J. Pharm. Pharmacol. 1997, 49: 706–711
Received November 11, 1996
Accepted February 17, 1997

© 1997 J. Pharm. Pharmacol.

Antiplatelet Effects of Some Aporphine and Phenanthrene Alkaloids in Rabbits and Man

CHE-MING TENG, CHEUNG-MEI HSUEH, YA-LING CHANG, FENG-NIEN KO, SHOEI-SHENG LEE* AND KARIN CHIUNG-SHEUE LIU*

*Pharmacological Institute and *School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan*

Abstract

Two aporphines (boldine and laurolitsine) and five phenanthrene alkaloids (litebamine, secoboldine, *N*-cyanosecoboldine, *N*-methylsecoglaucine and *N*-methylsecopredicentrine) were evaluated in-vitro for their ability to inhibit platelet aggregation.

All seven alkaloids inhibited aggregation of rabbit platelets and inhibited the release of ATP induced by arachidonic acid and collagen in rabbit platelets. Those aggregations induced by platelet-activating factor (PAF), thrombin, U46619 and ADP were inhibited by the three *N*-substituted secoboldine derivatives only. Thromboxane B_2 formation caused by arachidonic acid was also suppressed by these compounds. They did not affect the generation of [^{3}H]inositol monophosphate caused by collagen, PAF and thrombin in the presence of indomethacin. Platelet cyclic AMP level was unaffected by litebamine, but was increased by *N*-methylsecoglaucine. Litebamine suppressed the secondary aggregation, but not the primary aggregation, induced by ADP and adrenaline in platelet-rich plasma from man, whereas *N*-methylsecoglaucine inhibited both primary and secondary aggregation.

It is concluded that the antiplatelet effect of these seven aporphine and phenanthrene alkaloids is mainly a result of inhibition of thromboxane A_2 formation; *N*-methylsecoglaucine has additional antiplatelet activity as a result of increasing the levels of platelet cyclic AMP.

Aporphine alkaloids are widely distributed in the plant kingdom. Some aporphine alkaloids (dicentrine, discretamine, *N*-methylactinodaphnine, etc.) have recently been isolated from plant sources and shown to have α_1-adrenoceptor blocking action in vascular and other smooth muscles (Teng et al 1991; Ko et al 1993; Guh et al 1995). Inhibition of platelet aggregation by many aporphine alkaloids has also been observed (Chen et al 1995a, b, 1996a, b). However, the mechanisms of antiplatelet action of these alkaloids have not been determined. To study the structure–activity relationship, we compared the antiplatelet activity of some aporphine and related phenanthrene alkaloids and also tried to elucidate their mechanism of action.

Materials and Methods

Materials

Boldine was purchased from Sigma. Laurolitsine was isolated from *Phoebe formosana* (Lauraceae) (Lee et al 1993). Litebamine, secoboldine and *N*-cyanosecoboldine were prepared by chemical synthesis (Lee et al 1992). *N*-Methylsecopredicentrine and *N*-methylsecoglaucine were prepared from secoboldine by reductive *N*-methylation (HCHO/MeOH, $NaBH_4$), then *O*-methylation with diazomethane. TLC and NMR spectroscopy showed these compounds to be essentially pure. The chemical structures of the compounds are shown in Fig. 1. Thrombin (bovine) was purchased from Parke Davis and dissolved in 50% glycerol to give a stock solution of 100 NIH units mL^{-1}. Collagen (type 1, bovine Achilles tendon), obtained from Sigma, was homogenized in 25 mM acetic acid and then stored at $-70°C$. Platelet-activating factor (PAF), ADP, arachidonic acid, Dowex-1 (100–200 mesh: x8, Cl^{-1}) resin, myo-inositol, prostaglandin (PG) E_1, LiCl, EDTA, sodium citrate, bovine serum albumin (BSA), indomethacin and luciferase-luciferin were purchased from Sigma. Myo[2-^{3}H]inositol (10–20 Ci $mmol^{-1}$) was purchased from Amersham. Thromboxane B_2 and cyclic AMP RIA kits were obtained from New England Nuclear.

Correspondence: C. M. Teng, Pharmacological Institute, College of Medicine, National Taiwan University, No. 1 Jen-Ai Rd, Sect. 1, Taipei 10018, Taiwan.

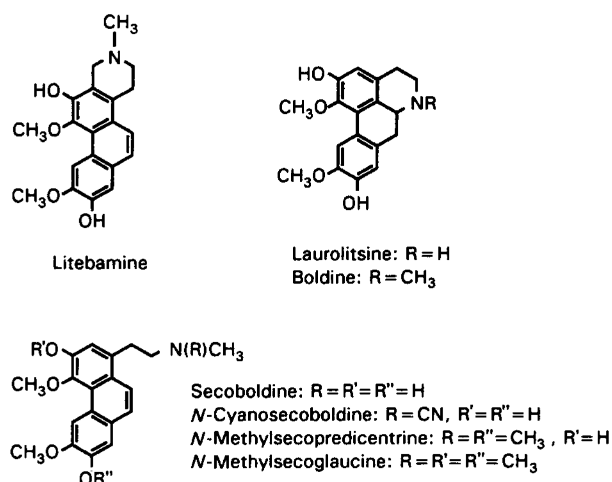

FIG. 1. Chemical structures of some aporphine and phenanthrene alkaloids.

Preparations of platelets

Blood was collected from the rabbit marginal ear vein and was mixed with EDTA to a final concentration of 6 mM. It was centrifuged for 10 min at 90 *g* and room temperature (21°C) and the supernatant was obtained as platelet-rich plasma (PRP). The PRP was further centrifuged at 500 *g* for 10 min. The platelet pellets were washed with Ca^{2+}-free Tyrode solution (containing 2 mM EDTA, 0·1 mg mL^{-1} apyrase and 3·5 mg mL^{-1} BSA) and centrifuged at 500 *g* for 10 min. The pellets were then washed with the above Tyrode solution without EDTA. After centrifugation under the same conditions the platelet pellets were finally suspended in Tyrode solution of composition (mM): NaCl (136·8), KCl (2·8), $NaHCO_3$ (11·9), $MgCl_2$ (1·1), NaH_2PO_4 (0·33), $CaCl_2$ (1·0) and glucose (11·2) containing 3·5 mg mL^{-1} BSA. PRP from man was obtained from the supernatant after centrifugation of blood mixed with 3·8% sodium citrate (1:9 to blood).

Platelet aggregation and ATP release reaction

Platelet aggregation was measured by the turbidimetric method as described by O'Brien (1962). ATP released from platelets was detected by the bioluminescence method as described by DeLuca & McElory (1978). Both the aggregation and release of ATP were measured simultaneously by a Lumi-aggregometer (Chrono-Log, USA) connected to two dual-channel recorders. One minute before the addition of the aggregation inducer, the platelet preparations were stirred at 1200 rev min^{-1}. To eliminate the effect of the solvent on the aggregation, the final concentration of dimethylsulphoxide was fixed at 0·5%. ATP of known concentration was used to calibrate the intensity of the bioluminescence. The absorbance of PRP or platelet suspension was taken as 0% aggregation and the absorbance of platelet-poor plasma or platelet-free Tyrode solution taken as 100% aggregation. Percent inhibition was expressed in some instances by assuming the value of the control (without any inhibitor) to be 100%.

Thromboxane B_2 assay

After challenge of platelets with the aggregation inducer for 6 min, 2 mM EDTA and 50 μM indomethacin were added. After centrifugation in an Eppendorf centrifuge (Model 5414) for 2 min, thromboxane B_2 in the supernatant was measured by radioimmunoassay (RIA).

Labelling of membrane phospholipids and measurement of the production of [^{3}H]inositol phosphate

The methods of Huang & Detwiler (1986a) and Neylon & Summer (1987) were modified in our laboratory (Ko et al 1996). All experiments were performed in the presence of 5 mM LiCl to inhibit inositol phosphate phosphatase. Because the levels of inositol bisphosphate and inositol trisphosphate were very low, we measured the inositol monophosphate as an index of the total inositol phosphate formation.

Cyclic AMP assay

The method of Karniguian et al (1982) was followed. Platelet suspension (10^9 platelets mL^{-1}) was warmed at 37°C for 1 min and then PGE_1 or aporphine derivatives was added with incubation for 3 min. The reaction was stopped by adding 10 mM EDTA followed immediately by boiling for 5 min. Upon cooling to 4°C, precipitated protein was sedimented and the supernatant was used to assay for cyclic AMP content by RIA.

Statistics

Results are expressed as the mean ± standard error of the mean (s.e.m.) and comparisons were made with Student's *t*-test. A probability of 0·05 or less was considered as indicative of significance.

Results

Effects of aporphine and phenanthrene alkaloids on platelet aggregation

Boldine, laurolitsine and litebamine (300 μM) markedly inhibited the aggregation of rabbit platelets induced by arachidonic acid (100 μM) and collagen (10 μg mL^{-1}), and slightly inhibited that induced by ADP (20 μM). However, they did not affect platelet aggregation induced by PAF (2 ng mL^{-1}), thrombin (0·1 units mL^{-1}) or U46619 (1 μM). In addition to arachidonic acid and collagen, those aggregations induced by PAF and U46619 were also inhibited by secoboldines. The *N*-substituted secoboldine derivatives, *N*-cyanosecoboldine, *N*-methylsecoglaucine and *N*-methyl-secopredicentrine (300 μM) completely or markedly inhibited platelet aggregation caused by arachidonic acid, collagen, ADP, PAF, thrombin and U46619 (Table 1).

Table 1. Inhibitory effects of aporphine and phenanthrene alkaloids on the aggregation of rabbit washed platelets induced by ADP, arachidonic acid, collagen, PAF, thrombin and U46619.

Treatment	% Inhibition					
	ADP	Arachidonic acid	Collagen	Platelet-activating factor	Thrombin	U46619
Boldine	19·7 ± 6·2†	100 ± 0·0†	80·5 ± 5·9†	5·3 ± 2·5*	1·6 ± 0·8	4·1 ± 1·1†
Laurolitsine	24·9 ± 2·3†	39·4 ± 17·6*	78·5 ± 9·2†	3·2 ± 2·5	2·3 ± 1·0*	4·2 ± 0·2†
Litebamine	36·8 ± 11·7†	92·8 ± 3·6†	71·5 ± 3·6†	13·9 ± 5·4*	0·0 ± 0·0	7·9 ± 3·0‡
Secoboldine	16·1 ± 1·2†	100 ± 0·0†	100 ± 0·0†	52·6 ± 10·9†	6·0 ± 2·3*	60·3 ± 18·1†
N-Cyanosecoboldine	98·1 ± 1·5†	96·7 ± 2·7†	87·9 ± 1·7†	100 ± 0·0†	6·1 ± 2·0‡	100 ± 0·0†
N-Methylsecoglaucine	100 ± 0·0†	100 ± 0·0†	100 ± 0·0†	100 ± 0·0†	91·7 ± 3·1†	100 ± 0·0†
N-Methylsecopredicentrine	100 ± 0·0‡	100 ± 0·0†	100 ± 0·0†	100 ± 0·0†	88·1 ± 2·2†	100 ± 0·0†

Rabbit washed platelets were pre-incubated with the test compounds (300 μM) or with dimethylsulphoxide (0·5%, control) at 37°C for 3 min. The inducer ADP (20 μM), arachidonic acid (100 μM), collagen (10 μg mL^{-1}), platelet-activating factor (2 ng mL^{-1}), thrombin (0·1 units mL^{-1}) or U46619 (1 μM) was then added. Percent inhibition of the control aggregation is presented as means ± s.e.m. (n = 3 or 4). *$P < 0·05$, ‡$P < 0·01$, † $P < 0·001$; significantly different from the respective control value.

Table 2. Effect of aporphine and phenanthrene alkaloids on the arachidonic acid-induced aggregation of rabbit washed platelets.

Concn (μM)	% Inhibition					
	Boldine	Litebamine	Secoboldine	*N*-Cyanosecoboldine	*N*-Methylsecoglaucine	*N*-Methylsecopredicentrine
300	100 ± 0·0†	–	100 ± 0·0†	–	100 ± 0·0†	100 ± 0·0†
150	79·8 ± 13·0†	100 ± 0·0†	71·1 ± 16·7†	–	93·3 ± 6·1†	72·5 ± 16·2†
60	7·8 ± 4·2	100 ± 0·0†	44·1 ± 17·6*	100 ± 0·0†	35·1 ± 1·4†	1·6 ± 0·7*
30	3·7 ± 1·0‡	35·2 ± 18·8	10·1 ± 4·0*	100 ± 0·0†	12·9 ± 0·7‡	1·2 ± 0·5*
15	–	16·7 ± 8·6	–	80·4 ± 12·6†	–	–
6	–	–	–	4·8 ± 1·7*	–	–

Rabbit washed platelets were pre-incubated with the test compounds at various concentrations or with dimethylsulphoxide (0·5%, control) for 3 min, then arachidonic acid (100 μM) was added to trigger the aggregation. Percent inhibition of control aggregation is presented as mean ± s.e.m. (n = 3–5). **P* < 0·05, ‡*P* < 0·01, † *P* < 0·001; significantly different from the respective control value.

Table 3. Effect of aporphine and phenanthrene alkaloids on PAF-induced aggregation of rabbit washed platelets.

Concn (μM)	% Inhibition		
	N-Cyanosecoboldine	*N*-Methylsecoglaucine	*N*-Methylsecopredicentrine
300	100 ± 0·0†	100 ± 0·0†	100 ± 0·0†
150	67·9 ± 12·8†	100 ± 0·0†	95·8 ± 3·6†
60	13·8 ± 6·3*	13·5 ± 1·3†	2·1 ± 1·1
30	10·3 ± 1·6†	3·9 ± 1·2‡	2·4 ± 1·4
15	10·2 ± 5·5	–	–
6	4·2 ± 0·4†	–	–

Rabbit washed platelets were pre-incubated with the test compounds at various concentrations or with dimethylsulphoxide (0·5%, control) for 3 min, then platelet-activating factor (2 ng mL^{-1}) was added to trigger the aggregation. Percent inhibition of the control aggregation is presented as means ± s.e.m. (n = 4 or 5). **P* < 0·05, ‡*P* < 0·01, † *P* < 0·001; significantly different from the control value.

The antiplatelet potency of aporphine and phenanthrene alkaloids was then compared in platelet aggregation induced by arachidonic acid (100 μM), and the potency of *N*-cyanosecoboldine, *N*-methylsecoglaucine and *N*-methylsecopredicentrine on PAF-(2 ng mL^{-1})-stimulated platelet aggregation was also evaluated. The results are shown in Tables 2 and 3, respectively. Among the drugs tested litebamine was the most potent inhibitor of arachidonic acid-induced platelet aggregation. It inhibited only the arachidonic acid- and collagen-induced aggregation, with a minimum effective concentration of approximately 15 μM and a maximum effective concentration of 60 μM. *N*-Methylsecoglaucine was the most potent inhibitor of PAF-induced aggregation; the minimum and maximum effective concentrations were approximately 30 and 150 μM, respectively.

Effects of litebamine and N*-methylsecoglaucine on ATP release*

Litebamine and *N*-methylsecoglaucine were then selected in subsequent experiments to study the properties and mechanisms of their antiplatelet action. The antiplatelet effects of these two compounds with the responding inducer were concentration-dependent. In addition to the aggregation, ATP release caused by the inducer was inhibited concentration-dependently (Fig. 2).

Effects of aporphine and phenanthrene alkaloids on thromboxane B_2 formation

Thromboxane B_2 formation in platelet suspension was measured 6 min after addition of the aggregation inducer. All the tested compounds (300 μM) markedly inhibited the thromboxane B_2 formation induced by arachidonic acid (100 μM) (Table 4). The effect of litebamine on thromboxane formation also showed that this inhibition is concentration-dependent.

Effects of litebamine and N*-methylsecoglaucine on phosphoinositide breakdown*

In [^{3}H]inositol-labelled rabbit washed platelets, collagen (10 μg mL^{-1}), PAF (2 ng mL^{-1}) and thrombin (0·1 units mL^{-1}) increased [^{3}H]inositol monophosphate formation to 1·9 ± 0·2, 2·8 ± 0·3 and 3·7 ± 0·1, respectively, times resting level in the presence of indomethacin (20 μM). Litebamine at 30 μM (2·3 ± 0·1, 2·9 ± 0·2 and 3·7 ± 1·7 fold, respectively) and *N*-methylsecoglaucine at 300 μM (2·0 ± 0·2, 2·6 ± 0·1 and 2·9 ± 0·2 fold, respectively) did not significantly affect the [^{3}H]inositol phosphate formation caused by the three inducers.

Effects of litebamine and N*-methylsecoglaucine on cyclic AMP formation*

The level of cyclic AMP in unstimulated platelets was 0·38 ± 0·08 pmol/(10^8 platelets). Prostaglandin E_1 at 1 μM or 10 μM increased the cyclic AMP level to 7·6 and 14·7 times, respectively, that at resting level. Litebamine (60–300 μM) did

Table 4. Effect of aporphine and phenanthrene alkaloids on arachidonic acid-induced thromboxane B_2 formation by rabbit washed platelets.

Treatment	Concn (μM)	Inhibition of thromboxane B_2 formation (%)
Boldine	300	94·3 ± 2·1 (6)*
Litebamine	60	96·3 ± 0·6 (5)*
	30	78·8 ± 6·5 (6)*
	15	77·1 ± 5·3 (6)*
Secoboldine	300	88·0 ± 7·2 (3)*
N-Cyanosecoboldine	30	97·2 ± 0·7 (6)*
N-Methylsecoglaucine	300	48·2 ± 8·7 (6)*
N-Methylsecopredicentrine	300	96·6 ± 0·8 (6)*

Rabbit washed platelets (4·5 × 10^8 mL^{-1}) were pre-incubated with the test compounds or with dimethylsulphoxide (0·5%, control) at 37°C for 3 min, then the inducer was added. Aggregation and thromboxane B_2 formation were terminated by EDTA (2 mM) and indomethacin (50 μM) 6 min after the addition of the inducer. Percent inhibition of control is presented as means ± s.e.m. (n). *$P < 0.001$; significantly different from the control value.

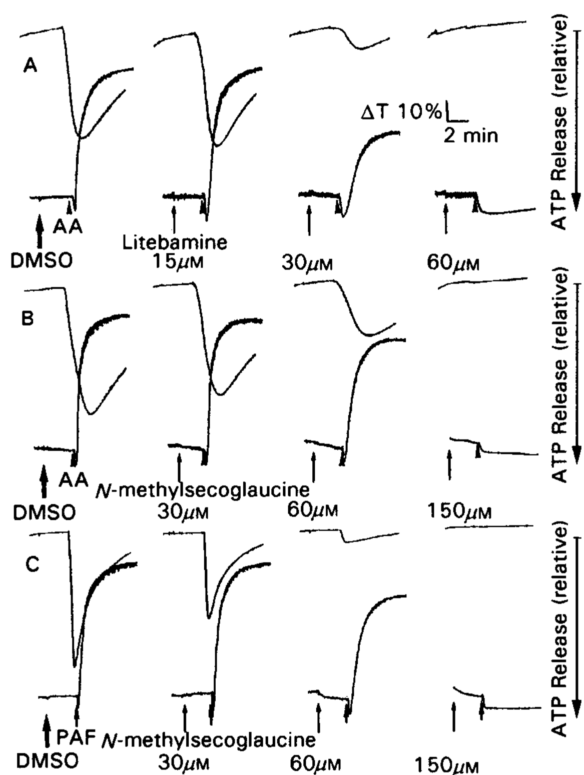

FIG. 2. Inhibitory effects of litebamine (A) and *N*-methylsecoglaucine (B, C) on arachidonic acid-induced (A, B) and PAF-induced (C) platelet aggregation of, and ATP release by, rabbit washed platelets. Rabbit washed platelets were incubated with litebamine or *N*-methylsecoglaucine at various concentrations or with dimethylsulphoxide (0·5%, control) for 3 min, then arachidonic acid (AA, 100 μM) or PAF (2 ng mL^{-1}) was added to trigger aggregation (upper traces) and ATP release (lower traces).

not have significant effect whereas *N*-methylsecoglaucine (30–300 μM) increased the platelet cyclic AMP level in a concentration-dependent manner (Table 5).

Table 5. Effect of litebamine and N-methylsecoglaucine on the cyclic AMP level of rabbit washed platelets.

Treatment	cAMP (pmol/(10^8 platelets))
Control	0·38 ± 0·08
Prostaglandin E_1	
1 μM	2·87 ± 0·37†
10 μM	5·60 ± 0·50†
Litebamine	
60 μM	0·34 ± 0·04
150 μM	0·37 ± 0·06
300 μM	0·33 ± 0·06
N-Methylsecoglaucine	
30 μM	0·44 ± 0·07
60 μM	0·71 ± 0·13*
150 μM	1·13 ± 0·23‡
300 μM	3·20 ± 0·49†

Various concentrations of the test compounds or of dimethylsulphoxide (0·5%, control) were pre-incubated with rabbit washed platelets at 37°C for 3 min; cAMP formation was stopped then by adding 10 mM EDTA and immediate boiling for 5 min. Values are presented as means ± s.e.m. (n = 5). *$P < 0.05$, ‡$P < 0.01$ and † $P < 0.001$; significantly different from the control value.

Effects of litebamine and N-methylsecoglaucine on the aggregation of PRP from man

Adrenaline (5 μM) and ADP (5 μM) caused biphasic aggregation in platelet-rich plasma from man. Secondary aggregation was suppressed concentration-dependently by litebamine whereas primary aggregation was not affected; adrenaline- and ADP-induced secondary aggregation was completely abolished at 30 and 60 μM, respectively. *N*-Methylsecoglaucine suppressed both the primary and secondary aggregation. This inhibition was also concentration-dependent (Fig. 3).

Discussion

Boldine, litebamine and laurolitsine inhibited the aggregation and release reaction of rabbit washed platelets. The degree of inhibitory effect was shown to be different depending upon the type of aggregation inducer. Arachidonic acid-induced aggregation was the most easily inhibited; that by collagen was less so, while those by ADP, PAF and thrombin were not affected.

Exogenous arachidonic acid can be converted to prostaglandin endoperoxides by platelet cyclo-oxygenase and then in turn converted by thromboxane synthase to thromboxane A_2, a very potent aggregating agent (Hamberg et al 1975). The antiplatelet action of boldine, litebamine and laurolitsine is probably a result of the inhibition of thromboxane A_2 forma-

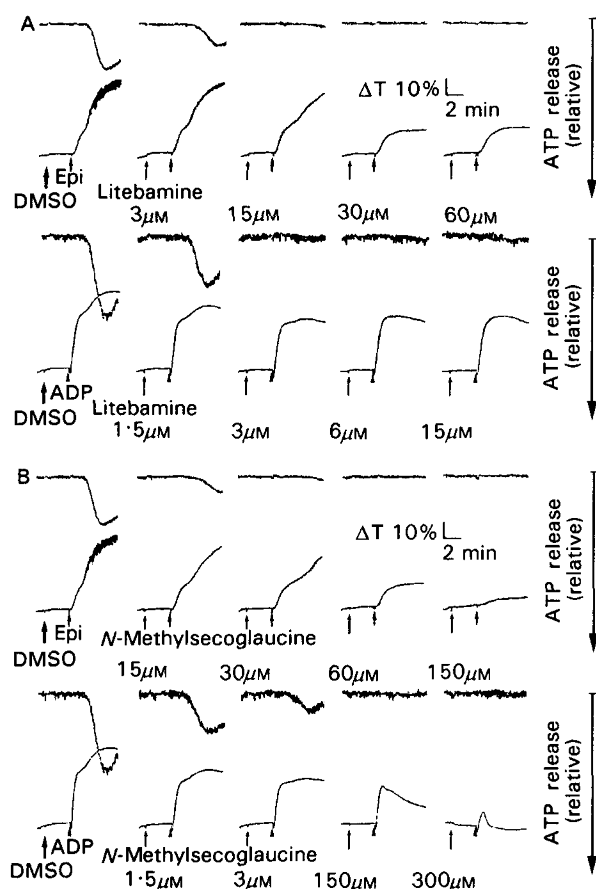

FIG. 3. Effects of litebamine (A) and *N*-methylsecoglaucine (B) on epinephrine- or ADP-induced aggregation of, and ATP release by, platelet-rich plasma from man. Platelet-rich plasma was incubated with litebamine or *N*-methylsecoglaucine at various concentrations or with dimethylsulphoxide (0·5%, control) for 3 min, then epinephrine (5 μM) or ADP (5 μM) was added to trigger aggregation (upper traces) and ATP release (lower traces).

tion. This is supported by the following evidence: platelet aggregation, ATP release and formation of thromboxane B_2, a stable metabolite of thromboxane A_2, induced by arachidonic acid were parallelly and concentration-dependently inhibited; in ADP- and adrenaline-induced aggregation of platelet-rich plasma from man, litebamine inhibited mainly secondary aggregation, which is known to be dependent on the formation of thromboxane A_2 and release of ADP (Huang & Detwiler 1986b); boldine, litebamine and laurolitsine either did not affect or only slightly inhibited the aggregation of rabbit platelets caused by ADP and PAF, both of which were known to activate platelets without causing formation of thromboxanes (Teng et al 1987).

N-Cyanosecoboldine, *N*-methylsecoglaucine and *N*-methylsecopredicentrine also significantly inhibited arachidonic acid- or collagen-induced platelet aggregation. Therefore, the antiplatelet action of these compounds is a consequence, at least partly, of the inhibition of thromboxane A_2 formation. However, they also inhibited ADP-, PAF-, thrombin- or U46619-induced platelet aggregation. This means that some other pathway(s), in addition to thromboxane formation, could be involved in the antiplatelet action. Phosphoinositide breakdown is an important pathway in signal transduction of agonist-induced platelet activation (Nishizuka 1984). This process generates two active products, diacylglycerol and inositol-1,4,5-trisphosphate (IP_3), which have roles as secondary messengers in triggering the activation of protein kinase C, in calcium mobilization from intracellular compartments, in phosphorylation of proteins and in causing the release reaction in platelets. In contrast, the elevation of cyclic AMP levels, either by stimulation of adenylate cyclase or by inhibition of cyclic AMP-phosphodiesterase, is the most potent mechanism of inhibition of platelet functions (Vane et al 1990). Elevated cyclic AMP inhibits most platelet responses including aggregation, ATP release and increase of intracellular Ca^{2+} concentration. *N*-Methylsecoglaucine did not affect the generation of [^{3}H]inositol phosphate formation caused by collagen, PAF and thrombin, but increased the platelet cyclic AMP level at a concentration similar to those needed to inhibit PAF-induced aggregation. Thus, *N*-substituted secoboldine derivatives might have dual antiplatelet actions—inhibition of thromboxane formation and increasing the cyclic AMP level. Whether the increase of cyclic AMP formation by *N*-methylsecoglaucine is a result of direct activation of adenylate cyclase or inhibition of phosphodiesterase needs further investigation.

Examining the structure–activity relationship we found that aporphine skeletons with lower polarity were more effective. Laurolitsine, which is more polar than boldine, has less antiplatelet activity. Moreover, the phenanthrene group as in secoboldine might contribute to their broader spectrum of antiplatelet activity by increasing cyclic AMP level, and *N*-substituted secoboldines showed higher potency.

Acknowledgements

This work was supported by a research grant from the National Science Council of Taiwan (NSC85-2622-B002-007).

References

Chen, C. C., Lin, C. F., Huang, Y. L., Ko, F. N., Teng, C. M. (1995a) Bioactive constituents from the flower buds and peduncles of *Lindera megaphylla*. J. Nat. Prod. 58: 1423–1425

Chen, I. S., Chen, J. J., Tsai, I. L., Chang, Y. L., Teng, C. M. (1995b) New *p*-quinonoid aporphine alkaloids and antiplatelet aggregation constituents of *Hernandia sonora*. Planta Medica 61: 537–539

Chen, K. S., Ko, F. N., Teng, C. M., Wu, Y. C. (1996a) Antiplatelet and vasorelaxing actions of some benzylisoquinoline and phenanthrene alkaloids. J. Nat. Prod. 59: 531–534

Chen, K. S., Wu, Y. C., Ko, F. N., Teng, C. M. (1996b) Antiplatelet and vasorelaxing action of some aporphinoids. Planta Medica 62: 133–136

DeLuca, M., McElory, W. D. (1978) Purification and properties of firefly luciferase. Methods Enzymol. 57: 3–15

Guh, J. H., Ko, F. N., Yu, S. M., Wu, Y. C., Teng, C. M. (1995) Pharmacological evaluation of *N*-methylactinodaphnine, a novel vascular α-adrenoceptor antagonist, isolated from *Illigera luzonensis*. Eur. J. Pharmacol. 279: 33–41

Hamberg, M., Svensson, J., Samuelsson, B. (1975) Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc. Natl Acad. Sci. USA 72: 2994–2998

Huang, E. M., Detwiler, T. C. (1986a) Effect of lithium on platelet phosphoinositide metabolism. Biochem. J. 236: 895–901

Huang, E. M., Detwiler, T. C. (1986b) Stimulus-response coupling mechanisms. In: Phillips, D. R., Shuman, M. A. (eds) Biochemistry of Platelets. Academic Press, New York, pp 1–68

Karniguian, A., Legrand, Y. J., Caen, J. P. (1982) Prostaglandins: specific inhibition of platelet adhesion to collagen and relationship with cAMP level. Prostaglandins 23: 437–457

Ko, F. N., Yu, S. M., Su, M. J., Wu, T. S., Teng, C. M. (1993) Pharmacological activity of (−)-discretamine, a novel vascular α-adrenoceptor and 5-hydroxytryptamine receptor antagonist, isolated from *Fissistigma glaucescens*. Br. J. Pharmacol. 110: 882–888

Ko, F. N., Yeh, L. J., Liang, H. C., Kuo, S. C., Teng, C. M. (1996) Mechanism of action of *p*-chlorobiphenyl on the inhibition of platelet aggregation. J. Pharm. Pharmacol. 48: 395–400

Lee, S. S., Lin, Y. J., Wu, Y. C., Chen, C. H. (1992) A facile semisynthesis of litebamine, a novel phenanthrene alkaloid, from boldine via a biogenetical approach. Tetrahedron Lett. 33: 6309–6310

Lee, S. S., Tsai, F. Y., Chen, I. S., Liu, K. C. S. (1993) Additional alkaloids from *Phoebe formosana*. J. Chin. Chem. Soc. 40: 209–212

Neylon, C. B., Summer, R. J. (1987) Stimulation of α_1-adrenoceptors in rat kidney mediate increased inositol phospholipid hydrolysis. Br. J. Pharmacol. 91: 367–376

Nishizuka, Y. (1984) Turnover of inositol phospholipids and signal transduction. Science 225: 1365–1370

O'Brien, J. R. (1962) Platelet aggregation II. Some results from a new method of study. J. Clin. Pathol. 15: 452–455

Teng, C. M., Chen, W. Y., Ko, W. C., Ouyang, C. (1987) Antiplatelet effect of butylidenephthalide. Biochim. Biophys. Acta 924: 375–382

Teng, C. M., Yu, S. M., Ko, F. N., Chen, C. C., Huang, Y. L., Huang, T. F. (1991) Dicentrine, a natural vascular α_1-adrenergic antagonist, isolated from *Lindera megaphylla*. Br. J. Pharmacol. 104: 651–656

Vane, J. R., Aanggard, E. E., Botting, R. M. (1990) Regulatory functions of the vascular endothelium. N. Engl. J. Med. 223: 27–36