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5-Aminoisoquinolinone, a potent inhibitor of poly (adenosine 5′-diphosphate ribose) polymerase, reduces myocardial infarct size

Nicole Wayman [a], Michelle C. McDonald [a], Andrew S. Thompson [b], Michael D. Threadgill [b], Christoph Thiemermann [a,*]

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

[b] *Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK*

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Abstract

This study investigates the effects of a novel, water-soluble inhibitor of the activity of poly (adenosine 5′-diphosphate ribose) polymerase, 5-aminoisoquinolinone [5-aminoisoquinolin-1(2*H*)-one], on (i) poly (adenosine 5′-diphosphate ribose) polymerase activity in rat cardiac myoblasts and (ii) the infarct size caused by regional myocardial ischaemia and reperfusion in the rat. Exposure of H9c2 cells to hydrogen peroxide (H_2O_2, 1 mM) caused a significant increase in poly (adenosine 5′-diphosphate ribose) polymerase activity and an 80–90% reduction in mitochondrial respiration (cellular injury). Pretreatment of these cells with 5-aminoisoquinolinone (0.003–1 mM) caused a concentration-dependent inhibition of poly (adenosine 5′-diphosphate ribose) polymerase activity (IC_{50}: ~4.5 μM, $n = 6–9$) and cell injury (EC_{50}: ~4.45 μM, $n = 9$). In a rat model of myocardial infarction, left anterior descending coronary artery occlusion (25 min) and reperfusion (2 h) resulted in an infarct size of 50 ± 3%. Administration (1 min before reperfusion) of 5-aminoisoquinolinone reduced myocardial infarct size in a dose-related fashion. Thus, 5-aminoisoquinolinone is a potent inhibitor of poly (adenosine 5′-diphosphate ribose) polymerase activity in cardiac myoblasts and reduces myocardial infarct size in vivo.

Keywords: Heart, cardiomyoblast; Reactive oxygen species; Poly (adenosine 5′-diphosphate ribose) polymerase; Infarct size, (rat)

1. Introduction

Poly (adenosine 5′-diphosphate ribose) polymerase [also known as poly (adenosine 5′-diphosphate ribose) synthetase; E.C. 2.4.2.30] is an ubiquitous, chromatin-bound enzyme (Ikai and Ueda, 1983). Activation of poly (adenosine 5′-diphosphate ribose) polymerase is triggered by single-strand breaks in DNA and subsequently catalyses the transfer of ADP-ribose moieties from NAD^+ to various nuclear proteins including histones and poly (adenosine 5′-diphosphate ribose) polymerase (automodification domain) itself (Ueda and Hayaishi, 1985). Continuous or excessive activation of poly (adenosine 5′-diphosphate ribose) polymerase produces extended chains of ADP-ribose on nuclear proteins and results in a substantial depletion of intracellular NAD^+ and subsequently ATP, which may ultimately cause cell death (Berger, 1985; Schraufstatter et al., 1986a,b; Hyslop et al., 1988; Thies and Autor, 1991). Radicals including superoxide anions, hydrogen peroxide or hydroxyl radicals cause strand breaks in DNA, activation of poly (adenosine 5′-diphosphate ribose) polymerase and depletion of NAD^+ and ATP in cultured cells (Berger, 1985; Schraufstatter et al., 1986a,b; Hyslop et al., 1988; Thies and Autor, 1991). There is good evidence that various chemically distinct inhibitors of poly (adenosine 5′-diphosphate ribose) polymerase activity (including 3-aminobenzamide, nicotinamide and 1,5-dihydroxyisoquinolinone [5-hydroxyisoquinolin-1(2*H*)one]) reduce the degree of tissue injury associated with regional ischaemia and reperfusion of the heart (Thiemermann et al., 1997; Zingarelli et al., 1997, 1998; Bowes et al., 1998b; Docherty et al., 1999), the brain (Eliasson et al., 1997), the gut (Cuzzocrea et al., 1997) and the kidney (Chatterjee et al., 2000). Most notably, the degree of tissue injury caused by ischaemia and reperfusion of the heart (Zingarelli et al., 1998; Walles et al., 1998; Grupp et al., 1999; Pieper et al.,

* Corresponding author. Tel.: +44-207-882-6119; fax: +44-207-251-1685.

E-mail address: c.thiemermann@mds.qmw.ac.uk (C. Thiemermann).

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2000) and brain (Eliasson et al., 1997) is attenuated in mice in which the gene for poly (adenosine 5′-diphosphate ribose) polymerase has been disrupted by gene targeting [poly (adenosine 5′-diphosphate ribose) polymerase knock out or −/− mice]. In contrast to certain isoquinoline derivatives, such as 1,5-dihydroxyisoquinoline and 3,4-dihydro-5-[4-(piperidin-1-yl)butoxy]isoquinolin-1(2*H*)-one (DPQ), 3-aminobenzamide is a weak inhibitor of poly (adenosine 5′-diphosphate ribose) polymerase activity that does not readily cross cell membranes (Rankin et al., 1989). Although 1,5-dihydroxyisoquinoline and DPQ are more potent inhibitors of poly (adenosine 5′-diphosphate ribose) polymerase activity than 3-aminobenzamide, 1,5-dihydroxyisoquinoline and DPQ both have to be dissolved in dimethylsulfoxide (10% w/v). Dimethylsulfoxide is a potent scavenger of hydroxyl radicals and recent studies indicate that dimethylsulfoxide itself is an inhibitor of poly (adenosine 5′-diphosphate ribose) polymerase activity (Banasik and Ueda, 1999). Thus, it is not surprising that dimethylsulfoxide itself reduces the organ injury in conditions associated with organ ischaemia and reperfusion (Wray et al., 1998; McDonald et al., 1999). Thus, there is still a great need for the development of potent, water-soluble inhibitors of poly (adenosine 5′-diphosphate ribose) polymerase activity. In 1991, Suto et al. reported that 5-aminoisoquinolin-1(2*H*)-one is a water-soluble inhibitor of poly (adenosine 5′-diphosphate ribose) polymerase activity in a cell-free preparation (enzyme purified 900-fold from calf thymus) (Suto et al., 1991; Arundel-Suto et al., 1991). As 5-aminoisoquinolinone is not commercially available and the reported syntheses are low yielding and unreliable (Wenkert et al., 1964; Suto et al., 1991), we have developed a new efficient method for its preparation (McDonald et al., 2000). We have then evaluated the effects of 5-aminoisoquinolinone on poly (adenosine 5′-diphosphate ribose) polymerase activity in rat cardiac myoblasts and investigated the effects of 5-aminoisoquinolinone on the infarct size caused by regional myocardial ischaemia and reperfusion in the anaesthetised rat.

2. Methods

2.1. Materials

Unless otherwise stated, all compounds were obtained from Sigma-Aldrich (Poole, Dorset, UK). Minimum essential medium (DMEM) was from Life Technology (Paisley, Lanarkshire, UK). Thiopentone sodium (Intraval Sodium®) was obtained from Rhône Mérieux (Harlow, Essex, UK). All stock solutions were prepared in nonpyrogenic saline (0.9% NaCl; Baxter Healthcare, Thetford, Norfolk, UK). Reagents for chemical synthesis were purchased from Aldrich Chemical (Poole, Dorset, UK). The synthesis of 5-aminoisoquinolinone has been reported in detail elsewhere (McDonald et al., 2000).

2.2. Cell culture

Rat cardiac myoblasts (H9c2 cells) were obtained from the American Type Culture Collection (Rockville, MD, USA) and grown to confluence in culture flasks containing minimum essential medium (DMEM) supplemented with L-glutamine (3.5 mM) and 10% foetal calf serum. Cells were passaged every 2 days—removed by treatment with trypsin (0.05%), EDTA (0.02%) and then cultured (37 °C, 5% CO_2) in 96-well (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide [MTT] assay) or 6-well plates [measurement of poly (adenosine 5′-diphosphate ribose) polymerase activity] (Falcon, UK) until they reached confluence. Cells were used at the following passage numbers: H9c2 cells (P_{1-15}).

2.3. Measurement of cell injury (MTT assay)

Cell viability was determined indirectly by measuring the mitochondrial-dependent reduction of MTT to formazan (i.e. mitochondrial respiration). Cells in 96-well plates were incubated with MTT [100 μl, 0.2 mg ml^{-1}, dissolved in phosphate buffer solution (PBS, pH 7.4)] for 60 min at 37 °C. MTT solution was removed by aspiration and cells were solubilised in 100 μl of dimethylsulfoxide. The amount of purple formazan formed was detected and quantified by measuring the absorbance of the solution at 550 nm using an Anthos Labtec microplate reader (Labtec, Uckfield, Sussex, UK). Results are expressed as mitochondrial respiration as a percentage of the control cells (i.e. those not exposed to hydrogen peroxide), which was taken as 100%. To elucidate the effects of 5-aminoisoquinolinone on the cell injury caused by H_2O_2, cells were preincubated (10 min, 37 °C) with 5-aminoisoquinolinone (0.003–1 mM) and then exposed to H_2O_2 (1 mM) for 4 h after which time cell injury/death was assessed.

2.4. Measurement of poly (adenosine 5′-diphosphate ribose) polymerase activity in rat cardiomyoblasts

We have previously reported that exposure of rat cardiac myoblasts to H_2O_2 causes a concentration-dependent (10 μM–10 mM) and time-dependent (1–6 h) increase in cell injury, which is secondary to an increase in poly (adenosine 5′-diphosphate ribose) polymerase activity in these cells (Bowes et al., 1999). Cells were preincubated with media containing the poly (adenosine 5′-diphosphate ribose) polymerase inhibitor 5-aminoisoquinolinone (0.003–1 mM). After a 10-min preincubation period, the cells were exposed to H_2O_2 (1 mM) for 60 min, and then collected to measure poly (adenosine 5′-diphosphate ribose) polymerase activity. Poly (adenosine 5′-diphosphate ribose) polymerase activity was measured as the ability of permeabilised cells to transfer the substrate [^{3}H]-NAD^+ onto nuclear proteins over a set time period as described by Schraufstatter et al. (1986a). Following the appropriate

treatment and duration, the media were aspirated before addition of fresh culture medium (400 μl); the cells were then scraped and transferred to Eppendorff tubes. Following centrifugation ($10\,000 \times g$, 10 s) and aspiration of media, the cells were resuspended in reaction buffer (56 mM Hepes buffer containing 28 mM potassium chloride, 28 mM sodium chloride, 2 mM magnesium chloride, 0.02% digitonin, and 125 nmol NAD^+ spiked with 0.5 μCi ml^{-1} [^{3}H-NAD^+], pH 7.5), vortexed for 5 s and incubated at 37 °C for 5 min. The reaction was terminated by addition of 200 μl of 50% trichloroacetic acid and the resultant precipitate was pelleted by centrifugation at $10\,000 \times g$ for 3 min. The protein pellet was washed twice with 50% trichloroacetic acid and was then solubilised in 200 μl 0.2 M NaOH/2% SDS (*N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propane-sulphonate) overnight at 37 °C in a shaking incubator (Luckham, Basingstoke, Hants, UK). The radioactivity incorporated into protein was determined by scintillation counting (Beckman Instruments, High Wycombe, Bucks, UK).

2.5. Coronary artery ligation in the rat in vivo

The care and use of animals in this work were in accordance with UK Home Office guidelines on the Animals (Scientific Procedures) Act 1986 and the European Community guidelines for the use of experimental animals. This study was carried out on 45 male Wistar rats (Tuck, Rayleigh, Essex, UK) weighing 250–320 g receiving a standard diet and water ad libitum. The method of coronary artery occlusion and reperfusion in the anaesthetised rat was performed as previously described (Zacharowski et al., 2000). All animals were anaesthetised with thiopentone sodium (Intraval®, 120 mg/kg i.p.) and anaesthesia was maintained by supplementary injections (~ 10 mg/kg i.v.) of thiopentone sodium as required. In rats, this anaesthetic protocol, which has been approved by the Home Office of the UK, leads to a long-lasting surgical anaesthesia. The rats were intubated and ventilated with a Harvard ventilator (inspiratory oxygen concentration: 30%; 70 strokes/min, tidal volume: 8–10 ml/kg). Body temperature was maintained at 38 ± 1 °C. The right carotid artery was cannulated and connected to a pressure transducer (Spectramed, P23XL) to monitor mean arterial blood pressure. The right jugular vein was cannulated for the administration of drugs. Subsequently, a lateral thoracotomy was performed and the heart was suspended in a temporary pericardial cradle. A snare occluder was placed around the left anterior descending coronary artery. After completion of the surgical procedure, the animals were allowed to stabilise for 15 min before left anterior descending coronary artery ligation. The coronary artery was occluded at time 0 by tightening of the occluder. This was associated with the typical haemodynamic (fall in mean arterial blood pressure) changes of myocardial ischaemia. After 25 min of acute myocardial ischaemia, the occluder was reopened to allow the reperfusion for 2 h. Heart rate and mean arterial blood pressure were continuously recorded on a four-channel Grass 7D polygraph recorder (Grass, MA, USA). The pressure rate index, a relative indicator of myocardial oxygen consumption (Baller et al., 1981), was calculated as the product of mean arterial blood pressure and heart rate, and expressed in mm Hg/min $\times 10^{-3}$. Following the 2-h reperfusion period, the coronary artery was reoccluded and Evans Blue dye (1 ml of 2% w/v) was injected into the left ventricle, via the right carotid artery cannula, to distinguish between perfused and non-perfused (area at risk) sections of the heart. The Evans Blue solution stains the perfused myocardium, while the occluded vascular bed remains uncoloured. The animals were killed with an overdose of anaesthetic and the heart excised. It was sectioned into slices of 3–4 mm, the right ventricular wall was removed, and the area at risk (pink) was separated from the nonischaemic (blue) area. The area at risk was cut into small pieces and incubated with *p*-nitroblue tetrazolium (0.5 mg/ml) for 20 min at 37 °C. In the presence of intact dehydrogenase enzyme systems (viable myocardium), *p*-nitroblue 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 weight of the area at risk. The following experimental groups were studied.

1. Left anterior descending coronary artery occlusion (25 min) and reperfusion (2 h) plus administration of vehicle (saline, 1 ml/kg i.v. bolus), starting 1 min before reperfusion and maintained throughout the reperfusion period (at 1 ml/kg/h) ($n = 12$).
2. Left anterior descending coronary artery occlusion and reperfusion plus administration of 5-aminoisoquinolinone (0.03 mg/kg i.v. bolus injection 1 min before reperfusion followed by an infusion of 0.03 mg/kg/h, $n = 11$).
3. Left anterior descending coronary artery occlusion and reperfusion plus administration of 5-aminoisoquinolinone (0.1 mg/kg i.v. bolus injection 1 min before reperfusion followed by an infusion of 0.1 mg/kg/h, $n = 9$).
4. Left anterior descending coronary artery occlusion and reperfusion plus administration of 5-aminoisoquinolinone (0.3 mg/kg i.v. bolus injection 1 min before reperfusion followed by an infusion of 0.3 mg/kg/h, $n = 6$).
5. Sham operation (no left anterior descending coronary artery occlusion) and infusion of vehicle ($n = 7$).

2.6. Statistical evaluation

All data are presented as mean ± S.E.M. of n observations, where n represents the number of animals or blood

samples studied. The IC_{50} values were calculated using a Graph Pad Prism Statistical Package (version 3.0). For repeated measurements (haemodynamics), a two-factorial analysis of variance (ANOVA) was performed. Data without repeated measurements were analysed by one-factorial ANOVA, followed by a Dunnett's test for multiple comparisons using a Graph Pad Prism Statistical Package (version 3.0). A *P*-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Effects of 5-aminoisoquinolinone on the impairment in mitochondrial respiration caused by hydrogen peroxide in rat cardiac myoblasts

Exposure of rat cardiac myoblasts to H_2O_2 (1 mM for 4 h) caused a substantial (~80%) reduction in mitochondrial respiration (Fig. 1). Pretreatment of these cells with 5-aminoisoquinolinone caused a concentration-dependent attenuation (EC_{50}: ~4.45 μM) of the impairment in mitochondrial respiration caused by H_2O_2 (Fig. 1).

3.2. Effects of 5-aminoisoquinolinone on the increase in poly (adenosine 5′-diphosphate ribose) polymerase activity caused by hydrogen peroxide in rat cardiac myoblasts

Exposure of rat cardiac myoblasts to H_2O_2 caused a significant increase in poly (adenosine 5′-diphosphate ribose) polymerase activity (Fig. 2). This increase in poly (adenosine 5′-diphosphate ribose) polymerase activity was attenuated by pretreatment of the cells with 5-aminoisoquinolinone in a concentration-dependent fashion (IC_{50}: ~4.5 μM).

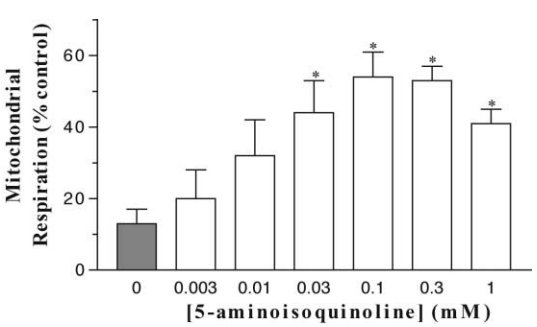

Fig. 1. The effect of 5-aminoisoquinolinone (0.003–1 mM, $n = 9$) on the impairment in mitochondrial respiration caused by H_2O_2 (1 mM, $n = 9$) in rat cardiac myoblasts. 5-Aminoisoquinolinone causes a concentration-dependent attenuation of the impairment in mitochondrial respiration caused by H_2O_2. Data are expressed as mean ± S.E.M. of *n* observations. * $P < 0.05$ when compared with H_2O_2 control (solid column).

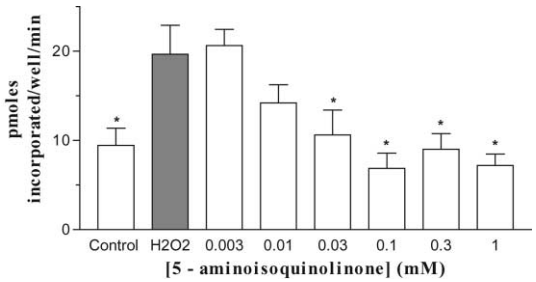

Fig. 2. The effect of (a) 5-aminoisoquinolinone (0.003–1 mM, $n = 6$) on the increase in poly (adenosine 5′-diphosphate ribose) polymerase activity caused by H_2O_2 (1 mM, $n = 9$) in rat cardiac myoblasts. 5-Aminoisoquinolinone causes a concentration-dependent attenuation of the increase in poly (adenosine 5′-diphosphate ribose) polymerase activity caused by H_2O_2. Data are expressed as mean ± S.E.M. of *n* observations. * $P < 0.05$ when compared with H_2O_2 control (solid column).

3.3. Effects of 5-aminoisoquinolinone myocardial infarct size in the rat

The mean values for the area at risk were similar in all animal groups studied and ranged from 47 ± 3% to 56 ± 2% ($P > 0.05$, Fig. 3A). In rats, which received the vehicle for 5-aminoisoquinolinone (saline), occlusion of the left anterior descending coronary artery for 25 min followed by reperfusion for 2 h resulted in an infarct size of 50 ± 3% ($n = 12$) of the area at risk. When compared with

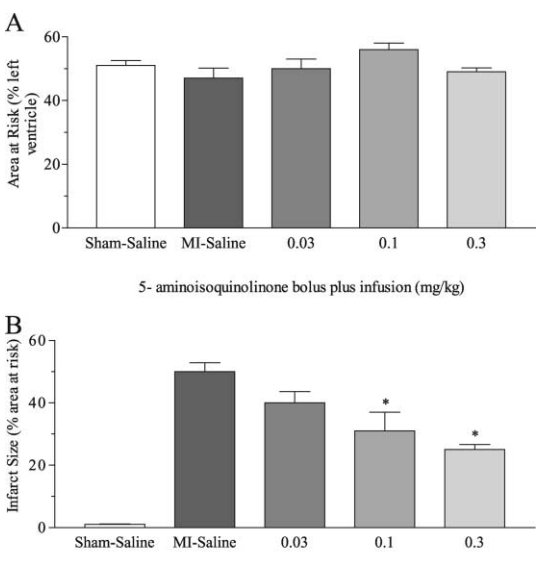

Fig. 3. (A) Area at risk and (B) infarct size in rats subjected to regional myocardial ischaemia (25 min) and reperfusion (2 h). Different groups of animals were subjected to the surgical procedure alone (no left anterior descending coronary artery occlusion, Sham, open column, $n = 7$) or subjected to left anterior descending coronary artery occlusion and reperfusion and treated with either vehicle (saline, control, darkest column, $n = 12$) or 5-aminoisoquinolinone (0.03 mg/kg bolus plus 0.03 mg/kg/h infusion, $n = 11$; 0.1 mg/kg bolus plus 0.1 mg/kg/h infusion, $n = 9$; 0.3 mg/kg bolus plus 0.3 mg/kg/h infusion, $n = 6$). * $P < 0.05$ when compared to control.

vehicle, administration of 5-aminoisoquinolinone (starting before and throughout the reperfusion period) resulted in a substantial, dose-related reduction in myocardial infarct size (Fig. 3B). Sham operation alone did not result in a significant degree of infarction in any of the animal groups studied ($< 2\%$ of the area at risk) (Fig. 3B).

3.4. Haemodynamic effects of 5-aminoisoquinolinone in rats subjected to myocardial ischaemia and reperfusion

Haemodynamic data, for example, mean arterial pressure (mm Hg), heart rate (bpm), and pressure rate index (mm Hg/min $\times 10^{-3}$) measured during the course of the experiments were similar in all groups studied (Table 1). In sham-operated rats (no left anterior descending coronary artery occlusion), administration of the highest dose of 5-aminoisoquinolinone used had no effect on any of the haemodynamic parameters measured (data not shown). In rats subjected to left anterior descending coronary artery occlusion and reperfusion that received either saline or 5-aminoisoquinolinone, mean values for mean arterial pressure and pressure rate index fell throughout the experiment (when compared to sham-operated animals, $P < 0.05$, Table 1). The mean values for mean arterial pressure or pressure rate index were, however, not significantly different between rats subjected to left anterior descending coronary artery occlusion and reperfusion, which were treated with either saline or 5-aminoisoquinolinone (Table 1).

4. Discussion

We have recently reported on a new, reliable and efficient synthesis of 5-aminoisoquinolin-1(2*H*)-one (McDonald et al., 2000). This method leads to a higher yield of 5-aminoisoquinolinone than that previously reported (Wenkert et al., 1964). Suto et al. have reported that 5-aminoisoquinolinone has an IC_{50} of 240 nM when evaluated in vitro in a cell-free system [isolated poly (adenosine 5′-diphosphate ribose) polymerase enzyme isolated from calf thymus] for inhibitory activity against poly (adenosine 5′-diphosphate ribose) polymerase, which is broadly comparable with other potent 5-substituted isoquinolinones (Suto et al., 1991; Watson et al., 1998). As the compound is a mimic of the nicotinamide moiety of the substrate NAD^+, it is conceivable that it may also inhibit other ADP-ribosyl transferases. We have previously examined the effect of 5-aminoisoquinolinone on the mono-ADP-ribosylating activity of diphtheria toxin and found that it had an IC_{50} of approximately 250 μM, indicating ca. 1000-fold selectivity for poly (adenosine 5′-diphosphate ribose) polymerase inhibition (McDonald et al., 2000).

Armed with this encouraging potency and enzyme selectivity in vitro and with the great advantage of very good water solubility of 5-aminoisoquinolinone in comparison with other isoquinolinones, we proceeded to investigate the effects of this compound on poly (adenosine 5′-diphosphate ribose) polymerase activity in rat cardiac myoblasts. Exposure of rat cardiac myoblasts to H_2O_2 (1 mM for 30

Table 1
Effects of 5-aminoisoquinolinone on the alterations in MAP, HR and PRI caused by occlusion (for 25 min) and reperfusion (2 h) of the LAD in the anaesthetised rat

Group	*n*		Occlusion (min)			Reperfusion (min)	
			Baseline	15	25	60	120
Sham–Saline	7	MAP	108 ± 11	102 ± 9	106 ± 12	93 ± 5[a]	83 ± 4[a]
		HR	430 ± 15	428 ± 17	428 ± 17	426 ± 10	424 ± 10
		PRI	46 ± 5	44 ± 4	45 ± 5	40 ± 3[a]	35 ± 2[a]
MI–Saline	12	MAP	101 ± 4	89 ± 5	85 ± 5	66 ± 5	52 ± 5
		HR	414 ± 10	420 ± 12	416 ± 12	406 ± 14	408 ± 13
		PRI	41 ± 2	37 ± 2	36 ± 3	27 ± 2	21 ± 2
5-Aminoisoquinolinone (0.03 mg/kg)	11	MAP	122 ± 7	109 ± 5	100 ± 7	79 ± 7	66 ± 4
		HR	440 ± 11	439 ± 9	437 ± 10	409 ± 10	423 ± 7
		PRI	54 ± 3	48 ± 3	44 ± 4	33 ± 3	28 ± 2
5-Aminoisoquinolinone (0.1 mg/kg)	9	MAP	102 ± 8	96 ± 8	96 ± 8	69 ± 5	63 ± 5
		HR	437 ± 10	436 ± 9	442 ± 9	429 ± 14	418 ± 18
		PRI	45 ± 4	42 ± 4	42 ± 4	30 ± 3	26 ± 2
5-Aminoisoquinolinone (0.3 mg/kg)	6	MAP	110 ± 8	105 ± 10	98 ± 10	67 ± 8	67 ± 9
		HR	450 ± 13	429 ± 23	439 ± 16	410 ± 13	418 ± 10
		PRI	49 ± 3	44 ± 3	43 ± 5	28 ± 4	28 ± 4

Different groups of animals were subjected to the surgical procedure alone (no left anterior descending coronary artery occlusion, Sham, $n = 7$) or subjected to left anterior descending coronary artery occlusion and reperfusion and treated with either vehicle (saline, control, $n = 12$) or 5-aminoisoquinolinone (0.03 mg/kg bolus plus 0.03 mg/kg/h infusion, $n = 11$; 0.1 mg/kg bolus plus 0.1 mg/kg/h infusion, $n = 9$; 0.3 mg/kg bolus plus 0.3 mg/kg/h infusion, $n = 6$).

[a] $P < 0.05$ when compared to control.

min) resulted in a twofold increase in poly (adenosine 5′-diphosphate ribose) polymerase activity. This increase in poly (adenosine 5′-diphosphate ribose) polymerase activity was attenuated by pretreatment of the cells with 5-aminoisoquinolinone in a concentration-dependent fashion (IC_{50}: ~4.5 μM). These findings demonstrate that 5-aminoisoquinolinone is a water-soluble and potent inhibitor of poly (adenosine 5′-diphosphate ribose) polymerase activity. When compared to 3-aminobenzamide, 1,5-dihydroxyisoquinoline [5-hydroxyisoquinolin-1(2*H*)-one] or nicotinamide, 5-aminoisoquinolinone is the most potent (water-soluble) inhibitor of poly (adenosine 5′-diphosphate ribose) polymerase activity which we have tested (Bowes et al., 1998a). The inhibition by 5-aminoisoquinolinone of the increase in poly (adenosine 5′-diphosphate ribose) polymerase activity in rat cardiac myoblasts was associated with a reduction in the impairment in mitochondrial respiration caused by H_2O_2 in these cells. Most notably, the EC_{50} of 5-aminoisoquinolinone for the prevention of the impairment in mitochondrial respiration caused by H_2O_2 was similar (EC_{50}: ~4.45 μM) to the IC_{50} for the inhibition of poly (adenosine 5′-diphosphate ribose) polymerase activity caused by 5-aminoisoquinolinone (IC_{50}: ~4.5 μM). This finding strongly suggests that the prevention by 5-aminoisoquinolinone of the cell death caused by H_2O_2 in rat cardiac myoblasts is due to the inhibition of poly (adenosine 5′-diphosphate ribose) polymerase activity in these cells.

In a separate study, we have then evaluated the effects of 5-aminoisoquinolinone on the infarct size caused by regional myocardial ischaemia (left anterior descending coronary artery occlusion for 25 min) and reperfusion (for 2 h) in the anaesthetised rat. We have previously reported (Zacharowski et al., 2000) that the model of coronary artery occlusion in the rat used here results in the classical histological signs of acute myocardial infarction: These included the occurrence of complete coagulation necrosis, cytoplasmic eosinophilia of cardiomyocytes, hyperaemia of blood vessels, extravasation of red blood cells and accumulation of polymorphic neutrophils in the border zone of the infarcted tissue (Zacharowski et al., 2000). We report here that 5-aminoisoquinolinone causes a substantial, dose-related reduction in myocardial infarct size (as determined by *p*-nitroblue tetrazolium staining). There is good evidence that less potent inhibitors of poly (adenosine 5′-diphosphate ribose) polymerase activity (including 3-aminobenzamide: 10 mg/kg, nicotinamide: 10 mg/kg and 1,5-dihydroxyisoquinoline: 3 mg/kg) reduce by ~30–50% the degree of tissue injury associated with regional myocardial ischaemia and reperfusion of the heart (Thiemermann et al., 1997; Zingarelli et al., 1997, 1998; Bowes et al., 1998b), the brain (Eliasson et al., 1997), the gut (Cuzzocrea et al., 1997) and the kidney (Chatterjee et al., 2000). Interestingly, a much larger reduction in cerebral infarct size (~80%) can be detected in mice in which the gene for poly (adenosine 5′-diphosphate ribose) polymerase has been disrupted by gene targeting [poly (adenosine 5′-diphosphate ribose) polymerase knock out or −/− mice] (Eliasson et al., 1997). Thus, it is possible that a much larger therapeutic benefit in conditions associated with ischaemia–reperfusion, can be obtained with more potent, water-soluble inhibitors of poly (adenosine 5′-diphosphate ribose) polymerase activity. The effects of 5-aminoisoquinolinone on the infarct size arising from ischaemia–reperfusion of the brain and kidney, therefore, warrant investigation.

One may argue that the reduction in cell death in both models of ischaemia reperfusion injury is due to the ability of 5-aminoisoquinolinone to scavenge reactive oxygen species. Moreover, the poly (adenosine 5′-diphosphate ribose) polymerase inhibitors 3-aminobenzamide and nicotinamide have been reported to possess radical-scavenging properties (Wilson et al., 1984; Althaus and Richter, 1987; Redegeld et al., 1992). However, there is good evidence that at concentrations below 10 mM, 3-aminobenzamide, nicotinamide and 1,5-dihydroxyisoquinolinone (which is an isoquinoline derivative and, hence, has a similar chemical structure to 5-aminoisoquinolinone) do not scavenge reactive oxygen species (Bowes et al., 1998a). This notion is supported by the finding that these agents are unable to attenuate the development of single-strand breaks in DNA, caused by reactive oxygen species in several cell types (Schraufstatter et al., 1986b).

3-Aminobenzamide is a weak inhibitor of poly (adenosine 5′-diphosphate ribose) polymerase activity that does not readily cross cell membranes (Rankin et al., 1989). Although 1,5-dihydroxyisoquinoline and DPQ are more potent inhibitors of poly (adenosine 5′-diphosphate ribose) polymerase activity than is 3-aminobenzamide, 1,5-dihydroxyisoquinoline and DPQ both have to be dissolved in dimethylsulfoxide. This may cause a substantial problem, as (i) dimethylsulfoxide itself is a potent scavenger of hydroxyl radicals (Ashwood-Smith, 1967), and (ii) dimethylsulfoxide itself inhibits poly (adenosine 5′-diphosphate ribose) polymerase activity (Banasik and Ueda, 1999). Thus, it is not surprising that dimethylsulfoxide itself reduces the organ injury in conditions associated with organ ischaemia and reperfusion (Wray et al., 1998; McDonald et al., 1999). Thus, 5-aminoisoquinolinone currently represents the best pharmacological tool to elucidate the role of poly (adenosine 5′-diphosphate ribose) polymerase in the pathophysiology of myocardial ischaemia–reperfusion injury and other diseases.

In conclusion, this study reports that 5-aminoisoquinolinone is a potent inhibitor of poly (adenosine 5′-diphosphate ribose) polymerase activity in rat cardiac myoblasts. We have also discovered that the administration (just before and during the reperfusion period) of 5-aminoisoquinolinone causes a substantial, dose-related reduction in myocardial infarct size as determined by macrochemical staining with *p*-nitroblue tetrazolium. Our results support

the hypothesis (Thiemermann et al., 1997) that inhibitors of poly (adenosine 5′-diphosphate ribose) polymerase activity may be useful in conditions associated with myocardial ischaemia and reperfusion.

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