Antitumor Activity of Brasilicardin A, a Novel Terpenoid Antibiotic from *Nocardia brasiliensis*

HISAYUKI KOMAKI[a,c], YASUSHI TANAKA[a], KATSUKIYO YAZAWA[b], HIROAKI TAKAGI[a,c], AKIKAZU ANDO[c], YOSHIHO NAGATA[c] and YUZURU MIKAMI[b,*]

[a] R & D Department, Higeta Shoyu Co., Ltd., 2-8, Chuo-cho, Choshi, Chiba 288-8680, Japan
[b] Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba 260-8673, Japan
[c] Department of Biotechnology, Graduate School of Science and Technology, Chiba University, 648, Matsudo, Matsudo, Chiba 271-8510, Japan

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During our continued search for bioactive metabolites from pathogenic microorganisms, we isolated and reported a novel tricyclic diterpenoid antibiotic, brasilicardin A (Fig. 1), from a strain of *Nocardia brasiliensis*[1,2]. In addition to the potent immunosuppressive activity of brasilicardin A in a mouse mixed lymphocyte reaction (MLR) assay system[1,2], our recent studies indicated that the antibiotic shows an interesting antitumor spectrum. In this paper, we report its *in vitro* and *in vivo* antitumor activity.

Brasilicardin A was prepared from a culture broth of *N. brasiliensis* IFM 0406 as described previously[1]. IC_{50} values against various cell lines were determined and compared with those of adriamycin. P388/ADR, P388, L1210 (mouse leukemia), P815 (mouse mastocytoma), CCRF-CEM, HL60, Jurkat, MOLT-4 (human leukemia), HeLa (human cervix carcinoma), KB (human oral epidermoid carcinoma), HEK-293 (human embryonic kidney) and COS-1 (SV40 transformed monkey kidney) cell lines were used in the present studies. Cytotoxicity assays were performed in a 96-well microplate; each well contained 10^4 cells and a variable amount of the test compounds in 0.2 ml of medium. The cells were cultured for 3~4 days and the cytotoxic activity was determined by the MTT colorimetric assay[3].

The IC_{50} values for brasilicardin A varied depending on the cell lines tested, ranging from 0.078 to 100 μg/ml (Table 1). P388 and P388/ADR cell lines were highly susceptible to brasilicardin A, with IC_{50} values of 0.22 and 0.078 μg/ml, respectively. Interestingly, the P388/ADR cell line was the most susceptible cell line to the antibiotic, followed by the parent P388 cell line. HeLa, MOLT-4 and CCRF-CEM cell lines belonged to less susceptible groups.

Table 1. *In vitro* cytotoxic activities.

cell line	IC_{50} (μg/ml)	
	brasilicardin A	adriamycin
P388/ADR*	0.078	1.0
P388	0.22	0.050
L1210	1.2	0.18
P815	11	0.14
CCRF-CEM	25	0.072
HL60	0.40	0.014
Jurkat	5.6	0.090
MOLT-4	29	0.037
HeLa	100	0.15
KB	3.2	0.69
HEK-293	6.3	0.057
COS-1	8.6	0.18

* Adriamycin-resistant P388

Fig. 1. Chemical structure of brasilicardin A.

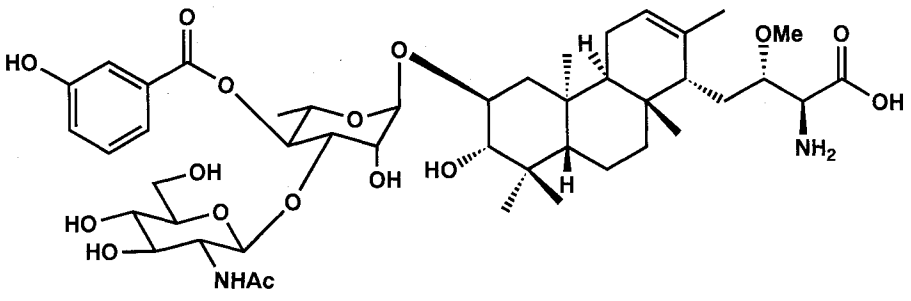

Table 2. *In vivo* antitumor activities on P388 or P388/ADR leukemia bearing mice.

implanted cell line	compound	dose[a] (mg/kg)	MST (SD)[b] (days)	T/C (%)	body weight on day 10 (g)
P388	control	-	10.4 (0.97)	100	24.8
	brasilicardin A	1	12.7 (2.28)**	123	25.9
		5	12.7 (2.75)*	123	26.3
		10	13.2 (2.22)**	128	27.3
	adriamycin	1	12.4 (4.58)**	120	23.2
		5	8.4 (1.84)	81	15.5
P388/ADR	control	-	11.1 (0.32)	100	26.8
	brasilicardin A	1	14.4 (2.46)**	130	26.2
		5	14.8 (3.35)**	133	27.3
		10	18.3 (3.17)**	165	26.4
	adriamycin	1	10.7 (1.75)	96	23.5
		5	7.0 (2.17)	63	-

[a] Each compound was administered intraperitoneally from day 0 to 9.
[b] Mean survival time (standard deviation)
* $p<0.05$ by t-test, *vs.* control.
** $p<0.01$ by t-test, *vs.* control.

On the other hand, adriamycin was active against all cell lines tested except for adriamycin resistant P388/ADR. To confirm the *in vitro* cell line specific activity of brasilicardin A against P388/ADR tumor cells, the antitumor activities of brasilicardin A against P388 and P388/ADR in mice were tested and compared with those of adriamycin. P388 or P388/ADR cells were implanted intraperitoneally (ip) at 5×10^5 cells/mouse into CDF_1 mice aged 6 weeks (weighting about 17 g). The treatment started 3 hours after the tumor implantation. Each test compound was administered ip from day 0 to day 9. Brasilicardin A showed a significant prolongation of mean survival time (MST) against P388 leukemia at doses of 1, 5 and 10 mg/kg for 10 consecutive days, giving T/C values of 123, 123 and 128%, respectively (Table 2). The antibiotic exhibited a remarkable prolongation of MST against P388/ADR, and their T/C values at the concentrations of 1, 5 and 10 mg/kg were 130, 133 and 165%. Although adriamycin showed antitumor activity against P388 tumor cells, no detectable antitumor effect against P388/ADR tumor cells was observed. These results indicated brasilicardin A is highly active against P388/ADR *in vivo* as well as *in vitro*. Furthermore brasilicardin A was found to be less toxic at effective doses such as 10 mg/kg because no body weight decrease was observed (Table 2).

It has been reported that the multidrug resistant mechanism of P388/ADR is due to the expression of P-glycoprotein, which acts by pumping antitumor agents out of cells[4]. Many immunosuppressive agents are known to reverse the multidrug resistance by inhibiting the activity of P-glycoprotein[5,6]. Since our previous studies showed that brasilicardin A has immunosuppressive activity, we are interested in the effect of brasilicardin A on P-glycoprotein, which was evaluated by monitoring its effect on the IC_{50} values of adriamycin against P388/ADR. Verapamil and cyclosporin A (CyA) were used as positive controls of P-glycoprotein inhibition. P388/ADR cells were cultured with various concentrations of adriamycin in the presence of brasilicardin A, verapamil or CyA for 72 hours followed by MTT assay, and their effects on the susceptibility to adriamycin were observed. The IC_{50} of adriamycin was 1.0 μg/ml in the absence of these test compounds (Table 3). However, in the presence of verapamil at concentrations of 1 and 10 μg/ml, the IC_{50} values of adriamycin decreased to 0.23 and 0.084 μg/ml, respectively. CyA was also found to decrease the IC_{50} values to 0.17 and 0.050 μg/ml, respectively. On the other hand, a significant decrease in the IC_{50} values of adriamycin with brasilicardin A was not observed. Therefore, these results indicate that brasilicardin A does not reverse the multidrug resistance in P388/ADR.

It is not clear why brasilicardin A exhibited more potent activity against P388/ADR than P388 tumor cells. Our

Table 3. Comparison of reversing effect of brasilicardin A on adriamycin-resistance in P388/ADR tumor cells with those of P-glycoprotein inhibitors.

compound	conc. (μg/ml)	IC_{50} [a] (μg/ml)
control	—	1.0
brasilicardin A	0.01	0.84
	0.1	—[b]
verapamil	1	0.23
	10	0.084
cyclosporin A	1	0.17
	10	0.050

[a] IC_{50} value of adriamycin alone, or in combination with tested compound against P388/ADR.

[b] IC_{50} value was not determined due to the direct cytotoxic effect of brasilicardin A.

ongoing studies using various tumor cells with different resistance genes may help us understand the selective activity of brasilicardin A against P388/ADR cells. Our recent preliminary study indicated that brasilicardin A induces G_1-phase arrest of P388 cells *in vitro*. Therefore, it is reasonable to consider that brasilicardin A has different mechanisms of immunosuppressive activity from those of reference drugs such as CyA, although further detailed studies are necessary to understand the association between G_1-arrest activity of brasilicardin A and the unique narrow cytotoxic spectrum of the antibiotic.

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