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Fructose-1,6 diphosphate as a protective agent for experimental ischemic acute renal failure

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Cold ischemia time is a risk factor for the development of acute renal failure in the immediate post-transplant period. In this study, we aimed to determine if intravenous fructose-1, 6-diphosphate (FDP), given before nephrectomy, attenuates renal cell injury in a cold ischemia model. Male adult Wistar rats were subjected to infusion of either FDP 350 mg/kg (group F, $n = 6$), an equal volume of 0.9% NaCl (group S, $n = 6$), an equal volume/osmolality of mannitol (group M, $n = 6$) or no infusion (group C, $n = 7$). Kidneys were then perfused *in situ* with Collins solution and nephrectomy was performed. Other kidney slices were stored in Collins solution at 4°C. Adenosine triphosphate (ATP) levels and lactate dehydrogenase (LDH) release were examined at 0, 24, 48 and 72 h. Other slices, obtained after 50 min immersion in Collins solution at 37°C, were frozen for characterization of cytoskeletal preservation using phalloidin-FITC staining. Apical fluorescence intensity of proximal tubule cells, indicative of the F-actin concentration, was measured in a fluorescence microscope interfaced with computer image analysis system. Adenosine triphosphate levels, after up to 72 h of tissue incubation, were higher ($P < 0.05$) in the FDP group when compared to other groups. In addition, LDH release was smaller ($P < 0.0001$) in the FDP group. The F-actin concentration of proximal tubule cells cells was greater in the FDP group ($P < 0.0001$). Results indicate that FDP is a useful tool to increase tissue viability in a rat kidney subjected to cold ischemia, by maintaining ATP cell content, decreasing LDH release and preventing microfilament disruption of proximal tubule cells.

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In cadaveric renal transplantation, cold ischemia time is a risk factor for the development of acute renal failure.[1] United States Renal Data Service data show that the frequency of delayed graft function (DGF) increases by 23.4% for every 6 h of cold ischemia, and is associated with diminished kidney allograft survival.[2]

DGF syndrome, like postischemic acute renal failure in the rat, is precipitated by complete interruption of the renal blood supply, causing severe cellular adenosine triphosphate (ATP) depletion. Cellular processes, which include protein synthesis, lipogenesis and membrane transport, become impaired when ATP is markedly depleted and ATP depletion causes early alterations in the actin cytoskeleton, resulting in loss of the membrane domains and cell detachment.[3] The preservation of ATP levels can be crucial, to maintain cell integrity and prolong cell survival.

Fructose-1,6-diphosphate (FDP) is an intracellular metabolite, that enhances carbohydrate utilization by stimulating glycolysis and, simultaneously, inhibiting gluconeogenesis.[4] The potential advantage of using FDP as the initial substrate is that, whereas the net yield of the anaerobic metabolism of one mole of glucose is two ATP moles, one mole of FDP metabolized in the same conditions would result in a net gain of four ATP moles since FDP does not require phosphorylation.[5,6]

It has been demonstrated, using ^{13}C-nuclear magnetic resonance spectroscopy, that exogenous FDP can cross the plasma membrane.[7–9] Labelled FDP was measured in blood, where it reaches the highest amount 10 min after administration. The hydrolytic activity of FDP was measured in organ extracts and was found to be maximal in the kidney.[10] Exogenous FDP, entering the cells, can stimulate the glycolytic pathway in three different ways (Figure 1): FDP can be hydrolyzed by fructose-1,6-diphosphatase, and increase fructose-6-phosphate levels, which can be used as a substrate for endogenous FDP production; FDP can activate phosphofructokinase, bypassing the acidosis-induced metabolic block; and FDP can directly stimulate the activity of piruvatekinase, increasing the production of pyruvate.

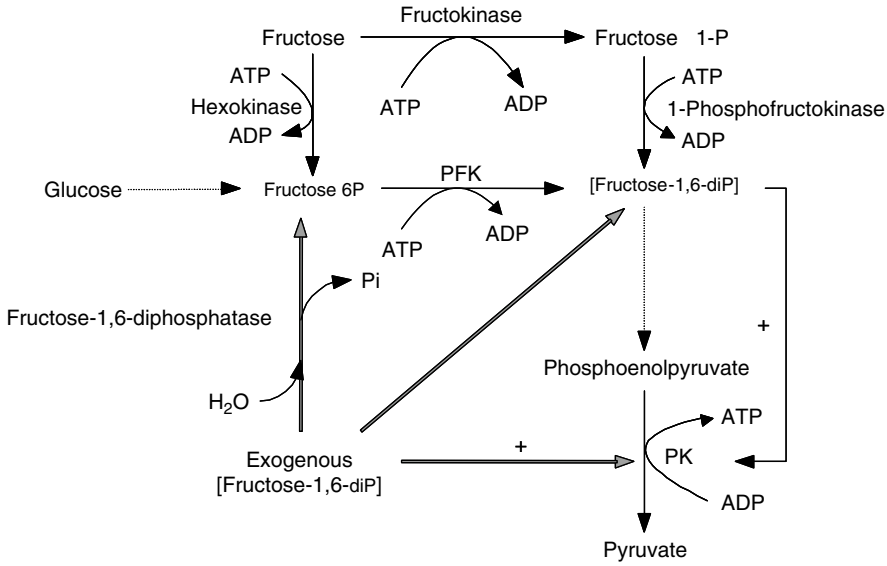


Figure 1 | The glycolytic pathway. Grey headed arrows indicate possible fates and actions of exogenous FDP in glucose metabolism.

For years, FDP has been used both clinically and experimentally during hypoxia and ischemia to facilitate metabolic recovery after hypoxic or ischemic insult. Pretreatment with FDP provides histologic and functional protection, 24 h after the insult, in rats subjected to 30 min of bilateral renal artery occlusion.[11] Post-treatment, with FDP infusion beginning 10 min after release of the renal artery clamps, also provided significant, although not complete, functional and histologic protection from renal damage.[12] FDP administration attenuates lung injury,[13] prevents reperfusion injury in rat intestines[14] and improves motility of spermatazoa.[15] Moreover, FDP prevents ischemia-induced brain damage in rabbits,[16] attenuates cyclosporin neprotoxicity[17] and also has a beneficial effect in patients with coronary artery disease.[18,19] FDP reduces postischemic reperfusion injury in heart,[20] liver,[21] and heart[22] transplantation, and reduces the mortality rate in experimental sepsis.[23,24]

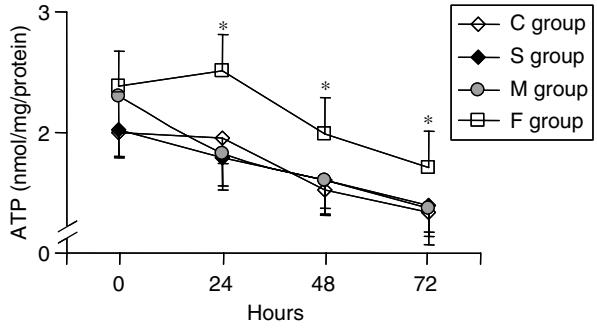
The present study intended to determine whether intravenous administration of FDP in rats, prior to nephrectomy, improves renal tissue preservation during cold ischemia.

RESULTS

According to the data, soon after the infusion of Collins solution, renal cells progressively lost viability.

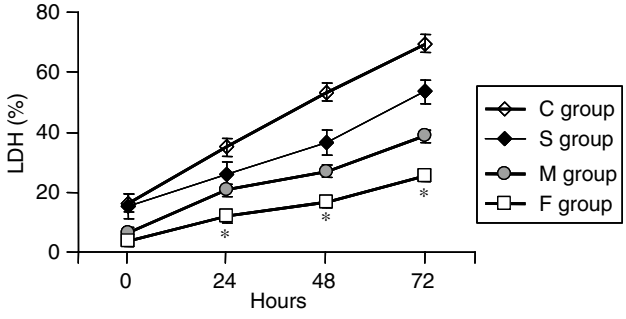
Figure 2 shows the cellular ATP content over 72 h. Throughout the entire procedure there was a significant decrease in all groups, but ATP content was better preserved in the FDP (F) group ($P<0.05$). FDP infusion led to a maintenance of cellular ATP content in the first 24 h followed by a gradual decrease not seen in other groups.

Figure 3 shows the lactate dehydrogenase (LDH) leakage from slices over 72 h. LDH leakage, taken as a measure of plasma membrane damage, was directly proportional to ischemia time. At time zero, LDH activity was already present in the supernatant of all groups, probably as a consequence of



*$P<0.05$ versus C, S and M

Figure 2 | ATP levels in renal slices over 72 h of tissue incubation. There was a significant decrease in the C, S and M groups over 72 h. In the F group, there was no decrease in ATP content in the first 24 h, followed by a progressive decrease but levels were kept higher in the F group in comparison to the C, S and M groups at 24, 48 and 72 h.



*$P<0.05$ versus C, S and M groups

Figure 3 | LDH release from renal slices into medium over 72 h of tissue incubation. Data represent mean ± s.e.m. There was a significant increase in LDH release in all groups. From 24 to 72 h the release was in the order of ($P<0.05$) C > S > M > F.