Supervisor: Prof. Dr. Elizabeth Rőth
Department of Experimental Surgery
Faculty of Medical Sciences, University of Pécs

Summary of Ph.D. thesis

Clinical and experimental evaluation of the cardio-protective properties of Fructose-1,6-diphosphate (FDP)

Dr. János Gál

Department of Anaesthesia and Intensive Therapy
Faculty of Medical Sciences, University of Pécs

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Introduction

Having entered the 21st century we are still confronted with the clinical problems and increased risk of perioperative morbidity and mortality associated with coronary artery disease (CAD). In the patient undergoing cardiac surgery, as well as the patient with increased risk of CAD undergoing non cardiac surgery, our goal is to prevent poor perioperative cardiac outcome that may compromise the patient's life, or quality of remaining life, after surgery. The mechanism of any adverse outcome can be broadly divided into problems related to either, or both, supply-demand imbalance and abnormal endothelial-myocyte functions. Therapeutic maneuvers, in the first condition, can be aimed to improve supply and/or decrease demand. Interventions aimed at reducing cellular dysfunction can also be divided into those related to ischaemia-reperfusion injury and/or inflammatory processes.

Classically the treatment of ischaemic heart disease revolves around increasing oxygen supply and decreasing oxygen demand. Preoperatively this is usually achieved with the use of oxygen, β blockade, and vasodilators. In the failing heart, this may be confounded by the use of inotropes which will increase myocardial work and oxygen requirement. In order to maintain viability and contractility, the myocyte must continually resynthesis high-energy phosphates (ATP). It is capable of doing this by using various substrates, namely carbohydrates, lipids, ketones or lactate (depending on physiological state and substrate availability). Metabolic support of the ischaemic and post ischaemic heart aims to improve the efficiency of oxygen and substrate utilization. Available therapeutic interventions are thus based on improving oxygen and metabolic component supply or preventing an increased demand.

The preferred substrate of cardiac muscle is free fatty acids (FFA). The FFA are metabolised to acyl CoA and then transported into the mitochondria. Here, they undergo β-oxidation producing acetyl-CoA which enters the TCA cycle for subsequent ATP production. Glycolysis breaks glucose to pyruvate (and provides a small amount of ATP) which enters the mitochondria where it is converted to Acetyl-CoA. Although FFA have a greater yield of ATP per mole of carbon atoms, glucose metabolism produces more ATP per mole of oxygen consumed when compared to FFA. Providing there is an

abundant supply of oxygen, the use of FFA is thus a more efficient substrate for energy production. During ischaemia there is an increased adrenergic response which acts to decrease insulin release and increase lypolysis and plasma FFA levels. Initially during ischaemia, there is a decrease in glucose and FFA oxidation and a concomitant increase in ATP production from glycolysis. This can only continue as long as glucose is provided to be metabolized. Ischaemia-induced upregulation of the anaerobic glycolytic pathway is also short-lived due to the accumulation of lactate, which inhibits the key glycolytic enzyme- phosphofructokinase (PFK) resulting in ATP depletion and eventual cell death. Accelerated glycolysis will generating pyruvate, together with a small amount of ATP, but pyruvat dehydrogenase is inhibited by the reducing equivalents from FFA metabolism. The uncoupling of glycolysis and glucose oxidation will induce a build up of lactate and protons eventually leading to intracellular acidosis. Excess protons are exchanged for sodium ions, which are, in turn, exchanged for calcium ions. This produces intracellular calcium overload which results in cellular, and specifically contractile, dysfunction.

Background

Most cardiac surgery requires cardiac standstill to enable surgical maneuvers. This is typically achieved by perfusing the heart, either via the coronary arteries, or both through the arteries and the coronary sinus, with a chilled, depolarizing cardioplegic solution; several recipes for such solution exist. The period of cardioplegia thus inevitably results in ischaemia, although the purpose of chilling the heart is with the intention of minimizing the energy requirements of the tissue during this period. Nonetheless, myocardial performance, in terms of cardiac output, is frequently obtunded as a results of this ischaemic injury. The fundamental problem in ischaemia is a demand for more energy than is produced. The central molecule in the provision of energy is ATP, which has two high-energy phosphate bonds, and has a storage form known as creatine phosphate. Ischaemia thus creates a problem of ATP generation in heart muscle (and other muscle).

During ischaemia, or when there is a sudden need for energy in excess of the oxygen supply, anaerobic metabolism (glycolysis) can proceed as normal. However, there is insufficient oxygen to support respiration. Thus, the products of glycolysis pile up faster than they can be cleared by respiration. The cell handles this by shunting the products of glycolysis into theformation of lactic acid (Lactate). Lactate has two important effects. First it is acidic, and if it remains in the cells then it has directly toxic properties of its own. Secondly, after an ischaemic period when aerobic metabolism has stopped, and glycolysis has continued, lactate actually inhibits even anaerobic metabolism because of an inhibitory effect on a crucial enzyme in the Embden-Meyerhof pathway. The enzyme is called phosphofructokinase (PFK), and its role is to convert fructose-6-phosphate into fructose-1,6-diphosphate (FDP), a reaction that involves investing the second of the two ATP molecules in the anaerobic part of the metabolism of glucose. Thus, for a while, glycolysis persist in the absence of respiration, but eventually even anaerobic metabolism fails in tissues that are not being perfused. Exogenous administration of FDP may restore anaerobic metabolism because it does not depend upon PFK for its metabolism. In contrast to glucose, which yields 2 molecules of ATP, net, for every molecule of glucose that is anaerobically metabolised, exogenous FDP yields four molecules of ATP, because FDP joins the Embden-Meyerhof pathway after the two ATP molecules have been invested. Furthermore FDP may be expected to differ from orthodox inotropes in terms of the influence on energy balance. Receptor-mediated positive inotropes usually increase work, and therefore oxygen demand by the heart through increasing ambient second messenger concentrations, or inositol phosphates. Exogenous FDP may be able to provide ATP without the need for an increased oxygen supply.

FDP as a Cytoprotective Agent

Fructose-1,6-diphosphate is an intermediary glycolytic pathway moiety which appears to reduce tissue injury in organs exposed to ischaemia-reperfusion. The cytoprotective effects are generally thought to be related to FDP enhancing anaerobic carbohydrate utilisation, and ATP production through activation of phosphofructokinase or, by serving as a high-energy substrate for anaerobic glycolysis. FDP enters the glycolytic pathway distal to the rate limiting enzyme (PFK), which is inhibited by negative feedback of lactate accumulation during anaerobic metabolism. FDP has been shown to increase ATP and creatine phosphate levels in ischaemic/reperfused myocardium. FDP, therefore, yields higher levels of ATP and ensure ongoing glycolysis, reducing the relative period of ischaemia. Anaerobic metabolism occurs in the myocardium during the period of aortic cross clamping, as well as in the pulmonary vascular bed on cessation of pulmonary artery flow during cardiopulmonary bypass (CPB).

FDP has been shown, in a variety of *in vitro* and *in vivo* experiments, to ameliorate ischaemic damage in several organs including heart, brain, kidney, and intestine. In patients with lower limb ischaemia, FDP was reported to improve blood flow, reduce pain at rest, and increase walking distance. In patients with sickle cell disease, FDP treatment decreased pain associated with acute vasoocclusive episode. Recently FDP has been reported to protect rat heart subjected to prolonged preservation (12 hours) in 4°C St. Thomas solution and subsequently reperfused (isoleted working rat heart model). These results of previous experiments suggest that FDP may hold promise as a cytoprotective agent in coronary artery bypass graft (CABG) surgery.

Aimes and results of Ph.D. project

The primary objective of our investigations was to examine the influence of FDP on myocardial performance after the obligatory ischaemic period, which accompanies aortic cross clamping during cardiopulmonary bypass.

Clinical investigations

In a one hundred twenty patients study FDP or 5% Dextrose (125 or 250 mg/kg) was administered over 30 min before cardioplegia and or added to the cardioplegic solution during CABG surgery followed or not by two 30 min infusions 2 and 6 hours after CABG. The study was done in 5 major stages and approved by the local Ethics Committee (Hillingdon Health Authority, Yiewsley, Middlesex, London, UK; Ethics Committee Submission No. 638). Written informed consent was used. Good Clinical Practice and Declaration of Helsinki compliance were maintained throughout. The study also received permission from the Medicines Control Agency (UK) and the Food and Drugs Administration (USA). Each stage was designed as an independent, parallel-group, double-blind, placebo-controlled clinical trial. The purpose of this trial was to determine the most promissing regime as defined by improvement of post surgical cardiac functions and other relevant parameters.

Results: The most favourable results were seen in the stage 4 dosing regimen, where pre-treatment of FDP was combined with FDP supplemented crystalloid cardioplegia. In this stage, the FDP-treated subjects, as compared to placebo, showed: significantly reduced the serum CK-MB levels at 2h, 4h and 6h post-CPB (five placebo-treated patients, and none of the FDP-treated subjects suffered a new subendocardial myocardial infarction); significantly higher left ventricular stroke work index at 6h, 12h and 18h post-CPB, and lower pulmonary vascular resistance indices at 6 and 18h post-CPB. Holter monitoring for the first 72h post-operatively demonstrated atrial fibrillation in 6,6%, FDP-treated, and in 33,3%, placebo-treated subjects.

The post-CPB inotropic support was required by 2 of 15 (13.3%) FDP-treated and 5 of 15 (33,3%), placebo-treated subjects. Furthermore, 2 placebo-treated subjects and no FDP-treated subjects required adrenaline support during the first 18 hours post-CPB.

The other dosing regimens did not show similar benefits. The fact that 250 mg/kg bolus infusion of FDP plus cardioplegia showed benefit over single bolus regimens, prompted us to investigate a multiple bolus strategy in stage 5 of this trial. In this phase, metabolic acidosis and slight impairment of ventricular function (as measured by LVSWI) in the early postoperative period was observed in the FDP-treated group. No detrimental effects were observed in these subjects. This problem of metabolic acidosis was subsequently overcome by decreasing the 2nd and 3rd dose of FDP to 125 mg/kg. With the exception of stage 5, all other stages showed favourable tolerability of FDP.

Assessment of Nucleotide degradation products by HPLC

FDP has been shown to confer myocardial protection during cardiac surgery and its cytoprotective properties are thought to be mediated through metabolic upregulation of the Embden-Meyerhof pathway distal to the key glycolytic enzyme, thereby restoring anaerobic glycolysis. Ischaemia and reperfusion during cardiac surgery inevitably produces myocardial metabolic changes, accompanied by adenine nucleotide degradation, that adversely affect postoperative mechanical function of the heart. Hence, the quantification of myocardial release of nucleotide catabolites provides useful information regarding myocardial preservation and the ability to recover from ischaemia. We investigated whether the improved myocardial preservation by FDP could be attributed to improved intermediary metabolism. The measurement of adenine nucleotide degradation products (directly linked to high energy phosphate turnover) provides a direct method of assessing myocardial metabolic changes.

IRB approval was obtained for this double blind, parallel-group, placebo controlled clinical trial. 12 consenting patients (LVEF > 45%) scheduled for elective CABG surgery received either 250 mg/kg FDP or 5% Dextrose (control) as intravenous pre-treatment, over a period of 30 minutes prior to cardiopulmonary bypass.

In the second stage, 10 consenting patients (LVEF > 45%) scheduled for elective CABG surgery received either 2.5 mM (1.4g/l) FDP or 5% Dextrose into the cardioplegic solution. Myocardial protection consisted of anterograde crystalloid cardioplegia, in conjunction with global and topical hypothermia. Arterial and coronary sinus blood was collected into liquid nitrogen at these time points; pre-aortic clamping (baseline), immediately following, 1, 5, 10, and 30 mins. after aortic declamping. 0.8 ml of blood was subsequently mixed with 0.8 ml perchloric acid (1.3 mM) and analysed by high performance liquid chromatography (HPLC).

Results: FDP and placebo groups did not differ in age, aorta cross-clamp or bypass duration. In the *first stage*, analysis of myocardial (coronary sinus - arterial) nucleotide metabolites indicated a substantial increase in the release of inosine and hypoxanthine during reperfusion in both the FDP and especially in the control groups. Compared to pre-aortic clamping (baseline) values, hypoxanthine and inosine concentrations were significantly elevated at 1 and 5 mins ($p < 0.05$) in the FDP group *vs.* 0, 1, 5 ($p < 0.01$) and 10 mins (all $p < 0.05$) in the control group, following aortic declamping. Adenine nucleotides undergo slow deamination in cardiomyoctes but rapid deamination and phosphorylysis to inosine and hypoxanthine in endothelial cells. These data suggest that FDP may contribute to myocardial and more specifically endothelial cytoprotection during the ischaemic insult of cardiac surgery, through a direct metabolic pathway.

In summary of the *second stage* of this investigation there was no evidence for protective effect of FDP-on nucleotide degradation between the treated and control group.

Coronary endothelial protection by FDP, in a rat Langendorrf model.

The endothelium plays a major role in controlling vascular tone, through the secretion of various vasoactive substances, of which the most potent vasodilator is nitric oxide (NO). Ischaemia has been shown to attenuate this vasodilatory capacity of the coronary arteries in response to endothelium-dependent vasodilators, following regional and global normothermic ischaemic arrest. Furthermore, the coronary microvasculature is known to undergo profound alteration following ischaemia-reperfusion injury, resulting in a reduction of coronary flow, or low reflow.

This postischaemic impairment of coronary flow could be due to morphological or functional abnormalities: for example cellular swelling and disruption and lumen plugging resulting in extravascular compression; interstitial oedema is known to occur in stunned myocardium and could also impair the coronary tone; ischaemic endothelial cells release vasoconstricting agents resulting in an impairment of normal coronary vessel regulation by the endothelial cells.

Numerous pre-clinical studies have shown improved mechanical function and a reduction in infarct size in isolated heart studies by FDP. Clinical studies suggest a similar benefit, with improved recovery of myocardial performance following CABG surgery. The improved glycolysis during ischaemia, as well as the beneficial effects on the microcirculation by FDP, suggests that FDP may promote coronary endothelial cytoprotection during ischaemia. Furthermore, our suggestion that FDP may also modulate coronary endothelial function, and thereby improve and favour recovery of myocardial function. No studies have explored the effect of FDP on coronary endothelial function.

In this study we investigated the role of FDP in providing coronary endothelial cytoprotection, as well as in modulating coronary endothelial function. We used an isolated rat heart model of ischaemia-reperfusion injury that is 4 hours of hypothermic cardioplegic arrest. All the investigations were performed in accordance with the Home Office Guidance on the Operation of Animals (Scientific Procedure) Act 1986, published by HMSO, London, United Kingdom.

In all these investigations, we used adult male Sprague-Dawley rats weighing between 300 and 450g. Investigations were done in batches, with equal placebo/control and FDP-treated animals in each batch.

Animals assigned to the investigate the early, metabolic effect of FDP, received either;

1, intravenous FDP (250 mg/kg), under general anaesthesia, followed by FDP (2.5 mM) supplemented Krebs-Henseleit coronary perfusate administered to the isolated heart up to the time of ischaemia. The control group received an equal volume of 5% D/W intravenously, with no further supplement to the Krebs-Henseleit coronary perfusate.

2, St Thomas cardioplegia supplemented with FDP (2.5 mM). The control group received no additive.

Results: *In the first group*, FDP-pre-treatment improved postischaemic hyperaemic and basal coronary flow (0 % vs. 40 %, placebo had > 25% reduction). Post-ischaemic coronary vasoconstriction to 5-HT reflects endothelial dysfunction. This was greater in placebo-treated hearts ($p<0.05$). These data suggest that FDP could reduce coronary low reflow that follows ischaemia. This is supported by the observed improvement in hyperaemic response following ischaemia, decrease in magnitude of reduction in post-ischaemic coronary flow, and improved percentage recovery of stimulated coronary flow to 5-HT and GTN, in the FDP pre-treated group. These beneficial effects can be mediated by endothelial cytoprotection during ischaemia, as suggested by the reduction in incidence of coronary vasoconstriction that follows post-ischaemic challenge with 5-HT. 5-HT an endothelial-dependent vasodilator results in coronary vasoconstriction in segments where the endothelium is denuded. This is mediated by smooth muscle serotonin receptors.

In summary of the *second group* of this investigation there was no evidence for protective effect of FDP-on endothelial dysfunction between the treated and control group.

FDP enhances cGMP release from human saphenous vein.

Our clinical studies, suggested a bi-phasic beneficial effect of FDP, in the setting of ischaemia-reperfusion injury following CPB. The early-onset effect was characterised by attenuation of pulmonary vasoconstriction and recovery of ventricular function in the immediate post-operative period, suggestive of FDP having beneficial effects on both the myocardium and the vasculature. This phase is aptly termed the "early, direct-metabolic effect" and most likely mediated by the ability of FDP to raise ATP levels during the critical period of ischaemia, as well as by the ability to improve the microcirculation. More intriguing, however, was the observation of a late-onset phase characterised by active pulmonary vasodilation and recovery of myocardial function. It is unlikely that this slower response is due to direct metabolic effects of FDP (K_{elim} = 2.77 ; $t_{1/2}$ ~ 15 minutes), therefore we termed this phase the "delayed, indirect-metabolic effect". Based on the time course of these events, it is our hypothesis that the delayed effect of FDP could be related to induced changes in genetic activation, either via transcriptional or translational / post-translational mechanisms.

The cytoprotective properties of FDP are thought to be mediated through metabolic upregulation of the Embden-Meyerhof pathway, thereby restoring anerobic glycolysis. Recent studies suggested additional mechanisms exist involving the NO-cGMP pathway. Knowing the central importance of the L-arginine-NO-cGMP pathway in vascular homeostasis, initial studies investigated the influence of FDP on vascular cGMP pathways as a possible mechanism of cytoprotection independent of intermediary metabolism.

Specific studies included; ***1,*** time and dose effects of FDP, ***2,*** endothelial dependency of FDP action, ***3,*** potential molecular mechanisms of FDP action.

The response of human vascular tissue to FDP, and the mechanism whereby FDP exerted this effect was investigated by quantifying vascular cGMP production as the endpoint. Saphenous vein was obtained from patients undergoing coronary artery bypass grafting surgery, dissected free of fat and cut into rings 4 mm in width. Rings were pre-incubated in oxygenated modified Krebs solution (this medium was used in all investigations).

To determine the contribution of ongoing endothelial NO production to tissue cGMP levels or to investigate the responsiveness of the cGMP pathway, the tissues were treated with L-NAME (10^{-4}M x 30 mins.) or sodium nitroprusside (SNP) (10^{-6}M x 15 mins.), respectively. To investigate FDP induced responses, rings were treated with increasing concentrations (1.25, 2.5, or 5 mM) of FDP and increasing time of incubation (2, 4 or 6h) with FDP. After treatments, 3-isobutyl-1-methylxanthine (IBMX, 10 mM x 20 mins.), a phosphodiesterase inhibitor was used to stop enzymatic reactions. Extract was then obtained from the tissue using HCl (200 µl, 0.1 N x 1h), which was then processed for routine radioimmunoassay using polyclonal cGMP antibody. All tests were done in duplicate and the cGMP results corrected for the wet weight of each ring and results are therefore expressed as pmol per gram wet weight of vascular tissue. Using the average of these duplicates, the results were then matched to paired control rings.

Results: Following incubation with FDP, saphenous vein showed a significant dose- and time-response related increase in cGMP levels. Maximal effects were observed following incubation with 2.5 mM FDP for 6-hours ($p < 0.05$). Other dose and time points resulted in an increase in cGMP levels, however, failed to show statistical significance.

Following confirmation that FDP mediates a late-onset vasodilatory effect through an increase in cGMP, the interaction between FDP and the L-arginine-NO-cGMP pathway in human saphenous vein rings was investigated. These investigations included, incubation of vascular rings with substances known to attenuate (N(G)-nitro-L-arginine methylester [L-NAME] or direct trauma to the endothelial layer) or enhance (bradykinin, L-arginine) the endothelial-dependent L-arginine-NO-cGMP pathway. In all tests where the endothelial layer was mechanically removed, there is potential of injury to the smooth muscle integrity. This was assessed and confirmed to be intact by SNP - a substance known to increase cGMP production via an endothelial -independent manner. Tests were repeated in parallel, in combination with FDP.

Results: When compared to matched control rings, SNP results in a significant increase in cGMP levels (19.7 fold increase; $p = 0.0003$; for all vascular rings). Similarly, a significant, but lesser, increase in cGMP levels (2.74 fold increase; $p < 0.05$; for all vascular rings) occurs following incubation with FDP. L-Name resulted in > 60% ($p < 0.01$) reduction in intracellular cGMP levels, supporting the notion that the L-arginine-NO-cGMP pathway is a major contributor to intracellular cGMP, through soluble guanylate cyclase. Agents known to enhance endothelial dependent NO production, include bradykinin and L-arginine, these showed insignificant increases in cGMP production. The combination of L-arginine and FDP showed a significant increase in cGMP ($p < 0.05$), whether this effect is one of synergism remains to be investigated.

In all tests where the endothelial layer was mechanically removed, SNP (an endothelial independent NO-donor) still showed a large increase in cGMP production (15.5 fold increaseincrease; $p = 0.004$; for all vascular rings), confirming integrity of the smooth muscle. cGMP production by bradykinin (positive control) and L-Name (negative control) did not differ from control values (136 % & 91.2 % of control, respectively; $p > 0.05$), confirming absence of stimulated or basal endothelial response. Importantly, in contrast to the increase in cGMP levels observed in vascular rings with intact endothelium which are incubated with FDP, we see that removal of the endothelium ablates this response (73% of control; $p > 0.05$). A similar ablation of response to FDP was also seen when vascular rings with intact endothelium, incubated in the presence of L-NAME.

These data suggest that FDP mediates an increase in cGMP production through an endothelial-dependent and nitric-oxide-dependent mechanism.

In the next phase we investigated whether this endothelial-dependent, NO-dependent increase in cGMP by FDP could be mediated by an induction mechanism involving protein transcription or translation / post translation pathways. This possibility was explored by pharmacological means using inhibition of transcription and protein synthesis. Vascular rings, with and without FDP (2.5 mM x 6- hours) were treated with actinomycin-D (10 μM; a transcription inhibitor) and cyclohexamide (100 μM; a translation inhibitor).

Results: Incubation of vascular rings with cyclohexamide or actinomycin-D resulted in a negligible reduction in cGMP levels when compared to control values (92.2 & 87.8%, respectively). Incubation of vascular rings with FDP resulted in an increased cGMP level (211%), however, this response is attenuated in the presence of cyclohexamide (69.9 % of FDP) and actinomycin-D (35.9 % of FDP; $p \leq 0.05$).

These data suggest that FDP mediates an increase in cGMP levels through an endothelial-dependent, NO-dependent pathway, which involves protein synthesis.

These results showed that FDP increased vascular cGMP content and could suggested FDP-induced upregulation of the NO-cGMP pathway. In comparison to the short half life of FDP ($t_{1/2}\beta = 15$ mins.) this delayed time course in increasing cGMP levels suggests that FDP may contribute to previously described 'late' effects on myocardial function preservation and reduction in pulmonary vascular resistance, possibly mediated by metabolic-independent mechanisms of action that facilitate endothelial cytoprotection.

Summary of main results

1. In our clinical investigations we observed beneficial effects of FDP, when used in combination both intravenously and in the cardioplegic solution. In contrast, there were no differences in clinical measures between the FDP-treated and control group when FDP was administered only intravenously or in the cardioplegia on its own.

2. Regarding nucleotide breakdown products, there were beneficial effects of FDP when used intravenously. However, there were no significant effects of FDP on these metabolites when FDP was used in the cardioplegic solution only.

3. Animal studies show that there is a coronary endothelial protection by intravenously administered FDP. Contrary, this was not observed following inclusion of FDP in the cardioplegia.

4. The data of the in vitro human rings experiments show that FDP elicits an increase in cGMP levels through an endothelium and NO-dependent pathway, which involves protein synthesis.

Conclusion

Current practice of scheduled coronary artery bypass graft surgery is associated with a certain degree of an archetypal ischaemia-reperfusion injury. FDP was protective against this injury, using consistent biochemical, haemodynamic and clinical end-points. For maximal benefit and reduced side effects, our clinical data suggests that FDP needs to be administered in combination (iv. plus into the cardioplegia), and in a dose range in the region of 250 mg/kg iv. However, this beneficial effect of FDP appears to be lost when used only intravenously or in the cardioplegic solution.

Similarly to our clinical measures and outcome data, using FDP only in cardiopplegia remained ineffective in preventing increased nucleotide breakdown following ischaemia and reperfusion. Furthermore, the in vitro data in a rat model of heart ischaemia-reperfusion also suggest inefficiency of FDP when used only in cardioplegia. Interestingly and contrary to the clinical measures some protection has been seen in nucleotide metabolism and in the in vitro Langendorrf experiments following intravenous administration of FDP.

The observed pulmonary vasodilation and improvement in ventricular function suggest that FDP exhibits beneficial effects on both the myocardium and the vasculature. These effects are likely mediated through two distinct mechanisms and time dependence. Our data suggest an early, direct metabolic effect - evidenced by the prevention of early postoperative pulmonary vasoconstriction, and by reduced elevation of myocardial adenine nucleotide metabolites immediately after the ischaemic period during CPB. Furthermore, there might be a delayed, indirect-metabolic effect (possibly involving a translational/transcriptional process) - supported by delayed onset of pulmonary vasodilation and recovery of myocardial function.

There are multiple pathways which might be involved in FDP-induced vascular relaxation. We have considered and provided a series of biochemical evidence that the upregulation of the L-arginine-nitric oxide-cGMP pathway could be a primary mechanism underlying this effect of FDP.

After elucidating potential novel mechanisms of FDP action we might need to reconsider strategies for future clinical investigation protocols involving FDP.

We suggest the following changes in future clinical studies,

1. Administration of FDP before starting CPB (minimum 6 hours earlier).

2. Continuing of the administration of FDP after aorta cross clamp off.

3. Using FDP in cardioplegic solution, should be excluded.

Furthermore we suggest to extend the clinical investigations to compare FDP to other drugs affecting the endothelial NO-cGMP pathway and old and novel cardioprotective strategies such as glucose-insulin-potassium infusion during CABG surgery.

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12. September, 2000. *Pécs*: Riedel B, Gál J, Royston D.: Is myocardial protection still necessary in off pump surgery? (III. International symposium on myocardial cytoprotection, accepted for **oral presentation.**)

Abstracts submitted for oral presentation

1. October, 2000. *San Francisco,* USA: Gál J, Riedel B, Marczin N, Royston D.: Heparine induced endothelial dysfunktion as quantified by cGMP radioimmunoassay. (Annual Meeting of the American Society of Anesthesiologists.)

2. December, 2000. *Singapore*: Gál J, Riedel B, Royston D.: FDP decreases cGMP level in vena saphena rings, incubating with human septic serum. (11th Congress of the Western Pacific Association of Critical Care Medicine.)