I.V. infusion of a drag-reducing polymer extracted from aloe vera prolonged survival time in a rat model of acute myocardial ischaemia†

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Background. I.V. infusion of drag-reducing polymers (DRPs) has been shown to improve survival time in animals subjected to hemorrhagic shock. We hypothesized that DRPs might prolong survival time in rats following acute myocardial ischaemia (AMI).

Methods. Sixteen adult male rats were anaesthetized and mechanically ventilated. An i.v. infusion of either Dextran-40 2.5% (Control, n=8) or Dextran-40 2.5% containing 50 μg ml⁻¹ of an aloe vera-based DRP (DRP, n=8) was initiated at 3.5 ml h⁻¹. The left anterior descending coronary artery was ligated. Blood pressure, skin-tissue perfusion, and heart rate were monitored and arterial blood samples were analysed.

Results. The mortality at 60 min following coronary ligation was 0% in the DRP group vs 50% in the control group (P=0.025). DRP-treated animals maintained higher mean arterial pressure [60.9 (5.1) vs 47.5 (5.1) mm Hg, P=0.004] and tissue perfusion [4.2 (3.4) vs 1.2 (0.5) TPU, P=0.029]. The DRP group trended towards better acid–base status with base excess [5.0 (1.7) vs 8.1 (5.1) mmol litre⁻¹, P=0.083] and pH [7.42 (0.07) vs 7.35 (0.02), P=0.03].

Conclusions. Administration of nanomolar concentrations of aloe vera-based DRP prolonged survival time in animals with AMI. DRPs may offer a novel method to treat organ/tissue hypoperfusion.

Br J Anaesth 2007; 98: 23–8

Keywords: drag-reducing polymer, aloe vera; heart, cardiac ischaemia; microcirculation; rat

Accepted for publication: August 14, 2006

Blood-soluble drag-reducing polymers (DRPs) added to blood at nanomolar concentrations have been shown to enhance tissue perfusion in various animal models.† These polymers have a large molecular size of over 10⁶ Daltons (Da) with a relatively linear structure. They possess the so-called drag-reducing capacity. The addition of very small amounts of these polymers to a fluid leads to a reduction in resistance of turbulent flow in pipes, thereby increasing flow rate at a constant pressure or by reducing driving pressure at a constant flow. This drag-reducing phenomenon was described by B. A. Toms (Toms effect) in 1948. The mechanisms behind the Toms effect are still not completely understood. In a mammalian circulatory system, the Toms effect most likely does not occur, because the flow in the blood vessels is not turbulent excluding the aorta and main branches in large animals and humans. Nevertheless, a number of animal studies have demonstrated that the i.v. injection of DRPs increased blood flow rate and decreased calculated peripheral vascular resistance without direct effects on blood viscosity or vascular smooth muscle tone. Recent animal studies have shown that the i.v. infusion

of DRPs prevented hypoxaemic damage in hepatocytes\(^1\) and improved the survival of the animals with severe haemorrhagic shock.\(^1\)

In this study, we hypothesized that the addition of DRPs into the systemic circulation would improve the homeostasis of an animal with cardiogenic shock thus prolonging the survival time of the animal. To test this hypothesis, the efficacy of a recently discovered DRP from aloe vera\(^1\) was investigated in a rat model of acute myocardial ischaemia (AMI).

**Methods**

**Preparation of DRP extracted from aloe vera**

Aloe DRP solutions were prepared as described previously.\(^1\) Briefly, mucilage was obtained from fresh leaves of aloe vera plant. Purification of the aloe DRP was performed using standard methods of precipitation with ethanol 100% and dissolving the precipitate in normal saline solution with gentamicin 0.25 mg ml\(^{-1}\) (Gentamicin sulfate, Elkins-Sinn, Inc., Cherry Hill, NJ, USA). Then, the preparation was dialysed against saline containing gentamicin for 48 h using a dialysis membrane with a 50,000 Da molecular weight cut-off (Spectra/Por membrane, Spectrum Laboratories, Rancho Dominguez, CA, USA). Size-exclusion chromatography (GPC–Triple Detector, Viscotek, Houston, TX, USA) and fluid dynamic and rheological characterizations were used to assure the polymer’s physicochemical properties providing its drag-reducing ability. The average molecular size of the DRP slightly varied from lot to lot and was \(~4–6\times10^5\) Da. Before each animal experiment, the polymer was dissolved in sterile saline with Dextran-40 2.5% (Gentran 40, Baxter Healthcare Corp., Deerfield, IL, USA) at a concentration of 50 \(\mu\)g ml\(^{-1}\). The control solution was saline with Dextran-40 2.5%.

**Acute coronary artery ligation model and infusion study protocol**

This pilot animal study was performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Sixteen male Sprague–Dawley rats [392 (53) g; Harlan Sprague–Dawley, Indianapolis, IN, USA] were anaesthetized with an i.m. injection of ketamine (70 mg kg\(^{-1}\), xylazine (8 mg kg\(^{-1}\)) and succinylcholine (0.4 mg kg\(^{-1}\)) were given. The heart was then exposed through a median sternotomy. A 7-0 polypropylene suture (Prolene, Ethicon Inc., Surmerville, NJ, USA) was then passed beneath the proximal left anterior coronary artery (LAD).

Fifteen minutes after sternotomy, the LAD was ligated proximally with a polypropylene suture manually tied. Throughout the procedure, all haemodynamic parameters were continuously monitored and automatically recorded. When the diastolic pressure of the right carotid artery decreased below 25 mm Hg during the experiment, Dextran-40 2.5% (0.5–1.0 ml) (without DRP) was given as a bolus through the tail vein cannula as volume therapy. The rats were considered to have died when the mean arterial pressure (MAP) decreased to less than 10 mm Hg despite volume therapy. For the surviving rats, a second arterial blood sample (0.2 ml) was obtained from the right carotid artery 60 min after ligation of the LAD.

To justify the reproducibility of the severe myocardial ischaemia in the rat model used in our study, preliminary experiments were performed to evaluate the ischaemic area of the heart that would result from LAD occlusion. Using six adult rats with the same anaesthetic preparation, proximal LAD ligation was performed as described. The rat was euthanized with an i.v. bolus injection of saturated potassium chloride. The heart was immediately explanted and a 14 G cannula was inserted into the ascending aorta for retrograde injection into the coronary arteries. After a flush injection of 1 ml of normal saline through the cannula, 1 ml of brilliant blue dye was injected manually in the same fashion. After the injection of the dye, the great vessels, the free wall of the right ventricle, and both atria were removed and discarded. The left ventricle along with the interventricular septum was then sharply dissected into the stained part with brilliant blue dye and the non-stained part,
which were weighed. The ischaemic area of this coronary artery ligation model was 39.6 (5.6)% wet weight of the left ventricle along with the interventricular septum. This confirmed that the model of AMI applied in our study was severe and well reproducible.

Data presentation and statistical analysis
Data were presented as mean (sd) and analysed using an unpaired Student’s t-test which compared DRP and control groups in haemodynamic parameters (heart rate, MAP and tissue perfusion). A paired Student’s t-test was used for intra-group comparison of arterial blood sample measurements [pH, $P_{\text{aO}}$, $P_{\text{aCO}}$, actual base excess (ABE), and Hct], while an unpaired Student’s t-test was used for inter-group comparisons. Comparison of the survival curves was performed with Log rank test. $P<0.05$ was considered statistically significant.

Results

Survival of the animals (Fig. 1)
Mortality within 60 min of myocardial ischaemia was 0% in DRP vs 50% in Control ($P=0.025$). Four of eight rats in the control group died within 60 min as a result of cardiogenic shock [the average time to death of the four animals was 36 (15) min].

Haemodynamics
Baseline haemodynamics did not show any statistically significant difference between DRP and Control groups. Heart rate was significantly lower in DRP group compared with that in Control at 30, 40 and 50 min after the coronary ligation (Fig. 2A). At 60 min following coronary arterial ligation, the DRP group maintained a higher MAP [60.9 (5.1) vs 47.5 (5.1) mm Hg, $P = 0.004$] (Fig. 2n) and skin-tissue perfusion [4.2 (3.4) vs 1.2 (0.5) TPU, $P = 0.029$] (Fig. 2c).

For the surviving animals, the total infusion volume at 60 min after the coronary ligation was 5.50 (0.35) ml (DRP=8) vs 6.68 (0.79) ml (Control=4) ($P=0.002$). This included volumes of the Dextran-40 2.5% solution of 0 ml (DRP=8) vs 1.39 (0.40) ml (Control=4) given as a bolus to maintain diastolic pressure of the right carotid artery above 25 mm Hg. The total amount of aloe polymer given to the DRP group was 76.9 (10.6) μg per 100 g of body weight ($n=8$).

Arterial blood sample analysis
There was a statistically higher pH and a lower $P_{\text{aCO}}$ in the DRP group. However, other arterial blood gas indices did not show any significant difference between the two groups (each group=8) (Table 1).

Among the survivors at 60 min, the DRP group showed a trend towards better acid–base status in the arterial blood [ABE $-5.0$ (1.7) vs $-8.1$ (5.1) mmol litre$^{-1}$, $P=0.083$; pH 7.42 (0.07) vs 7.35 (0.02), $P=0.034$] (Table 2).

Discussion
In this study, we demonstrated that i.v. infusion of a DRP solution preserved MAP, tissue perfusion, and resulted in better acid–base status after AMI by ligation of the LAD coronary artery in the rat model. All rats in the DRP infusion group survived, while half of the control group died from congestive heart failure within the 60 min study period. In this experiment, the infusion of either DRP solution or vehicle in the control group was initiated 40 min prior to acute coronary ischaemia. This prophylactic loading with DRP ameliorated the derangement of haemodynamic parameters caused by AMI.

This study demonstrated the effectiveness of the new DRP, which was derived from aloe vera. The drag-reducing capacity and beneficial haemodynamic effects of the aloe vera-based polymer have been recently confirmed as comparable with high molecular weight (MW=3500 kDa) polyethylene glycol (PEG-3500).1 It has been demonstrated that this aloe vera-based DRP extended the survival time of rats subjected to lethal haemorrhage to the same degree as PEG-3500.1 In those experiments, the effect of DRPs (both aloe-based and PEG-3500) was compared with that of the vehicle (saline with dextran-40 2.5%) and the PEG-200 (polyethylene glycol with MW 200 kDa, also mixed with dextran-40 2.5%). PEG-200 does not possess drag-reducing properties. These control solutions, without drag-reducing capacity, failed to improve survival time in haemorrhaged animals.1 As aloe-based DRP and PEG-3500 are chemically different with the only similar property being the ability to reduce flow resistance, those results supported the
hypothesis that the primary mechanism responsible for the haemodynamic effects caused by DRPs is physical in nature. In the study presented here, a Dextran-40 2.5% (non-drag reducing polymer) solution was used in the control group, therefore, the beneficial effect in the DRP group over the control group was attributed to the aloe-based DRP which was added to the infusion solution at a minute concentration.

In this study, the i.v. infusion of DRP was initiated before the coronary artery ligation. The intention was to investigate the prophylactic effect of the DRP loading on AMI. Interestingly, MAP increased after the initiation of DRP infusion and became significantly higher than that of the control group immediately before coronary ligation. Skin-tissue perfusion also started to increase in the DRP
group prior to the coronary ligation, although the difference did not reach statistical significance. This reaction of the cardiovascular system to the DRP infusion might have played an important role in animal survival. It was previously shown that i.v. injection of nanomolar concentrations of DRPs significantly increased the number of functioning capillaries and the velocity of RBC flow in capillaries in normal and diabetic rats. One can hypothesize that DRPs significantly increased the number of functioning capillaries, and velocity of their flow in microvessels. However, the exact mechanism behind this difference is not yet understood. Initial arterial blood gas analysis showed a slightly higher pH and a lower $P_{aCO_2}$ in the DRP group with statistical significance. These were unexpected findings, as the experiment was performed with a single ventilation setting and the randomization was conducted where the surgeon was blinded to the infusion solution. It is very unlikely that this difference in pH and $P_{aCO_2}$ contributed to the difference in the survival time of the two groups, as both groups were in the same state of respiratory alkalosis.

The exact mechanism of action of DRPs in vivo is still unclear. Although several hypotheses have been proposed including the reduction of pressure loss in the arterial trees, facilitation of the relocation of red blood cells closer to the capillary wall, and increase of plasma mixing in capillaries, the mechanism remains to be studied. Several limitations of the current preliminary study are noteworthy. First, all measurements beyond 30 min after the LAD ligation were difficult to compare between two groups because control animals began to die at this time-point. The haemodynamic measurements in the surviving animals beyond 30 min should be considered as supplemental evidence of the effect of DRP. Second, the breadth of haemodynamic measurements during the experiment were limited. Additional parameters, such as cardiac output, systemic vascular resistance, or preload independent left ventricular contractility, could provide important mechanistic information. Third, infant size at the completion of the experiment was not measured because of the extended period of hypotension prior to the early mortality in the control group. It was a concern that this significant hypotensive period before death in the control group might artificially increase the area of infarct in the myocardium. It is anticipated that in future experiments a sub-lethal model will be used to compare the area of myocardial infarction at the end of the experiment. Fourth, the length of the observation period was limited in 60 min post coronary ligation, therefore, the question remains whether the DRP simply extends survival time beyond 60 min or is truly effective at increasing survival per se.

To date, DRPs have not been used in the clinical setting. Their side-effects, immunogenicity, toxicity, pharmacodynamics and pharmacokinetics remain to be studied. Animal studies, including the current one, suggest the potential benefits of DRP applications in a variety of clinical situations, especially where prevention of hypoxaemia and improvement of tissue perfusion are desired. For example, prevention or treatment of ischaemic vascular disease as this study indicated, resuscitation of trauma victims or haemorrhagic shock patients and maintenance of tissue perfusion of organ/tissue grafts in transplantation or in plastic surgery.

In summary, this study demonstrated that i.v. administration of a DRP improved haemodynamics, thereby prolonging survival time in a rat model of AMI. This study further confirms that DRPs hold promise as a novel therapeutic modality for the treatment and prevention of organ/tissue hypoperfusion, and warrant further investigation.
Acknowledgement
This study was supported in part by grants to M.V.K. from the William G. McGowan Charitable Fund and the Jewish Healthcare Foundation. The authors would also like to thank Dr Arkady Uraysh for his technical assistance.

References