Thromboxane Receptor Blockade with SQ 30,741 Improves Post-Ischemic Myocardial Function in Anesthetized Dogs (42955)

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Abstract. **This study was conducted to determine whether the thromboxane A_2 receptor antagonist SQ 30,741 can improve post-ischemic recovery of cardiac function in anesthetized dogs. Saline or SQ 30,741 was infused throughout a 15-min coronary occlusion and 5 hr of reperfusion. Ischemic regional cardiac function was determined using subendocardial ultrasonic crystals. Despite no differences in collateral blood flow or reperfusion flow, SQ 30,741 significantly improved ventricular segmental shortening at all times measured during reperfusion. At 5 hr after the initiation of reperfusion, segmental shortening was 3 ± 16 and 44 ± 10% of baseline values for saline and SQ 30,741 groups, respectively. These results implicate thromboxane receptor activation in the pathogenesis of myocardial stunning, and thromboxane antagonists may be useful in mitigating this functional deficit.** [P.S.E.B.M. 1989, Vol 192]

The thromboxane A_2 (TxA_2)/prostaglandin endoperoxide receptor antagonists SQ 29,548 and SQ 30,741 (structures shown in Fig. 1) have been shown to be effective in reducing myocardial infarct size in canine models of coronary occlusion and reperfusion (1, 2) as well as in other models of myocardial ischemia (3). In a model of reversible ischemia and reperfusion in dogs, SQ 29,548 also significantly improved the return of contractile function in the affected region (4). SQ 29,548 is an experimental compound which has not been developed for use in the clinic, but SQ 30,741 has the potential of becoming a clinically useful TxA_2 antagonist and is now in Phase II clinical trials for the treatment of myocardial ischemia. Because of the importance of improving post-ischemic contractile function in patients, we wanted to determine whether SQ 30,741 could also improve function in stunned myocardial tissue. This is important not only for potential clinical utility, but also to determine whether another TxA_2 antagonist with a chemical structure different from that of SQ 29,548 can improve function. This would help to confirm that the beneficial effects of SQ 29,548 are not due to non-TXA_2-related or nonspecific effects. This is especially of interest because of the recent findings of Farber *et al.* (5) that TxA_2 antagonist BM 13.505 did not improve ischemic regional function in the dog, although the TxA_2 synthetase inhibitor dazmagrel significantly attenuated stunning. The authors suggested that TxA_2 was not involved in the pathogenesis of myocardial stunning. Thus, the purpose of this study was to determine whether SQ 30,741 can significantly improve postischemic contractile function in a canine model of brief coronary occlusion and reperfusion.

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Materials and Methods

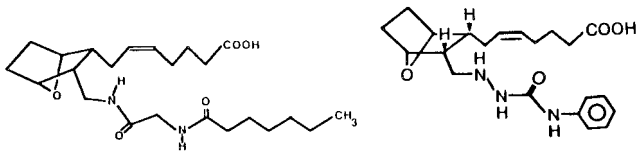
Adult anesthetized (iv pentobarbital sodium, 30 mg/kg) mongrel dogs (10–15 kg) were instrumented with a Mikro-Tip catheter transducer (Millar Instruments) in the left carotid artery and fluid-filled catheters were placed in the left femoral artery and vein for collection of blood and iv drug infusions, respectively. The animals were placed on artificial respiration and then subjected to a left thoracotomy. The left anterior descending coronary artery (LAD) was isolated proximal to its first branch and a left atrial catheter was implanted for the injection of dye (determination of area at risk) or radioactive microspheres. Segmental shortening in the LAD and left circumflex coronary artery perfused regions were determined using piezoelectric crystals (Triton Technology Inc.) implanted into the subendocardium of these two regions. The crystals in each pair were placed approximately 10 mm apart. The technique of measuring segmental shortening with the use of ultrasonic crystals has been described in more detail previously (6, 7).

The animals were divided into two groups: (i) animals receiving iv saline throughout the experiment (n = 10), and (ii) animals receiving iv SQ 30,741 (5 mg/kg + 5 mg/kg/hr, n = 8) throughout the experiment. SQ 30,741 is a potent and selective TXA_2/prostaglandin endoperoxide antagonist which has been described previously (8). Pretreatment with drug or saline began 15 min before LAD occlusion. The LAD was occluded with surgical silk and remained occluded for 15 min. Reperfusion was begun by complete and rapid release of the coronary ligature, and the animals were followed for 5 hr postreperfusion. Using a reference blood flow method, myocardial blood flow was measured using ^{141}Ce-, ^{51}Cr-, or ^{46}Sc-labeled microspheres (3M Co., 15 ± 3 μm) at 10 min into the LAD occlusion and 15 and 60 min after the initiation of reperfusion. Hemodynamic and blood gas variables were measured throughout the experiment. Normoxia and eucapnia were maintained by adjusting the respirator. At the end of the experiment, patent blue violet dye was injected into the left atrial catheter while the LAD was perfused at the animals' existing pressure for determination of the area at risk.

The segment shortening data were normalized as previously described (6, 9). End diastolic length and end systolic length were determined and shortening was expressed as a percentage of pre-ischemia, pre-drug shortening. For the analysis of blood flow, subepicardial and subendocardial pieces from the ischemic (in the area at risk) and nonischemic regions and the reference flow samples were counted for radioactivity in a Beckman Autogamma 8000 gamma counter. Four transmural pieces of the myocardium (0.5–1.0 g) were taken from the area at risk in the region around the crystals and the flows were calculated as the mean of these pieces. At then end of the experiment, the subendocardial position of the crystals was confirmed and the ends of the crystals were found to be 1.12 ± 0.32 cm from the epicardial surface. Differences between treatments were determined using a Student's t test. For percentage of shortening, these data were transformed for statistical analysis using the arcsine transformation (10) because of their deviation from normality. All data are presented as mean ± SE and significance was set at $P < 0.05$.

Results

Throughout the experiment, no changes in arterial blood pressure or heart rate were noted as shown in Table I. Only one animal fibrillated (vehicle group) upon reperfusion and it was promptly defibrillated and recovered. Absolute segmental shortening was similar in both groups (19.9 ± 2.1 and 22.0 ± 2.9% for vehicle and SQ 30,741-treated groups, respectively) in the region which was to be ischemic (LAD perfused region). The shortening data presented in Figure 2 have been normalized for baseline values as described in Materials and Methods. As can be seen in Figure 2, LAD occlusion resulted in significant myocardial dysfunction such that systolic bulging was noted in both groups. Upon reperfusion, segmental shortening rebounded in both groups toward baseline, although shortening was significantly improved in drug-treated animals compared with saline-treated animals. After 5–10 min of reperfusion, shortening diminished in both groups, although SQ 30,741 significantly blunted this functional decline. In saline-treated animals, little segmental shortening was noted and did not significantly recover during the 5-hr reperfusion interval. In this group, some animals began to recover while other deteriorated, accounting for the high variability observed. In SQ 30,741-treated



SQ 30,741 SQ 29,548

Figure 1. The chemical structures of SQ 29,548 and SQ 30,741.

Table I. The Effect of SQ 30,741 on Hemodynamic Variables before and after LAD Occlusion

	Pre-drug	Post-drug	Occlusion	Minutes postreperfusion							
				1	5	10	15	30	45	60	300
Systolic blood pressure (mm Hg)											
Saline (n = 10)	114 ± 6[a]	114 ± 5	103 ± 7	104 ± 5	111 ± 5	111 ± 3	114 ± 3	115 ± 2	118 ± 3	109 ± 12	118 ± 5
SQ 30,741 (n = 8)	106 ± 4	105 ± 2	102 ± 6	107 ± 4	105 ± 3	106 ± 2	107 ± 10	105 ± 3	110 ± 3	115 ± 10	109 ± 3
Diastolic blood pressure (mm Hg)											
Saline (n = 10)	92 ± 6	97 ± 6	82 ± 7	82 ± 5	92 ± 5	90 ± 3	91 ± 2	92 ± 2	96 ± 2	95 ± 2	90 ± 5
SQ 30,741 (n = 8)	81 ± 4	78 ± 3	77 ± 7	81 ± 4	79 ± 3	80 ± 2	81 ± 2	84 ± 2	85 ± 2	88 ± 2	77 ± 5
Heart rate (beats/min)											
Saline (n = 10)	156 ± 7	157 ± 6	152 ± 8	146 ± 8	151 ± 9	155 ± 7	156 ± 7	158 ± 5	158 ± 4	158 ± 6	169 ± 4
SQ 30,741 (n = 8)	158 ± 3	157 ± 4	154 ± 6	150 ± 8	150 ± 7	154 ± 5	156 ± 4	159 ± 3	158 ± 3	166 ± 4	163 ± 5

[a] All values are mean ± SE.

animals, segmental shortening was maintained at approximately 40% of baseline throughout the reperfusion period and was significantly higher compared to saline controls at all times measured. Absolute segmental shortening was relatively constant in the non-ischemic region in both groups during the experiment (18.1 ± 1.1 and 16.7 ± 1.9% pre-ischemia and 15.0 ± 1.1 and 13.9% at the end of the experiment for vehicle and drug-treated groups, respectively).

Myocardial blood flow data are shown in Table II. Flow in the ischemic region during LAD occlusion was significantly lower compared to the non-ischemic region, particularly in the subendocardial region. Collateral flow was similar in both groups. During reperfusion, flow initially returned toward those levels seen in the non-occluded region at 15 min postreperfusion, and no differences were seen between treatment groups. At 60 min, reflow into the subendocardial region was significantly reduced compared to the non-ischemic region, and these values were similar in both groups. The tissue samples for the ischemic regions were taken from the center of the area at risk. The area at risk as measured using planimetry was similar in size for both groups (33 ± 2 vs 32 ± 3% of the left ventricular area

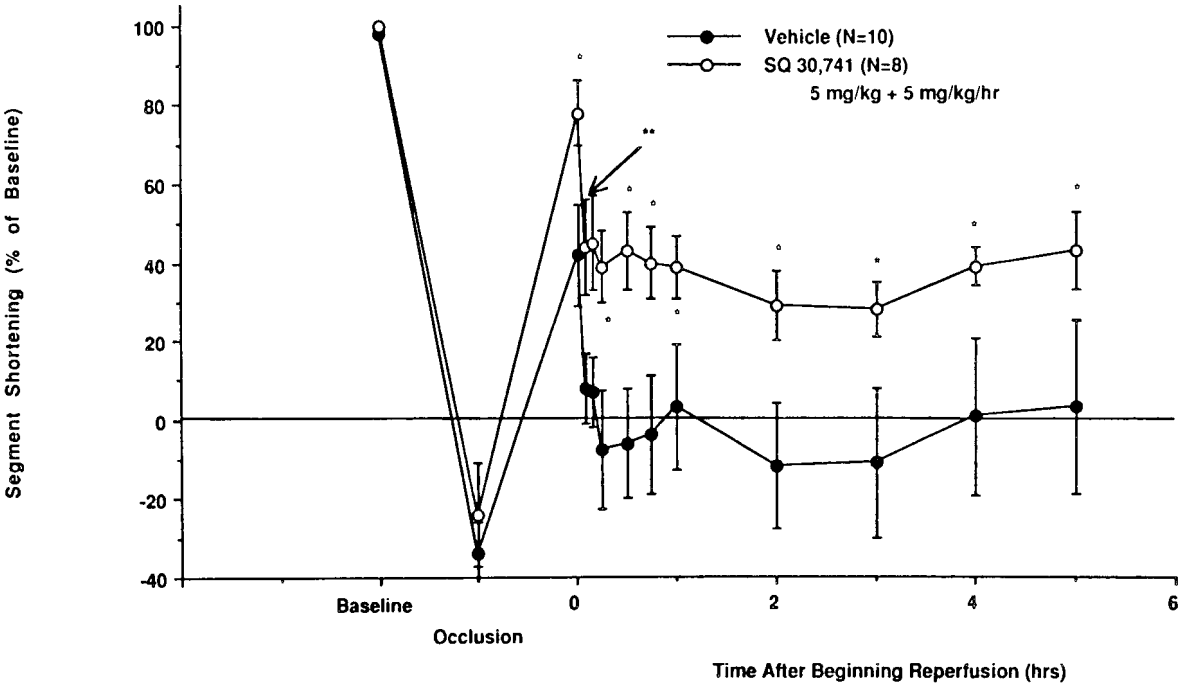


Figure 2. The effect of SQ 30,741 on segmental shortening as a percent of baseline values following a brief coronary artery occlusion. Significant systolic bulging was noted during occlusion in both vehicle (saline) and SQ 30,741 groups. Despite reperfusion, marked ventricular dysfunction was noted, particularly in the vehicle group. At all times measured during reperfusion, SQ 30,741 resulted in a significant improvement in regional function (*$P < 0.05$).

Table II. The Effect of SQ 30,741 on Ischemic and Post-Ischemic Myocardial Blood Flows (ml/min/100 g)

	Nonoccluded Region			Occluded Region		
	Occlusion	Reperfusion		Occlusion	Reperfusion	
		15 min	60 min		15 min	60 min
Subepicardium						
Vehicle (n = 4–10)	138 ± 10[a]	120 ± 11	120 ± 15	27 ± 5[b]	90 ± 10	90 ± 5
SQ 30,741 (n = 4–8)	139 ± 11	130 ± 15	107 ± 6	29 ± 4[b]	104 ± 14	95 ± 5
Subendocardium						
Vehicle (n = 4–10)	148 ± 13	118 ± 11	115 ± 4	12 ± 3[b]	89 ± 5	73 ± 10[b]
SQ 30,741 (n = 4–8)	127 ± 9	130 ± 15	114 ± 8	12 ± 2[b]	107 ± 6	63 ± 2[b]

[a] All values are mean ± SE.
[b] Significantly different from its respective nonoccluded region flow ($P < 0.05$).

at risk for vehicle and drug groups, respectively). The ischemic regional crystals were always found to be in the center of the area at risk.

Discussion

Brief coronary occlusions which are not sufficiently severe to cause myocardial necrosis can still result in profound contractile dysfunction which can last for hours or days (11–13). The precise mechanism for this myocardial stunning is not entirely clear, but pharmacologically induced improvements in reperfusion function have suggested some possible mechanisms. Some of the sequelae of reperfusion injury may aggravate ischemic injury, one example being oxygen-derived free radical emission (14, 15). Several studies have indicated that free radical scavengers or inhibitors of xanthine oxidase can enhance reperfusion contractile function (14, 15). A recently published study from our laboratory has shown that the TxA_2 antagonist SQ 29,548 can improve reperfusion function and that at least some of this beneficial effect was seen during reperfusion itself (4). In the same study we also showed that diltiazem was effective in improving reperfusion function, but had little of its beneficial effects during reperfusion. Diltiazem was probably improving reperfusion function by reducing the severity of the ischemia during occlusion itself, and this was probably due to an improvement in the O_2 supply/demand balance. Other compounds such as nifedipine and nicorandil, which are thought to reduce the severity of ischemia via improvements in O_2 supply/demand parameters, have also been shown to improve reperfusion function (9, 16).

The model of postreperfusion contractile dysfunction used in this study has been used extensively before and has been found to result in reproducible myocardial stunning (12, 14). As has been observed previously, occlusion of the LAD in our study resulted in systolic bulging and, immediately upon reperfusion, contractile function returned toward pre-ischemia values (16). After several minutes, myocardial function decreased such that no shortening was later observed in the reperfusion period in vehicle-treated animals, and this has also been previously observed (16).

The selective TxA_2 antagonist SQ 29,548 has been shown previously to reduce post-ischemic myocardial dysfunction in dogs (4). This finding suggests that TxA_2 release during ischemia and reperfusion may be contributing to the myocardial stunning seen after temporary coronary occlusion. In contrast, data published by Farber *et al.* (5) have indicated that the TXA_2 receptor antagonist BM 13.505 does not improve post-ischemic function. Although we do not know the precise reason for the differences between the two studies, it is possible that non-TXA_2 receptor-blocking activities of SQ 29,548 are acting to improve function. For this reason we tested the effects of another TxA_2 antagonist, SQ 30,741, in a dose higher than that known to result in > 95% TxA_2 receptor blockade in the dog as measured by reversal of U-46619-induced platelet shape change responses (2).

SQ 30,741 was found to significantly improve post-ischemic function in the present study. It is known that TxA_2 is released during ischemia and reperfusion in such models (17, 18) and probably acts to aggravate the ischemia. Studies with TxA_2 synthetase inhibitors and receptor antagonists have shown that they can reduce the severity of ischemia in a variety of ischemia models, which strongly suggests a role for TxA2 in the pathogenesis of myocardial ischemia (1–3). We have found that with two different TxA_2 antagonists, blockade of the TxA_2 receptors is associated with an improved reperfusion function. We do not know whether SQ 30,741 is exerting its protective effects during the occlusion or reperfusion period, although our study with SQ 29,548 showed that protection was still seen when it was given only during reperfusion. We do know that SQ 30,741 was not increasing collateral flow in these animals. The area at risk for the two groups was not significantly different. Since no changes in peripheral hemodynamic status were noted, it does not seem as if changes in O_2 demand secondary to an improved hemodynamic profile caused the reduced severity of ischemia.

We do not know precisely why SQ 29,548 and SQ 30,741 reduce reperfusion stunning, but it seems that reperfusion injury may be mediated in part by TxA_2. It is not known whether TxA_2 has direct cardiodepressant effects, although it has been suggested that it may, particularly in concert with leukotrienes (19). It may also be possible that TxA_2 causes the release of other mediators which could directly influence myocardial stunning. It appears that SQ 30,741 does not mitigate stunning by affecting collateral or reperfusion flow. Unfortunately, we do not know what happens to microregional flow distribution, and future studies should be conducted to determine oxygen extraction or consumption in the ischemic region to determine whether some flow maldistribution is occurring. We also do not know what is happening to flow reserve in the heart but SQ 30,741 has been previously shown to improve this in more severe models of ischemia (20). Preservation of ATP or energy sparing during the ischemia per se is not a likely mechanism as SQ 29,548 also preserved reperfusion function and cell viability when given only during reperfusion (4, 21). SQ 30,741 is known to be a selective TxA_2/endoperoxide receptor antagonist (2, 22). SQ 30,741 has been previously shown to inhibit a variety of dose-dependent responses to a TxA_2/PGH_2 mimetic, U-46619, including platelet aggregation and shape change responses (2, 23), and tracheal smooth muscle contraction (22). A high affinity and competitive interaction of SQ 30,741 for platelet TxA_2/PGH_2 receptors has been found using receptor binding techniques (24). Selectivity of antagonism has been shown

and concentrations of SQ 30,741 which completely block U-46619-mediated responses do not block reactivity to KCl, serotonin, histamine, carbachol, prostaglandin E_2 and $F_{2\alpha}$, and leukotriene (25). SQ 30,741 has not been shown to have agonist activity nor to alter the activity of prostaglandin synthetic enzymes (26). All of this pharmacologic work was done at the Squibb Institute for Medical Research. Thus, it appears that SQ 30,741 should be working via its efficacy as a TxA_2 antagonist. It is clear that further work needs to be done to elucidate the physiologic mechanism of action of SQ 30,741.

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