Addition of dextran sulfate to blood cardioplegia attenuates reperfusion injury in a porcine model of cardiopulmonary bypass[☆]

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Abstract

Objective: Contact of blood with artificial surfaces and air as well as ischemia/reperfusion injury to the heart and lungs mediate systemic and local inflammation during cardiopulmonary bypass (CPB). Activation of complement and coagulation cascades leads to and accompanies endothelial cell damage. Therefore, endothelial-targeted cytoprotection with the complement inhibitor and endothelial protectant dextran sulfate (DXS, MW 5000) may attenuate CBP-associated myocardial and pulmonary injury. **Methods:** Eighteen pigs (DXS, $n = 10$; phosphate buffered saline [PBS], $n = 8$) underwent standard cardiopulmonary bypass. After aortic cross-clamping, cardiac arrest was initiated with modified Buckberg blood cardioplegia (BCP), repeated after 30 and 60 min with BCP containing either DXS (300 mg/10 ml, equivalent to 5 mg/kg) or 10 ml of PBS. Following 30 min reperfusion, pigs were weaned from CPB. During 2 h of observation, cardiac function was monitored by echocardiography and invasive pressure measurements. Inflammatory and coagulation markers were assessed regularly. Animals were then sacrificed and heart and lungs analyzed. **Results:** DXS significantly reduced CK-MB levels (43.4 ± 14.8 ng/ml PBS, 35.9 ± 11.1 ng/ml DXS, $p = 0.042$) and significantly diminished cytokine release: TNFalpha (1507.6 ± 269.2 pg/ml PBS, 222.1 ± 125.6 pg/ml DXS, $p = 0.0071$), IL1beta (1081.8 ± 203.0 pg/ml PBS, 110.7 ± 79.4 pg/ml DXS, $p = 0.0071$), IL-6 (173.0 ± 91.5 pg/ml PBS, 40.8 ± 19.4 pg/ml DXS, $p = 0.002$) and IL-8 (304.6 ± 81.3 pg/ml PBS, 25.4 ± 14.2 pg/ml DXS, $p = 0.0071$). Tissue endothelin-1 levels were significantly reduced (6.29 ± 1.90 pg/100 mg PBS, 3.55 ± 1.15 pg/100 mg DXS $p = 0.030$) as well as thrombin—anti-thrombin formation (20.7 ± 1.0 μg/ml PBS, 12.8 ± 4.1 μg/ml DXS, $p = 0.043$). Also DXS reduced cardiac and pulmonary complement deposition, neutrophil infiltration, hemorrhage and pulmonary edema (measured as lung water content, $81 \pm 3\%$ vs $78 \pm 3\%$, $p = 0.047$), indicative of attenuated myocardial and pulmonary CPB-injury. Diastolic left ventricular function (measured as dp/dt_{min}), pulmonary artery pressure (21 ± 3 mmHg PBS, 19 ± 3 mmHg DXS, $p = 0.002$) and right ventricular pressure (21 ± 1 mmHg PBS, 19 ± 3 mmHg DXS $p = 0.021$) were significantly improved with the use of DXS. **Conclusions:** Addition of DXS to the BCP solution ameliorates post-CPB injury and to a certain extent improves cardiopulmonary function. Endothelial protection in addition to myocyte protection may improve post-CPB outcome and recovery.

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Keywords: Ischemia; Reperfusion; Complement inhibition; Cytoprotection; Endothelium

1. Introduction

In addition to surgical trauma, the use of cardiopulmonary bypass (CPB) itself is associated with an acute pro-inflammatory response [1] mainly caused by contact activation of blood with artificial surfaces and air, shear stress as well as ischemia/reperfusion (IR) injury. Clinically, myocardial damage ranging from stunning to necrosis with low output syndrome and atrial fibrillation may occur, leading to multiorgan failure in severe cases.

Activation of the coagulation and complement system, leukocytes, endothelial cells and other pro-inflammatory mediators, contribute to organ damage [2]. Complement inhibition [3] and technical modifications such as ultrafiltration devices to remove excess water and pro-inflammatory mediators [4], may improve clinical outcome to a certain extent. However, the problem of CPB-associated inflammation and in particular IR-injury is not entirely prevented by their use. Furthermore, cardiac arrest induced with cardioplegic solution for the purpose of myocardial preservation during CPB does not specifically protect the endothelium. IR-

induced endothelial cell activation with shedding of its natural anticoagulant and anti-inflammatory surface glyco-calyx layer [5] may, in part, contribute to CPB-associated tissue damage. Restoration of an anti-inflammatory and anti-coagulant environment through functional replacement of the shed heparan sulfate proteoglycan (HSPG) may therefore attenuate cardio-pulmonary dysfunction. To this purpose, low molecular weight dextran sulfate (DXS, MW 5000), a sulfated glycosaminoglycan analog and potent inhibitor of complement [6,7] and coagulation [8] that has been shown to bind to HSPG-denudated endothelium [7] and to ameliorate acute myocardial IR-injury in vivo [9], was tested in a porcine model of CPB. We hypothesized that DXS would preserve cardiovascular and pulmonary function by complement inhibition and endothelial cell protection in CPB mediated inflammation and IR-injury.

2. Materials and methods

Care and use of animals in the present study were in compliance with the European Convention on Animal Care, the respective Swiss national guidelines, and approved by the Animal Care Committee of the Canton of Berne, Switzerland.

2.1. Animals and anesthesia

Eighteen large white pigs (60 ± 3 kg) were premedicated with intramuscular ketamine (20 mg/kg) and xylazine (2 mg/kg), followed by intravenous administration of midazolam (0.5 mg/kg) and atropine (0.05 mg/kg), and were intubated and mechanically ventilated with a volume-controlled ventilator (Servo 900, Siemens AG, Solna, Sweden) with 5 cm H_2O end-expiratory pressure. Anesthesia was maintained with continuous intravenous infusion of pentobarbital (8 mg/kg/h), fentanyl (0.03 mg/kg/h), and pancuronium chloride (1 mg/kg/h) for muscle relaxation.

Arterial, central venous and Swan-Ganz catheters were introduced into the internal and external jugular veins and carotid artery respectively. The urinary bladder was catheterized through a midline incision, and urinary output measured regularly. Rectal and central venous blood temperatures (thermistor in pulmonary artery catheter) were measured continuously. A heating pad was used to maintain the body temperature between 37.5–38.5 °C, except during CPB-induced hypothermia.

2.2. Surgical procedure

A full midline sternotomy and pericardiotomy was performed. Methylprednisolone (Solu-Medrol, Pfizer, Canada, 1 g) was administered intravenously and the pigs were heparinized with 400 IU/kg of heparin. Activated clotting time (ACT) was controlled regularly using high-range ACT cartridges (Medtronic, Minneapolis, MN) and an automatized ACT analyzer (Medtronic HemoTec, Parker, CO). If necessary additional heparin was administered to keep ACT above 250 s. A 5-F high-fidelity pressure-sensitive tip transducer (Millar Instruments; Houston, TX) was introduced through the base of the brachiocephalic trunk into the left ventricle. Data were recorded with the

PowerLab system (Chart 5.2, PowerLab, ADInstruments Castle Hill, Australia).

2.3. Cardiopulmonary bypass (CPB)

A minimal extracorporeal circulation (MECC) system comprising a membrane oxygenator (Quadrox, Jostra Inc., Hirrlingen, Germany) and a centrifugal pump (Jostra Inc.) with a priming volume of 600–800 ml was used. Standard aortic and bicaval cannulation was performed. A 7-F catheter was inserted across the right atrium into the coronary sinus for blood sampling throughout the experiment. Extracorporeal circulation was performed with moderate systemic hypothermia (32 °C) and a non-pulsatile mean flow rate of 3.3 (2.6–3.6) l/min/m^2. Ringer solution or hydroxyethyl starch was used for volume substitution. Antegrade cold modified Buckberg blood cardioplegia (BCP, using *Cardiople-gin* as the crystalloid cardioplegic solution) was administered after aortic cross-clamping, initiating a period of 60 min of cardiac arrest. Cold BCP (8 °C) was repeated after 30 min, and warm BCP (35 °C) 30 min thereafter. To the two repeat-cardioplegia solutions a total of either 10 ml phosphate buffered saline (PBS, *n* = 8) or 10 ml of low molecular dextran sulfate in PBS (DXS, MW 5000, Fluka Chemie, Buchs, Switzerland, 300 mg, equivalent to a total of 5 mg/kg body weight, *n* = 10) were added. The total volume of cardioplegia solution used was weight-adapted and similar in both groups (1420 ± 520 ml and 1330 ± 330 ml). After aortic declamping a reperfusion phase of 30 min was initiated followed by weaning off CPB and a post-CPB observation phase of 120 min. The animals were then sacrificed (intravenous bolus of potassium chloride) and the heart and lungs excised for further analysis.

2.4. Experimental groups

All experimenters were blinded with regard to treatment regimen. Randomization of the animals into the two groups was done prior to the experimental work, using a randomization code (DXS = 0, PBS = 1), created by a random number generator (SAS, version 9.1.2, SAS Institute Inc., Cary, NC, USA). The samples (DXS solution or PBS) were prepared according to the randomization output and stored at −20 °C until use. Randomization and sample preparation was performed by an independent laboratory technician. Prior to premedication of the animals, the corresponding vial was allocated to the pig (sequential number of vial = sequential number of the pig). All 18 consecutively enlisted pigs were treated according to this protocol. No animal was initially excluded. Two experiments (DXS = 1, PBS = 1) were terminated 1 h prematurely due to hemodynamic deterioration after sudden, intractable arrhythmia and electromechanical dissociation, respectively.

2.5. Hemodynamic monitoring and management

Heart rate, carotid and pulmonary arterial blood and central venous pressures were recorded continuously. Right ventricular and left atrial pressures were measured invasively. Left ventricular pressure as well as dp/dt_{max} and dp/dt_{min} were recorded with a 5-F high-fidelity pressure-

sensitive tip transducer (Millar Instruments). Unless recorded continuously, all functional data were measured at following time points: baseline, 30 min after cessation of CPB and every 30 min thereafter. Mean arterial pressure was kept at a minimum of 50 mmHg throughout the procedure, by adjusting fluid balance (with hydroxyethyl starch) and noradrenalin, 5 μg boli, followed by continuous intravenous infusion if pressure could not be maintained.

2.6. Echocardiography

Epicardial echocardiography was obtained using a transesophageal (TEE) probe (ACUSON ultrasound system, Siemens, Malvern, PA, USA) placed retrocardially to obtain following views according to the standard TEE views: two and four chamber views, transgastric midpapillary short axis view and transgastric two chamber view. Left ventricular ejection fraction was measured using the biplane method (or modified Simpson method). Measurements were obtained at baseline and simultaneously with other hemodynamic measurements.

2.7. Complement and coagulation

Blood samples were collected (EDTA plasma and serum) at baseline, after going on CPB, after 30 and 60 min on CPB, after 30 min weaning, and every 30 min thereafter. Samples were kept on ice until centrifugation and stored at −80 °C until further analysis.

Classical pathway complement activity (venous samples) was determined by standard CH50 assay [10].

Activated partial thromboplastin time (aPTT, venous samples) was measured using Dade Actin FS reagent in a standard coagulation assay.

Thrombin—anti-thrombin III (TAT) complexes (coronary sinus) were measured by micro-enzyme immunoassay (Enzygnost Micro, Behringwerke AG, Marburg, Germany).

2.8. Ischemic and inflammatory markers

Circulating troponin I and creatine kinase (CK-MB fraction) were determined by enzyme immunoassays (AxSYM microparticle enzyme immunoassay platform, Abbott laboratories, Abbott Park, IL, USA) in coronary sinus blood samples. Both assays were tested for cross-reactivity to porcine troponin I and CK-MB. The effect of DXS in concentrations <1 mg/ml (in the fluid phase) was tested and found to be negligible in the multiplex suspension array setting described below in detail (data not shown).

TNFalpha, IL-1beta, IL-6 and Il-8 were measured by sandwich immunoassay using the Luminex fluorescent bead technology. Matching antibody pairs ('DuoSet') specific for porcine antigens were purchased from R&D Systems, Minneapolis, MN, USA, and included anti-TNFalpha, anti-IL-1beta, anti-IL-6 and anti-IL-8. The capture antibodies were coupled to carboxylated beads using the Bio-Plex Amine Coupling Kit (Bio-Rad Laboratories, Hercules, CA). The biotinylated counterparts were used as the detection antibodies. Analysis was done with the Bio-Plex system (Bio-Rad Laboratories). Data analysis was done with Bio-Plex Manager version 4.0 software (Bio-Rad Laboratories) with five-parametric curve fitting.

2.9. Endothelin-1 (ET-1)

Plasma and tissue ET-1 levels (coronary sinus samples) were determined by specific radioimmunoassay after solid phase extraction on C18 reverse phase columns as previously described by Shaw et al. [11].

2.10. Histology and immunostaining

Representative samples from the heart and lungs were fixed in 4% buffered formaldehyde, paraffin-embedded, and 3 μm sections stained with hematoxylin-eosin. Neutrophil tissue numbers were determined by counting neutrophils in 10 randomly selected high power viewing fields from various samples of each experiment. Five μm sections were cut from all snap frozen tissue samples, air-dried, acetone fixed, hydrated and labeled using a two/three-step indirect immunofluorescence technique. The following antibodies were used: rabbit anti-human C1q, C3b/c and C4b/c (Dako); goat anti-human C6 (Quidel) cross-reactivity with the respective porcine antigens was verified. Secondary antibodies were goat anti-rabbit IgG(H + L)-FITC (Southern Biotechnology Associated), rabbit anti-mouse Ig-FITC (Dako) and biotinylated goat anti-rat IgG (Southern Biotechnology) followed by streptavidin-FITC (Amersham Biosciences). Samples from all experiments were stained and graded for complement deposition: 0: no staining, 1: minimal focal or diffuse staining, 2: moderately strong staining, 3: extensive staining.

2.11. Tissue water content

Water content of the lungs was determined through desiccation of the lungs at 100 °C for 48 h. Tissue water content was calculated using the formula [(wet weight − dry weight)/wet weight] × 100.

2.12. Statistics

Non-parametric tests were used for data analyses because of the relatively low number of data points rendering assumption for Gaussian distribution of the data rather speculative despite the fact that common tests did not reject the hypothesis of normal distribution. Differences between the two groups were compared by Mann—Whitney U-test. For parameters measured over time, baseline values were subtracted from the end-point measurements, to account for random differences between the two groups at baseline. All analyses were two-sided and differences were considered statistically significant with a p value of <0.05. Analyses involved baseline and end point measurements only, as significant between-group differences (in biochemical markers) were expected at these time points. Repeated, multiple testing was not performed to avoid corresponding problems with type I errors.

According to Little's test, missing values (max 15%) in the datasets were all missing completely at random (MCAR). The method of the last observation carried forward (LOCF) was used for imputation of missing data.

SAS Version 9.1.2 (SAS Institute Inc., Cary, NC, USA) and SPSS Version 12.0.1 (SPSS Inc., Chicago, IL, USA) software
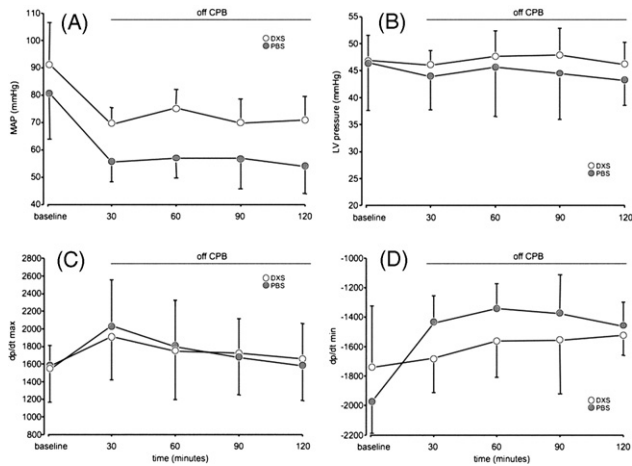
Fig. 1. Mean arterial pressure (MAP, A) and left ventricular (LV, B). Non-significant trend towards improved MAP and LV pressure in the DXS-treated animals during the post-CPB period. Monitoring of systolic (dp/dt_{max}, C) and diastolic function (dp/dt_{min}, D), recorded by Millar catheter. Data are mean $\pm$ standard deviation.
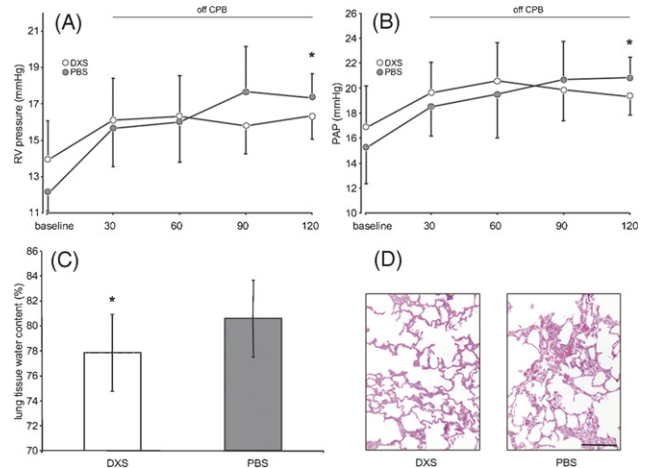

Fig. 2. Right ventricular (RV) pressure (A) and pulmonary artery pressure (PAP, B). Significant elevation at the end of the experiment in the PBS group as compared to the DXS group ($^{*}p = 0.021$ and $^{*}p = 0.002$, respectively). Significantly lower water content in the lungs (C, $^{*}p = 0.047$) correlates with decreased pulmonary damage (hemorrhage, neutrophil infiltration, edema) in the DXS-treated animals (D). Data are mean $\pm$ standard deviation. Scale bar represents 100 μm.

were used for all analyses. Data (corrected for hematocrit, where appropriate) are presented as mean $\pm$ standard deviation.

3. Results

3.1. Hemodynamics

Mean arterial pressure (MAP) at baseline was comparable in both groups. At 30 min post-CPB, MAP was significantly lower as compared to baseline in both groups. At the end of the observation period, differences between both groups were not significant (71 $\pm$ 9 mmHg for DXS vs 54 $\pm$ 10 mmHg for PBS; $p = 0.368$, Fig. 1A). The total amount of noradrenalin (246 $\pm$ 295 μg for DXS, 311 $\pm$ 255 μg for PBS) or infused hydroxyethyl starch (725 $\pm$ 572 ml for DXS, 1035 $\pm$ 893 ml for PBS) needed to maintain the minimal target MAP of 50 mmHg until the end of the experiment did not differ significantly between the two groups ($p = 0.261$ and $p = 0.318$, respectively). Following CPB and administration of hydroxyethyl starch, hematocrit was significantly reduced from average baseline values of 28.8 $\pm$ 1.8% to end point values of 21.6 $\pm$ 2.2% ($p < 0.005$). Baseline as well as end point hematocrit values did not differ significantly between the two groups ($p = 0.423$ and $p = 0.189$, respectively).

Mean left ventricular (LV) (Fig. 1B) as well as left atrial (LA) pressure did not change significantly throughout the post-CPB phase and were not significantly different from baseline values in both groups (46 $\pm$ 4 mmHg for DXS vs 43 $\pm$ 5 mmHg for PBS; $p = 0.669$).

Systolic function, measured as dp/dt_{max} was slightly, though not significantly, increased in both groups 30 min off CPB and remained relatively stable throughout the remaining post-CPB period (Fig. 1C, $p = 0.989$).

Diastolic function, measured as dp/dt_{min}, was impaired in PBS controls during the post-CPB period as compared to baseline ($p = 0.057$ baseline vs end of experiment) and did

not fully recover until the end of the experiment, whereas diastolic function remained relatively unchanged in the DXS group (Fig. 1D, $p = 0.782$).

RV (Fig. 2A) and pulmonary artery pressures (Fig. 2B) post-CPB were increased in both groups as compared to baseline, and were significantly higher in the PBS controls as compared to the DXS group at the end of the experiment ($p = 0.021$ and $p = 0.002$, respectively).

Episodes of atrial fibrillation occurred significantly less frequently in the DXS as compared to the PBS group (total episodes of arrhythmia: 9 times in the DXS group vs 18 times in the PBS group, $p = 0.006$, not shown).

3.2. Echocardiography

LV ejection fraction remained stable and within normal values during the whole procedure. No significant between-group differences were noted at the end of the observation period (68 $\pm$ 11 mmHg for DXS vs 70 $\pm$ 4 mmHg for PBS; $p = 0.341$; $p = 0.341$, results not shown). LV regional wall motion was preserved and similar in both groups.

3.3. Histology

Myocardial samples from both groups very focally revealed changes consistent with IR damage including focal wavy fibers and contraction bands, and were accompanied by neutrophil infiltration (not shown). The severity of the changes was more pronounced in samples from the PBS group.

Samples of lung tissue revealed comparable non-specific atelectatic changes in the lower lobes in both groups. Neutrophil accumulation was observed in edematous septa as well as in lung alveoli, particularly at sites of focal hemorrhage. The extent of infiltration and edema was more pronounced in PBS controls as compared to DXS treated animals (Fig. 2C).
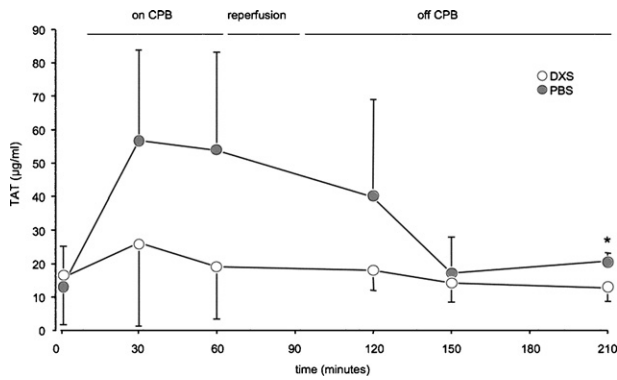
Fig. 3. Thrombin anti-thrombin (TAT) in plasma samples. Rapid increase in TAT production during CPB in the PBS group, with slow recovery post-CPB. Significantly less TAT in the DXS group at the end of the observation period ($^*p$ = 0.043). Data are mean $\pm$ standard deviation.

3.4. Lung water content

Water content of the lungs was significantly higher in the PBS controls as compared to the DXS treated animals (81 $\pm$ 3% vs 78 $\pm$ 3%, p = 0.047) (Fig. 2C). This finding correlated with increased histological signs of tissue edema in samples from the PBS experiments (Fig. 2D).

3.5. Soluble coagulation and complement parameters

Baseline aPTT was comparable in both groups (DXS 32.8 $\pm$ 4.4 s vs PBS 39.4 $\pm$ 16.5 s; p = 0.317). Values remained above 300 s in both groups following heparin administration (not shown).

Thrombin anti-thrombin (TAT) levels markedly increased in the PBS group during CPB and values were slow to recover, whereas levels remained largely unchanged throughout in the DXS group, with significantly less TAT complexes than in the PBS group at the end of the observation period (12.8 $\pm$ 4.1 μg/ml for DXS vs 20.7 $\pm$ 1.0 μg/ml for PBS; p = 0.043, Fig. 3).

CH50 values (assessment of classical complement pathway inhibition), decreased comparably in both groups and were lower at the end of the experiment as compared to baseline values. Values were comparable between both groups (55 $\pm$ 33% for DXS vs 68 $\pm$ 24% for PBS; p = 0.648, results not shown).

3.6. Markers of ischemia

Plasma troponin I values increased in both groups throughout the experiment, but differences were not statistically significant at the end of the observation period (48.6 $\pm$ 21.0 μg/ml for DXS vs 56.8 $\pm$ 18.70 μg/ml for PBS; p = 0.175, Fig. 4A). CK-MB values continuously increased in both groups, with significantly increased values in the PBS as compared to the DXS group at the end of the experiment (peak levels 35.9 $\pm$ 11.1 ng/ml for DXS vs 43.4 $\pm$ 14.8 ng/ml for PBS; p = 0.042, Fig. 4B).

3.7. Markers of inflammation

Plasma TNFalpha, IL-1beta, IL-6 as well as IL-8 levels increased in both groups during the course of the experiment,
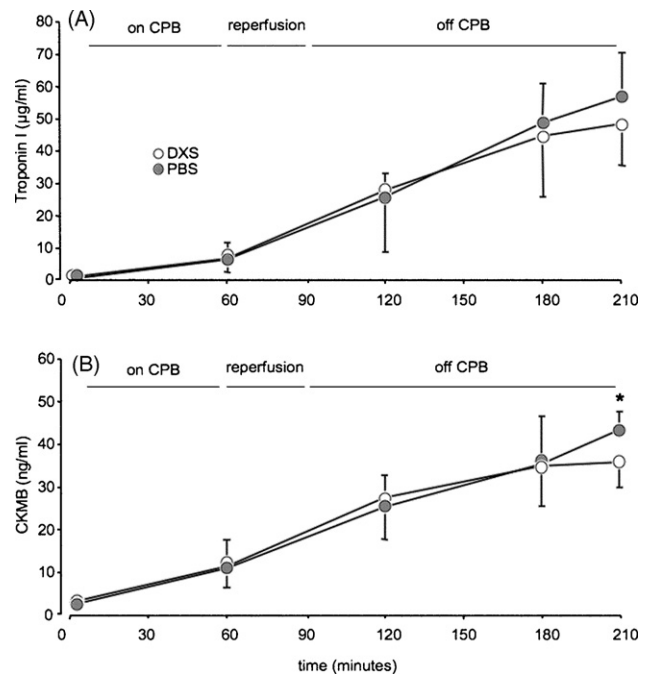

Fig. 4. Troponin I (A) and creatine kinase (CK-MB, B) levels in coronary sinus plasma samples. No significant differences at the end experiment between the groups for troponin I levels. Significantly reduced levels of CK-MB in the DXS group at the end of the observation period ($^*p$ = 0.042) Data are shown as mean $\pm$ standard deviation.
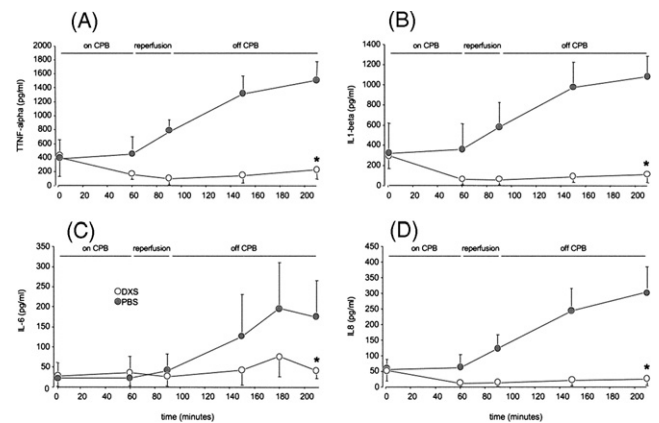

Fig. 5. TNFalpha, interleukin (IL-) 1beta, IL-6 and IL-8 levels in plasma samples. Significantly elevated levels of all cytokines at the end of the observation period as compared to PBS controls (TNFalpha: $^*p$ = 0.0071, IL-1beta: $^*p$ = 0.0071, IL-6: $^*p$ = 0.002, IL-8: $^*p$ = 0.0071). Data are mean $\pm$ standard deviation.

and were significantly increased in the PBS controls as compared to the DXS group at the end of the reperfusion period (TNFalpha: 222.1 $\pm$ 125.6 pg/ml for DXS vs 1507.6 $\pm$ 269.2 pg/ml for PBS, p = 0.0071; IL-1beta: 110.7 $\pm$ 79.4 pg/ml for DXS vs 1081.8 $\pm$ 203.0 pg/ml for PBS, p = 0.0071; IL-6: 40.8 $\pm$ 19.4 pg/ml for DXS vs 173.0 $\pm$ 91.5 pg/ml for PBS; p = 0.002, IL-8: 25.4 $\pm$ 14.2 pg/ml for DXS vs 304.6 $\pm$ 81.3 pg/ml for PBS, p = 0.0071; Fig. 5A—D).
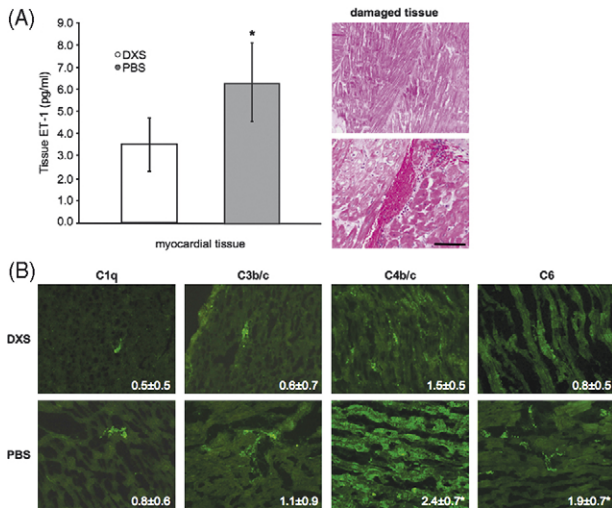
Fig. 6. Significantly more endothelin-1 (ET-1) in myocardium in the PBS group as compared to the DXS group ($^*p = 0.030$). Data are mean $\pm$ standard deviation. Immunofluorescence staining for complement deposition (B). Grading scores (mean $\pm$ standard deviation) are indicated for each complement component ($^*p = 0.004$ for C4b/c and $^*p = 0.001$ for C6). All images are representative of minimum four scored sections per experiment. Scale bar: 100 μm.

3.8. Plasma and tissue endothelin-1

Plasma ET-1 levels increased moderately in both groups during the experiment and were comparable between both groups at the end of the observation period (8.33 ± 1.27 pg/ml for DXS vs 9.33 ± 1.57 pg/ml for PBS; $p = 0.406$, not shown). Significantly less ET-1 was detected in myocardial tissue in the DXS group as compared to PBS controls (3.55 ± 1.15 pg/100 mg wet tissue for DXS vs 6.29 ± 1.90 pg/100 mg wet tissue in the PBS group $p = 0.030$, Fig. 6A).

3.9. Immunostaining

In the DXS group myocardial complement deposition (C1q, C4b/c, C3b/c, and C6) was reduced as compared with PBS controls. Corresponding grading scores were: C1q: 0.5 ± 0.5 for DXS vs 0.8 ± 0.6 for PBS, $p = 0.150$; C4b/c: 1.5 ± 0.5 for DXS vs 2.4 ± 0.7 for PBS, $p = 0.004$; C3b/c: 0.6 ± 0.7 for DXS vs 1.1 ± 0.9, $p = 0.120$; C6: 0.8 ± 0.5 for DXS vs 1.9 ± 0.7 for PBS, $p = 0.001$, Fig. 6B. Similarly also in lung tissue, complement levels (C1q, C4b/c, C3b/c and C6) in the DXS group were markedly reduced as compared to samples obtained for the PBS control group (not shown).

4. Discussion

The aim of this study was to assess measurable effects of low molecular weight dextran sulfate (DXS) added to standard blood cardioplegia in a setting similar to clinical open-heart surgery. We have shown previously that DXS functions as an (endothelial) cytoprotectant [7,8,13] and attenuates myocardial reperfusion injury in vivo [9]. Our hypothesis that DXS may preserve cardiovascular and pulmonary function by complement inhibition and endothe-

lial cytoprotection in CPB-mediated inflammation and IR-injury has, at least in part, been confirmed by our findings.

With respect to cardiac function, DXS improved diastolic function in comparison to PBS controls. Diastolic dysfunction is frequently observed following open-heart surgery, may have serious clinical implications and be difficult to treat. Systolic function however, as measured by conductance catheter and echocardiography, was unaffected. This may be because systolic function remained essentially unaffected throughout the experiment. Furthermore, echocardiography may not be sensitive enough here to assess differences and changes in ejection fraction of less than 5—10%. It therefore remains speculative what, if any, effects DXS may have on hearts with obviously impaired systolic LV function. Whilst the reduction in inflammation (discussed below) may not always correlate with improvement in clinical parameters and outcome, a few such possible correlations are highlighted in the following paragraphs.

DXS significantly reduced episodes of atrial fibrillation in this short-term post-CPB follow-up. From a clinical standpoint, atrial fibrillation is still an important source of postoperative morbidity and prolonged hospital stay, occurring in up to 50% of patients after CPB [12]. The prospect of reducing this rate is therefore desirable. Diminished local cardiac inflammation following DXS use may partly be responsible for the observed reduction of atrial fibrillation. Indeed, the incidence of atrial fibrillation correlates with inflammation [13]. Nevertheless, an important proportion of hemodynamically relevant episodes of atrial fibrillation manifest themselves 48—72 h postoperatively. In the short post-CBP follow-up in the current study, no predictions can be made as to the possibly continued, indirect positive anti-arrhythmic effect DXS may have had in a later post-CBP phase. DXS administration is associated with significant attenuation of myocardial damage and complement deposition and evidently reduced CK-MB levels. Presently, although a trend towards less troponin I in the DXS group was observed, the differences did not reach statistical significance; possibly because of a too short observation period, the peak for troponin release being slightly delayed as compared to CK-MB.

With respect to the lungs, DXS treated animals revealed significantly less neutrophil infiltration, complement deposition and edema than controls, indicative of reduced inflammation and possibly endothelial damage. As a positive consequence thereof, pulmonary artery as well as right ventricular (RV) pressures were significantly less elevated after CPB in the DXS group compared to PBS controls.

Lung injury following CPB is multifactorial and includes sequelae associated with IR-injury, changes in the integrity of the bronchoalveolar architecture and infiltration by inflammatory cells [14]. Such injury represents an important cause of postoperative morbidity with high mortality, and may lead to respiratory failure. Indirect attenuation of pulmonary artery pressures, either by reducing the cuffing effect on the arterioles provoked by accumulated lung water, and/or preservation of diastolic LV function by DXS may therefore prove critical in a clinical setting. Furthermore, preservation of perioperative RV function by DXS may be central to ensure optimal postoperative recovery. Indeed, the use of DXS (MW 8000) during CPB in a pediatric

population has been shown to ameliorate immediate post-CPB respiratory index [15].

A minimal extracorporeal circulation (MECC) system was used in our experiments. This system has proven to be safe and may also reduce morbidity as well as CPB-associated inflammation in humans [16]. Whilst MECC may not yet be as established as conventional ECC, precisely because of its reduced pro-inflammatory potential it was utilized in this study. Although differences in reduction of the anti-inflammatory potential of a substance may be more difficult to elucidate when the level of inflammation itself is possibly lower than in a conventional ECC system, any differences that are observed may be of even greater value. Indeed, DXS modulated several components of inflammation and coagulation.

Thrombin levels, measured as thrombin anti-thrombin (TAT) complexes, rapidly increase after CPB [17]. Reduction of thrombin generation by anti-thrombin III ameliorates IR-injury by attenuating leukocyte recruitment [18]. Inhibition of overall total thrombin production by DXS correlated with reduced cardiac and pulmonary neutrophil recruitment and may provide one mechanism by which damage was ameliorated.

Systemic complement inhibition in CPB, including soluble complement receptor type 1 [19] has proven beneficial in ameliorating post-CPB recovery. C5b-9 directly contributes to IR injury and lower levels are associated with reduced complications and better recovery post-CPB [20]. Furthermore, C5b-9 levels correlate with lung water accumulation and recovery of LV wall motion [19]. DXS reduced tissue deposition of all complement components, including C5b-9 in heart and lungs.

IL-6, an acute-phase inflammatory cytokine, is secreted by activated endothelial cells, amongst others. Plasma IL-6 is markedly increased following CPB and its production is stimulated by thrombin [21]. Reduction of plasma IL-6, possibly also in relation with a partial reduction in thrombin production by DXS, may therefore play an important role in curbing post-CPB inflammation. Furthermore, DXS also significantly reduced TNFalpha, IL-1beta and IL-8 levels, cytokines in part known to be activated and injurious during CPB and cardiac surgery in general.

Not only high plasma ET-1, but also elevated tissue ET-1 levels have been shown to predispose to alterations in pulmonary function and myocardial contractility [22]. Focally reduced tissue ET-1 levels after DXS treatment may therefore have contributed to partly improved cardio-pulmonary function post-CPB.

Of note, methylprednisolone (equivalent to 30 mg/kg) was administered to all animals prior to the experiment to prevent vasoplegia and hypotension as a reaction to CPB. Whilst various clinical studies [23], have shown a positive effect of methylprednisolone on reduction of inflammatory responses, the effect on clinical outcome is not so clear and certain patient populations may not necessarily benefit from its use [24]. Whilst a certain overall reduction in inflammation may be attributable to the use of methylprednisolone in this model, any methodological bias would be present in all experiments, including the controls. Whether DXS, in the applied dose, would suffice to reduce the inflammatory response in an equivalent manner in a protocol lacking methylprednisolone would have to be tested in further experiments.

4.1. Study limitations

As with all experimental models, the animals used in this CPB study were healthy. Furthermore, with limited numbers in each group, the power of this study is reduced. Additionally, the observation period post-CPB may have been too short to elucidate potentially significant, clinically relevant differences between the two groups. The use of hydroxyethyl starch may influence inflammation. However, comparable amounts were used in both groups, whereby observed differences between the groups are unlikely to be due to its use. With the aim to mimic a clinically realistic situation, we chose to adjust the use of vasoactives and volume to maintain a mean pressure of 50 mmHg, in effect however taking into account the limited expressiveness of these pressure-based functional measurements and the possibility of masking subtle influences of DXS upon function. Indeed load independent determination of cardiac function using a pressure-volume platform would have been able to provide more consistent and possibly more reliable data. However, despite these limitations, we made several reliable observations in a double-blinded pilot study with settings closely mimicking clinical reality. Indeed, in the complex setting of CBP-associated injury and inflammation, even small advances in ameliorating aspects of post-CBP damage may be of relevance for optimizing future treatment strategies.

In summary, the addition of low molecular weight dextran sulfate to standard blood cardioplegia significantly reduces local complement activation in the heart and lungs as well as the release of pro-inflammatory mediators. Furthermore, DXS in part improved the hemodynamic situation by ameliorating diastolic LV function and RV— and pulmonary artery pressures post-CPB. Whilst DXS has been used in a few patient trials [25], including CPB in children, [15] larger randomized clinical trials would be needed before judging the possible importance of the use of DXS in altering current clinical practice. However, in light of the fact that DXS at the current dose was not associated with any adverse events and was well tolerated, it remains to be substantiated whether a more aggressive treatment regimen, evaluated in a longer post-CBP follow-up and ultimately analyzed in human trials, may more markedly and sustainably improve post-CPB cardiac function and outcome.

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