determination

Groups of 10 female $CD2F_1$ mice were injected ip with antibiotics. The LD_{50} values were calculated by the method of LITCHFIELD-WILCOXON[11] based on mortality 2 weeks after administration.

Results

The LD_{50}'s of safracins in normal $CD2F_1$ mice by ip administration are shown in Table 1. The LD_{50}'s were 196 mg/kg with safracin A and 7.3 mg/kg with safracin B. In the region of these doses, death occured within 3 days after administration of safracin A and in 2 to 8 days with safracin B. The main toxic signs before death were weight loss and emaciation.

Tables 2 and 3 show the effect of safracins administered ip on the life span of mice inoculated ip with P388 and L1210 leukemias, respectively. These antibiotics were administered ip to mice bearing P388 leukemia on the following 4 different schedules; a single dose on day 1 only, every 4th day on days 1, 5 and 9, and daily on days 1~5 and 1~9. In therapy of P388 leukemia, a single dose of safracin A or B on day 1 only gave T/C values of 133% or lower. On other schedules, the maximum T/C values were 140~159% with 50 or 100 mg/kg/day safracin A and were 161~

Table 1. LD_{50}'s of safracins A and B in female $CD2F_1$ mice.

Antibiotic	LD_{50} in mg/kg (range, $P=0.05$)
Safracin A	196 (180~214)
Safracin B	7.3 (6.1~8.7)

Safracins were given ip.

Table 2. Effect of safracins administered ip on the life span of mice inoculated ip with P388 leukemia.

Antibiotic	Dose (mg/kg/day)	T/C (%) on the following schedules			
		Day 1 only	Every 4th day on days 1, 5 and 9	Daily on days 1~5	Daily on days 1~9
Safracin A	5	118	118	135	146
	10	114	118	142	142
	25	112	123	147	149
	50	112	127	149	159
	100	127	140	Toxic	Toxic
	250	Toxic	Toxic		
Safracin B	0.005				121
	0.01				128
	0.025			130	138
	0.05	110	123	128	142
	0.1	108	122	139	149
	0.25	115	139	161	165
	0.5	124	144	161	197
	1	128	165	161	179
	2.5	133	Toxic	Toxic	Toxic
	5	Toxic			

No mice survived over 30 days after inoculation. Median survival times of control groups were 10.4~11.5 days.

Table 3. Effect of safracins administered ip on the life span of mice inoculated ip with L1210 leukemia.

Antibiotic	Dose (mg/kg/day)	Mean survival time (days)	T/C (%)
Safracin A	0	7.2	100
	5	8.4	117
	10	8.6	119
	25	8.8	122
	50	9.0	125
	100	7.4	103
	250	2.2	Toxic
Safracin B	0	7.7	100
	0.05	8.6	112
	0.1	9.2	119
	0.25	10.2	132
	0.5	11.0	143
	1	12.2	158
	2.5	5.0	Toxic

Antibiotics were given daily on days 1~5. No mice lived over 30 days after inoculation.

Table 4. Effect of safracins administered ip on the life span of mice inoculated ip with B16 melanoma.

Antibiotic	Dose (mg/kg/day)	Median survival time (days)	T/C (%)
Safracin A	0	21	100
	5	26	124
	10	28	133
	25	31	148
	50	28	133
	100	6	Toxic
Safracin B	0	17	100
	0.05	24	141
	0.1	27	159
	0.25	30	176
	0.5	34	200
	1	13	Toxic

Safracins were given daily on days 1~9. No mice survived over 50 days after inoculation.

197% with 0.5 or 1 mg/kg/day safracin B.

Safracins were found to be active against L1210 leukemia, but this leukemia was somewhat less sensitive to these antibiotics than P388 leukemia. Namely, the maximum T/C values achieved by daily doses on days 1~5 were 125% with safracin A and 158% with safracin B.

Table 4 shows the effect of safracins administered ip on the life span of mice inoculated ip with B16 melanoma. Safracins were given once daily on days 1~9. Safracin B was also more effective than

Table 5. Effect of safracins administered sc on the life span of mice implanted sc with P388 leukemia.

Antibiotic	Dose (mg/kg/day)	Median survival time (days)	T/C (%)	50-Day survivors
Safracin A	0	15.8	100	0/11
	10	24.5	155	1/5
	25	28.5	180	2/5
	50	>49.8	>315	3/5
	100	37.5	237	2/5
	250	3.1	Toxic	0/5
Safracin B	0	15.8	100	0/11
	0.1	23.2	147	0/5
	0.25	28.0	177	0/5
	0.5	24.5	155	1/5
	1	>50.0	>316	4/5
	2.5	>50.0	>316	4/5

Antibiotics were given every 4th day on days 1, 5 and 9 in the vicinity of the implantation site.

safracin A in therapy of this tumor. Maximum T/C vaiues were 148% for safracin A and 200% for safracin B.

Table 5 shows the effect of safracins administered sc against P388 leukemia implanted sc. More than 50% of leukemic mice lived over 50 days following sc administration on days 1, 5 and 9.

In the case where the same iv route was used for antibiotics and P388 leukemia cells, the safracins had no effect even when administered as little as 5 minutes after tumor implantation (data not shown).

Although data are not shown, neither significant prolongation of the life span of tumor-bearing mice nor inhibition of growth of tumors was obtained with safracins when different routes were used for drug administration and tumor implantation (ip administration for sc-implanted B16 melanoma and Lewis lung carcinoma, and iv, sc and oral administration for ip-implanted leukemias). In these experiments, 10~250 mg/kg/day safracin A and 0.1~5 mg/kg/day safracin B were given daily on days 1~5 or 1~9. The highest doses were lethal when administered parenterally, but not orally.

Discussion

The present studies demonstrate that safracins A and B, new antibiotics isolated from *P. fluorescens* A2-2 cultures, are active against the mouse tumors, L1210 and P388 leukemias and B16 melanoma. Interestingly, toxic and therapeutic doses of safracin B were much lower than those of safracin A, in spite of the fact that they are very closely related analogs, *i.e.*, safracin B is 21-hydroxysafracin A (Fig. 1). In addition, safracin B was more effective in increasing the life span of tumor-bearing mice than safracin A. These results are consistent with the importance of the α-carbinol structure for antitumor activity documented with other antitumor antibiotics.[5,6,10]

When safracins were given ip to mice implanted ip with P388 leukemia, intermittent (every 4th day on days 1, 5 and 9) or daily (days 1~5 and 1~9) treatments were more effective than a single dose on day 1 only for prolonging their life span. Accordingly, further studies on activity of safracins were made with these intermittent or daily dosage schedules. Safracins administered *via* the same route for tumor implantation were effective against ip-implanted B16 melanoma or sc-implanted P388 leukemia. However, these antibiotics were found to be inactive in the experimental systems when different routes of injection were used for antibiotics and tumor cells (ip administration for sc-implanted tumors, and iv, sc and oral doses for ip-inoculated tumors). These results may imply that safracins do not distribute well into tissues away from the site of administration. In the case where the same iv route of injection for antibiotics and cells was used, safracins did not increase the life span of mice bearing P388 leukemia even when administered immediately after cell implantation. This is probably due to a rapid decrease

in the safracin level in the blood after iv administration. To clarify the reasons for this route dependency, pharmacokinetic studies in animals are under way.

As mentioned above, structurally safracins belong to saframycin family of antibiotics.[3,4] The present results, together with the findings of ARAI *et al.*,[5,6] indicate that safracin B is almost as effective therapeutically as the most active saframycins against mouse tumors such as L1210 and P388 leukemias.

ARAI *et al.*[5,6] have proposed that 14-methoxysaframycin B and 21-hydroxysaframycin B (saframycin S) differ in their mode of action: For the latter it involves formation of an antibiotic-DNA complex through a reaction of the α-carbinolamine with guanine moiety of cellular DNA; the former has no such active site, though it possesses significant but less potent antitumor activity. Similarly, safracin A and 21-hydroxysafracin A (safracin B) seem to differ in the mode of action, because safracin A exhibited an exponentially decreasing dose-survival curve following a shoulder region at lower concentrations whereas safracin B showed a biphasic curve like bleomycin.[13,14] Furthermore safracin B preferentially inhibited RNA synthesis, while safracin A inhibited DNA, RNA and protein syntheses nonspecifically in Chinese hamster ovary cell cultures (T. OKUMOTO *et al.*, unpublished data).

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