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Effects of MK-447 on platelet shape change, aggregation, and ATP release by collagen, ADP, and stable analogue of thromboxane A_2 in rabbit platelets

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KEY WORDS MK-447; collagen; adenosine diphosphate; thromboxane A_2; blood platelets; platelet aggregation; adenosine triphosphate; indomethacin

ABSTRACT

AIM: To investigate the effects of MK-447 on platelet shape change, aggregation, and ATP release by collagen (Col), ADP, and stable analogue of thromboxane A_2 (STA_2) in rabbits. **METHODS**: Platelet shape change and aggregation were quantified in light transmission by turbidimetric method and release reaction was assessed by the amount of ATP in platelet-rich plasma (PRP). **RESULTS**: (1) MK-447 100 - 700 $\mu mol \cdot L^{-1}$ caused only the shape change, which was not inhibited by indometacin 3 $\mu mol \cdot L^{-1}$. Platelet shape changes by Col, ADP, and STA_2 were reduced ($P < 0.01$) after the addition of MK-447. The lag phase was prolonged ($P < 0.01$) in Col and shortened ($P < 0.01$) in ADP. (2) MK-447 reduced the aggregation by Col 5 $mg \cdot L^{-1}$ ($P < 0.01$), and enhanced that by ADP 0.3 - 10 $\mu mol \cdot L^{-1}$ and STA_2 0.1 - 3 $\mu mol \cdot L^{-1}$ ($P < 0.01$). (3) The release reaction by STA_2 1 - 3 $\mu mol \cdot L^{-1}$ was also increased ($P < 0.01$). The effects of MK-447 on STA_2 were not inhibited by S-145. **CONCLUSION**: MK-447 induced the platelet shape change, and showed the dual effects, inhibition or enhancement, on the actions by different aggregating agents.

INTRODUCTION

MK-447 (2-aminomethyl-4-*t*-butyl-6-iodophenol hydrochloride) as an anti-inflammatory agent possessed the dual effects on prostaglandin (PG) endoperoxide biosynthesis by acting as a tryptophan-like cofactor of PG hydroperoxide synthase[1-3]. In the presence of MK-447, the production of epoprostenol (PGI_2) was stimulated in isolated rat aorta without the effect on thromboxane (TX) generation in blood platelets[4]. Collagen-induced aggregation was not inhibited by MK-447 300 $\mu mol \cdot L^{-1}$, but inhibited by its analog[5]. Our previous results showed that thrombin-induced aggregation and release reaction were enhanced by MK-447 due to the synergistic effect of intracellular calcium mobilization[6]. The present study attempted to know MK-447 effects on platelet shape, aggregation, and release reaction elicited by collagen (Col), adenosine diphosphate (ADP), and an analog of thromboxane A_2 (STA_2).

MATERIALS AND METHODS

Agents Lucifferin-lucifferase (Sigma Chemical Co) 40 $mg \cdot L^{-1}$ for measuring the secretion of ATP was dissolved in saline before use. ATP (Sigma) 1 $mmol \cdot L^{-1}$ was dissolved in distilled water, stored at -20 ℃, and diluted with saline to 1 $\mu mol \cdot L^{-1}$ before use. MK-447 (Merck, Sharp & Dohome) was dissolved in saline 10 $g \cdot L^{-1}$, kept at 4 ℃, and diluted with Tris-buffered saline before use. STA_2 and S-145[7] were kindly provided from Shionogi and Ono Pharmaceutical Co, respectively. ADP, HEPES, and Col were from Sigma Co.

Preparation of platelet-rich plasma (PRP)[6].

Platelet aggregation Platelet aggregation was quantified in light transmission through PRP by turbidimetric method[8].

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Platelet release reaction The release reaction was assessed by the luminescence of ATP released in the medium from dense granules during platelet aggregation[6,9].

Statistical analysis All data were from at least 6 rabbits $\bar{x} \pm s$ and compared by t test.

RESULTS

Effects on Col-induced changes MK-447 concentration-dependently induced platelet shape changes without aggregation, and this effect of MK-447 was not affected by indometacin 3 $\mu mol \cdot L^{-1}$. The shape change, aggregation, and ATP secretion by Col 5 $mg \cdot L^{-1}$ were reduced by the pretreatment of PRP with MK-477 100 – 700 $\mu mol \cdot L^{-1}$ in a concentration-dependent manner at 10 min before the addition of Col. The lag phases (duration of shape change) were prolonged. MK-447-induced shape change was well correlated negatively with the effects of MK-447 on Col ($r^2 = 0.988$, $P < 0.01$). (Tab 1)

When PRP was incubated with MK-447 for 2 or 5 min, the effects of MK-447 on Col were less potent than those for 10 min (Tab 2).

Effects on ADP-induced changes After the PRP was pretreated with MK-447 100 – 700 $\mu mol \cdot L^{-1}$ at 10 min before ADP, the lag phase of aggregation was shortened, the shape change was reduced, and the aggregation was enhanced without the effect on ATP secretion. (Tab 3)

Effects on STA_2-induced changes STA_2 0.1 – 3 $\mu mol \cdot L^{-1}$ concentration-dependently caused platelet shape change and aggregation, which were accompanied with ATP release when STA_2 reached 1 – 3 $\mu mol \cdot L^{-1}$. The shape change was reduced, but both aggregation and ATP release were enhanced after the addition of MK-447 700 $\mu mol \cdot L^{-1}$ 10 min prior to STA_2. In the presence of S-145 100 $nmol \cdot L^{-1}$, the effects of MK-447 on STA_2 were not blocked. (Tab 4)

Tab 2. Effects of incubation time on platelet aggregation by Col 5 $mg \cdot L^{-1}$ in the presence of MK-447 in rabbit PRP. $n = 12$ preparations from 6 rabbits in each group. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

MK-447/$\mu mol \cdot L^{-1}$	Aggregation at different incubation time/%		
	2 min	5 min	10 min
0	72 ± 8	70 ± 12	69 ± 7
300	69 ± 9[a]	67 ± 8[a]	65 ± 10[a]
500	56 ± 7[b]	49 ± 4[c]	41 ± 6[c]
700	52 ± 11[c]	37 ± 14[c]	10.4 ± 2.8[c]

DISCUSSION

Platelet shape change is an important and essential early event during platelet activation by some agents like MK-447, thrombin, serotonin, and ADP[6,10]. Our results demonstrated that MK-447 induced the shape change without aggregation and ATP release in rabbit platelets, which was involved in intracellular Ca^{2+} release[6] and not related to cyclooxygenase products. It was also shown that once blood platelets underwent the shape change by MK-447, other

Tab 1. Effects of MK-447 on Col-induced shape change, aggregation, and ATP secretion in rabbit PRP. $n = 28 – 36$ preparations from 6 rabbits. $\bar{x} \pm s$. $^aP > 0.05$ *vs* MK-447 only. $^fP < 0.01$ *vs* control.

Groups	n	MK-447/$\mu mol \cdot L^{-1}$				
		0	100	300	500	700
MK-447						
Shape change/%	31		1.9 ± 1.1	4.7 ± 1.3	10 ± 3	11 ± 6
Indometacin 3 $\mu mol \cdot L^{-1}$	28		1.8 ± 1.3[a]	4.2 ± 1.1[a]	10 ± 4[a]	11 ± 4[a]
Col 5 $mg \cdot L^{-1}$						
Shape change/%	30	7.8 ± 2.2	7.9 ± 2.4	8.4 ± 2.5	2.4 ± 1.8[f]	0.7 ± 0.4[f]
Lag phase/s	30	84 ± 12	102 ± 8	96 ± 14	120 ± 18[f]	156 ± 24[f]
Aggregation/%	30	68 ± 8	67.5 ± 2.6	63 ± 4.6	43 ± 42[f]	4.8 ± 2.6[f]
ATP release/μmol	30	0.82 ± 0.16	0.79 ± 0.14	0.52 ± 0.15[f]	0.24 ± 0.14[f]	0

Tab 3. Effects of MK-447 on platelet shape change and aggregation by ADP 0.3 – 10 μmol·L^{-1} in rabbit PRP. n = 16 – 18 preparations from 6 rabbits. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Groups	n	MK-447/μmol·L^{-1} 0	100	300	500	700
Shape change/%	18	8±3	7.6±1.7	4.5±2.2^c	2±3^c	1.1±0.8^c
Lag phase/s	18	41±8	33±4^b	21±3^c	1.8±2.8^c	4.4±1.8^c
Aggregation/%						
ATP 0.3 μmol·L^{-1}	16	1.4±0.6	9±6^c	11±8^c	19±11^c	23±10^c
ATP 1 μmol·L^{-1}	18	19±4	34±3^c	41.8±2.3^c	49.2±2.9^c	52±3^c
ATP 3 μmol·L^{-1}	17	34±5	47±6^c	53.6±1.4^c	61±4^c	64.0±2.8^c
ATP 10 μmol·L^{-1}	16	50±4	56.8±1.1^c	65±5^c	71±4^c	73±7^c

Note: Shape change and lag phase were measured with ADP 3 μmol·L^{-1}.

Tab 4. Effects of MK-447 700 μmol·L^{-1} on STA_2-induced aggregation and ATP release in the absence or presence of S-145 100 nmol·L^{-1} in rabbit PRP. n = 7 – 13 preparations from 6 rabbits. $\bar{x} \pm s$. $^cP < 0.01$ *vs* STA_2. $^dP > 0.05$ *vs* MK-447.

	n	Stable of analogue of thromboxane A_2/μmol·L^{-1} 0.1	0.3	1	3
Shape change/%					
STA_2	13	0	2.1±2.2	7.8±2.1	8±3
MK-447	10	0	0	1.1±1.8^c	1.2±1.4^c
S-145 + MK-447	7	0	0	1.0±2.6^d	1.4±2.1^d
Aggregation/%					
STA_2	13	0	0	22.4±2.4	45±4
MK-447	10	4.5±1.8^c	32±4^c	66.6±2.8^c	64.4±2.9^c
S-145 + MK-447	7	7.3±3.5^d	27±6^d	59±6^d	62±4^d
ATP release/μmol					
STA_2	13			0.04±0.03	0.22±0.07
MK-447	10			0.58±0.14^c	1.09±0.14^c
S-145 + MK-447	7			0.54±0.26^d	1.15±0.23^d

aggregating agents, such as thrombin[6], ADP, and STA_2, could not cause the shape change again until MK-447-induced shape change was recovered, after MK-447, thrombin, ADP, and STA_2 directly activated platelets resulting in the acceleration of platelet activation.

Pretreatment of PRP with MK-447 enhanced the aggregation by ADP and STA_2, which was accordant with the effect of MK-447 on thrombin[6] and similar to that of 5-HT on ADP[10] and lower concentration of STA_2[11]. The possible mechanisms about the enhancement of aggregation by MK-447 were due to the synergistic effect of intracellular Ca^{2+} mobilization and the increase in platelet dense granule secretion including endogenous 5-HT[12]. In the present experiment, ADP 10 μmol·L^{-1} induced a full aggregation because of the saturate effect of fibrinogen receptor, but it was still enhanced by MK-447 under this condition without the increase in release reaction, suggesting that more fibrinogen receptors might be exposed via intracellular Ca^{2+} release by MK-447. In addition, TXA_2 receptors were not involved in the effects of MK-447 on STA_2, so, other unknown mechanism might be associated with the effects of MK-447 on STA_2 (there was no influence of MK-447 on STA_2 in the presence of S-145). In contrast with the results mentioned above, the aggregation and release reaction by Col were inhibited by MK-477 preincubation when its concentration was over 300 μmol·L^{-1}, this result was consistent with the previous report[5] in which the inhibitory effect of MK-447 300 μmol·L^{-1} on Col was not found, but it was observed in this study with higher concentration of MK-447 and the incubation time was also related.

Taken together our previous and present results, it was concluded that MK-447 caused a sustained platelet shape change and the complex effects on aggregation and release reaction, in which, at least, intracellular Ca^{2+} release was involved in its enhancement, but the inhibitory effect of MK-447 on Col was not explained with these experiments.

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MK-447 对兔血小板中胶原、ADP 及血栓素 A_2 稳定类似物诱导的血小板变形、聚集和腺苷三磷酸释放的影响

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关键词 MK-447；胶原；腺苷二磷酸；血栓素 A_2；血小板；血小板聚集；腺苷三磷酸；吲哚美辛

目的：研究 MK-447 对胶原、ADP 及血栓素 A_2 稳定类似物(STA_2)诱导的血小板变形、聚集和释放反应的影响. **方法**：浊度法评价血小板变形和聚集反应，测定富含血小板血清中 ATP 的量确定释放反应. **结果**：(1) MK-447 诱导血小板变形，不被吲哚美辛抑制. 预置 MK-447 可使胶原、ADP 及 STA_2 的血小板变形能力下降，时程延长. (2) MK-447 抑制胶原的聚集反应，并使 ADP 和 STA_2 聚集增强. (3) 胶原和 STA_2 的释放反应可被 MK-447 抑制和增强. MK-447 对 STA_2 的作用与 S-145 无关. **结论**：血小板变形在其激活早期发挥重要作用. MK-447 诱导血小板变形，并对不同聚集剂的作用表现为抑制和增强的双重影响.

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