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Blockade of Thromboxane and the Prevention of Eicosanoid-Induced Sudden Death in Mice[1] (42190)

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Abstract. We studied the effects of thromboxane-receptor antagonism and thromboxane synthetase inhibition in a thrombotic model of sudden death in mice. Intravenous injection of arachidonic acid (AA; 80 mg/kg) or the prostaglandin–endoperoxide analog U-46,619 (2.3 mg/kg) results in sudden death in approximately 90% of the animals. Pretreatment with the thromboxane receptor antagonist SQ-29,548 (0.3–10 mg/kg) protects dose-dependently against AA and U-46,619-induced sudden death. In contrast, CGS-13,080, a thromboxane synthetase inhibitor, shows a dose-dependent beneficial effect in AA-induced sudden death only. Although PTA_2 has partial thromboxane agonistic properties in the rabbit, it protected the mice against AA-induced sudden death, thus demonstrating TxA_2 antagonistic properties in this species. These data emphasize the importance of thromboxane A_2 as a major mediator of arachidonic acid-induced sudden death and the effectiveness of thromboxane-receptor antagonists in endoperoxide-induced sudden death.

Silver *et al.* (1) demonstrated that intravenous injection of arachidonic acid (AA) in rabbits led to sudden death within a few minutes. Intravenous injection of AA or the prostaglandin–endoperoxide analog U-46,619 into mice produced a response similar to rabbits, which leads to death within 2–3 min (2, 3). Platelet thrombi, trapped in the pulmonary microcirculation, are a key aspect of the sudden death (1, 2). Thromboxane (Tx) A_2 is quantitatively a very prominent platelet eicosanoid (4) product formed and acts as a vasoconstrictor and potent proaggregatory agent. It was shown to be the primary mediator of AA-induced sudden death in rabbits (5). The plasma concentrations of TxB_2, the chemically stable degradation product of TxA_2, have been shown to be significantly elevated after AA challenge (6, 7). Pharmacologic pretreatment of rabbits with glucocorticoids (8), nonsteroidal anti-inflammatory drugs (9), thromboxane synthetase inhibitors (6), or thromboxane-receptor antagonists (3, 5, 10) prevents either the increase in plasma TxB_2 concentrations or the actions of thromboxanes, and protects against sudden death. The studies of Myers *et al.* (3) suggest that TxA_2 is also a major mediator of sudden death in mice.

In this study, we used a modification of the sudden death model in mice (2) by working with anesthetized mice (3) without introducing an intravenous catheter, but by performing the intravenous injection into the tail and penile veins. The antithrombotic potential of SQ-29,548, a new specific thromboxane-receptor antagonist (11) was evaluated. We also tested the thromboxane synthetase inhibitor CGS-13,080 (12) and the thromboxane-receptor antagonist pinane thromboxane A_2 (PTA_2) (13). PTA_2 is a thromboxane analog with thromboxane-receptor antagonistic activities in human, cat, and guinea pig platelets and in dog, cat, and guinea pig coronary arteries. However, PTA_2 does not exert any antiaggregatory activity in rabbit platelets and actually constricts rabbit coronary arteries, behaving as a partial thromboxane agonist in this species (14). Therefore, its antagonistic properties *in vivo* to inhibit AA-induced sudden death were examined in the mouse.

Materials and Methods. Male CS-1 mice with a weight range of 29–33 g were purchased from Charles River Breeding Company (Wilmington, Mass.) and were kept under controlled environmental conditions with food and water intake *ad libitum*. Mice were anes-

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TABLE I. PREVENTION OF ARACHIDONIC ACID (AA, 80 mg/kg)-INDUCED SUDDEN DEATH IN MICE BY PRETREATMENT WITH PINANE THROMBOXANE (PTA_2)

Pretreatment	Stimulus	Survivors/animals tested	% Survival	Significance
Vehicle	Vehicle	11/14	79	—
PTA_2 (3 mg/kg)	Vehicle	4/5	80	N.S.
Vehicle	AA	2/24	8	$P < 0.005$*
PTA_2 (1 mg/kg)	AA	0/3	0	—
PTA_2 (3 mg/kg)	AA	6/9	67	$P < 0.005$**

Note. PTA_2 (1 or 3 mg/kg) or its vehicle (ethanol: 50 μl) was injected into the tail vein 2 min prior to injection of arachidonic acid (80 mg/kg) or its vehicle (Na_2CO_3, 10 m*M*: 100 μl) into the penile vein. N.S. = not significant from vehicle/vehicle group.

* Compared to vehicle/vehicle group.

** Compared to vehicle/AA group.

thetized (3) with sodium pentobarbital (75 mg/kg, ip). Pinane thromboxane A_2 obtained from Biomol Company, Philadelphia, Pennsylvania, was dissolved in ethanol and stored at −30°C as a 2 mg/ml stock solution in ethanol. SQ-29,548 was provided by the Squibb Institute for Medical Research (Princeton, N.J.) and dissolved at a concentration of 10 mg/ml in ethanol. CGS-13,080, a gift from Ciba-Geigy Corporation (Summit, N.J.), was dissolved at a concentration of 10 mg/ml in 0.9% NaCl and adjusted to pH 8.0 with sodium hydroxide. Further dilutions of the drugs were made in their respective solvents. Drug solutions or their respective vehicles were injected into the tail vein at a volume of 50 μl and concentrations of 0.3, 1, 3, and 10 mg/kg. Arachidonic acid (Nu Chek, Elysian, Minn.) was dissolved in 10 m*M* Na_2CO_3 at a concentration of 25 mg/ml. U-46,619 (Upjohn Co., Kalamazoo, Mich.) was dissolved in ethanol at a concentration of 1.4 mg/ml. Two minutes after the drug pretreatment (3), 100 μl of AA solution containing 80 mg/kg (i.e., 2.5 mg per mouse), its vehicle or 50 μl of U-46,619 containing 2.3 mg/kg (i.e., 70 μg per mouse), or its vehicle were injected into the dorsal vein of the penis. Animals living 60 min after the AA challenge were considered survivors. Statistical comparisons between the groups were performed by using χ^2 test according to Snedecor (15) and $P < 0.05$ was regarded as being significant.

Results. Injection of AA into anesthetized mice usually produced lethality in 1 to 3 min. The survival rate following AA alone was only 8% (2 of 24; Table I) after a 1-hr observation period. This is significantly different ($P < 0.005$) from the 79% (11 of 14; Table I) and 92% (12 of 13) survival rate observed in mice 1 hour after iv injection of the vehicles employed (ethanol or saline, 50 μl each and Na_2CO_3, 10 m*M*, 100 μl). Arachidonic acid-induced sudden death in mice was characterized by respiratory arrest, convulsions, and cardiac arrest. Pretreatment of the animals with the thromboxane-receptor antagonist SQ-29,548 (Fig. 1) exerted no direct toxic effect itself (11 of 12 survived; Fig. 1), similar to its vehicle (11 of 14; Table I). Moreover, SQ-29,548 protected significantly and dose dependently against AA-induced sudden death (Fig. 1). At 1 mg/kg of SQ-29,548, 38% of the

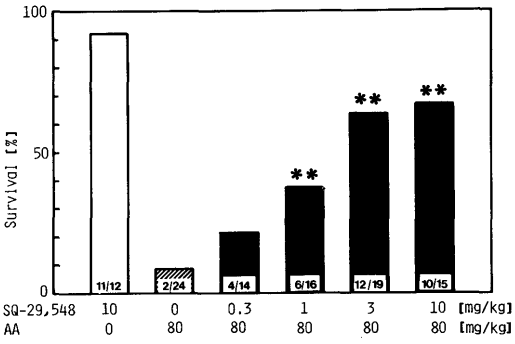

FIG. 1. Survival rates in mice injected with arachidonic acid (AA: 80 mg/kg) alone, or with AA + SQ-29,548 (0.3 to 10 mg/kg). As a control, SQ-29,548 (10 mg/kg) was given alone (first bar). Survival was significantly improved in the drug-treated groups at 1 mg/kg ($P < 0.01$), 3, and 10 mg/kg ($P < 0.005$) relative to the group receiving AA (80 mg/kg). Numbers inside bars indicate the number of mice studied in each group.

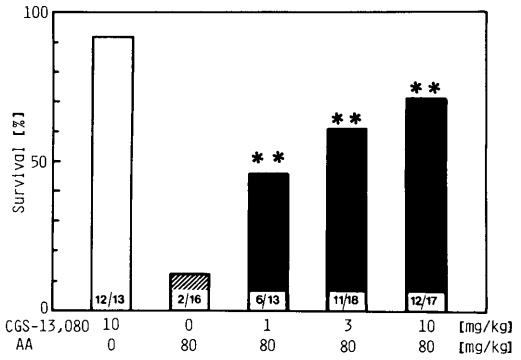

FIG. 2. Survival rates in mice injected with arachidonic acid (AA) alone, or with AA + CGS-13,080 (1 to 10 mg/kg). As a control CGS-13,080 (10 mg/kg) was given alone (first bar). All doses (1, 3, and 10 mg/kg) of this thromboxane synthetase inhibitor improved survival ($P < 0.005$) relative to the group receiving AA (80 mg/kg). The numbers inside bars indicate the number of mice studied in each group.

mice survived, at 3 mg/kg of SQ-29,548 63% survived, and at 10 mg/kg 67% of the animals survived arachidonic acid. These effects are significantly different ($P < 0.01$–0.005) from the survival rate in the untreated AA group (2 of 24; Fig. 1). Pinane thromboxane A_2, which has both agonist and antagonist properties in different species, did not protect against sudden death at 1 mg/kg, but did give a 67% survival rate at a dose of 3.0 mg/kg ($P < 0.005$, Table I). When the animals were pretreated with the thromboxane synthetase inhibitor, CGS-13,080, the incidence of sudden death in response to AA was also significantly reduced (Fig. 2). CGS-13,080 at a dose of 10 mg/kg injected prior to the vehicle for AA did not exert any direct toxic effect. At this dose, 92% (12 of 13) survived (Fig. 2) as compared to a 92% survival rate in control mice given only the vehicle for CGS-13,080 and AA. Moreover, in mice challenged with AA, CGS-13,080 showed a dose-dependent protective effect after injection of 1, 3, or 10 mg/kg with survival rates of 46, 61 or 71% ($P < 0.005$; Fig. 2). Thus, inhibition of thromboxane synthesis with CGS-13,080 or antagonism of thromboxane A_2 with SQ-29,548 or PTA_2 effectively protects against arachidonic acid-induced sudden death in mice. When U-46,619 was used as the sudden death-inducing agent, 14% (2 of 14) and 15% (2 of 13) of the vehicle-treated animals survived (Table II). This was significantly different from mice treated with SQ-29,548 (10 mg/kg), CGS-13,080 (10 mg/kg) or their respective vehicles and the vehicle for U-46,619 ($P < 0.005$; Table II). Pretreatment of mice with SQ-29,548 (10 mg/kg) resulted in significant protection from sudden death exerting a survival rate of 67% (8 of 12; Table II). In contrast, mice pretreated with CGS-13,080 at 10 mg/kg did not show a significant protection with 3 of 12 animals surviving (25%).

Discussion. We have extended the original findings of Kohler *et al.* (2) that arachidonic acid-induced sudden death in mice is a valid

TABLE II. EFFECT OF SQ-29,548 and CGS-13,080 ON PROSTAGLANDIN–ENDOPEROXIDE ANALOG (U-46,619; 2.3 mg/kg)—INDUCED SUDDEN DEATH IN MICE

Pretreatment	Stimulus	Survivors/animals tested	% Survival	Significance
Vehicle a	Vehicle	11/14	79	—
Vehicle b	Vehicle	7/7	100	—
SQ-29,548 (10 mg/kg)	Vehicle	7/8	87	—
CGS-13,080 (10 mg/kg)	Vehicle	5/6	83	—
Vehicle a	U-46,619	2/14	14	$P < 0.005$*
Vehicle b	U-46,619	2/13	15	$P < 0.005$**
SQ-29,548 (10 mg/kg)	U-46,619	8/12	67	$P < 0.01$***
CGS-13,080 (10 mg/kg)	U-46,619	3/12	25	N.S.

Note. SQ-29,548 (10 mg/kg) or its vehicle (vehicle a = ethanol 50 μl) and CGS-13,080 (10 mg/kg) or its vehicle (vehicle b = NaCl 50 μl; pH 8.0) were injected into the tail vein 2 min prior to injection of U-46,619 (2.3 mg/kg) or its vehicle (ethanol, 50 μl) into the penile vein. N.S. = not significantly different from vehicle a/U-46,619 group.

* Compared to vehicle a/vehicle group.

** Compared to vehicle b/vehicle group.

*** Compared to vehicle a/U-46,619 group.

and useful model for the investigation of antithrombotic drugs. In modifying the originally described method, we performed the intravenous injection of the drugs into the tail vein and AA into the dorsal penile vein, which allowed safe administration by direct visual control with only minor surgical trauma. We have also confirmed the pivotal role of TxA_2 in the mouse model of sudden death (3, 16). SQ-29,548, a newly synthesized specific thromboxane receptor antagonist inhibits platelet aggregation *in vitro* induced by the prostaglandin–endoperoxide 9,11-azo PGH_2 (11). The vasoconstrictor effects of 9,11-azo PGH_2 and $PGF_{2\alpha}$ are dose-dependently diminished by SQ-29,548 in isolated strips of guinea pig trachea and rat aorta, whereas the vasoconstrictor effects of serotonin and norepinephrine are not affected (11). The dose-dependent protective effect of SQ-29,548 in AA and U-46,619-induced sudden death in mice demonstrates that the compound is also an effective inhibitor of proaggregatory and vasoconstrictor thromboxane-type eicosanoids *in vivo*. This agreed with results obtained with another less specific TxA_2 antagonist (3) in the mouse model and with reports about the efficacy of SQ-29,548 in the AA-induced sudden death in rabbits (17). CGS-13,080, which exerts antithrombotic effects *in vivo* comparable to SQ-29,548, is known to be a potent and selective inhibitor of thromboxane synthesis. CGS-13,080 does not antagonize the vasoconstrictor actions of TxA_2 (12), prostaglandin–endoperoxides or other eicosanoids on vascular smooth muscle, nor does it protect in U-46,619-induced sudden death where a preformed Tx-agonist is already present. Therefore, the results presented provide further evidence of TxA_2 being the major pathophysiologic mediator in AA-induced sudden death in mice.

Although the dose of AA or the prostaglandin–endoperoxide analog required for induction of sudden death in mice is much higher than that required for rabbits (2, 3, 5–8, 10), the pathophysiology of the eicosanoid sudden death seems to be comparable. Therefore, the mouse model is regarded as an interesting alternative to the sudden death model in rabbits (2, 3, 16). The mouse model has been used to study the *in vivo* antithrombotic effects of nonsteroidal and steroidal anti-inflammatory agents (2, 18), cyclooxygenase inhibitors (2), TxA_2 synthetase inhibitors (16), and TxA_2-receptor antagonists (3). It may be particularly relevant in the evaluation of species differences of drug actions. In this regard, PTA_2, which is a partial thromboxane agonist in rabbits, and is an effective thromboxane receptor antagonist in several other species (14), significantly protected against AA-induced sudden death in mice. This suggests that PTA_2 acts as an antagonist rather than agonist in the mouse.

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