中国药理学报 *Acta Pharmacologica Sinica* 1991 Jul ; 12 (4) : 331-335

Effects of CI-930 on hemostasis, thrombosis, and AA-induced hemodynamic reaction[1]

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ABSTRACT In mice, CI-930 0.5-2 mg · kg^{-1} ip not only prolonged the tail bleeding time but also protected the mice from sudden thromboembolic death induced by arachidonic acid (AA, 100 mg · kg^{-1}, iv) or TXA_2/PGH_2 mimetic U46619 (200 μg · kg^{-1}, iv). CI-930 0.625 and 2.5 mg · kg^{-1} iv exhibited a dose-dependent inhibitory effect on thrombus formation in rat arteriovenous shunt. All these effects of CI-930 were more potent than those of dazoxiben, a known antiplatelet drug. In rabbit, AA 0.75 mg · kg^{-1} iv caused a rapid and marked increase in pulmonary vascular resistance and a concomitant sharp decrease in cardiac output and carotid arterial pressure. CI-930 itself 0.5 mg · kg^{-1} iv resulted in a long-lasting fall in carotid arterial pressure, systemic vascular resistance, and a slight decrease in cardiac output. In addition, CI-930 protected rabbit from all the harmful hemodynamic responses to the occlusion of pulmonary microcirculation, which was induced by AA. The results suggest that CI-930 possess a potent antihemostatic, antithrombotic, and probably antihypertensive effects on experimental animals.

KEY WORDS CI-930; dazoxiben; arachidonic acids; bleeding time; pulmonary artery; thrombosis; thromboembolism; blood pressure; vascular resistance; cardiac output

The cardiotonic agent CI-930 (4,5-dihydro-6-[4-(1*H*-imidazol-1-yl) phenyl]-5-methyl-3(2*H*)-pyridazinone) is a phosphodiesterase Ⅲ inhibitor[1]. It possesses inotropic activities in both isolated myocardial strips and intact animals[2]. In patients with severe congestive heart failure, CI-930 improved their cardiac performance with a moderate decrease in systemic and pulmonary arterial pressures[3,4]. In recent studies from our laboratory[5], we have demonstrated that CI-930 possessed powerful inhibitory effects on platelet aggregation and thromboxane A_2 (TXA_2) biosynthesis from platelets and neutrophils with a corresponding increase in PGE_2, $PGF_{2\alpha}$, and PGD_2 production. In this study, we examined the effects of CI-930 on hemostasis, thrombosis, and rabbit hemodynamic responses to AA trying to find out whether CI-930 possesses antithrombotic properties in several thrombosis models.

Received 1990 Nov 15 Accepted 1991 May 9

[1] Project supported by the Nationl Natural Science Foundation of China, № 3880741

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MATERIALS AND METHODS

Animals Animals used were ♂ Kunming strain mice weighing 21.4 ± SD 2.0 g, New Zealand rabbits weighing 3.0 ± 0.3 kg, and ♂ Wistar rats weighing 311 ± 42 g.

Chemicals CI-930 (Department of Medical Chemistry in our College) and dazoxiben (Shanghai Zhaohui Pharmaceutical Factory) were freshly dissolved in normal saline. TXA_2/PGH_2 mimetic U46619, a gift from the Upjohn Company, was dissolved in ethanol, stored at -20℃ and diluted in distilled water containing 0.2% Na_2CO_3. Sodium arachidonate solution was prepared by dissolving arachidonic acid (Sigma, >99% pure) in Na_2CO_3 100 mmol · L^{-1}. Final dilutions for injection were made in normal saline before use.

Tail bleeding time in conscious mice The tail bleeding time test was essentially performed as previously described[6] Twenty min after ip CI-930 or dazoxiben, the bleeding

time was measured. Bleeding time exceeding 10 min were recorded as > 600 s.

Thrombus formation *in vivo* Male Wistar rats were anesthetized with sodium pentobarbitone 40 mg · kg^{-1} ip. An extra-corporeal shunt was made between the right jugular vein and left carotid artery[7]. The circulation of blood was established 20 min after iv CI-930 or dazoxiben. Two thrombi were formed in each rat and total weight of the two thrombi were used for statistical analysis.

Acute pulmonary thromboembolic death in mice Acute pulmonary thromboembolic death was induced in ♂ mice by iv AA (100 mg · kg^{-1}) or U46619 (200μg · kg^{-1}) into the tail vein[8,9]. CI-930 or dazoxiben were injected by ip 30 min before challenge with AA or U46619. The mice were observed for signs of respiratory distress and the time of death. Mice which lived for 30 min after iv AA or U46619 were considered as survivors.

Hemodynamic responses in rabbits Rabbits were anesthetized by iv urethane 1-1.2 g · kg^{-1}. After endotracheal intubation, ventilation with air was maintained using a respiratory pump (frequency: 50 times · min^{-1}, tidal valume: 20 ml). Catheters were placed in the right jugular vein and left carotid artery for administration of drugs and measurement of arterial blood pressure (BP) respectively. Another catheter with a blunted needle was directly inserted into the pulmonary artery for the measurement of pulmonary arterial BP[10]. An electromagnetic flow probe (5-6 mm lumen) connecting the flow meter (Model MFV-1200) was placed around ascending aorta for the determination of cardiac output (CO, ml · min^{-1}). Arterial BP, pulmonary arterial BP, CO, and electrocardiogram (lead II) were continuously recorded via an eight-channel ink-jet recorder on a polygraph system (Model RM-6000, Nihon Kohden).

Statistical analysis All measurement data are expressed as $\bar{x} \pm$ SD. Differences between the means were analyzed with t test. Survival data (enumeration data) were analyzed by Fisher's exact probability test.

RESULTS

Effects of CI-930 and dazoxiben on mice tail bleeding time CI-930 ip prolonged the tail bleeding time significantly compared with control group in conscious mice. The antihemostatic effect of CI-930 was dosedependent (Tab 1). CI-930 1 mg · kg^{-1} ip made the tail bleeding time > 600 s in 8 of 17 mice. A known thromboxane synthetase inhibitor, dazoxiben, 4 mg · kg^{-1} ip prolonged the tail bleeding time from 25 ± 13 to 63 ± 48 s.

Tab 1. Effects of CI-930 and dazoxiben on tail bleeding time in mice. $\bar{x} \pm$ SD. $^{*}P > 0.05$, $^{***}P < 0.01$ vs control.

	Dose, mg · kg^{-1}	n	Bleeding time, s
Control	–	29	25 ± 10
CI-930	0.5	15	40 ± 20***
	1	17	400 ± 223***
	2	13	485 ± 146***
Dazoxiben	2	16	25 ± 13*
	4	9	63 ± 48***

Effects of CI-930 and dazoxiben on acute pulmonary thromboembolic death in mice AA 50 mg · kg^{-1} or U46619 100μg · kg^{-1} iv caused sudden death in 1/3 or 2/5 mice respectively. Less than 1 min after iv a lethal dose of AA (100 mg · kg^{-1}) or U46619 (200 μg · kg^{-1}), All the mice began to exhibit almost the same symptoms characterized by obvious respiratory distress and cyanosis. This was soon followed by bulging of the eyes, convulsive seizures, and cessation of respiration. There was more than 95% mortality within 3 min. CI-930 (0.5, 1, 2 mg · kg^{-1} ip) decreased the degrees of respiratory distress and the mortality (Tab 2) in a dose-dependent

manner. Dazoxiben 2 mg · kg^{-1} ip was ineffective to protect the mice from sudden death induced by both AA and U46619.

Tab 2. Effects of CI–930 and dazoxiben on AA (100 mg · kg^{-1} iv) or U46619 (200 μg · kg^{-1} iv)–induced acute thromboembolic death in mice. Data were analyzed by Fisher's exact probability test. $^{*}P>0.05$, $^{***}P<0.01$ *vs* control.

	Dose, mg · kg^{-1}	Mice died / mice dosed AA	U46619
Control	–	15 / 15 (100%)	12 / 12 (100%)
CI–930	0.5	5 / 11 (45%)***	5 / 11 (45%)***
	1	3 / 14 (21%)***	3 / 11 (27%)***
	2	1 / 14 (7%)***	2 / 12 (17%)***
Dazoxiben	2	9 / 9 (100%)*	8 / 8 (100%)*
	4	9 / 10 (90%)*	

Effects of CI–930 and dazoxiben on thrombus formation *in vivo* Insertion of a cotton thread into the extracorporeal shunt between the carotid artery and jugular vein caused an increase in the weight of the thread due to deposition of thrombus. CI–930 (0.625–2.5 mg · kg^{-1}) and dazoxiben (2.5 mg · kg^{-1}) iv reduced the thrombus weight. (Tab 3). CI–930 2.5 mg · kg^{-1} was more potent than dazoxiben with 59.3% inhibition of control. The inhibitory effect of CI–930 and dazoxiben on thrombus formation was dose–related.

Effects of CI–930 on rabbit hemodynamic

Tab 3. Effect of CI–930 and dazoxiben on thrombus formation in rat extracorporeal shunt. $\bar{x}$ ± SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

	Dose, mg · kg^{-1}	*n*	Thrombus weight, mg
Control	–	9	59 ± 12
CI–930	0.16	6	60 ± 8*
	0.63	5	45 ± 10**
	2.5	6	24 ± 4***
Dazoxiben	0.63	6	64 ± 12*
	2.5	5	42 ± 10**

responses to pulmonary microcirculation occlusion induced by AA AA (0.75 mg · kg^{-1}) iv caused a marked increase in mean pulmonary arterial BP, pulmonary vascular resistance, and a sharp decrease in CO and mean carotid arterial BP (Tab 4). These responses reached a peak 1–2 min after AA iv and lasted 3–5 min. CI–930 itself 0.5 mg · kg^{-1} iv caused a long–lasting decrease in mean carotid arterial BP, CO, and systemic vascular resistance, but had no effect on pulmonary vascular resistance. About 20 min after iv CI–930, the rabbits challenged with AA showed only a small and transient rise in pulmonary vascular resistance and a slight decrease in CO and mean carotid arterial BP. There were significant differences between AA group and CI–930+AA group demonstrating a protective effect of CI–930 on AA–challenged rabbits. Heart rate tended to decrease by AA but tended to increase by CI–930 ($P>0.05$).

DISCUSSION

The effects of CI–930 which consists of prolonging the tail bleeding time in mice, inhibiting the thrombus formation in rat, and protecting the mice from acute pulmonary thromboembolic death were more potent than another antiplatelet drug dazoxiben, a thromboxane synthetase inhibitor. These results indicate that CI–930 possesses powerful *antihemostatic* and *antithrombotic effects in* experimental models and may be more useful than dazoxiben in preventing and treating thromboembolic disorders.

The observation that U46619, a TXA_2 / PGH_2 analogue, produced the same respiratory distress in mice as AA did strongly suggested that prostaglandin endoperoxides and TXA_2, the main metabolites of AA via cyclooxygenase, were mainly involved in platelet aggregation and pulmonary thromboembolism in mice. The evidence that CI–930 protected the mice from sudden

Tab 4. Rabbit hemodynamic responses to iv AA (0.75 mg · kg^{-1}, $n=9$), iv CI–930 (0.5 mg · kg^{-1}, $n=10$), and iv AA 20 min after iv CI–930 ($n=7$). $\bar{x}\pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control. $^{+}P<0.05$ *vs* iv AA group.

		Control	1 min	2 min	3 min	5 min	10 min	20 min
PAP_m (kPa)	AA	2.2±0.3	3.6±0.5***	3.1±0.3***	2.7±0.4***	2.4±0.3*	2.23±0.17*	2.27±0.24*
	CI–930	2.3±0.4	2.1±0.3*	2.0±0.3*	1.9±0.3**	1.85±0.27**	1.87±0.28**	2.0±0.3*
	CI–930+AA	2.0±0.3	2.3±0.4*	2.3±0.4*	2.03±0.28*	1.9±0.4*	1.9±0.4*	2.0±0.4*
CAP_s (kPa)	AA	14.7±0.9	10.1±2.5***	9.3±1.4***	10.9±1.2***	12.7±2.4**	13.5±2.4*	14.8±1.5*
	CI–930	14.8±1.9	12.5±0.8***	11.7±0.8***	11.7±0.8***	11.3±0.9***	11.2±1.1***	10.9±0.8***
	CI–930+AA	10.9±0.8	10.5±0.9*	9.2±1.5***	9.3±1.3***	9.6±0.9***	10.9±1.3*	11.6±1.2*
CAP_d (kPa)	AA	10.5±0.9	6.1±1.9***	6.0±1.2***	7.1±1.9***	8.3±2.3**	9.7±2.3*	10.7±1.5*
	CI–930	10.4±1.9	5.9±1.2***	5.7±1.1***	5.9±1.1***	5.7±1.2***	6.4±1.6***	6.3±1.6***
	CI–930+AA	6.3±1.6	5.6±1.2*	4.8±0.7**	4.9±0.8*	5.5±1.5*	6.7±1.7*	7.1±1.5*
CAP_m (kPa)	AA	11.9±0.9	7.3±2.1***	7.2±1.2***	8.4±1.6***	9.7±2.3**	10.9±2.3*	12.1±1.3*
	CI–930	11.9±1.9	8.1±0.9***	7.7±0.8***	7.7±0.7***	7.6±1.1***	8.0±1.3***	7.9±1.2***
	CI–930+AA	7.9±1.2	7.2±0.9*	6.3±0.8***	6.4±0.9**	7.1±1.5*	8.1±1.6*	8.5±1.5*
CO ml · min^{-1}	AA	340±45	225±51***	127±51***	172±56***	261±61***	311±59*	333±51*
	CI–930	346±65	330±57*	297±43*	286±37*	271±44***	279±46**	284±41**
	CI–930+AA	284±41	257±51*	224±50^{**+}	249±42*	261±38*	289±43*	299±35*
$PAP_m \cdot CO^{-1}$ kPa · L^{-1} · min^{-1}	AA	6.8±1.1	17±6***	27±13***	15±6***	10±4**	8.1±2.1*	7.5±1.6*
	CI–930	6.9±1.3	6.8±1.5*	7.1±1.5*	6.8±1.5*	7.1±1.5*	7.1±1.6*	6.9±1.5*
	CI–930+AA	6.9±1.5	10±3***	11±3^{***+}	9.2±2.1^{**+}	7.9±1.7*	6.9±1.7*	6.8±1.6*
$CAP_m \cdot CO^{-1}$ kPa · L^{-1} · min^{-1}	AA	34±8	41±14*	45±10**	44±14*	40±12*	35±8*	34±7*
	CI–930	36±6	25±4**	26±4***	28±4***	28±4***	29±5***	28±4***
	CI–930+AA	28±4	29±6*	29±5^{**+}	28±5*	28±4*	28±5*	29±5*
HR beats · min^{-1}	AA	272±32	267±36*	256±40*	253±37*	259±41*	262±40*	256±34*
	CI–930	268±47	283±43*	290±43*	283±43*	286±39*	278±43*	277±40*
	CI–930+AA	277±40	268±36*	259±42*	261±43*	264±42*	264±39*	263±42*

PAP_m = mean pulmonary arterial pressure; CAP = carotid arterial pressure (systolic, diastolic, mean); CO = Cardiac output; HR = Heart rate.

thromboembolic death induced by U46619 indicated that CI–930 also possessed an antagonistic effect against U46619. Whether or not CI–930 is a TXA_2 / PGH_2 receptor antagonist awaits further study.

The purpose of this rabbit hemodynamic test was to provide an analysis of the antagonistic effect of CI–930 against AA *in vivo*. The rabbit hemodynamic responses to AA were all correlated with formation of aggregated platelet masses and occlusion of the microcirculation in lung. The protective effect of CI–930 on AA–challenged rabbit provided further evidence that CI–930 was effective in prevention and treatment of thromboembolic disorders. Furthermore, the evidence that CI–930 produced a long lasting decrease in systemic vascular resistance strongly demonstrated that CI–930 possessed a vasodilating effect *in vivo*. This may be useful in treatment

of hypertension especially hypertension with congestive heart failure.

ACKNOWLEDGMENTS U46619 was a gift from Dr J E Pike of Upjohn Co in Kalamazoo MI; CI-930 and dazoxiben were provided by Prof WAN Wei-Qin and WEI Jin-You respectively in our College.

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CI-930 对止血、血栓形成和 AA 引起的血流动力学改变的影响

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提要 CI-930 0.5-2 mg·kg^{-1} ip 延长小鼠尾出血时间，保护 AA (100 mg·kg^{-1} iv)和 U46619 (200 μg·kg^{-1} iv)引起的血栓栓塞性卒死. CI-930 0.625, 2.5 mg·kg^{-1} iv 还抑制大鼠体内动脉血栓的形成. AA 0.75 mg·kg^{-1} iv 增加兔 PAP_m, 降低 CO. CI-930 0.5 mg·kg^{-1} iv 降低 PAP_m 和体循环阻力并能对抗 AA 对兔血流动力学的有害影响. 提示 CI-930 具有抗止血，抗血栓形成及可能的抗高血压作用.

关键词 CI-930; 达唑氧苯; 花生四烯酸; 出血时间; 肺动脉; 血栓形成; 血栓栓塞; 血压; 血管阻力; 心输出量