PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **188**, 504–508 (1988)

# The Role of Thromboxane $A_2$ in Reperfusion Injury (42769)

GARY J. GROVER, PAUL G. SLEPH, AND CHARLES PARHAM

*Department of Pharmacology, Squibb Institute for Medical Research, Route 206 & Provinceline Road, P.O. Box 4000, Princeton, New Jersey 08543-4000*

*Abstract.* Thromboxane $A_2$ ($TXA_2$) receptor antagonists can limit infarct size in models of coronary occlusion and reperfusion, but it was unknown if these compounds can mitigate reperfusion injury. Anesthetized open chest dogs were subjected to left circumflex coronary (LCX) occlusion for 90 min. Two minutes before reperfusion, the dogs were given iv saline (0.9% NaCl) or the $TXA_2$ antagonist SQ 29,548 (0.2 mg/kg + 0.2 mg/kg/hr). Reperfusion was instituted for 5 hr at which time infarct size was determined. Regional myocardial blood flow was determined before, during, and after occlusion. SQ 29,548 treatment resulted in a significant reduction in infarct size (57 ± 7 and 34 ± 8% of the left ventricular area at risk infarcted in the saline and SQ 29,548 groups, respectively). No differences in collateral flow during occlusion were observed between groups, but SQ 29,548 treatment resulted in a significantly higher subendocardial reperfusion flow (54 ± 10 and 93 ± 14 ml/min/100g for the saline and SQ 29,548 groups, respectively). Thus, $TXA_2$ seems to play a role in exacerbating reperfusion injury and $TXA_2$ receptor blockade may have potential as a mode of therapy for ischemia–reperfusion damage. 

Thromboxane $A_2$ ($TXA_2$) has been shown to be released in a variety of experimental myocardial ischemia models and in some forms of ischemia in humans (1–3). While $TXA_2$ is released during the occlusive event, reperfusion is associated with a marked release of $TXA_2$, though it is unknown if this is due to an increased synthesis of $TXA_2$ or a washout phenomenon (2). $TXA_2$ has a host of physiological effects, many of which may aggravate myocardial ischemia, including coronary vasoconstriction and enhanced platelet aggregation (4). Inhibition of $TXA_2$ synthesis has been shown to reduce the severity of myocardial ischemia in models of occlusion without reperfusion, indicating a deleterious role for $TXA_2$ during ischemia (5, 6). Recently selective $TXA_2$ receptor antagonists have been developed which should not affect prostanoid production in parallel pathways (7, 8) and these compounds have been shown to reduce the severity of myocardial ischemia in animal models (9–11).

In a recent study from our laboratory, we showed that a $TXA_2$ receptor antagonist, SQ 30,741, significantly limited infarct size in a model of occlusion and reperfusion with a concomitant increase in subendocardial reperfusion flow (10). This compound was infused during occlusion and reperfusion and thus it was unknown if the beneficial effect of $TXA_2$ antagonism on infarct size reduction and subendocardial reflow was occurring during the occlusion per se or during reperfusion. Thus, the purpose of this study was to determine if antagonism of $TXA_2$ receptors can result in myocardial infarct size reduction and an improved subendocardial reflow when given immediately before reperfusion.

**Methods.** Mongrel dogs of either sex (12–16 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg). A catheter was placed into the right femoral vein for pentobarbital infusion and another was placed into the left femoral artery for collection of blood samples for blood gas and regional blood flow determinations. A Millar Mikrotip catheter pressure transducer was placed into the left femoral artery and was advanced into the aortic arch for the measurement of arterial blood pressure. An endotracheal tube was placed into the trachea and the animals were artificially respired such that eucapnia was maintained.

A left thoracotomy was performed at the fifth intercostal space and the heart was exposed. The left circumflex coronary artery (LCX) was isolated proximal to its first branch and a silk suture was placed around it for later occlusion. A catheter was placed into the left atrial appendage for dye and radioactive microsphere injection.



0037-9727/88 $1.50

The animals were allowed to stabilize for 15–30 min at which time an arterial blood sample was removed anaerobically for measurement of blood gases using a Radiometer (ABL 3, Copenhagen) blood gas analyzer. Arterial blood pressure, heart rate, and ECG were measured. Regional myocardial blood flow was then determined using $^{141}Ce$, $^{51}Cr$, or $^{85}Sr$-labeled microspheres (3M Co., 15 ± 3 μm) using a reference flow method (12). The animals were subjected to LCX occlusion for 90 min and hemodynamic variables were monitored throughout this period. Regional blood flow was again determined at 40 min post-LCX occlusion. Two minutes before reperfusion, the animals were divided into two groups: (1) animals receiving an iv infusion of saline ($n$ = 9) throughout the reperfusion period and (2) animals receiving an iv infusion of SQ 29,548 (0.2 mg/kg + 0.2 mg/kg/hr, $n$ = 7) throughout the reperfusion period. SQ 29,548 is a selective $TXA_2$/prostaglandin endoperoxide receptor antagonist (8). The dose of SQ 29,548 chosen was previously shown to result in a 1000-fold shift in the dose–flow response in various vascular tissues to the $TXA_2$ mimetic U-46619 (9). At 90 min postocclusion, reperfusion was instituted for 5 hr. Hemodynamic and blood gas parameters were monitored throughout the reperfusion period. Regional myocardial blood flow was determined at 1 hr postreperfusion.

At the end of the experiment, the LCX was rapidly cannulated and perfused at the animals' existing pressure with Ringer's lactate solution (sodium 130 meq, potassium 4 meq, calcium 2.7 meq, chloride 109 meq, lactate 28 meq) so that the previously occluded bed was perfused. Patent blue violet dye (1 mg/kg of a 10 mg/ml solution) was injected into the left atrial catheter and the heart was quickly excised and washed in isotonic saline. The dye was used to delineate the area at risk. The area at risk was that area not stained with the dye.

The atria were trimmed away leaving only the ventricles. The ventricles were then cut transversely into 0.5 cm slices. The borders of the area at risk (no dye) were delineated and separated and the slices were incubated at 37°C for 30 min in a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) in phosphate-buffered saline.

The ventricular slices were then dried and both sides were carefully traced on clear transparencies. The area at risk and the infarcted region were demarcated on these transparencies. The areas of interest were then determined using planimetric techniques. The infarct size was expressed as a percentage of the left ventricular (LV) and total (LV + RV) area at risk. Tissue samples were taken from the subepicardial and subendocardial halves from the ischemic (central ischemic region) and nonischemic regions for determination of blood flow. Radioactivity was determined in the tissue and reference blood samples on a Beckman Autogamma 8000 gamma counter.

Changes in hemodynamic, blood flow, and infarct size variables with respect to time were analyzed using a one-way analysis of variance and multiple comparisons were done using the Newman–Keuls procedure. Differences between saline and drug treatments were determined using a $t$ test. Significance was set at $P < 0.05$ and all values were expressed as the means ± SE.

**Results.** Hemodynamic data for saline- and SQ 29,548-treated animals during occlusion and reperfusion are shown on Table I. Arterial blood pressures and heart rate were similar for both groups before LCX occlusion. Systolic pressure remained constant throughout the experiment for the saline- and SQ 29,548-treated animals. Diastolic pressure remained constant during coronary occlusion but tended to decrease later during the reperfusion in both groups. Heart rate remained relatively constant for both groups throughout the experiment.

The data showing the effect of SQ 29,548 on myocardial infarct size are shown on Fig. 1. Infarct size is expressed as a percentage of the LV area at risk and the LV + RV area at risk. Infarct size was significantly decreased in animals receiving SQ 29,548 during the reperfusion. Infarct size was reduced approximately 45% with SQ 29,548 treatment. The areas at risk were similar in size for both groups (40 ± 3 and 43 ± 7% of the LV for saline- and SQ 29,548-treated animals, respectively).

Regional myocardial blood flows are shown for saline- and drug-treated groups on Table II. Preocclusion flows were similar in all regions for both groups. At 40 min post-

TABLE I. EFFECT OF SALINE OR SQ 29,548 (0.2 mg/kg + 0.2 mg/kg/hr) GIVEN ONLY DURING REPERFUSION ON HEMODYNAMIC VARIABLES BEFORE AND DURING LCX OCCLUSION AND REPERFUSION

| | | Occlusion | | Reperfusion | | | |
|---|---|---|---|---|---|---|---|
| | Control | 8 min | 40 min | 10 min | 1 hr | 2 hr | 5 hr |
| Systolic blood pressure (mm Hg) | | | | | | | |
| Saline ($n$ = 9) | 106 ± 11 | 108 ± 6 | 117 ± 7 | 117 ± 6 | 112 ± 6 | 111 ± 10 | 107 ± 3 |
| SQ 29,548 ($n$ = 7) | 104 ± 6 | 98 ± 7 | 100 ± 5 | 101 ± 4 | 106 ± 7 | 102 ± 6 | 99 ± 5 |
| Diastolic blood pressure (mm Hg) | | | | | | | |
| Saline ($n$ = 9) | 82 ± 5 | 79 ± 5 | 82 ± 5 | 80 ± 6 | 76 ± 5 | 77 ± 5 | 60 ± 5* |
| SQ 29,548 ($n$ = 7) | 76 ± 5 | 70 ± 5 | 75 ± 5 | 74 ± 4 | 77 ± 6 | 76 ± 6 | 60 ± 7 |
| Heart rate (beats/min) | | | | | | | |
| Saline ($n$ = 9) | 154 ± 5 | 155 ± 8 | 161 ± 8 | 152 ± 9 | 160 ± 7 | 168 ± 6 | 172 ± 6 |
| SQ 29,548 ($n$ = 7) | 163 ± 9 | 180 ± 12 | 163 ± 13 | 164 ± 14 | 156 ± 8 | 166 ± 10 | 162 ± 5 |

*Note.* All values are means ± SE.
* Significantly different compared to its paired control preocclusion value ($P < 0.05$).

occlusion, flows were significantly reduced in the ischemic (LCX) region to similar levels in both groups. The reduction in flow in this region was particularly marked in the subendocardial region. During occlusion, flows in the nonischemic region remained unchanged for both groups. At 1 hr postreperfusion, subepicardial ischemic regional flow returned to control levels in both groups. In the subendocardial region, flow returned only to approximately 65% of preocclusion values in saline-treated animals. Subendocardial reflow was significantly higher in animals treated with SQ 29,548 compared to the saline group and was approximately 20% higher than its paired preocclusion value. Flows in the nonischemic region remained constant for both groups during reperfusion.

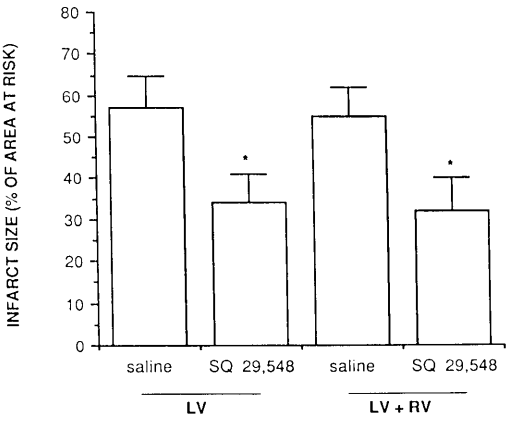


FIG. 1. The effect of SQ 29,548 (0.2 mg/kg + 0.2 mg/kg/hr; $n$ = 7) or vehicle ($n$ = 9) given only during reperfusion on myocardial infarct size. Infarct size is expressed as the percentage of the left ventricular (LV) or left ventricular + right ventricular (LV + RV) areas at risk. SQ 29,548 resulted in a significant reduction in infarct size (* $P < 0.05$) compared to that of animals given saline during reperfusion.

**Discussion.** Previous studies have indicated that $TXA_2$ release during myocardial ischemia may act to further aggravate the deleterious effects of the ischemia (5, 6, 9–11). This was initially studied using $TXA_2$ synthetase inhibitors which are more selective than cyclooxygenase inhibitors, but still may affect prostanoid production in parallel metabolic pathways (5–7). Recently, selective $TXA_2$ receptor antagonists which may give us a more selective tool for studying the effects of $TXA_2$ under normal and pathological conditions (8) have been developed. $TXA_2$ synthesis inhibitors may affect production of parallel prostanoid metabolites, thus clouding interpretation of data (7). Several investigators have shown that $TXA_2$ receptor antagonists can reduce the severity of myocardial ischemia (9–11). In two studies, SQ 29,548 was shown to reduce several indices of myocardial ischemia in rats and cats in models of total coronary occlusion without reperfusion (10, 13). In previous studies, we have shown the $TXA_2$ receptor antagonists SQ 29,548 and SQ 30,741 to reduce infarct size in a model of LCX occlusion and subsequent reperfusion (9, 14). Along with this infarct size reduction, a significantly improved

TABLE II. THE EFFECT OF SQ 29,548 (0.2 mg/kg + 0.2 mg/kg/hr, REPERFUSION ONLY) ON REGIONAL MYOCARDIAL BLOOD FLOW BEFORE, DURING, AND AFTER LCX OCCLUSION

| | Nonoccluded region | | | Occluded region | | |
|---|---|---|---|---|---|---|
| | Control | 40 min postoccl | 1 hr postreperf (postdrug) | Control | 40 min postoccl | 1 hr postreperf (postdrug) |
| Subepicardium (ml/min/100 g) | | | | | | |
| Saline ($n$ = 9) | 95 ± 7 | 96 ± 9 | 79 ± 9 | 78 ± 7 | 16 ± 5* | 73 ± 14 |
| SQ 29,548 ($n$ = 7) | 85 ± 7 | 92 ± 19 | 99 ± 20 | 75 ± 7 | 18 ± 5* | 78 ± 10 |
| Subendocardium (ml/min/100 g) | | | | | | |
| Saline ($n$ = 9) | 89 ± 8 | 97 ± 10 | 78 ± 9 | 82 ± 8 | 8 ± 3* | 54 ± 10* |
| SQ 29,548 ($n$ = 7) | 92 ± 5 | 98 ± 17 | 94 ± 4 | 79 ± 8 | 5 ± 2* | 93 ± 14† |

*Note.* All values are means ± SE.
* Significantly different compared with its respective preocclusion value ($P < 0.05$).
† Significantly different compared with its respective saline group value ($P < 0.05$).

subendocardial reperfusion flow at 1 hr postreperfusion was noted with $TXA_2$ blockade. It was also shown that the subendocardial reflow was significantly and inversely correlated with infarct size. Since the thromboxane receptor antagonists were administered throughout the experiment, it was unknown if any of the beneficial effects of these agents occurred during the reperfusion period. $TXA_2$ blockade does exert some beneficial effect during coronary occlusion as most investigators have studied these compounds in models of coronary occlusion without reperfusion but the role of $TXA_2$ release and blockade of its receptors in reperfusion injury has not previously been investigated.

In the present study we found that SQ 29,548 infused immediately before reperfusion resulted in a significant reduction in infarct size and a concomitant improvement in subendocardial reflow. Thus it appears that $TXA_2$ release during reperfusion may be one mediator of reperfusion injury and may aggravate such events as the no-reflow phenomenon. Few compounds which can protect myocardial tissue when given only during reperfusion have been found. Calcium antagonists such as verapamil have been found to be effective only when given early before or after coronary occlusion (15). A study by Reynolds *et al.* (16) showed that when propranolol was given only during reperfusion, significant myocardial salvage was observed. Jolly *et al.* (17) showed that the combined administration of superoxide dismutase and catalase seemed to be effective during the early reperfusion period.

The precise physiological mechanism for the mitigation of reperfusion injury with TXA blockade via SQ 29,548 is presently unknown. It may be related to alleviation of TXA-mediated vascular tone. We observed a significant increase in subendocardial reflow with $TXA_2$ blockade. It should be noted that in unpublished studies, SQ 29,548 does not affect myocardial flow or peripheral hemodynamic status in nonischemic dogs, indicating that the compound does not have a large nonspecific effect and that in normal animals, $TXA_2$ tone does not exist to any degree. Similar results on reflow were noted for diltiazem in a model of ischemia and reperfusion nearly identical to ours (18). It is of interest however that doses of ibuprofen which resulted in myocardial preservation did not result in a significantly improved regional reflow (19). This may be related to its effects on other arachidonic acid metabolites or its relatively less potent myocardial protection when compared with our present study. $TXA_2$ release during reperfusion may also be inducing platelet or neutrophil aggregate formation and thus aggravating microvascular obstruction and degradation seen during reperfusion (4, 20). Preliminary evidence indicates that SQ 29,548 significantly reduces neutrophil infiltration during myocardial ischemia (Dr. J. Mehta, personal

communication). Mitigation of these events via $TXA_2$ blockade may result in reduced reperfusion injury. The contribution of $TXA_2$ in causing or potentiating oxygen-derived free radical release is presently unknown.

Thus, mitigation of $TXA_2$-induced reactions in ischemic and reperfused myocardial tissue seems to result in some degree of protection. This protective effect can occur without increases in collateral flow during occlusion per se and appears to work at least in part by alleviating some reperfusion-induced event. Since some compounds seem to be effective only during the occlusion itself, multiple methods of pharmacological attack on ischemia and reperfusion seem possible. It may prove feasible to combine drugs such as calcium antagonists (21) which have beneficial effects during occlusion with drugs which may mitigate reperfusion injury and obtain additive effects.


1. Lewy RI, Wiener L, Walinsky P, Lefer AM, Silver MJ, Smith JB. Thromboxane release during pacing-induced angina pectoris: Possible vasoconstrictor influence on the coronary vasculature. Circulation **61**:1165–1171, 1980.
2. Michael LH, Hunt JR, Lewis RM, Entman ML. Myocardial ischemia. Platelet and thromboxane concentrations in cardiac lymph and the effects of ibuprofen and prostacyclin. Circ Res **59**:49–55, 1986.
3. Schmitz JM, Apprill PG, Buja LM, Willerson JT, Campbell WB. Vascular prostaglandin and thromboxane production in a canine model of myocardial ischemia. Circ Res **57**:223–231, 1985.
4. Ogletree ML. Overview of physiological and pathological effects of thromboxane $A_2$. Fed Proc **46**:133–138, 1987.
5. Smith EF, Lefer AM, Smith JB. Influence of thromboxane inhibition on the severity of myocardial ischemia in cats. Canad J Physiol Pharmacol **58**:294–300, 1980.
6. Burke SE, DiCola G, Lefer AM. Protection of ischemic cat myocardium by CGS-13080, a selective potent thromboxane $A_2$ synthesis inhibitor. J Cardiovasc Pharmacol **5**:842–847, 1983.
7. Bertele V, Falanga A, Tomasiak M, Chiabrando C, Cerletti C, DeGastano G. Pharmacologic inhibition of thromboxane synthetase and platelet aggregation: Modulating role of cyclo-oxygenase products. Blood **63**:1460–1466, 1984.
8. Ogletree ML, Harris DN, Greenberg R, Haslanger MF, Nakane M. Pharmacological actions of SQ 29,548, a novel selective thromboxane antagonist. J Pharmacol Exp Ther **234**:435–441, 1985.
9. Grover GJ, Schumacher WA. Effect of the thromboxane receptor antagonist SQ 29,548 on infarct size in dogs. J Cardiovasc Pharmacol **11**:29–35, 1988.
10. Hock CE, Brezinski ME, Lefer AM. Anti-ischemic actions of a new thromboxane receptor antagonist, SQ 29,548 in acute myocardial ischemia. Eur J Pharmacol **122**:213, 1986.
11. Schror K, Thiemermann C. Treatment of acute myocardial ischemia with a selective antagonist of thromboxane receptors (BM 13.177). Brit J Pharmacol **87**:631–637, 1986.
12. Dole WP, Jackson DL, Rosenblatt JI, Thompson WL. Relative error and variability in blood flow measurements with radiolabelled microspheres. Amer J Physiol **243**:H370–H378, 1982.
13. Brezinski ME, Yanagisawa A, Lefer AM. Cardioprotective actions of specific thromboxane receptor antagonists in acute myocardial ischemia. J Cardiovasc Pharmacol **9**:65, 1987.
14. Grover GJ, Schumacher WA, Simon M, Parham C, Goldenberg H. Effect of the thromboxane $A_2$ antagonist SQ 30,741 on infarct size and myocardial blood flow during myocardial ischemia. J Cardiovasc Pharmacol, in press, 1988.
15. Lo H, Kloner RH, Braunwald E. Effect of intracoronary verapamil on infarct size in the ischemic reperfused canine heart: Critical importance of timing of treatment. Amer J Cardiol **56**:672–677, 1985.
16. Reynolds RD, Burmeister WE, Gorczynski RJ, Dickerson DD, Mathews MP, Lee RJ. Effects of propranolol on myocardial infarct size with and without coronary artery reperfusion in the dog. Cardiovasc Res **15**:411–420, 1981.
17. Jolly SR, Kane WJ, Bailie MB, Abrams GD, Lucchesi BR. Canine myocardial reperfusion injury. Its reduction by the combined administration of superoxide dismutase and catalase. Circ Res **54**:277–285, 1984.
18. Bush LR, Romson JL, Ash JL, Lucchesi BR. Effects of diltiazem on extent of ultimate myocardial injury resulting from temporary coronary artery occlusion in dogs. J Cardiovasc Pharmacol **4**:286–296, 1982.
19. Romson JL, Bush LR, Jolly SR, Lucchesi BR. Cardioprotective effects of ibuprofen in experimental regional and global ischemia: J Cardiovasc Pharmacol **4**:187–196, 1982.
20. Spagnuolo PR, Ellner JJ, Hassid A, Dunn MJ. Thromboxane $A_2$ mediates augmented polymorphonuclear leukocyte adhesiveness. J Clin Invest **66**:406–414, 1980.
21. Klein HH, Schubothe M, Nebendahl K, Kreyzer K. The effect of two different diltiazem treatments on infarct size in ischemic, reperfused porcine hearts. Circulation **69**:1000, 1984.


Received February 29, 1988. P.S.E.B.M. 1988, Vol. 188.
Accepted May 10, 1988.