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Characterization of the antiplatelet effects of (2*S*)-5-methoxy-6-methylflavan-7-ol from Draconis Resina

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Abstract

(2*S*)-5-methoxy-6-methylflavan-7-ol (MMF) was purified from Draconis Resina and its in vitro effects on various aspects of platelet reactivity were examined. Results indicated that MMF dose dependently inhibited aggregation of washed rabbit platelets induced by collagen, arachidonic acid, ADP, U46619 or platelet-activating factor (PAF), with IC_{50} values of 17.2, 49.8, 179.8, 109.6, and 189.2 μM, respectively. Concomitantly, MMF also dose dependently suppressed ATP release by platelets activated by these stimulants. The increase in intracellular free calcium ($[Ca^{2+}]_i$), elicited by these activating agents, was inhibited by MMF as reflected by fura-2 fluorescence measurements. However, MMF had no effects on the cyclic AMP level of platelets. In addition, MMF inhibited the arachidonic acid-induced thromboxane B_2 and prostaglandin D_2 formation in intact platelet suspensions or homogenized platelet lysates. This study provided evidence that MMF is an antiplatelet agent whose activity is likely related to cyclooxygenase inhibition and suppression of $[Ca^{2+}]_i$ increase. © 1998 Elsevier Science B.V.

Keywords: (2*S*)-5-Methoxy-6-methylflavan-7-ol; Draconis Resina; Platelet, washed, rabbit; Aggregation; Cyclooxygenase inhibition; Ca^{2+}, intracellular

1. Introduction

Draconis Resina (Dragons's Blood) is a red resin collected from *Daemonorops draco* Blume (Cardillo et al., 1971; Kiangsu Institute of Modern Medicine, 1977). In Chinese medicine, Draconis Resina is a major component of the well-known hemostatic preparation 'Yun-Nan-Bai-Yao'. Although only the 'hemostatic' effect has been systematically investigated (Kiangsu Institute of Modern Medicine, 1977), the 'vasoactive–antithrombotic' activity of Draconis Resina is considered an important potential application in Chinese medicinal practice (Kiangsu Institute of Modern Medicine, 1977). It would be interesting to study the pharmacological effects of Draconis Resina, which reputedly possesses both 'vasoactive–antithrombotic' and 'hemostatic' activities. With regard to information pertaining to the active components of Draconis Resina, their isolation and characterization have been reported (Arnone and Nasini, 1989, 1990; Cardillo et al., 1971; Merlini and Nasini, 1976), but few pharmacological studies have been performed. The present study was therefore initiated to evaluate the antithrombotic activity of the Draconis Resina component, (2*S*)-5-methoxy-6-methylflavan-7-ol (MMF, Fig. 1).

Preliminary studies from our laboratories have demonstrated that Draconis Resina possesses a certain amount of toxicity, hemostatic activity, some analgesic effect and vasoactive–antithrombotic potency (Tsai et al., 1995). During previous attempts at isolation of active components from Draconis Resina, MMF was found to be a major component of the chloroform extract and shown to possess antiplatelet activity (Tsai et al., 1995). In this study, attempts were made to elucidate the underlying mechanisms of its effects on platelet responses.

2. Materials and methods

2.1. Purification of (2S)-5-methoxy-6-methylflavan-7-ol (MMF) from Draconis Resina

MMF was isolated from Draconis Resina in our laboratories according to a previously reported method (Tsai et al., 1995). In brief, Draconis Resina (30 g) was pulverized and extracted with chloroform. The chloroform extract was

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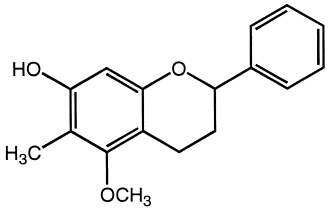

Fig. 1. Structure of (2*S*)-5-methoxy-6-methylflavan-7-ol (MMF).

chromatographed on a silica gel (70–230 mesh) column and successively eluted with *n*-hexane, chloroform, acetone and methanol. The chloroform eluate (11.8 g) was in turn chromatographed on a silica gel column (230–400 mesh) and eluted with *n*-hexane/acetone (2:1) to yield 7 fractions. The active fraction was further purified by preparative thin-layer chromatography on silica gel, using *n*-hexane/chloroform (4:1) as solvent system, to yield MMF (0.85 g) as white crystals. MMF was identified by comparison of its infrared (IR), mass (MS) and nuclear magnetic resonance (NMR) spectra with previously reported data (Cardillo et al., 1971). The MMF crystal also had the same melting point (122–124°C) as that reported by Cardillo et al. (1971). The purity of the purified compound was estimated to be over 98% MMF based on the clear spectral data and sharp melting point.

2.2. Preparation of washed platelet suspension

Washed platelets were obtained from rabbits as previously described (Ardlie et al., 1970, 1971). In brief, rabbit blood was collected from the marginal ear vein into tubes containing one-sixth volume of acid-citrate-dextrose as anticoagulant. The blood was centrifuged at $200 \times g$ for 15 min at room temperature. The platelet-rich plasma was mixed with 1/40 volume of EDTA (final concentration 5 mM) and re-centrifuged at $1000 \times g$ for 12 min. The supernatant was discarded and the platelet pellet was suspended in modified Ca^{2+}-free Tyrode's buffer (137 mM NaCl, 2.8 mM KCl, 2 mM $MgCl_2$, 0.33 mM NaH_2PO_4, 5 mM glucose, 10 mM HEPES) with 0.35% bovine serum albumin, heparin (50 unit/ml) and apyrase (1 unit/ml). Following incubation at 37°C for 20 min, the washed platelet pellet was resuspended in Tyrode's buffer containing 1 mM Ca^{2+}. The platelet numbers were counted by using a hemacytometer and adjusted to 3.5×10^8 platelets/ml. To eliminate or minimize any possible effects of the solvent, the final concentration of the vehicle dimethyl sulfoxide (DMSO) in the platelet suspension was fixed at 0.5%.

2.3. Platelet aggregation and ATP release reaction

Aggregation and ATP release were determined either separately or simultaneously. Aggregation was measured by a turbidimetric method (O'Brien, 1962). The PACKS-4 aggregrometer (Helena Laboratories, Beaumont, TX, USA) was used. Transmission of washed platelet suspension was assigned 0% aggregation while transmission through Tyrode's buffer was assigned 100% aggregation. ATP release was detected by the bioluminescence method as described by DeLuca and McElory (1978). Alternatively, aggregation and ATP release were simultaneously determined, using a Lumi-aggregrometer (1020B, Payton Sci., Scarborough, ONT, Canada) connected to two dual-channel recorders. Platelets (0.5 ml) were pre-incubated with 2.5 μl of the vehicle DMSO (0.5%) to serve as control or MMF for 2 min and then stimulated with 2.5 μl of stimulants of aggregation such as collagen, arachidonic acid, U46619, ADP, PAF or thrombin.

2.4. Malondialdehyde measurement

Malondialdehyde measurement was modified from the method described by Yagi (1975). Four minutes following challenge with the aggregation stimulants, 0.5 ml of platelet suspension was mixed with equal volumes of cold 20% trichloroacetic acid to stop all reactions. Following centrifugation at $10\,000 \times g$ for 3 min, the supernatant was reacted with 10 mM 2-thiobarbituric acid in a boiling water bath for 15 min. The malondialdehyde/2-thiobarbituric acid reactant was extracted by *n*-butanol and the concentration determined by measuring fluorescence (Ex = 532 nm, Em = 553 nm). The amount of malondialdehyde was calculated according to the standard equation.

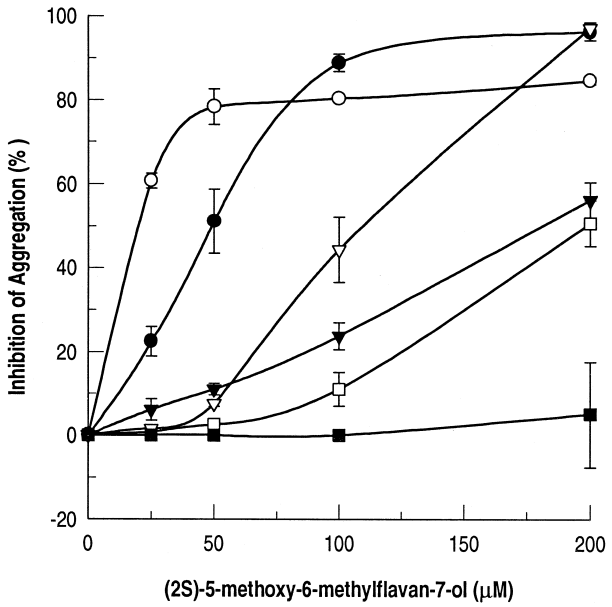

Fig. 2. Concentration-dependent inhibition curves for the effect of MMF on platelet aggregation induced by 20 μg/ml collagen (○), 100 μM arachidonic acid (●), 1 μM U46619 (▽), 20 μM ADP (▼), 2 nM PAF (□) and 0.1 unit/ml thrombin (■). For methods, see text. Each point represents the means and standard deviations from six independent experiments.

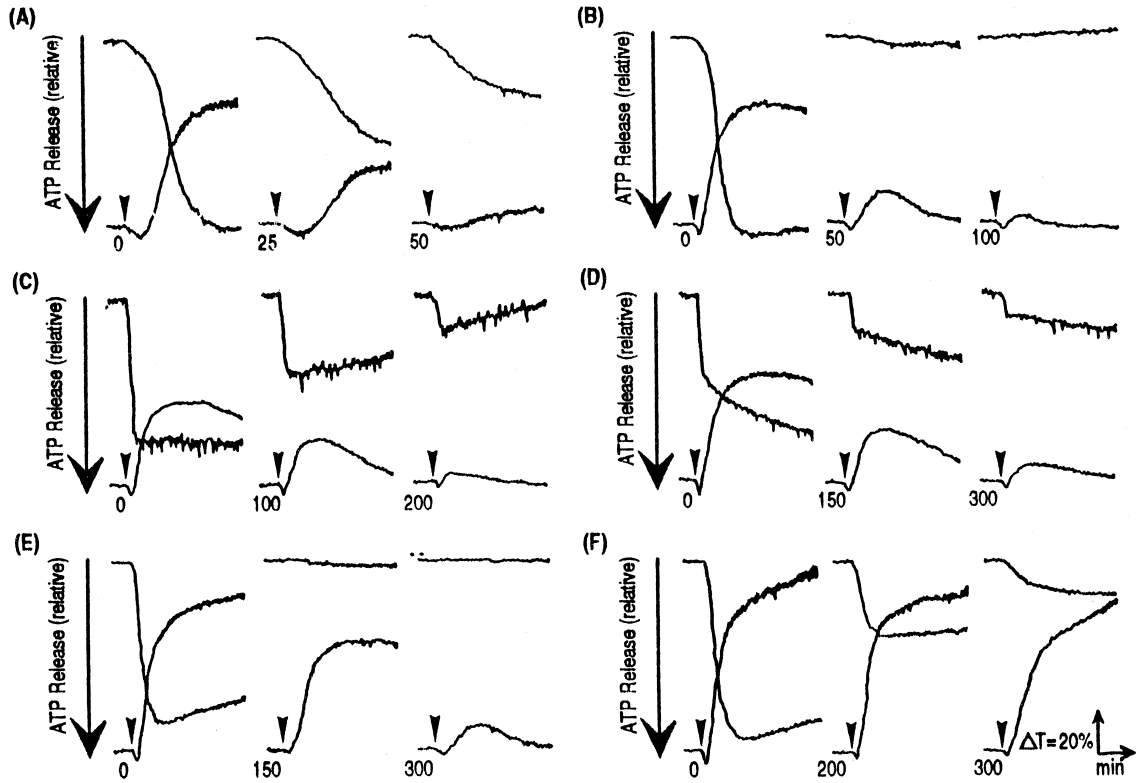

Fig. 3. The aggregation and ATP release time-courses of washed rabbit platelets preincubated with 0.5% DMSO (0) or various μM (numeral) of MMF for 2 min followed by (A) 20 μg/ml collagen, (B) 100 μM arachidonic acid, (C) 1 μM U46619, (D) 20 μM ADP, (E) 2 nM PAF and (F) 0.1 unit/ml thrombin (arrow head) to trigger cellular responses. The results shown are representative of six independent experiments. The concentrations were chosen on the basis of the IC_{50} of each agonist and 2 × those concentrations to give the maximal effects.

2.5. Thromboxane B_2 and prostaglandin D_2 assay

These assays were performed as described by Teng et al. (1988). Four minutes following challenge of the platelets (0.5 μl) with the aggregation stimulants, EDTA (2 mM) and indomethacin (50 mM) were added to stop the reactions. After centrifugation at 10 000 × g for 3 min, thromboxane B_2 and prostaglandin D_2 contents in the platelet supernatants were assayed, using thromboxane B_2 and prostaglandin D_2-methoxime EIA kits according to the procedures provided by the manufacturer (Cayman). In experiments in which platelet lysates were used, the platelets were disrupted by freeze–thawing and sonication (Kasahara and Hinkle, 1977). The platelet lysates were challenged with the aggregation stimulants and their thromboxane B_2 and prostaglandin D_2 contents determined by the same procedure described above.

2.6. Cyclic AMP evaluation

The evaluation procedure was as described by Yu et al. (1992). The 37°C pre-warmed platelet suspension (0.5 ml) was mixed with purified MMF, prostaglandin E_1 or DMSO. After 6 min of stimulation, EDTA (10 mM) was added to the sample, which was then boiled in water for 5 min. Following centrifugation at 10 000 × g for 3 min, aliquots of the supernatant were taken for the determination of their cyclic AMP levels, using an EIA kit (Cayman).

2.7. Intraplatelet Ca^{2+} measurement

The platelets were loaded with the fluorescent dye fura-2 by incubating washed rabbit platelets with 1 mM of fura-2-acetoxymethyl ester (fura-2-AM) at 37°C for 30 min. The platelets were then resuspended in modified Ca^{+2}-free Tyrode's buffer to a concentration of 2×10^8 cells/ml. In each experiment, 1 ml of platelet suspension was equilibrated with an equal volume of 2 mM Ca^{2+}-con-

Table 1
Effect of MMF on arachidonic acid-induced malondialdehyde formation in washed rabbit platelets

	μM	Malondialdehyde formation (μM)	Platelet aggregation (%)
DMSO	0	2.5 ± 0.2	60.1 ± 7.6
MMF	50	1.6 ± 0.2[a]	19.6 ± 1.3[b]
	100	1.3 ± 0.2[a]	4.1 ± 3.8[b]

Values are presented as means ± S.D. ($n = 6$). Significance of differences between experimental and control (DMSO) groups: [a]$P < 0.01$, [b]$P < 0.001$.

Table 2
Effect of MMF on thromboxane B_2 formation induced by arachidonic acid and collagen in washed rabbit platelets

	μM	100 μM arachidonic acid		20 μg/ml collagen	
		Thromboxane B_2 (ng/ml)	Inhibition (%), thromboxane B_2/aggregation	Thromboxane B_2 (ng/ml)	Inhibition (%), thromboxane B_2/aggregation
0.5% DMSO	0	233.5 ± 25.0		183.6 ± 14.3	
MMF	25	159.6 ± 24.8[a]	31.6/20.0	35.1 ± 3.9[b]	80.9/69.7
	50	121.9 ± 29.0[b]	47.8/59.1	12.6 ± 3.7[c]	93.1/78.3
	100	37.1 ± 16.9[c]	84.1/92.5	2.3 ± 2.3[c]	98.7/80.0
Indomethacin	2	16.5 ± 5.7[c]	92.9/100	0.4 ± 0.1[c]	99.8/83.0

The thromboxane B_2 level in resting platelets was 0.4 ± 0.1 ng/ml. Values are presented as means ± S.D. ($n = 4$). Significance of differences between experimental and control groups: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

taining buffer at 37°C for 3 min. Then, small portions (2.5–10 μl) of DMSO, MMF and the various aggregation stimulants were added and the changes in fluorescence with time were recorded. During measurements, the platelet suspension was kept at 37°C and continuously stirred. Fluorescence was recorded at Ex = 340, 380 nm and Em = 510 nm, using an F-4500 spectrofluorometer (Hitachi, Tokyo, Japan) with a multi-wavelength time-scan program. $[Ca^{2+}]_i$ was calculated as described previously by Grynkiewicz et al. (1985).

2.8. Statistical analysis

The statistical significance of differences between control and MMF treatment was evaluated using Student's *t*-test. A *P* value of 0.05 or less was considered as statistically significant.

2.9. Materials

The MMF was isolated from Draconis Resina in our laboratories according to the method described above. Apyrase, heparin, fibrinogen, bovine serum albumin, ADP, collagen (type I) and thrombin (bovine) were purchased from Sigma (St. Louis, MO, USA). Arachidonic acid, U46619 and PAF were obtained from Biomol Res. Lab., (Polymouth Meeting, PA, USA). DMSO, trichloroacetic acid, 2-thiobarbituric acid, solvents and silica gel were purchased from Merck (Darmstadt, Germany). EIA kits for thromboxane B_2, prostaglandin D_2 and cyclic AMP were purchased from Cayman (Ann Arbor, MI, USA). Fura-2-AM and pluronic F-127 were obtained from Molecular Probes (Eugene, OR, USA).

3. Results

3.1. Effect of MMF on platelet aggregation and ATP release

MMF induced a concentration-dependent inhibition of aggregation of washed rabbit platelets induced by a variety of activators including collagen (20 μg/ml), arachidonic acid (100 μM), U46619 (1 μM), ADP (20 μM) and PAF (2 nM) (Fig. 2). The IC_{50} values of MMF on collagen-, arachidonic acid-, U46619-, ADP- and PAF-induced platelet aggregation were 17.2, 49.8, 109.6, 179.8 and 189.2 μM, respectively. At the higher concentration of 400 μM, MMF inhibited 43.9 ± 9.4% of thrombin-induced platelet aggregation (data not shown).

Incubation of washed rabbit platelet suspension for 30 min with MMF (≤ 200 μM) did not lead to aggregation. Prolonging the pre-incubation to beyond 2 min did not

Table 3
Inhibitory effect of MMF on arachidonic acid-induced thromboxane B_2 formation in homogenized platelet lysates

	Thromboxane B_2 (ng/ml)
Resting	3.2 ± 0.1[a]
DMSO + arachidonic acid	41.2 ± 3.3
MMF (50 μM) + arachidonic acid	9.8 ± 1.3[a]
μMF (100 μM) + arachidonic acid	5.6 ± 0.9[a]
Indomethacin (20 μM) + arachidonic acid	3.0 ± 0.1[a]

Values are means ± S.D. ($n = 4$). Significance of differences between experimental and control (DMSO + arachidonic acid) groups: [a]$P < 0.001$.

Table 4
Effect of MMF and various inhibitors on prostaglandin D_2 formation induced by arachidonic acid in platelet suspensions or homogenized lysates

	Prostaglandin D_2 (ng/ml)	
	Intact platelet	Platelet lysate
Resting	0.03 ± 0.01[c]	0.02 ± 0.01[c]
DMSO + arachidonic acid	52.9 ± 4.0	31.0 ± 1.0
MMF (50 μM) + arachidonic acid	23.7 ± 2.1[a]	22.1 ± 1.8[a]
MMF (100 μM) + arachidonic acid	16.0 ± 0.5[b]	14.3 ± 1.6[b]
Indomethacin (20 μM) + arachidonic acid	23.0 ± 2.9[a]	15.2 ± 0.3[b]
Imidazole (1 mM) + arachidonic acid	125.3 ± 0.6[c]	61.5 ± 2.1[b]

Values are means ± S.D. ($n = 4$). Significance of differences between experimental and control (DMSO + arachidonic acid) groups: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

Table 5
Effect of MMF on platelet cyclic AMP level

	μM	Cyclic AMP level (pmol/10^8 platelets)
Resting		0.5 ± 0.1
MMF	100	0.5 ± 0.1
Prostaglandin E_1	10	5.6 ± 0.3[a]

Values are means $\pm$ S.D. ($n = 4$). Significance of difference as compared with resting values: [a]$P < 0.001$.

enhance the anti-aggregatory effects. The response to 100 μM arachidonic acid of platelets pre-treated with MMF for the relatively long period of 30 min could be restored by washing the platelets twice with the suspending buffer (data not shown).

ATP release from washed rabbit platelets induced by these agonists was also inhibited by MMF. In fact, the inhibition of ATP release was greater than the inhibition of aggregation. MMF-induced inhibition of ATP release was concentration-dependent and paralleled its inhibitory effect on aggregation (Fig. 3).

For comparison, the anti-aggregation effects of two known cyclooxygenase inhibitors (aspirin and indomethacin) were also examined. The IC_{50} values of MMF and aspirin on arachidonate-induced aggregation were 49.8 and 35.7 μM, considerably higher than the 0.3 μM of indomethacin.

3.2. Effect of MMF on malondialdehyde and thromboxane B_2 formation

Concomitantly with aggregation, malondialdehyde formation in washed rabbit platelets was measured 4 min after the addition of arachidonic acid. Malondialdehyde formation and aggregation induced by arachidonic acid were inhibited by MMF (Table 1) Both of these effects were concentration-dependent and paralleled each other.

When platelets were stimulated with 100 μM arachidonic acid and 20 μg/ml of collagen for 4 min, the amount of thromboxane B_2 was significantly raised from 0.4 ± 0.1 ng/ml to 233.5 ± 25.0 and 183.6 ± 14.3 ng/ml (mean $\pm$ S.D.), respectively (Table 2). Also shown in Table 2, thromboxane B_2 formation induced by arachidonic acid or collagen was inhibited by MMF and 2 μM indomethacin. The inhibitory effect of MMF on thromboxane B_2 formation was concentration-dependent and paralleled that on aggregation. Basal levels of thromboxane B_2, however, were not affected by MMF (25–100 μM) (data not shown). Under the same conditions, 100 μM arachidonic acid induced a rise in thromboxane B_2 in homogenized platelet lysate (Table 3). Although the amount of thromboxane B_2 formed in homogenized platelet lysate induced by arachidonic acid was less than that in intact platelets, the inhibitory effects of indomethacin and MMF

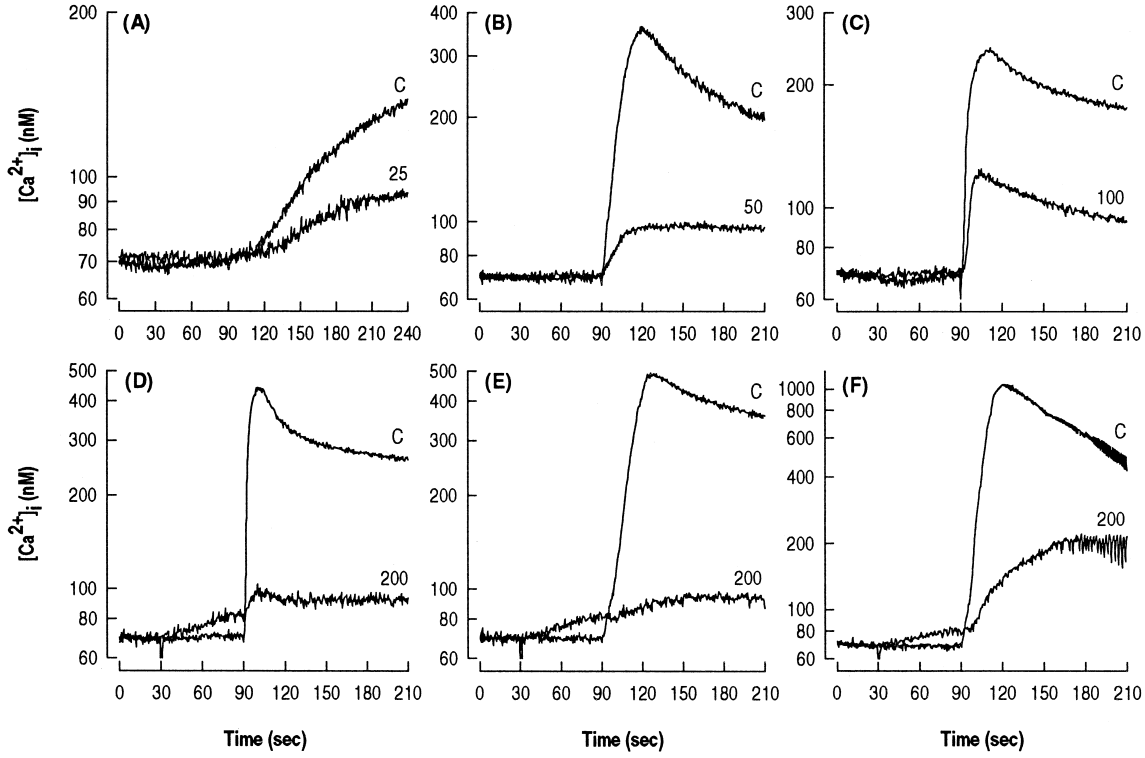

Fig. 4. The time-courses of $[Ca^{2+}]_i$ increase in fura-2-loaded platelets pretreated with 0.5% DMSO (0) or various μM (numeral) of MMF for 30 s and then activated by (A) 20 μg/ml collagen, (B) 100 μM arachidonic acid, (C) 1 μM U46619, (D) 20 μM ADP, (E) 2 nM PAF and (F) 0.1 unit/ml thrombin at 90 s to trigger $[Ca^{2+}]_i$ increase. The results shown are representative of four independent experiments. With the exception of thrombin stimulation, for which the concentration was randomly chosen, the other test concentrations were simply the IC_{50} of each agonist.

on the thromboxane B_2 formation in homogenized platelet lysate induced by arachidonic acid were similar (Table 3).

3.3. Effect of MMF on prostaglandin D_2 formation

In washed rabbit platelets or in homogenized platelet lysate, addition of arachidonic acid induced a rise in prostaglandin D_2 from the basal non-detectable level to about 50 or 30 ng/ml, respectively (Table 4). The prostaglandin D_2 formation induced by arachidonic acid could be inhibited by indomethacin and MMF. By contrast, the prostaglandin D_2 formation induced by arachidonic acid in washed rabbit platelet suspension or homogenized platelet lysate was enhanced about two-fold in the presence of 1 mM of the thromboxane B_2 synthetase inhibitor imidazole (Needleman et al., 1977).

3.4. Effect of MMF on the cyclic AMP levels of platelets

While 100 μM of MMF exerted very significant inhibitory effects on platelet activation, the cyclic AMP level was not significantly affected (Table 5). By comparison, 10 μM of prostaglandin E_1 increased the cyclic AMP level in platelets from 0.5 ± 0.1 pmol/10^8 platelets to 5.6 ± 0.3 pmol/10^8 platelets (Table 5).

3.5. Effect of MMF on the $[Ca^{2+}]_i$ of platelets

All six platelet aggregation activators (collagen, arachidonic acid, U46619, ADP, PAF, thrombin) tested induced an $[Ca^{2+}]_i$ increase in fura-2-loaded washed rabbit platelets although the patterns and the magnitudes of the increases were different (Fig. 4). The IC_{50} values of MMF, with reference to its anti-aggregation effects, were used to characterize their effects on $[Ca^{2+}]_i$ increase. As shown in Fig. 4, these agonist-induced $[Ca^{2+}]_i$ increases were inhibited by MMF. The extent of inhibition in almost all cases was greater than 50% (Fig. 4). Furthermore, the inhibitory effects on $[Ca^{2+}]_i$ were more potent when compared to the corresponding inhibitory effects on aggregation.

4. Discussion

The results from the present study indicated that MMF reversibly inhibited platelet activation induced by a variety of agents. The diversity in the activation pathways of these agents and in the effectors involved provided opportunities for studying the loci of inhibitory actions of MMF. Among the agents tested, MMF was most potent in inhibiting responses mediated via arachidonic acid metabolism (i.e., those induced by collagen or arachidonic acid) (Figs. 2 and 3). These results suggested that MMF might act on the plasma membrane of platelets, affecting arachidonic acid metabolism and some common pathways in signal transduction.

With regard to arachidonic acid-related effectors, it is well known that thromboxane A_2 is an important mediator of the aggregation and release reaction of platelets (Weiss, 1983). Thromboxane A_2 formation can be reflected by malondialdehyde production or thromboxane B_2 formation (Hamberg et al., 1975; Smith et al., 1976). The aggregation and ATP release of platelets induced by exogenous arachidonic acid are due to thromboxane A_2 formation (Hamberg et al., 1975). In our study, inhibition of malondialdehyde and thromboxane B_2 formation by MMF paralleled the inhibition of aggregation and ATP release in washed rabbit platelets stimulated by arachidonic acid (Tables 1 and 2). Likewise indomethacin, a cyclooxygenase inhibitor, almost completely inhibited both thromboxane B_2 formation and the aggregation of platelets induced by arachidonic acid (Table 2). Both MMF and indomethacin exerted greater inhibitory effects on thromboxane B_2 formation than platelet aggregation induced by collagen. This lent support to the proposal that thromboxane A_2 formation was the major mediator in collagen-induced platelet aggregation (Siess, 1989; Siess et al., 1983). In as much as inhibitors of cyclooxygenase or thromboxane synthetase can all suppress thromboxane B_2 formation from arachidonic acid, the possible role MMF may play in inhibiting arachidonic acid-metabolism becomes of interest. In the homogenized platelet lysate, thromboxane B_2 formation in the presence of arachidonic acid was inhibited by MMF and indomethacin (Table 3). Similar to indomethacin, but not imidazole, MMF inhibited prostaglandin D_2 formation in the presence of arachidonic acid (Table 4). Thus, MMF may be an inhibitor of cyclooxygenase rather than thromboxane synthetase. Compared to the inhibitory potency of indomethacin, the inhibitory potency of MMF on arachidonate-induced aggregation was 165 times lower. To eliminate the possible binding effect of bovine serum albumin on MMF, the experiments were repeated in bovine serum albumin-free Tyrode's buffer. However, instead of the inhibitory potency being enhanced, observations actually indicated a slight downward shift in activity. Nonetheless, comparison of IC_{50} values indicated the IC_{50} of MMF on arachidonate-induced aggregation of about 49.8 μM was comparable to that of 35.7 μM for aspirin.

Other than the collagen- and arachidonic acid-induced responses, the U46619-, ADP-, PAF- and thrombin-stimulated platelet aggregatory and secretory responses were also inhibited by MMF. U46619 acts as a thromboxane A_2 mimetic that is believed to induce platelet responses by activating thromboxane A_2 receptors directly via the phospholipase C-signal cascade (Ahn et al., 1988, Liel et al., 1987). ADP appears to trigger platelet responses by increasing $[Ca^{2+}]_i$ directly (Susanna et al., 1994). The exact mechanism underlying the actions of PAF or thrombin is unclear but the final common pathway for all of these activators is an increase in $[Ca^{2+}]_i$. Our experimental results showed that MMF inhibited the elevation of $[Ca^{2+}]_i$ caused by these inducers (Fig. 4). Since MMF inhibited

the effects of all of these agents to some extent, mechanisms in addition to cyclooxygenase inhibition might also exist. The importance of cyclic AMP in modulating platelet reactivity is well established (White and Gerrand, 1978). Elevated cyclic AMP inhibits most platelet responses, including a decrease in $[Ca^{2+}]_i$ due to uptake of Ca^{2+} into the dense tubular system and/or extrusion of Ca^{2+} from cells (Phillips and Shuman, 1986; Zavoico and Feinstein, 1984). As shown in Table 5, MMF did not induce any increase in cyclic AMP formation in washed rabbit platelets. Therefore, the inhibition of the $[Ca^{2+}]_i$ increase by MMF was not via cyclic AMP elevation. It has been reported that phosphoinositide breakdown is another important pathway, and that it may be a primary event in agonist-induced platelet activation (Berridge, 1984; Nishizuka, 1984). This phosphoinositide breakdown in platelets produces IP_3, leading to Ca^{2+} mobilization from the dense tubular system and to a $[Ca^{2+}]_i$ increase in platelets. The $[Ca^{2+}]_i$ change in platelets can also involve Ca^{2+}-specific channels and pumps in the plasma membrane (Tsai et al., 1988). Whether the inhibition of the $[Ca^{2+}]_i$ increase by MMF is due to the inhibition of phosphoinositide breakdown or to interference with the normal functioning of the Ca^{2+} transport system on the plasma membrane awaits further investigation.

In conclusion, the results from the present study demonstrated that MMF, a pure compound isolated from Draconis Resina, possesses anti-platelet actions. The anti-aggregation effect of MMF appeared in part to be due to the inhibition of thromboxane formation via the inhibition of cyclooxygenase, with the inhibition of the increase in $[Ca^{2+}]_i$ being a common factor. Draconis Resina has been demonstrated to possess toxicity, hemostatic activity, some analgesic effect and vasoactive–antithrombotic potency (Tsai et al., 1995). The same study also suggested that MMF, an active principle isolated from it, only accounted for part of the antithrombotic activity of Draconis Resina and that there appeared to be major active components other than MMF present in Draconis Resina (Tsai et al., 1995). Active principles possessing different and at times seemingly opposing effects are often found in the same herbal medicines. Discrete combinations of these principles may offer the advantage of normalizing abnormal body functions without severe side effects. Thus, the broad actions of Draconis Resina may offer certain potential therapeutic advantages.

Acknowledgements

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