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Ranolazine, a partial fatty acid oxidation inhibitor, reduces myocardial infarct size and cardiac troponin T release in the rat

Kai Zacharowski [a,*], Brent Blackburn [b], Christoph Thiemermann [c]

[a] *Department of Cardiac, Vascular and Inflammation Research, The William Harvey Research Institute, St. Bartholomew's and The Royal London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, UK*

[b] *CV Therapeutics, 3172 Porter Drive, Palo Alto, CA, 94304, USA*

[c] *Department of Experimental Medicine and Nephrology, The William Harvey Research Institute, St. Bartholomew's and The Royal London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, UK*

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Abstract

Ranolazine reduces cellular acetyl-CoA content via inhibition of fatty acid β-oxidation and activates pyruvate dehydrogenase. This metabolic switch increases ATP production per mole of oxygen consumed, reduces the rise in lactic acid and acidosis, and maintains myocardial function under conditions of reduced myocardial oxygen delivery. It is still unclear whether ranolazine causes a reduction of (i) infarct size and (ii) cardiac troponin T release, in a male Wistar rat model of left anterior descending coronary artery occlusion (25 min) and reperfusion (2 h). Rats were subjected to saline infusion ($n = 12$) or ranolazine (bolus injection: 10 mg/kg plus infusion: 9.6 mg/kg/h, $n = 12$), 30 min prior to left anterior descending coronary artery occlusion–reperfusion, respectively. Ranolazine caused a significant reduction in myocardial infarct size of approximately 33% compared to saline control ($P < 0.05$). In addition, infusion of ranolazine significantly attenuated the release of cardiac troponin T into the plasma from 65 ± 14 (controls) to 12 ± 2 ng/ml. This study demonstrates for the first time that ranolazine significantly reduces (i) infarct size and (ii) cardiac troponin T release in rats subjected to left anterior descending coronary artery occlusion–reperfusion.

Keywords: Ranolazine; Myocardial infarct size; Cardiac troponin T; Ischaemia; Reperfusion; (Rat)

1. Introduction

During the early hours of acute myocardial infarction, there is an increased utilization of fatty acids, as it is the preferred energy source in the ischaemic zone of the heart (Vetter et al., 1974). Two factors that influence the relative increase in myocardial fatty acid consumption are the increased circulating levels of free fatty acids and ischaemia-induced suppression of glucose oxidation. Adrenergic stimulation increases triglyceride lypolysis to increase free fatty acid levels. The heart is known to increase its consumption of free fatty acids under conditions of increased circulating levels of free fatty acids (Opie, 1995). Under conditions of poor oxygen supply, the metabolic intermediates that support oxidative phosphorylation of ADP accumulate, which in turn reduce pyruvate dehydrogenase activity, the enzyme that regulates entry of carbons from glucose, in the form of pyruvate, into the citric acid cycle and the electron transport chain (Stanley et al., 1997). Although an equivalent of a fatty acid would yield more ATP than the same equivalent of glucose, fatty acids are inherently less efficient in its utilisation of oxygen, since more oxygen is required to yield an equivalent amount of ATP than glucose oxidation. Furthermore, the suppression of glucose oxidation results in increased lactic acid formation and tissue acidosis. Therapeutic approaches to redress the imbalance between fatty acid oxidation and glucose oxidation that occurs during an ischaemic challenge, have included suppression of fatty acid lipolysis (Russell and Oliver, 1978) and infusions of glucose, insulin and K^+ (Fath-Ordoubadi and Beatt, 1997). We report herein a new approach to metabolic management of myocardial infarction and ischaemia with the partial fatty acid oxidation inhibitor, ranolazine.

Ranolazine modulates the metabolism that occurs in ischaemic myocytes by activating pyruvate dehydrogenase activity to promote glucose oxidation (Clarke et al., 1993, 1996; McCormack et al., 1996). Ranolazine is thought to

* Corresponding author. Tel.: +44-20-7882-5785; fax: +44-20-7882-6104.

E-mail address: k.zacharowski@mds.qmw.ac.uk (K. Zacharowski).

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switch substrate utilisation from fatty acids to glucose and, hence, to improve the efficiency of oxygen utilization and limit the production of lactic acid (Wyatt et al., 1995). This mechanism of action of ranolazine may explain its anti-ischaemic action, in the absence of any haemodynamic effects in human and animal models. Several studies with extracted enzymes and isolated rat heart mitochondria suggests that ranolazine activates pyruvate dehydrogenase indirectly, because ranolazine has no effects on (i) pyruvate dehydrogenase kinase, (ii) pyruvate dehydrogenase phosphatase and (iii) pyruvate dehydrogenase catalytic activity (Clarke et al., 1996). Nevertheless, it has been shown that ranolazine reduces cellular acetyl-CoA content in the presence of fatty acids via inhibition of fatty acid β-oxidation, and leads to decreased levels of acetyl-CoA and activation of pyruvate dehydrogenase (McCormack et al., 1996). By increasing the metabolic flux via pyruvate dehydrogenase, ranolazine limits the ischaemia-induced rise in lactic acid and increases ATP production per mole of oxygen consumed. Thus, under conditions of reduced myocardial oxygen delivery (e.g. ischaemia), ATP generation is preserved and myocardial function is maintained (Allely and Alps, 1990; Clarke et al., 1993; Gralinski et al., 1994; McCormack et al., 1996).

There is good evidence that ranolazine exerts beneficial effects in animal models of experimental myocardial ischaemia and in patients with angina pectoris (Allely and Alps, 1990; Cocco et al., 1992; Gralinski et al., 1994; Jain et al., 1990; Pepine and Wolff, 1999; Wang et al., 1999; Wolff and Investigators, 2000). For example, in the recently completed *M*onotherapy *A*ssessment of *R*anolazine *I*n *S*table *A*ngina (MARISA) trial (randomised, placebo-controlled, four-period crossover study), there was a dose-dependent increase in exercise duration, time to ST-segment change, and time to angina without clinically meaningful effects on blood pressure and heart rate. Agents which delay the onset of ischaemic tissue injury of the heart (such as ranolazine, organic nitrates and β-adrenoceptor antagonists) may or may not cause a significant reduction of the ultimate infarct size, arising from acute myocardial ischaemia–reperfusion. It is still unclear, whether ranolazine causes a significant reduction in myocardial infarct size. Recently, it has been demonstrated that ranolazine attenuates the release of the creatine kinase subunit muscle-brain (MB) in baboons subjected to regional myocardial ischaemia–reperfusion (Allely and Alps, 1990). Although this data suggests that ranolazine does reduce infarct size in this species, the respective results are difficult to interpret, as the number of experiments was limited and creatine kinase MB does not have a high tissue specificity (Allely and Alps, 1990; Jaffe et al., 2000). In subsequent studies, Lucchesi and colleagues have demonstrated that ranolazine does not reduce the infarct size caused by regional myocardial ischaemia–reperfusion in the dog (Black et al., 1994). The same investigators, on the other hand, have demonstrated that ranolazine causes a substantial protection against the ischaemia–reperfusion injury caused by global myocardial ischaemia–reperfusion in the isolated perfused heart of the rabbit (Gralinski et al., 1994). It is, however, not known whether ranolazine can reduce ischaemia–reperfusion injury in the rat heart. We investigated whether ranolazine exerts beneficial effects in a rat model of regional myocardial ischaemia–reperfusion, assessed by the determination of (i) myocardial infarct size and (ii) cardiac troponin T release. Cardiac troponin T is part of the contractile apparatus of striated musculature. Although the overall function of cardiac troponin T is the same in all striated muscles, cardiac troponin T originating from cardiac tissue clearly differs from skeletal muscle. Therefore, the determination of cardiac troponin T is a very important tool in the diagnosis of the occurrence of myocardial cell necrosis, for instance in acute myocardial infarction, myocarditis, unstable angina pectoris and the monitoring of reperfusion interventions (Christenson et al., 1998; Hamm et al., 1992; Katus et al., 1989, 1991; Ravkilde et al., 1993).

2. Methods

2.1. Myocardial ischaemia–reperfusion

In this study, male Wistar rats (Tucks, Reyleigh, Essex, UK) weighing 240–350 g were used. They received a standard diet and water ad libitum. All procedures were carried out in accordance with the Home Office *Guidance on the Operation of Animals (Scientific Procedures) Act 1986*, published by Her Majesty's Stationery Office (London) and the Association for Assessment and Accreditation of Laboratory Animal Care guidelines.

Rats were anaesthetised using thiopentone sodium (120 mg/kg i.p.), and anaesthesia was maintained with supplementary doses of thiopentone sodium as required. The trachea was cannulated, and artificial respiration was maintained using a Harvard ventilator with a frequency of 70 strokes/min, a tidal volume of 8–10 ml/kg, an inspiratory oxygen concentration of 30% and a positive end-expiratory pressure of 1–2 mm Hg, resulting in pCO_2 values of 36–44 mm Hg and pO_2 values over 150 mm Hg (Horstick et al., 1999; Zacharowski et al., 1999b,c). The right carotid artery was cannulated to monitor mean arterial blood pressure and heart rate. Pressure rate index was calculated as the product of mean arterial blood pressure and heart rate, and expressed in mm Hg/min/10^3. The right jugular vein was cannulated for the administration of drugs.

The method of coronary artery occlusion–reperfusion in the rat was performed as previously described (Zacharowski et al., 1999b,c). The chest was opened by a left-side thoracotomy, the pericardium was incised, and a suture and occluder were placed around the left anterior descending coronary artery. After completion of the surgical pro-

cedure, the animals were allowed to stabilise for 30 min before left anterior descending coronary artery ligation. At the end of the occlusion period (25 min), the occluder was released, allowing the reperfusion of the previously ischaemic myocardium for 2 h.

2.2. Determination of area at risk and infarct size

After re-occluding the left anterior descending coronary artery, Evans blue dye (1 ml of 2% w/v) was administered i.v. in order to determine between ischaemic (area at risk) and non-ischaemic myocardium (area not at risk). Subsequently, the heart was cut into four to five horizontal slices, the right ventricular wall was removed, and the area at risk (pink) was separated from the non-ischaemic (blue) area. The area at risk was cut into small pieces and incubated with *p*-nitro-blue tetrazolium (0.5 mg/ml) for 20 min at 37°C, to distinguish between ischaemic and infarcted tissue (Zacharowski et al., 1999b,c), while the area not at risk was incubated with saline. In the presence of intact dehydrogenase enzyme systems (normally perfused myocardium), *p*-nitro-blue tetrazolium forms a dark blue formazan, while areas of necrosis lack dehydrogenase activity and, therefore, fail to stain (Nachlas and Shnitka, 1963). Pieces were separated according to staining and weighed to determine the infarct size as a percentage of the area at risk.

2.3. Measurement of the plasma levels of cardiac troponin T

At the end of the myocardial ischaemia–reperfusion experiment, a blood sample (1 ml) was obtained from the carotid cannula and centrifuged to obtain plasma. The plasma supernatants were removed and stored frozen until assayed. The concentration of cardiac troponin T was determined by the short-turn-around-time (STAT) assay (provided by Boehringer Mannheim, Germany) using an Elecsys® System 2010 (Zacharowski et al., 1999c).

2.4. Exclusion criteria

The following exclusion criteria were set: (1) an area at risk of less than 30% of the left ventricle and greater than 60%; (2) death prior to completion of ischaemia–reperfusion protocol; (3) death of the rat due to ventricular fibrillation that could not be reversed by cardiac massage; (4) mean arterial blood pressure of less than 60 mm Hg (e.g. due to cardiac failure).

2.5. Experimental groups

1. Saline bolus injection (2.4 ml/kg) and infusion (2.4 ml/kg/h), starting 30 min prior to left anterior descending coronary artery occlusion and maintained throughout the reperfusion ($n = 12$).
2. Ranolazine bolus injection (10 mg/kg) and infusion (9.6 mg/kg/h), starting 30 min prior to left anterior descending coronary artery occlusion and maintained throughout the reperfusion ($n = 12$).
3. Sham-operated controls, no occlusion of the left anterior descending coronary artery for 25 min and 2 h of reperfusion ($n = 4$).

The *n*-numbers refer to rats that survived until the end of the experiment. The number of rats which died in the individual groups were as follows: group 1, $n = 1$; group 2, $n = 1$; and group 3, $n = 0$.

2.6. Drugs and materials

Unless otherwise stated, all compounds were obtained from Sigma. Ranolazine was dissolved in saline and was obtained from CV Therapeutics, Palo Alto, CA, USA. Thiopentone sodium (Intraval) was obtained from May & Baker.

2.7. Statistical analysis

Data are reported as mean ± SEM of *n* observations. One or two-way analysis of variance (ANOVA) followed

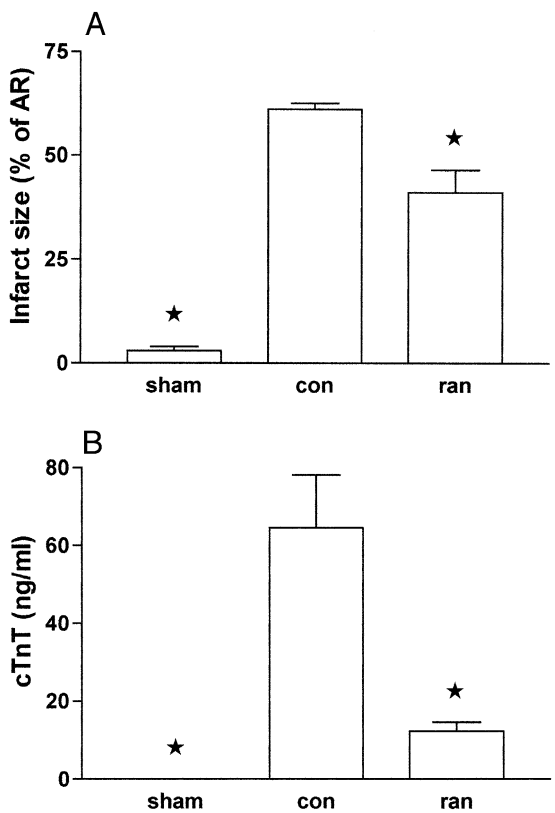

Fig. 1. Left anterior descending coronary artery occlusion–reperfusion (25 min–2 h) in the anaesthetised rat. (A) Infarct size (expressed as percent of the area at risk, AR). (B) Alterations in the plasma levels of cardiac troponin T (cTnT). ★$P < 0.05$ vs. control.

Table 1
Effects of ranolazine on mean arterial blood pressure (MAP), heart rate (HR) and pressure rate index (PRI) in rats subjected to left anterior descending coronary artery (LAD) occlusion and reperfusion
Data are means (SEM); MAP (mm Hg), HR (beats/min), PRI (mm Hg/min/10^3).

Group		Before treatment and LAD occlusion (min)	LAD occlusion (min)			LAD reperfusion (min)	
		−30	0	15	25	85	145
Sham ($n=4$)	MAP	126 (12)	128 (14)	121 (9)	114 (9)	106 (7)	100 (8)[a]
	HR	415 (6)	415 (5)	405 (6)	397 (12)	383 (11)	378 (15)
	PRI	52 (5)	53 (6)	49 (4)	45 (4)	41 (4)	38 (3)
Control ($n=12$)	MAP	125 (6)	122 (5)	112 (6)	108 (6)	88 (6)[b]	79 (4)[b]
	HR	440 (15)	443 (12)	435 (9)	425 (10)	442 (12)	428 (12)
	PRI	55 (4)	55 (3)	50 (4)	47 (3)	40 (3)[b]	34 (2)[b]
Ranolazine ($n=12$)	MAP	117 (6)	115 (6)	99 (7)	93 (5)	80 (6)[b]	71 (3)[b]
	HR	382 (10)[a]	378 (10)[a]	388 (7)[a]	387 (7)[a]	372 (6)[a]	361 (9)[a]
	PRI	45 (3)	44 (3)	38 (3)	36 (2)[a,b]	30 (2)[a,b]	25 (1)[a,b]

[a] $P < 0.05$ vs. control.
[b] $P < 0.05$ vs. time point −30 min.

by a Bonferroniïs test for multiple comparisons was used to compare groups. A P value of < 0.05 was considered statistically significant.

3. Results

3.1. Effects of ranolazine on infarct size and cardiac troponin T release caused by myocardial ischaemia–reperfusion

The mean values for the area at risk were $48 \pm 2\%$ (control), $48 \pm 1\%$ (ranolazine) or $47 \pm 3\%$ (sham), respectively. In rats which had received an infusion of saline, left anterior descending coronary artery occlusion–reperfusion resulted in an infarct size of 61% of the area at risk (control). When compared to vehicle, infusion of ranolazine caused a significant reduction in infarct size of approximately 33% (Fig. 1A). Sham operation alone did not result in a significant degree of infarction (Fig. 1A). In rats which had received an infusion of saline, left anterior descending coronary artery occlusion–reperfusion resulted in a release of cardiac troponin T into the plasma of 65 ng/ml (control). When compared to vehicle, infusion of ranolazine resulted in a significant reduction in the plasma concentrations of cardiac troponin T (12 ng/ml, Fig. 1B). Sham operation alone did not result in a significant increase in the plasma levels of cardiac troponin T.

3.2. Effects of ranolazine on mean arterial blood pressure, heart rate and pressure rate index measured during myocardial ischaemia–reperfusion

The mean values for the mean arterial blood pressure were similar in all animal groups studied at the time points 30, 0, 15, 25 and 85 min. However, at time point 145 min, sham rats had a significant higher mean arterial blood pressure when compared to control rats (Table 1). In rats which had received ranolazine, the heart rate was reduced during the experimental period (Table 1). The mean values for the pressure rate index were reduced in ranolazine-treated rats at the time points 25, 85 and 145 min (Table 1). In rats subjected to left anterior descending coronary artery occlusion and reperfusion, which received either saline or ranolazine, mean values for mean arterial blood pressure and pressure rate index fell significantly through the reperfusion period (Table 1).

4. Discussion

This is the first report that ranolazine reduces infarct size and the release of cardiac troponin T in rats that have been subjected to myocardial ischaemia–reperfusion. In this study, we have used two different and independent methods of measuring myocardial cell necrosis, namely staining for tissue viability using *p*-nitro-blue tetrazolium and measuring the release of cardiac troponin T into the plasma. Using both of these techniques, we provide convincing evidence that ranolazine causes a significant reduction in myocardial infarct size. Clearly, in all groups studied, there were no significant differences in body weight, heart weight or area at risk, suggesting that the beneficial effects of ranolazine were not related to differences in the amount of myocardial tissue sampled.

In the presence of intact dehydrogenase enzyme systems (viable myocardium), *p*-nitro-blue tetrazolium forms a dark blue formazan, while areas of necrosis lack dehydrogenase activity and therefore do not stain (Nachlas and Shnitka, 1963). To ensure that those sections of the area at risk, which failed to stain with *p*-nitro-blue tetrazolium, do indeed represent "necrotic" myocardium, we have previously carried out an extensive morphological analysis using both light microscopy and electron microscopy

(Zacharowski et al., 1999a). In addition to measuring myocardial cell necrosis by *p*-nitro-blue tetrazolium staining, determination of plasma levels of enzymes/proteins, which are released by cardiac myocytes, may also be used as a reliable indicator of tissue necrosis. Cardiac troponin I or cardiac troponin T are the specific recommended markers for the diagnosis of myocardial injury (Jaffe et al., 2000). The improved tissue specificity of cardiac troponin I or cardiac troponin T, compared with lactate dehydrogenase, creatine kinase, creatine kinase MB and aspartate aminotransferase, is well established. Clinical trials have demonstrated that when conjoint myocardial and skeletal muscle injury is present, the improved specificity of cardiac troponin I or cardiac troponin T reduces the number of false-positive results while maintaining high sensitivity (Adams et al., 1993; Katus et al., 1991).

This study has demonstrated beneficial effects of ranolazine in a rat model of myocardial ischaemia–reperfusion. Ranolazine partially inhibits the metabolism of fatty acids in ischaemic myocytes to reduce the intra-mitochondrial load of the metabolic intermediates acetyl-CoA and NADH that act to suppress pyruvate dehydrogenase activity (Clarke et al., 1993, 1996; McCormack et al., 1996). Furthermore, McCormack and colleagues have demonstrated in isolated rat hearts (working or Langendorff modes), under a variety of different conditions, that ranolazine stimulated glucose oxidation and reduced palmitate oxidation. Under normoxic, ischaemic or reperfusion conditions after global ischaemia, the effects of ranolazine on glucose oxidation were always present, whereas the effects on palmitate oxidation were not always observed. The authors suggested that the primary effect of ranolazine is to stimulate glucose oxidation, and that the other effects such as glycolysis and/or palmitate oxidation could be secondary as a result of metabolic compensation. In this study, we have not determined the effect of ranolazine on fatty acid levels neither in the blood nor in the myocardium.

Ranolazine, therefore, indirectly stimulates pyruvate dehydrogenase activity to promote glucose oxidation (Wyatt et al., 1995). Recently, it has been demonstrated that ranolazine attenuates palmitoyl-L-carnitine-induced mechanical dysfunction and metabolic derangement in the isolated perfused rat heart (Maruyama et al., 2000). These effects were concentration-dependent (5–20 μM), and ranolazine, in the absence of palmitoyl-L-carnitine, did not affect mechanical function or energy metabolism (Maruyama et al., 2000). Thus, under conditions of reduced myocardial oxygen delivery such as ischaemia, ATP generation is preserved, myocardial function is maintained (Allely and Alps, 1990; Clarke et al., 1993; Gralinski et al., 1994; McCormack et al., 1996) and tissue necrosis might be delayed. In a separate study, the plasma concentrations of ranolazine were measured in rats subjected to the same bolus and infusion regime as in the infarction study (Blackburn B, unpublished observation). According to these results, the dose of ranolazine can be estimated to be 7–10 μM, and is therefore similar to the one used by Maruyama and colleagues in their isolated perfused rat heart study (Maruyama et al., 2000).

During several experiments, differences in haemodynamic parameters were observed between the groups (saline vs. ranolazine). It should be noted that in rats subjected to 25 min left anterior descending coronary artery occlusion, the heart rate was lower in the ranolazine group at baseline (before drug infusion) and throughout the whole experimental period; however, the change from baseline was not statistically different within each treatment arm. These findings demonstrate that ranolazine has no effects on heart rate. The mean values for mean arterial blood pressure in ranolazine-treated rats were similar at all time points, compared to the respective control group. Therefore, a significant lower heart rate in the ranolazine study group (baseline until the end of the experiment) propagates the lower values to the pressure rate index (the product of heart rate and mean arterial blood pressure). It is unlikely that the moderate difference in heart rate between the groups accounts for the reduction in cardiac troponin T plasma level or infarct size, since one of us previously demonstrated that a similar reduction in heart rate in rats subjected to ischaemia–reperfusion injury was not associated with a significant reduction in myocardial infarct size (Gralinski et al., 1994).

In summary, ranolazine (bolus injection and infusion throughout the experiment) caused a significant reduction in myocardial infarct size, and attenuated the release of cardiac troponin T in rats subjected to left anterior descending coronary artery occlusion–reperfusion. These findings suggest that ranolazine may also exert beneficial effects in patients suffering from myocardial infarction. In this respect, new clinical trials should be conducted.

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References

Adams, J.E.d., Abendschein, D.R., Jaffe, A.S., 1993. Biochemical markers of myocardial injury: is MB creatine kinase the choice for the 1990s? Circulation 88, 750–763.

Allely, M.C., Alps, B.J., 1990. Prevention of myocardial enzyme release by ranolazine in a primate model of ischaemia with reperfusion. Br. J. Pharmacol. 99, 5–6.

Black, S.C., Gralinski, M.R., McCormack, J.G., Driscoll, E.M., Lucchesi, B.R., 1994. Effect of ranolazine on infarct size in a canine model of regional myocardial ischemia/reperfusion. J. Cardiovasc. Pharmacol. 24, 921–928.

Christenson, R.H., Duh, S.H., Newby, L.K., Ohman, E.M., Califf, R.M., Granger, C.B., Peck, S., Pieper, K.S., Armstrong, P.W., Katus, H.A.,

Topol, E.J., 1998. Cardiac troponin T and cardiac troponin I: relative values in short-term risk stratification of patients with acute coronary syndromes. GUSTO-IIa Investigators Clin. Chem. 44, 494–501.

Clarke, B., Spedding, M., Patmore, L., McCormack, J.G., 1993. Protective effects of ranolazine in guinea-pig hearts during low-flow ischaemia and their association with increases in active pyruvate dehydrogenase. Br. J. Pharmacol. 109, 748–750.

Clarke, B., Wyatt, K.M., McCormack, J.G., 1996. Ranolazine increases active pyruvate dehydrogenase in perfused normoxic rat hearts: evidence for an indirect mechanism. J. Mol. Cell. Cardiol. 28, 341–350.

Cocco, G., Rousseau, M.F., Bouvy, T., Cheron, P., Williams, G., Detry, J.M., Pouleur, H., 1992. Effects of a new metabolic modulator, ranolazine, on exercise tolerance in angina pectoris patients treated with beta-blocker or diltiazem. J. Cardiovasc. Pharmacol. 20, 131–138.

Fath-Ordoubadi, F., Beatt, K.J., 1997. Glucose–insulin–potassium therapy for treatment of acute myocardial infarction: an overview of randomized placebo-controlled trials. Circulation 96, 1152–1156.

Gralinski, M.R., Black, S.C., Kilgore, K.S., Chou, A.Y., McCormack, J.G., Lucchesi, B.R., 1994. Cardioprotective effects of ranolazine (RS-43285) in the isolated perfused rabbit heart. Cardiovasc. Res. 28, 1231–1237.

Hamm, C.W., Ravkilde, J., Gerhardt, W., Jorgensen, P., Peheim, E., Ljungdahl, L., Goldmann, B., Katus, H.A., 1992. The prognostic value of serum troponin T in unstable angina. N. Engl. J. Med. 327, 146–150.

Horstick, G., Berg, O., Heimann, A., Darius, H., Lehr, H.A., Bhakdi, S., Kempski, O., Meyer, J., 1999. Surgical procedure affects physiological parameters in rat myocardial ischemia: need for mechanical ventilation. Am. J. Physiol. 276, H472–H479.

Jaffe, A.S., Ravkilde, J., Roberts, R., Naslund, U., Apple, F.S., Galvani, M., Katus, H., 2000. It's time for a change to a troponin standard. Circulation 102, 1216–1220.

Jain, D., Dasgupta, P., Hughes, L.O., Lahiri, A., Raftery, E.B., 1990. Ranolazine (RS-43285): a preliminary study of a new anti-anginal agent with selective effect on ischaemic myocardium. Eur. J. Clin. Pharmacol. 38, 111–114.

Katus, H.A., Remppis, A., Looser, S., Hallermeier, K., Scheffold, T., Kubler, W., 1989. Enzyme linked immuno assay of cardiac troponin T for the detection of acute myocardial infarction in patients. J. Mol. Cell. Cardiol. 21, 1349–1353.

Katus, H.A., Schoeppenthau, M., Tanzeem, A., Bauer, H.G., Saggau, W., Diederich, K.W., Hagl, S., Kuebler, W., 1991. Non-invasive assessment of preoperative myocardial cell damage by circulating cardiac troponin T. Br. Heart J. 65, 259–264.

Maruyama, K., Hara, A., Hashizume, H., Ushikubi, F., Abiko, Y., 2000. Ranolazine attenuates palmitoyl-L-carnitine-induced mechanical and metabolic derangement in the isolated, perfused rat heart. J. Pharm. Pharmacol. 52, 709–715.

McCormack, J.G., Barr, R.L., Wolff, A.A., Lopaschuk, G.D., 1996. Ranolazine stimulates glucose oxidation in normoxic, ischemic, and reperfused ischemic rat hearts. Circulation 93, 135–142.

Nachlas, M.M., Shnitka, T.K., 1963. Macroscopic identification of early myocardial infarct by alterations in dehydrogenase activity. Am. J. Pathol. 43, 379–405.

Opie, L.H., 1995. Substrate and energy metabolism of the heart physiology and pathophysiology of the heart. In: Sperelakis, N. (Ed.), Kluwer Academic Publishing, Dordrecht, Chapter 20.

Pepine, C.J., Wolff, A.A., 1999. A controlled trial with a novel anti-ischemic agent, ranolazine, in chronic stable angina pectoris that is responsive to conventional antianginal agents. Ranolazine Study Group Am. J. Cardiol. 84, 46–50.

Ravkilde, J., Horder, M., Gerhardt, W., Ljungdahl, L., Pettersson, T., Tryding, N., Moller, B.H., Hamfelt, A., Graven, T., Asberg, A. et al., 1993. Diagnostic performance and prognostic value of serum troponin T in suspected acute myocardial infarction. Scand. J. Clin. Lab. Invest. 53, 677–685.

Russell, D.C., Oliver, M.F., 1978. Effect of antilipolytic therapy on ST segment elevation during myocardial ischaemia in man. Br. Heart J. 40, 117–123.

Stanley, W.C., Lopaschuk, G.D., Hall, J.L., McCormack, J.G., 1997. Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions: potential for pharmacological interventions. Cardiovasc. Res. 33, 243–257.

Vetter, N., Strange, R.C., Adams, W., Oliver, M.F., 1974. Initial metabolic and hormonal response to acute myocardial infarction. Lancet 1, 284–289.

Wang, J.X., Maruyama, K., Murakami, M., Endo, T., Komatsu, H., Akahane, M., 1999. Antianginal effects of ranolazine in various experimental models of angina. Arzneim.-Forsch. 49, 193–199.

Wolff, A.A., Investigators, f.t.M., 2000. MARISA: monotherapy assessment of ranolazine in stable angina. J. Am. Coll. Cardiol. 35 (Suppl. A), 408A.

Wyatt, K.M., Skene, C., Veitch, K., Hue, L., McCormack, J.G., 1995. The antianginal agent ranolazine is a weak inhibitor of the respiratory complex I, but with greater potency in broken or uncoupled than in coupled mitochondria. Biochem. Pharmacol. 50, 1599–1606.

Zacharowski, K., Olbrich, A., Otto, M., Hafner, G., Thiemermann, C., 1999a. Effects of the prostanoid EP3-receptor agonists M&B 28767 and GR 63799X on infarct size caused by regional myocardial ischaemia in the anaesthetized rat. Br. J. Pharmacol. 126, 849–858.

Zacharowski, K., Olbrich, A., Piper, J., Hafner, G., Kondo, K., Thiemermann, C., 1999b. Selective activation of the prostanoid EP(3) receptor reduces myocardial infarct size in rodents. Arterioscler., Thromb., Vasc. Biol. 19, 2141–2147.

Zacharowski, K., Otto, M., Hafner, G., Chatterjee, P.K., Thiemermann, C., 1999c. Endotoxin induces a second window of protection in the rat heart as determined by using *p*-nitro-blue tetrazolium staining, cardiac troponin T release, and histology. Arterioscler., Thromb., Vasc. Biol. 19, 2276–2280.