# Sanguinarine Non- Versus Re-Circulation During Isolated Heart Perfusion - A Jekyll and Hyde Effect?

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## Abstract

*Aims* In isolated rat heart perfusion experiments, drug administration occurs via retrograde perfusion. This can be done in the non-recirculating mode (coronary effluent is discarded), or recirculating mode (coronary effluent is collected and reused). It was recently observed in our lab while using sanguinarine, an MKP-1 inhibitor, that there were differences in outcomes depending on the mode of recirculation used.

*Methods and Results* Hearts from control (C); diet-induced obese (DIO) Wistar rats and their age matched controls (AMC) were perfused on the rig. Hearts received buffer (control), insulin, sanguinarine, insulin + sanguinarine combination or methanol (vehicle) for 15mins pre- and 10mins post-ischemia in either a non- or re-circulating manner. Hearts were subjected to 15mins global ischemia and 30mins reperfusion. Mechanical function was documented pre- and post-ischemia When not-recirculated, sanguinarine alone and in combination with insulin in C, DIO and AMC groups, caused a significant decrease in functional recovery during reperfusion. However, when the coronary effluent was recirculated, hearts perfused with sanguinarine or sanguinarine + insulin exhibited a significant recovery in function when compared with their non-recirculation counterparts ($p<0.01$). No differences were seen with either control, insulin nor vehicle hearts.

*Conclusion* Sanguinarine elicited a vast improvement in perfusion outcomes when recirculated compared to non-recirculation. Since this was seen during perfusion only

when sanguinarine was present, it is possible that recirculating reperfusion of the drug caused profound changes in its composition. More investigation is needed into the mechanisms involved. Thus caution should be exercised by researchers when designing a perfusion protocol for drug research.

## Introduction

The Langendorff perfusion technique has been used extensively for decades in cardiovascular research, especially when there is a need to administer drugs to the heart during perfusion [1]. This can be done in either a recirculating or non-recirculating mode [2, 3]. Because of the large volumes of perfusion fluid required during an experimental protocol, and the high cost of some drugs that are used, working protocols often operate in the recirculating mode [4], however this is not always the case [5].

Sanguinarine is an alkaloid derived from the roots of Sanguinaria canadendid and has been found to activate AMP-activated protein kinase (AMPK) [6] and inactivate mitogen-activated protein kinase phosphatase-1 (MKP-1) [7]. In our laboratory sanguinarine is used as an MKP-1 inhibitor, in a study to elucidate the involvement of MKP-1 in insulin stimulated cardioprotection. It is known that insulin offers protection to the heart during ischaemia/reperfusion and increases post ischaemic recovery [8]. However, the role of MKP-1 in this pathway is unknown under normal circumstances as well as in obesity and insulin resistance.

In our study, sanguinarine was initially administered in the non-recirculating mode; however, due to the high costs involved, the perfusion was switched to the recirculating mode. To our surprise, we observed that, when the drug was administered in the recirculating mode, it yielded different results

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from when it was given in the non-recirculating mode. This prompted us to pursue this phenomenon to distinguish whether the effect was seen with all the drug interventions we used or only with sanguinarine.

Our aim was thus to establish whether the perfusion mode (recirculating or non-recirculating) had an effect on functional recovery after ischaemia. Hearts from young control rats, dietary induced obese rats and their age matched controls were perfused in the presence of sanguinarine, insulin and/or the vehicle (methanol).

## Methods

### Animals

Male Wistar rats were used. Rats weighing between 200-220 g served as controls. A separate group of rats was divided into two subgroups and either fed a high caloric diet for 16 weeks (dietary induced obese, DIO) or used as the age matched controls (AMC) which received standard rat chow. The project was approved by the Committee of Experimental Animal Research of the Faculty of Health Sciences, Stellenbosch University and complied with the guidelines of the revised South African National Standard for the Care and Use of Animals for Scientific Purposes (SANS 10386, 2008 – P08/ 05/005) and the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. The rats were allowed free access to food and water, and maintained in Stellenbosch University's Central Research Facility at 22 °C with a 12 h day/night cycle.

### Isolated Heart Perfusion Methodology

Animals were injected with an overdose of Euthenase (sodium pentobarbitone, 0.12 mg per gram body weight). Once animals were anaesthetized and completely unresponsive as confirmed by foot pinching, hearts were excised, arrested in cold (4°C) Krebs-Henseleit buffer (KHB, 119 mM NaCl, 25 mM NaHCO$_3$, 4.75 mM KCl, 1.185 mM KH$_2$PO$_4$, 0.6 mM MgSO$_4$, 0.6 mM NaSO$_4$,1.25 mM CaCl$_2$.2H$_2$O, 10 mM glucose) and mounted on a working rat heart perfusion system. The isolated hearts were perfused with KHB equilibrated with 95%O$_2$/5%CO$_2$ at 37 °C. After 10mins stabilisation (retrograde perfusion), the heart was switched to perfusion in working mode for 20mins and mechanical function documented [(coronary flow (CF), aortic output (AO), cardiac output (CO = CF + AO), heart rate (HR), aortic systolic pressure (SP) and aortic diastolic pressure (DP)]. Total work (TW) was calculated as a function of the cardiac output and systolic pressure (TW = COxSPx0.0022). The hearts were then perfused retrogradely for 15mins either with KHB (Control), or with the following interventions:

Insulin (1mIU/ml), sanguinarine (2 μM), Insulin (1mIU/ml) + sanguinarine (2 μM) (S + I), Methanol (vehicle control for sanguinarine) (0.01 %).

Global ischaemia was then induced by stopping perfusion of the retrogradely perfused heart for 15 min. The temperature was maintained at 36.6 °C. Hearts were reperfused and treated with KHB (control) or the abovementioned interventions for a further 10mins in retrograde mode, and then in working heart mode for a further 20mins and mechanical function again documented. The percentage recovery was then calculated by expressing the post ischaemic total work as a percentage of the pre- ischaemic total work.

### Statistical Analyses

Data was analysed using Microsoft GraphPad Prism. The one way analysis of variance ANOVA was used for comparison between all groups. All values are expressed as mean ± standard error of the mean (SEM). N values were between 6 and 8 for all groups. A p-value smaller than 0.05 was considered significant.

## Results

### Control Hearts

When the total work (TW) recovery of the 200–220 g control rats was analysed it was seen that non–recirculation versus recirculation of control (61.9±6.7 vs 48.1±4.9 %), insulin (46.6±5.4 vs 53.6±5.2 %); methanol (vehicle) (63.9±7.02

**Table 1** Table to show all values for total work recovery in control (200 g), DIO and AMC rats

|  |  | Non recirculating | Recirculating |
|---|---|---|---|
| CONTROL (200 g) n=6-8 | Control | 48.1±4.9 | 61.9±6 |
|  | Insulin | 53.6±5.2 | 46.6±5.4 |
|  | Methanol | 46.1±4.8 | 63.9±7.02 |
|  | Sanguinarine | 18.5±5.9 | 46.1±3.1 * |
|  | S + I | 38±8.7 | 37.02±9.3 |
| DIO n=6-8 | Control | 40±9.8 | 50.5±21.9 |
|  | Insulin | 73.2±10.8 | 95.5±4.5 |
|  | Methanol | 86±4.02 | 61.1±17.3 |
|  | Sanguinarine | 8.5±6.4 | 67.1±4.6 * |
|  | S + I | 18.3±7.7 | 47.1±7 * |
| AMC n=6–8 | Control | 56.01±6.7 | 35.8±30.7 |
|  | Insulin | 83.6±3.9 | 87.8±2.3 |
|  | Methanol | 84.1±10.1 | 78.8±12.6 |
|  | Sanguinarine | 13.1±13.1 | 84.2±5.6 * |
|  | S + I | 7.2±3.9 | 69.1±8.7 * |

* = p<0.05 in comparison to non-recirculated values.

vs 46.1±4.8 %) and S+I (38.3±8.7 vs 37.02±9.3 %) hearts showed no difference in total work recovery. However, when sanguinarine recirculation was compared to non–recirculation the total work recovery was significantly improved, with non–recirculation values (18.5±5.9 %) being significantly lower than recirculated functional recovery (46.1±3.1 %) ($p < 0.01$) (Table 1).

## DIO and AMC Hearts

In both DIO and AMC hearts, neither control, insulin nor methanol differed in TW recovery between non–recirculated and recirculated modes of perfusion. DIO (non recirculated vs recirculated): control: 40±9.8 vs 50.5±21.9 %; insulin: 73.2± 10.8 % vs 95.5±4.5 %; methanol: 86.4±5.02 vs 61.1±17.3 % and AMC: control: 56.01±6.7 vs 35.8±30.7 %; insulin: 83.6 ±3.9 vs 87.8±2.3 %; methanol: 84.1±10.1 vs 78.8±12.6 %. However both sanguinarine alone and in combination with insulin had increased TW recovery in the recirculated as compared to the non–recirculated perfusion modes. DIO (non–recirculated vs recirculated): sanguinarine: 8.5±6.4 vs 67.1±4.6 %; S+I: 18.3±7.7 vs 47.1±7 % and AMC sanguinarine: 13.1±13.1 vs 84.2±5.6 %; S+I: 7.2±3.9 vs 69.1±8.7 % ($p < 0.01$) (Table 1).

## Discussion

The data show that in the case of sanguinarine the mode of reperfusion has a profound effect on functional recovery during reperfusion; with recirculation showing improved recovery compared to non–recirculation. This was not evident in any of the other interventions used. While there were differences between interventions e.g. insulin improved functional recovery in the older animals, the focal point of this article is to show how variances in outcomes can occur with the same drug with different modes of delivery.

It has been shown that coronary effluent from hearts which have undergone prior ischaemic preconditioning contained cytoprotective factors that, when transferred to other hearts, offers protection [9]. However to our knowledge our study is novel in that the hearts were not preconditioned, the coronary effluent was used on the same hearts by which it was produced and sanguinarine was the only drug used which caused this effect. This could be due to the fact that sanguinarine is rapidly metabolised by the heart into an innocuous compound during recirculation, and only when the heart has a constant supply of the drug (as occurs during perfusion in the non recirculation mode) is it able to effect changes. It has been demonstrated that rats metabolise sanguinarine into non toxic dihydrosanguinarine in vivo [10]. The results obtained in our in vitro study, suggest that the isolated heart can metabolize this cytotoxic compound. While a small increase in coronary flow was observed with

sanguinarine administration (results not included), this occurred in both recirculation and non–recirculation modes, meaning that an increase in circulation is not the reason for the phenomenon seen.

The value of these observations lies in the fact that the perfusion mode can affect the outcome of a study and should be taken into account by the researcher when planning a protocol. In this study the recirculation showed improved recovery, however the opposite could be true for other drugs.

In conclusion, researchers, pharmaceutical companies, drug developers and clinicians should be wary of this possibility when planning pre–clinical laboratory research and extrapolating the results towards clinical trials.

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