ANTITUMOR ACTIVITY OF 13-METHYL-BERBERRUBINE DERIVATIVES

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The antitumor activity of several 13-alkyl-berberrubine and newly synthesized ester derivatives, using experimental tumors (S-180, NF sarcoma, Ehrlich carcinoma and L-1210) in mice was tested. For chemotherapeutic evaluation, the therapeutic indices and ratios were determined. Our present findings confirmed that in berberrubine derivatives, the 9-demethyl structure was essential for the manifestation of antitumor activity and demonstrated that ester derivatives also exhibit a remarkable antitumor activity, and the 13-methyl derivatives of both berberrubine and palmatrubine alkaloids showed high antitumor activity.

Keywords — 13-alkyl-berberrubine; 13-methyl-berberrubine 9-esters; 13-methyl-palmatrubine; antitumor activity; therapeutic index; therapeutic ratio

INTRODUCTION

Using the total packed cell volume (TPCV) method, we previously found that berberrubine (9-demethyl-berberine) derivatives exhibited remarkable antitumor activity against sarcoma-180 (S-180), and discussed the correlation between their chemical structure and antitumor activity.¹⁾ Our findings led us to conclude that the 9-demethyl structure of the alkaloid was essential for the manifestation of antitumor activity.

In the present investigation, we examined the antitumor activity of several 13-alkyl-berberrubine and newly synthesized ester derivatives, using experimental tumors (S-180, NF sarcoma, Ehrlich carcinoma and L-1210) in mice. We selected the highly active compounds and estimated their LD₁₀ values. For chemotherapeutic evaluation, the therapeutic indices and ratios were determined.

MATERIALS AND METHODS

Compound Samples—These were synthesized and kindly provided by Messrs. Y. Sawa and F. Shimada of the Kanebo Medical Laborato-

ries (Osaka). The administered sample dose ranged from 1000 mg/kg to 3 mg/kg; administration was intraperitoneal (*i.p.*) or peroral (*p.o.*), as indicated in the relevant illustrations.

TPCV Method^{1,2)}—Seven-day-old S-180 or Ehrlich carcinoma ascites (0.05 ml, approximately 10⁷ cells) was *i.p.* transplanted into female ddY mice weighing 20 ± 2 g, and 24 h thereafter, the *i.p.* administration of the respective compound in physiological saline was started and continued for 5 consecutive days. On day 7 the mice were sacrificed by cervical dislocation and the TPCV of the tumor cells was measured and the growth rate (T/C, %) was determined.

Solid Tumor Systems—S-180 Solid Tumor: Seven-day-old S-180 ascites (0.05 ml, approximately 10⁷ cells) was subcutaneously (*s.c.*) transplanted into the right groin of female ddY mice weighing 20 ± 2 g; 24 h thereafter, the *i.p.* administration of the respective sample in physiological saline was started and continued for 5 consecutive days. On day 10 the mice were sacrificed and the weight of the solid tumor in sample-treated mice was compared with that of the control to determine T/C %.

TABLE I. *Antitumor Effect of 13-Substituted Berberrubine and Palmatrubine Derivatives in S-180-Bearing Mice*

Compound	Dose						
	R ₁	R ₂	X	30 mg/kg T/C & Res.		10 mg/kg T/C & Res.	
Berberrubine (9-Demethyl-berberine)	H	—	—	1	+++	29	++
13-Methyl-berberrubine	CH ₃	—	—	0	+++	9	+++
13-Ethyl-berberrubine	C ₂ H ₅	—	—	38	++	62	+
13- <i>n</i> -Propyl-berberrubine	C ₃ H ₇	—	—	68	—		
13-Allyl-berberrubine	CH ₂ = CHCH ₂	—	—	117	—		
13-Ethoxycarbonyl- berberrubine	C ₂ H ₅ OCO	—	—	105	—		
13-Methyl-berberrubine phosphate	CH ₃	H	H ₂ PO ₄	0	+++	42	+
13-Methyl-9-acetyl- berberrubine chloride	CH ₃	CH ₃ CO	Cl	0	+++	2	+++
13-Methyl-9- (<i>p</i> -methoxybenzoyl)- berberrubine chloride	CH ₃	CH ₃ OC ₆ H ₄ CO	Cl	0	+++	78	—
13-Methyl-9-cinnamoyl- berberrubine chloride	CH ₃	C ₆ H ₅ CH= CH-CO	Cl	0	+++		
13-Methyl-palmatrubine (13-Methyl-9-demethyl- palmatine)	CH ₃	—	—	0	+++	53	+
13-Methyl-palmatrubine chloride	CH ₃	H	Cl	14	++	107	—
13-Methyl-palmatrubine phosphate	CH ₃	H	H ₂ PO ₄	8	+++	106	—
13-Methyl-2-demethyl- palmatrubine chloride	CH ₃	H	Cl	0	+++	18	+++

Legend: The respective samples in physiological saline were *i.p.* administered daily for 5 consecutive days starting 24 h after S-180 ascites (10^7 cells) transplant. The total packed cell volume (TPCV) was measured on day 7.

Criteria:

	—	+	++	+++
T/C%:	100—66	65—41	40—11	10—0

T = TPCV of sample-treated mice.

C = TPCV of untreated S-180 bearing controls.

berberrubine

palmatrubine

Nakahara-Fukuoka Sarcoma: A 20 mg piece of a 10-day-old solid tumor of Nakahara-Fukuoka (NF) sarcoma³⁾ was *s.c.* transplanted into the back of female ddY mice weighing 20 ± 2 g; 24 h thereafter, the *i.p.* administration of the respective sample in physiological saline was started and continued for 5 consecutive days. On day 10 the mice were sacrificed, the tumor was weighed and its growth rate (T/C%) determined.

Survival Studies—Seven-day-old leukemia L-1210 (1×10^6 cells) or S-180 (approximately 10^7 cells) ascites was *i.p.* transplanted into female BDF₁ mice weighing 19 ± 2 g or female ddY mice weighing 20 ± 2 g, respectively; 24 h thereafter, the *i.p.* administration of the respective sample in physiological saline was started and continued for 5 consecutive days. The average survival time of the treated mice was compared with that of the control mice to obtain the increase in life span (ILS) values.

RESULTS AND DISCUSSION

As reported in our previous paper,¹⁾ the 9-

demethyl structure of berberrubine is essential for the manifestation of antitumor activity. Among the 13-alkyl-berberrubine derivatives tested, 13-methyl-berberrubine exhibited the highest antitumor activity; 13-ethyl-berberrubine was slightly, and other alkyl derivatives were not active.

Therefore, 13-methyl-9-ester derivatives were synthesized and the antitumor activity was compared with that of berberrubine *per se*. Among the derivatives, 13-methyl-9-acetyl-berberrubine chloride exhibited remarkable antitumor activity. 13-Methyl-palmatrubine derivatives were also prepared; 13-methyl-palmatrubine and 13-methyl-2-demethyl-palmatrubine chloride exhibited strong antitumor activity.

Next, we selected the most highly active compounds against S-180 ascites tumor and tested their effects on solid S-180, NF sarcoma and L-1210. As shown in Table II, in solid S-180 tumor, the T/C of 13-methyl-9-acetyl-berberrubine chloride (70 or 50 mg/kg) was 66%; at

doses of 100 mg/kg, that of 13-methyl-berberrubine was 58% and that of 13-methyl-palmatrubine was 82%. While in this series of compounds tested, 13-methyl-9-acetyl-berberrubine chloride at doses of 70 and 50 mg/kg was active against solid NF sarcoma tumors, the other compounds exhibited a lower level of antitumor activity.

These compounds were also tested, using the TPCV method, for their antitumor effects following *p.o.* administration to S-180 ascites-bearing mice. As shown in Table II, the 5-day administration of 1000 mg/kg/d resulted in a 50% T/C for 13-methyl-berberrubine and 13-methyl-9-acetyl-berberrubine chloride, and in a 39% T/C for 13-methyl-berberrubine chloride. At doses of 500 mg/kg/d, their T/C values were

69%, 76% and 92%, respectively. These three most active compounds against S-180 ascites were tested for their ability to increase the life span of S-180 ascites or L-1210-bearing mice. The *i.p.* administration of 100 mg/kg 13-methyl-berberrubine resulted in a survival of more than 60 d in five of eight S-180 ascites-bearing mice; the ILS was greater than 300%. Similarly, three of eight S-180 ascites-bearing mice treated with 70 mg/kg/d 13-methyl-9-acetyl-berberrubine chloride survived for more than 60 d (ILS > 300%).

The therapeutic index represents LD_{10}/ED_{90} by the TPCV method, using S-180 and Ehrlich carcinoma ascites. Their LD_{10} values were estimated as follows: the respective sample was *i.p.* administered once a day for five consecutive

TABLE II. *Antitumor Effect of Berberrubine and Palmatrubine Derivatives in S-180, NF-Sarcoma or L-1210 Bearing Mice*

Compound	Tumor		Sarcoma 180										NF Sarcoma					L-1210		
	Method	Ascites (T.P.C.V.)	Solid (tumor weight)										Solid (tumor weight)					Ascites (survival)		
	Route	<i>p.o.</i>	<i>i.p.</i>										<i>i.p.</i>					<i>i.p.</i>		
	Dose mg/kg	1000	500	300	250	200	150	100	70	50	200	150	100	70	50	100	50	30		
13-Methyl-berberrubine (13-Methyl-9-demethyl-berberine)	50	69	125					58					72				18			
13-Methyl-berberrubine phosphate	47		76	91	109							79	69				18	14	6	
13-Methyl-berberrubine chloride	39	92		80	73	105					105		93				12		14	
13-Methyl-9-acetyl-berberrubine chloride	50	76	97						66	66				66	62		45	43	33	
Berberrubine chloride	103	117						102				45					61		28	
13-Methyl-palmatrubine		80						82			47		91				69		14	

Legend: The respective samples in physiological saline were administered as indicated for 5 consecutive days starting 24 h after S-180 (10^7 cells), NF sarcoma (20 mg) or L-1210 (1.0×10^6 cells) transplant. TPCV was measured on day 7; tumor weight was determined on day 10. The numerical values represent T/C%. Increase in life span (ILS, %) was determined by average survival time.

days to non-tumor bearing mice and the animals were observed for two weeks. The therapeutic ratio was obtained by dividing the optimal drug dose by that dose which gave an increased life span of 30% in L-1210-bearing BDF₁ mice.

For 13-methyl-berberrubine, the LD₁₀ was 135 mg/kg, the therapeutic index in S-180 was approximately 15; in Ehrlich carcinoma it was 6.5 and its therapeutic ratio was less than 1 in L-1210 (Table III). While 13-methyl-berberrubine phosphate exhibited higher therapeutic indices of 17 and 11 in S-180 and Ehrlich carcinoma, respectively, its therapeutic ratio was also less than 1 in L-1210. The LD₁₀ of 13-methyl-9-acetyl-berberrubine chloride was approximately 90 mg/kg. While its therapeutic indices in S-180 (11) and Ehrlich carcinoma (5) were slightly lower than those of 13-methyl-berberrubine,

this compound exhibited a higher therapeutic ratio (4) with L-1210 as shown in Table III.

The LD₁₀ of 13-methyl-palmatrubine was approximately 320 mg/kg; the therapeutic indices in S-180 and Ehrlich carcinoma were 16 and 10, respectively; in L-1210 the therapeutic ratio was 2.5. As the acetyl derivatives of 13-methyl-berberrubine were found to provide better results than the parent compound, 13-methyl-9-acetyl-palmatrubine was synthesized. However, it exhibited lower activity and proved to be inferior to the parent compound.

Furthermore, the ED₉₀ values of 13-methyl-berberrubine derivatives were equal to or lower than those of berberrubine *per se* in S-180 and Ehrlich carcinoma and the above toxicity tests revealed that the indices of the 13-methyl-berberrubine derivatives were higher

TABLE III. *Therapeutic Evaluation of Berberrubine and Palmatrubine Derivatives*

Compound	Tumor					
	Sarcoma 180		Ehrlich		L 1210	
	ED ₉₀	Index	ED ₉₀	Index	ILS ₃₀	Ratio
Berberrubine phosphate	20	6	60	2	60-40	2-3
9-N-Methylcarbamoyl-berberrubine chloride	20	3.5	20	3.5	35	2
13-Methyl-berberrubine	9	15	21	6.5		< 1
13-Methyl-berberrubine phosphate	18	17	28	11		< 1
13-Methyl-9-acetyl-berberrubine chloride	8	11	18	5	20	4
Palmatrubine chloride	200	1	220	1	100	2
13-Methyl-palmatrubine	20	16	32	10	40	2.5
2-Demethyl-palmatrubine chloride	12.5	12	30	5		< 1

Index = LD₁₀/ED₉₀ in T.P.C.V. method.

Ratio = optimal dose/ILS 30% dose.

than those of berberrubine *per se*. Similar observations were made with respect to palmatrubine *per se* and the 13-methyl-palmatrubine derivatives.

While berberrubine exhibited an inhibitory effect on S-180 and Ehrlich carcinoma ascites tumors, palmatrubine did not, suggesting that the presence of the methylene dioxy group in the molecules is essential for the manifestation of antitumor activity. On the other hand, the 13-methyl derivatives of both berberrubine and palmatrubine exhibited high antitumor activity and their therapeutic indices were high.

Our present findings confirm that in berberubine derivatives, the 9-demethyl structure is essential for the manifestation of antitumor activity and demonstrated that ester derivatives also exhibit a remarkable antitumor activity. Furthermore, our present data illustrate that the

13-methyl derivatives of both berberrubine and palmatrubine alkaloid showed high antitumor activity.

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