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Effects of salicylic acid on post-ischaemic ventricular function and purine efflux in isolated mouse hearts

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Abstract

Acetyl salicylic acid (aspirin) is one of the most widely used drugs in the world. Various plasma concentrations of aspirin and its predominant metabolite, salicylic acid, are required for its antiarthritic (1.5–2.5 mM), anti-inflammatory (0.5–5.0 mM) or antiplatelet (0.18–0.36 mM) actions. A recent study demonstrated the inhibitory effects of both aspirin and salicylic acid on oxidative phosphorylation and ATP synthesis in isolated rat cardiac mitochondria in a dose-dependent manner (0–10 mM concentration range). In this context, the present study was conducted to determine the effects of salicylic acid on inosine efflux (a potential biomarker of acute cardiac ischaemia) as well as cardiac contractile function in the isolated mouse heart following 20 min of zero-flow global ischaemia. Inosine efflux was found at significantly higher concentrations in ischaemic hearts perfused with Krebs buffer fortified with 1.0 mM salicylic acid compared with those without salicylic acid (12575 ± 3319 vs. 1437 ± 348 ng ml^{-1} min^{-1}, mean $\pm$ SEM, $n = 6$ per group, $p < 0.01$). These results indicate that 1.0 mM salicylic acid potentiates 8.8-fold ATP nucleotide purine catabolism into its metabolites (e.g. inosine, hypoxanthine). Salicylic acid (0.1 or 1.0 mM) did not appreciably inhibit purine nucleoside phosphorylase (the enzyme converts inosine to hypoxanthine) suggesting the augmented inosine efflux was due to the salicylic acid effect on upstream elements of cellular respiration. Whereas post-ischaemic cardiac function was further depressed by 1.0 mM salicylic acid, perfusion with 0.1 mM salicylic acid led to a remarkable functional improvement despite moderately increased inosine efflux (2.7-fold). We conclude that inosine is a sensitive biomarker for detecting cardiac ischaemia and salicylic acid-induced effects on cellular respiration. However, the inosine efflux level appears to be a poor predictor of the individual post-ischaemic cardiac functional recovery in this *ex vivo* model.

Keywords: *Inosine, hypoxanthine, salicylic acid, acute cardiac ischaemia, contractile function, HPLC*

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Introduction

Aspirin (acetyl salicylic acid, ASA) is one of the most widely used drugs in the world and has been used for many years for its analgesic, antipyretic, anti-inflammatory and

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antiplatelet (blood thinning) properties (Evans et al. 1968, Schrör 1997, Vane & Botting 2003). ASA (160 or 325 mg dose) is routinely administered with other cardiac medications as part of initial emergency treatments to patients presenting with chest pain and potential acute myocardial infarction (MI) (Feldman & Cryer 1999, Abarbanell et al. 2001, Antman et al. 2004, Kosowsky 2006). Aspirin is known to inhibit platelet aggregation at the site of the cardiac thrombosis with a 325 mg dosage equating to ~0.36 mM blood concentration levels in an individual with ~70 kg body weight and ~5 litres total blood volume.

Aspirin's mechanism of action is well documented with interactions causing irreversible inhibition of both cyclooxygenase isoenzymes (COX-1, COX-2) (Smith & Dawkins 1971, Schrör 1997). The mechanism for ASA inhibiting platelet aggregation is through irreversible acetylation of the COX-1 enzyme which blocks synthesis of thromboxane A_2, a platelet aggregator and vasoconstrictor (Vane & Botting 2003). Other reported medical uses of ASA require higher blood concentration levels (e.g. a rheumatoid arthritis patient may require 1.5–2.5 mM ASA and an anti-inflammatory dose may require sodium salicylate levels of 0.5–5 mM) (Smith & Dawkins 1971, Nulton-Persson et al. 2004). In humans, ASA is rapidly metabolized (half-life ~3–4 h) and excreted via phase I metabolism (60% via deacetylation to salicylic acid (SA)) and phase II metabolism (~40% via conjugated products) (Rowland & Riegelman 1968).

In addition, a recent publication reported inhibitory effects of both ASA and SA on rat cardiac mitochondrial respiration (Nulton-Persson et al. 2004). These authors found that both ASA and SA could reduce NADH supply to the electron transport chain in isolated rat cardiac mitochondria, thus reducing ATP synthesis (via inhibition of oxidative phosphorylation). They also demonstrated a negative dose–response effect from both ASA and SA (0–10 mM concentration range) on cardiac mitochondrial respiration under non-ischaemic conditions. Other research was performed demonstrating the inhibitory effects of ASA and SA on xanthine oxidase, which would inhibit enzymatic conversion of hypoxanthine to xanthine and uric acid (Carlin et al. 1985, Masuoka & Kubo 2004). In this context, our present study was designed to examine further the effects of SA (0, 0.1 and 1.0 mM) on ATP catabolic by-products (e.g. inosine and hypoxanthine) along with an indirect evaluation of purine nucleoside phosphorylase (PNP) enzyme activity (indicated by inosine/hypoxanthine conversion ratio). We hypothesized that higher concentration levels of SA (e.g. 1.0 mM) coupled with periods of acute cardiac ischaemia may potentiate the ischaemic adverse effects on heart tissue via increased uncoupling of oxidative phosphorylation and subsequently enhanced efflux of ATP catabolic by-products such as inosine, which has been proposed in our recent publication (Farthing et al. 2006) as a potential biomarker of acute cardiac ischaemia.

Methods and materials

Chemicals, standards and Krebs buffer solution

All experimental chemicals were purchased and solutions prepared as per our recent published work (Farthing et al. 2006). Briefly, ACS grade or better purity hypoxanthine, xanthine, trifluoroacetic acid (TFA) and methanol (Optima) were purchased from Acros Organics (Fair Lawn, NJ, USA). Salicylic acid, adenosine, inosine, uric acid, sodium chloride, sodium bicarbonate, potassium chloride,

magnesium sulfate, monobasic potassium dihydrogen phosphate, dextrose, ethylenediaminetetraacidic acid (EDTA) and calcium chloride were all purchased from Sigma-Aldrich (St Louis, MO, USA).

Stock and working standards of adenosine, inosine, hypoxanthine, xanthine and uric acid (100 μg ml^{-1}) were prepared in deionized water and stored at 4°C as per work (Farthing et al. 2007). The working standards were maintained at −20°C along with the mouse Krebs buffer perfusate samples and demonstrated stability for at least 6 months. The Krebs buffer solution consisted of either 0, 0.1 or 1.0 mM SA and 118.5 mM NaCl, 25.0 mM $NaHCO_3$, 11.1 mM $C_6H_6O_6$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 0.5 mM EDTA and 2.5 mM $CaCl_2$ using in-house prepared deionized water (final pH 7.4 and continuously gassed with 95%O_2:5%CO_2 during the isolated heart experiments). For high performance liquid chromatography (HPLC) analysis, deionized water (18 MΩ) used was produced and filtered using the US Filter Purelab Ultra deionized water system (Lowell, MA, USA).

Langendorff isolated mouse heart preparation and experimental protocols

All animal experimental conditions were similar to our published work (Xi et al. 1998, Farthing et al. 2006). Adult male mice (ICR strain) were used for all cardiac ischaemia experiments with morphometric characteristics, baseline cardiac function, and ANOVA results presented in Table I. The protocol for groups II, III and IV consisted of 30 min of stabilization, 20 min of zero-flow global ischaemia and 30 min of reperfusion (Figure 1). Time-matched normoxic perfusion (for 80 min) was carried out for the control group and group I. Briefly, the mice were anaesthetized; hearts quickly isolated and cannulated onto a Langendorff apparatus within 3 min. Following the 30 min of stabilization period, global ischaemia was accomplished by stopping heart perfusion inflow for 20 min. Upon heart reperfusion, approximately 1.5 ml samples of Krebs buffered perfusate from the isolated mouse hearts were collected at predetermined time points (0, 1, 3, 5, 10 and 20 min) into plastic bullet centrifuge tubes and immediately frozen at −20°C. At the end of each experiment, the heart was removed from the Langendorff system and quickly weighed.

Our present study measured simultaneously both cardiovascular parameters (e.g. coronary flow rate, heart rate and cardiac developed force) and efflux of ATP catabolic by-products (e.g. inosine and hypoxanthine evaluated in Krebs perfusate samples) for each of the experimental groups. The control group consisted of six aerobically perfused hearts which were not subjected to ischaemia and used non-SA Krebs buffer

Table I. Morphometric characteristics and baseline cardiac function of the adult mice (ICR strain). Values are mean±SEM.

	Control ($n=6$)	Group I ($n=6$)	Group II ($n=6$)	Group III ($n=6$)	Group IV ($n=6$)
Body weight (g)	42.2±1.3	37.0±0.9	38.7±2.1	41.5±2.7	36.1±1.3
Heart wet weight (mg)	258±6	243±16	242±14	252±18	258±20
Heart rate (bpm)	368±23	340±26	345±23	368±37	373±17
Developed force (g)	0.81±0.19	1.06±0.27	1.12±0.12	1.09±0.23	0.77±0.16
Rate-force product ($g\times$bpm)	308±80	361±109	372±49	411±107	287±65
Coronary flow (ml min^{-1})	2.3±0.2	2.3±0.3	1.7±0.1	2.1±0.2	2.7±0.3

No significant difference between the groups were found for the listed parameters (one-way ANOVA).

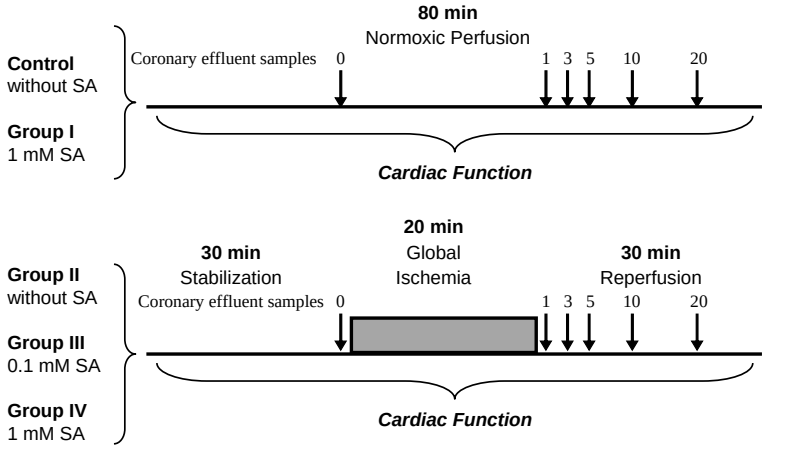

Figure 1. Protocol for animal experiments depicting experimental groups, salicylic acid (SA) level, ischaemic condition and coronary effluent sample time points.

(Farthing et al. 2006). Group I consisted of six hearts which were not subjected to global cardiac ischaemia; however, the Krebs buffer solution contained 1.0 mM SA to evaluate the effects of the highest tested concentration of SA on ATP catabolism and PNP activity. Group II consisted of six hearts that were subjected to global cardiac ischaemia with the Krebs buffer solution not containing SA to evaluate the effects of ischaemic conditions on ATP catabolic by-products. To determine if the effects of SA are dose-dependent in the hearts undergoing global cardiac ischaemia, group III consisted of six hearts which were subjected to Krebs buffer solution containing 0.1 mM SA and group IV consisted of six hearts subjected to 1.0 mM SA.

HPLC-UV conditions and mobile phase

All instrument conditions were performed as previously reported (Farthing et al. 2007). Briefly, the HPLC-UV method used a Phenomenex® Onyx™ monolithic C_{18} analytical column (20 cm $\times$4.6 mm I.D., 130 Å) and Onyx™ C_{18} guard column (5 cm $\times$4.6 mm I.D.) (Torrance, CA, USA). The mobile phase gradient consisted of aqueous trifluoroacetic acid (0.1% TFA in deionized water, pH 2.2, v/v) and methanol with time course (1–20% methanol linear gradient over 10 min). The mobile phase flow rate was 1.0 ml min^{-1} with operating pressure of $\sim$84 bar at ambient column temperature. A 15 µl direct injection of the Krebs buffer perfusate sample was made with optimal UV wavelength absorption of 250 nm used for inosine and hypoxanthine detection.

Data computation and statistics

The HPLC data acquisition and component computations were performed using TotalChrom™ Workstation software (Perkin Elmer™, Norwalk, CT, USA). Statistics utilizing ANOVA to compare the multigroup experimental results was performed using MS Excel (Microsoft®, Seattle, WA, USA) and post hoc analysis (Dunnett, Tukey) using JMP 6.0 (SAS Institute Inc., Cary, NC, USA) with $\alpha = 0.05$ and $p < 0.05$ demonstrating significance. Correlation evaluation was performed using

GraphPad Prism 4 (San Diego, CA, USA). For determining total effluxed inosine, the area under the curve (AUC) was calculated utilizing trapezoidal rule computations and MS Excel on HPLC results (0–20 min time point perfusate samples).

Results and discussion

Chromatography and method validation

Figure 2 shows representative chromatograms for: (A) low standard of 250 ng ml^{-1} hypoxanthine and inosine in deionized water; (B) standard of 2000 ng ml^{-1} each of hypoxanthine (RT ~5.2 min), uric acid (RT ~5.6 min), xanthine (RT ~6.7 min), adenosine (RT ~10.3 min) and inosine (RT ~10.5 min) in deionized water; (C) group I (no ischaemia + 1.0 mM SA) perfusate sample collected at 51 min of aerobic perfusion; (D) group II (ischaemia + 0 mM SA) perfusate sample collected at 1 min of reperfusion; (E) group III (ischaemia + 0.1 mM SA) perfusate sample collected at 1 min of reperfusion; and (F) group IV (ischaemia + 1.0 mM SA) perfusate sample collected at 1 min of reperfusion.

As demonstrated by the chromatograms, the HPLC method provided sufficient sensitivity (Figure 2A) and selectivity (Figure 2B) for each of the ATP catabolic

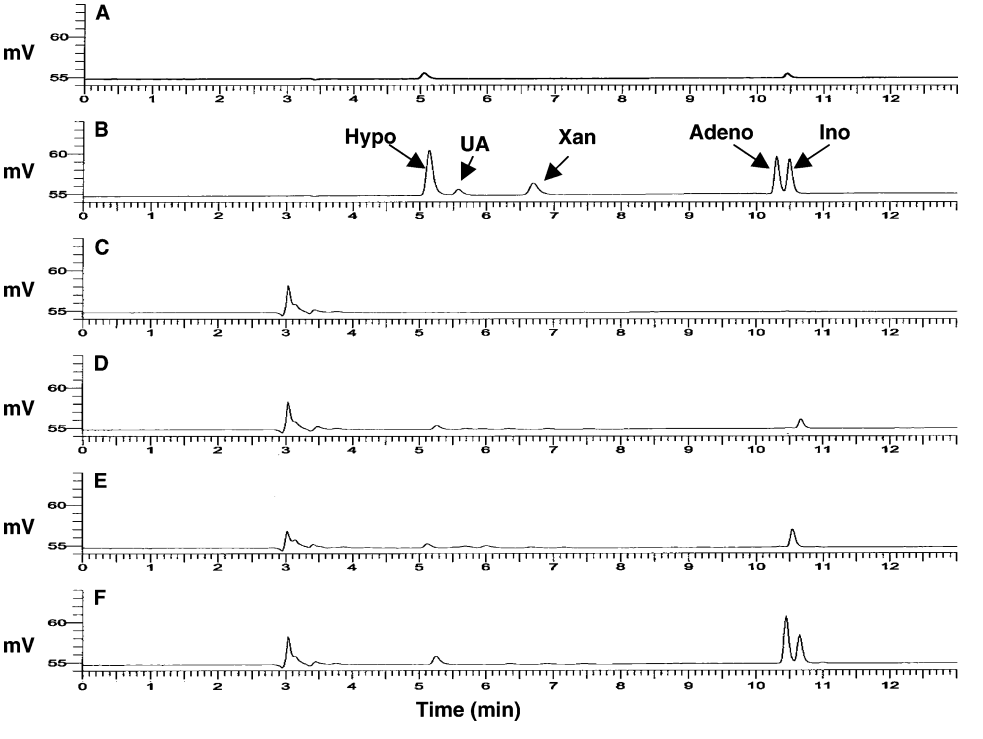

Figure 2. Chromatograms representing (A) low standard of 250 ng ml^{-1} hypoxanthine (Hypo, RT 5.2 min) and inosine (Ino, RT 10.6 min) in deionized water, (B) standard of 2000 ng ml^{-1} each of hypoxanthine, uric acid (UA, RT 5.6 min), xanthine (Xan, RT 6.7 min), adenosine (Adeno, RT 10.3 min) and inosine in deionized water, (C) group I mouse perfusate sample (no ischaemia and 1.0 mM SA), (D) group II perfusate sample (20 min global ischaemia and no SA), (E) group III mouse perfusate sample (20 min global ischaemia and 0.1 mM SA) and (F) group IV mouse perfusate sample (20 min global ischaemia and 1.0 mM SA).

by-products. Figure 2C–F demonstrates how changes in experimental conditions (ischaemia and SA concentration levels) resulted in increased concentration levels of inosine and hypoxanthine effluxed from the heart. Particularly Figure 2E and 2F demonstrates higher levels of ATP catabolic by-products suggesting that the presence of SA in the Krebs buffer exacerbates ATP uncoupling only in the ischaemic mouse hearts. It is also noteworthy that the heart subjected to ischaemia under 1.0 mM SA (group IV, Figure 2F) had elevated levels of ATP catabolic by-product adenosine, which is the nucleoside precursor to inosine and indicative of the largest total amount of ATP catabolic by-products effluxed due to the effect of 1.0 mM SA on the ischaemic heart. All chromatograms obtained from animal reperfusates using SA in the Krebs buffer solution lacked detectable levels of xanthine and uric acid components, thus supporting published research citing ASA and SA inhibitory effects on the xanthine oxidase enzyme (Carlin et al. 1985, Masuoka & Kubo 2004).

Method validation was performed as described in our previous publication (Farthing et al. 2007) and in summary, the method demonstrated excellent linearity of the calibration standards (0.25–5 μg ml^{-1}, $R > 0.9990$) with detection limits of 100 ng ml^{-1} for both inosine and hypoxanthine components. Method accuracy and precision for inosine and hypoxanthine was determined using quality control samples ($n = 15$) with acceptable combined intra-day and inter-day component accuracy ($\pm 6\%$ error) and precision ($\pm 8.1\%$ CV). To demonstrate component stability, re-injections of the animal perfusate samples were made after sitting on the HPLC autosampler overnight at laboratory ambient temperature and again after long-term storage. Both inosine and hypoxanthine in animal perfusate demonstrated excellent stability overnight on the HPLC autosampler and for more than 6 months when stored at $-20^{\circ}C$.

Effect of SA on purine efflux

Table II lists typical inosine and hypoxanthine levels for the mouse hearts representing each of the five experimental groups. As shown in Figure 3, the control group without both cardiac ischaemia and SA (Farthing et al. 2006) and group I (non-ischaemic hearts but using 1.0 mM SA, the highest tested SA concentration) did not have detectable amounts of ATP catabolic by-products. However, inosine levels for groups II, III and IV (all underwent global ischaemia) had significantly elevated total inosine efflux of 1437 ± 348, 3872 ± 900 and $12\,575 \pm 3319$ ng ml^{-1} min^{-1}, respectively (mean $\pm$ SEM, Figure 3), i.e. groups II ($p < 0.05$), III ($p < 0.05$) and IV ($p < 0.01$) as compared with the control group. These inosine results demonstrate that SA concentration levels increased ATP catabolism under our conditions of acute global cardiac ischaemia with groups III and IV mean total effluxed inosine levels potentiated above group II by approximately 2.7-fold and 8.8-fold, respectively.

It should be noted that we did not observe any ATP catabolic by-products from group I animals (1.0 mM SA and non-ischaemic). Based on published work (Cronstein et al. 1994, Nulton-Persson et al. 2004), we suspected that in the presence of 1.0 mM SA we might observe low concentration levels of ATP catabolic by-products even under non-ischaemic conditions. However, we did not observe either inosine or hypoxanthine in detectable levels from any perfusate samples from this experimental group. One possible explanation is that these authors used cardiac mitochondrial preparations for their studies and not the entire heart tissue as did our study. They reported 1.0 mM SA uncoupling oxidative phosphorylation by approximately 20%

Table II. Typical inosine and hypoxanthine concentration levels and ino/hypo ratios under various experimental conditions (control and global cardiac ischaemia) and concentration levels of salicylic acid in Krebs buffer (0, 0.1 and 1.0 mM).

Sample	Ischaemia	Reperfusion time (min)	Hypoxanthine (uM)	Inosine (uM)	Inosine/hypoxanthine ratio	Krebs salicylic acid level
Control	No	0	0	0	N/A	0 mM SA
		1	0	0	N/A	
		3	0	0	N/A	
		5	0	0	N/A	
		10	0	0	N/A	
		20	0	0	N/A	
Group I	No	0	0	0	N/A	1.0 mM SA
		1	0	0	N/A	
		3	0	0	N/A	
		5	0	0	N/A	
		10	0	0	N/A	
		20	0	0	N/A	
Group II	Yes	0	0	0	N/A	0 mM SA
		1	1.10	2.13	1.9	
		3	0.29	0.56	1.9	
		5	0	0.15	N/A	
		10	0	0.11	N/A	
		20	0	0	N/A	
Group III	Yes	0	0	0	N/A	0.1 mM SA
		1	1.32	3.80	2.9	
		3	0.44	1.04	2.4	
		5	0	0.78	N/A	
		10	0	0.52	N/A	
		20	0	0	N/A	
Group IV	Yes	0	0.44	0.19	0.4	1.0 mM SA
		1	3.01	18.68	6.2	
		3	1.25	2.09	1.7	
		5	0.74	1.12	1.5	
		10	0	0	N/A	
		20	0	0	N/A	

(using oxygen electrode and α-ketoglutarate dehydrogenase enzyme analysis) which may not produce high enough concentrations of ATP metabolites to activate the normally dormant adenosine deaminase and PNP enzymes, thus inosine and hypoxanthine would not be produced and detected. Using SA and ischaemic conditions together would combine the effects of SA (ATP decoupling) and ischaemia (blocks ATP synthesis) and lead to significant reduction in cellular ATP concentration and the potentiated effluxed levels of inosine observed in our present study.

Effect of SA on cardiac function

The bar charts in Figure 4 (mean $\pm$SEM) demonstrate the effects of SA on cardiac functional parameters (cardiac developed force (DF), heart rate (HR) and rate-force product (RFP)). No significant differences were found in HR for all experimental conditions. For DF and RFP, significance ($p < 0.05$) was demonstrated between groups III and IV relative to each other but neither group was statistically different

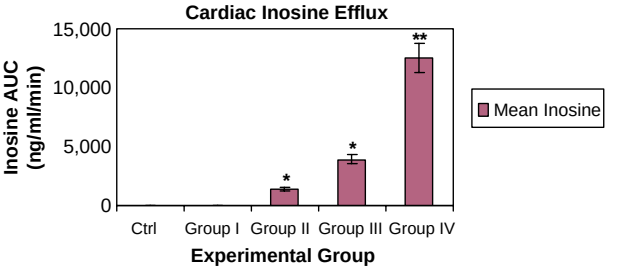

Figure 3. Bar chart representing total effluxed inosine (mean+SEM) for control (Ctrl), group I, group II, group III and group IV experimental conditions. Groups II, III and IV demonstrated statistical significance (*$p < 0.05$, **$p < 0.01$) from the control and group I. Ctrl (no ischaemia and no SA), group I (no ischaemia and 1.0 mM SA), group II (20 min global ischaemia and no SA), group III (20 min global ischaemia and 0.1 mM SA) and group IV (20 min global ischemia and 1.0 mM SA).

than the control. Cardiac DF and RFP were increased (beneficial) at 0.1 mM SA level but adversely affected at 1.0 mM SA level. Theses results demonstrate that under conditions of acute global cardiac ischaemia, higher levels of SA in the Krebs buffer exhibited an increasing relationship on effluxed inosine; however, a beneficial then adverse effect on DF and RFP was observed. These results have confirmed the previously reported positive inotropic effects of ASA on cardiac contractility through inhibiting COX enzymes and in turn prostaglandin synthesis (Karmazyn 1986) and through its modulating effects on cellular calcium levels (Molderings & Schümann 1987).

Non-linear relationship of inosine efflux and cardiac contractile function following ischaemia

Figure 5 is a correlation plot which demonstrates the lack of correlation between total effluxed inosine (in groups II, III and IV) and DF or RFP, with r^2 (coefficient of determination) values of 0.52 and 0.59, respectively. This non-linear relationship can be explained by an apparent beneficial effect of 0.1 mM SA on heart contractility, yet an adverse effect at 1.0 mM SA while total effluxed inosine increased with increasing

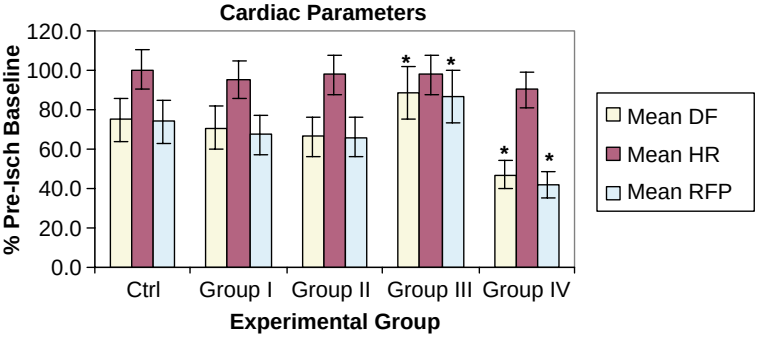

Figure 4. Bar chart representing mean±SEM for cardiac developed force (DF), heart rate (HR) and rate-force product (RFP) at the end of 30 min reperfusion for each of the experimental groups. DF and RFP on groups III and IV demonstrated statistical significance (*$p < 0.05$) between each other but neither demonstrated significance from control (Ctrl) and group I. Ctrl (no ischaemia and no SA), group I (no ischaemia and 1.0 mM SA), group II (20 min global ischaemia and no SA), group III (20 min global ischaemia and 0.1 mM SA) and group IV (20 min global ischaemia and 1.0 mM SA).

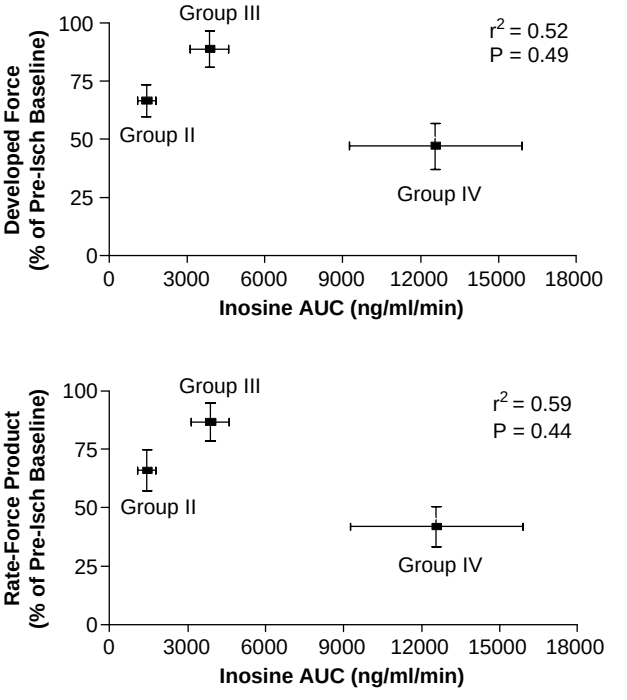

Figure 5. Correlation plot demonstrating lack of significant linear correlation between total effluxed inosine (AUC) and cardiac developed force (DF) or rate-force product (RFP) in the ischaemic mouse hearts under various SA concentrations (0, 0.1 and 1.0 mM). Group II (20 min global ischaemia and no SA), group III (20 min global ischaemia and 0.1 mM SA) and group IV (20 min global ischaemia and 1.0 mM SA).

SA concentration levels. Therefore whereas the inosine efflux level could serve as a sensitive biomarker for acute cardiac ischaemia, it appears to be a poor predictor of the individual post-ischaemic cardiac functional recovery at least in this *ex vivo* model.

Effect of SA on PNP activity

Figure 6 illustrates the ATP catabolic by-products resulting from acute cardiac ischaemic conditions. Normally dormant enzymes in heart tissue (e.g. adenosine deaminase, purine nucleoside phosphorylase) are activated due to cellular build-up of ATP catabolic by-products (e.g. ADP, AMP). Under our experimental conditions, inosine and hypoxanthine were the primary effluxed by-products of ATP catabolism. In observation of the larger amounts of total inosine effluxed in our 1.0 mM SA perfusate samples, we wanted to investigate if SA had a negative effect (inhibitory) on PNP enzyme activity which might account for the potentiated effluxed inosine levels.

The PNP enzyme activity under ischaemic conditions can be indirectly determined by calculating the conversion of inosine to hypoxanthine ratio on each perfusate sample. Table II lists the inosine (μM)/hypoxanthine (μM) (ino/hypo) conversion ratio for the experimental groups. In group II (ischaemia, 0 mM SA), the ino/hypo conversion ratio was constant (~1.9) and reproducible from all animals in this group. Under group IV experimental conditions (ischaemia, 1.0 mM SA), the ino/hypo conversion ratios were not constant with the perfusate sample collected at 1 min of

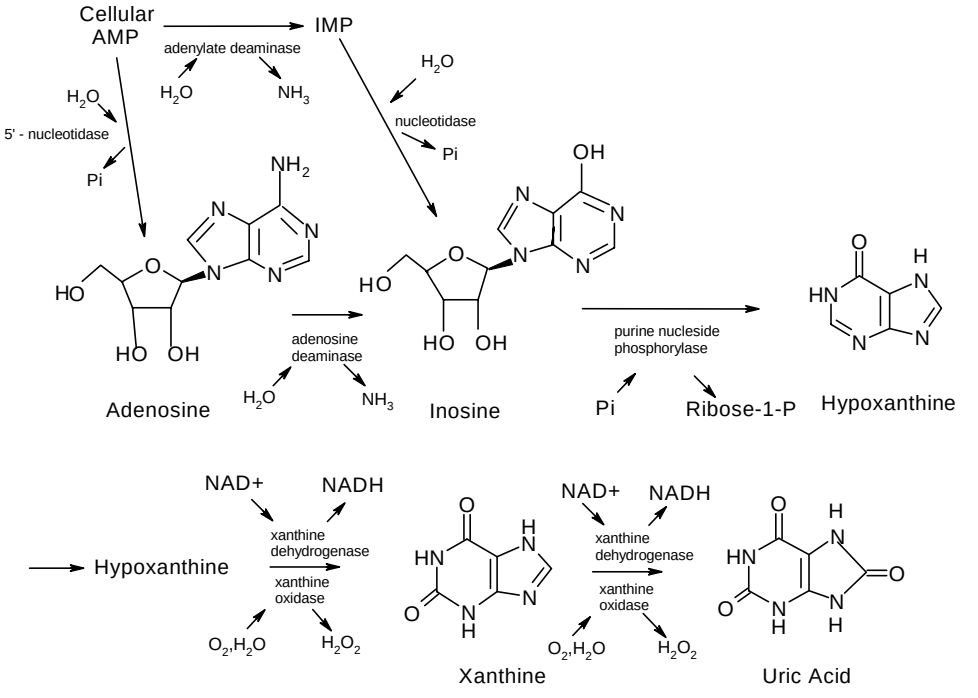

Figure 6. Biochemical pathway of ATP catabolic by-products resulting from global acute cardiac ischaemia.

reperfusion having the largest ino/hypo ratio, and at the subsequent time points returning to a more constant ino/hypo conversion ratio (~1.6) (Figure 7).

These results can be explained by two effects occurring simultaneously when using both SA and ischaemic conditions. Cardiac ischaemic (anaerobic) conditions can cause ATP catabolism as demonstrated in our previous work (Farthing et al. 2006), while SA has been reported to cause decoupling of cardiac mitochondrial respiration (Nulton-Persson et al. 2004). Both situations may contribute to the increase in ATP catabolic by-products as heart muscle contractions require significant amounts of

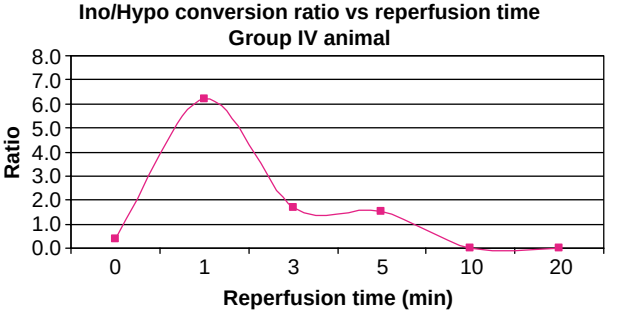

Figure 7. Plot of inosine (μM) to hypoxanthine (μM) (ino/hypo) conversion ratio versus reperfusion time (min). The plot represents data from one animal heart preparation in group group IV to 1 mM SA and ischaemic experimental conditions. Ino/hypo conversion ratio is highest in the 1 min reperfusion sample and returns to a constant ratio before dropping to zero as aerobic conditions presumably deactivate ADA and PNP enzymes in the cardiac myocytes.

ATP as its primary energy source. This leads to cellular build-ups of ADP and AMP metabolic by-products as ATP is not able to be regenerated (via salvage pathway) or synthesized, thus ATP catabolism occurs to eliminate the cellular build-up of by-products. As aerobic conditions to the heart tissue was re-established via reperfusion, the ino/hypo conversion ratio returns to a more constant conversion ratio (e.g. $\sim$1.6). The conversion ratio drops to zero as aerobic conditions should deactivate ADA and PNP enzymes in the cardiac myocytes, thus inhibiting ATP catabolism (Figure 7).

Potential clinical relevance

Since both ASA and SA have been reported to uncouple mitochondrial respiration under aerobic conditions and in turn to inhibit ATP synthesis (Nulton-Persson et al. 2004) and our current study demonstrates a potentiated ATP catabolism by SA under cardiac ischaemia, it is possible that current use of ASA for medical emergency treatment in acute cardiac ischaemic situations (e.g. acute MI) may potentially increase the ischaemia-caused ATP catabolism and inosine efflux. While standardized doses of ASA (160–325 mg) are used to inhibit platelet aggregation at the site of thrombus as part of the treatment for acute MI, the higher 325 mg dose equates to $\sim$0.36 mM ASA blood concentration levels. Even with its significant protein binding (e.g. albumin), the augmented free drug levels of ASA and its metabolite SA may have an inhibitory effect on cardiac ATP production during the periods of acute cardiac ischemia. The SA-induced enhancement of inosine efflux should also be taken into consideration when we analyze and interpret the patient's plasma inosine level as a potential biomarker for acute cardiac ischaemia (Farthing et al. 2006, 2007).

Whereas there are certainly positive benefits associated with using ASA as part of the treatment for acute MI patients to inhibit platelet aggregation at the site of thrombus, higher doses of ASA used for other medical conditions including analgesia (0.5 mM plasma salicylate) and rheumatoid arthritis (1.5–2.5 mM plasma salicylate) may actually aggravate the ischaemic effects on heart tissue metabolism and ventricular contractile function, if the patients who have been using higher dose of ASA suddenly encounter acute cardiac ischaemic events. This animal research on isolated mouse hearts utilized SA levels of 0.1 and 1.0 mM, which were slightly lower but similar to expected blood concentration levels in human patients utilizing aspirin as treatment for the above described medical conditions (e.g. acute MI, rheumatoid arthritis). We suggest that further laboratory and clinical studies are warranted on the apparent adverse effects of higher concentration levels of ASA and SA on ATP catabolism under acute cardiac ischaemia, given the knowledge of how widespread ASA is used for its other medical benefit (e.g. analgesia, rheumatoid arthritis) and the possibility of those patients may one day experience acute cardiac ischaemic events.

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