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INVITED REVIEW

ISCHEMIA AND REPERFUSION: EFFECT OF FRUCTOSE-1,6-BISPHOSPHATE†

GIUSEPPE LAZZARINO, BARBARA TAVAZZI and DONATO DI PIERRO

Department of Experimental Medicine and Biochemical Sciences, II University of Rome, "Tor Vergata", and Centro Interuniversitario per lo Studio del Danno da Ipossia e Iperossia, Rome, Italy

and

BRUNO GIARDINA

Institute of Chemistry and Centro CNR sulla Chimica dei Recettori, Catholic University "Sacro Cuore", Rome, Italy

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Several lines of evidence indicating a close relationship among ischemia, concentration of high-energy metabolites and onset of the "oxygen paradox" in reperfused tissues have been published. In this framework, we have recently studied the effects of exogenous fructose-1,6-bisphosphate on energy metabolism and on oxygen free radical damages of isolated rat heart subjected to anoxia and reoxygenation. In comparison with control groups, hearts perfused in the presence of 5 mM fructose-1,6-bisphosphate throughout the different perfusion conditions showed higher concentrations of energy metabolites at the end of anoxia, most of which were normalized after reperfusion. Furthermore, in comparison with control hearts, a reduction of tissue malondialdehyde and of lactate dehydrogenase release in the perfusate was observed in fructose-1,6-bisphosphate-perfused hearts. In this article we review most of the available data concerning the ability of fructose-1,6-bisphosphate to protect from ischemia and reperfusion damage outlining those recent findings which contributed both to clarify the pharmacological profile of the drug and to give an insight in its probable mechanism of action.

KEY WORDS: reperfusion injuries, energy metabolism, oxygen free radicals, fructose-1,6-bisphosphate, isolated rat heart.

ENERGY METABOLISM AND OXYGEN FREE RADICALS INJURY IN REPERFUSED TISSUES

The main source of energy production in respiring tissues is represented by oxygen-dependent mitochondrial metabolic cycles which provide most of the ATP that allows

Please address all correspondence to: Dr. Giuseppe Lazzarino, Department of Experimental Medicine and Biochemical Sciences, II University of Rome "Tor Vergata", Via O. Raimondo, 00173 Rome (Italy). Phone #: 6/72592110. Fax #: 6/7234793.

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the cells to accomplish the energy-requiring processes necessary for their own survival. The early metabolic event during ischemic and anoxic phenomena is the rapid and pronounced depletion of ATP and creatine phosphate (CrP)[1] with a consequent increase in their degradation products, such as ADP, AMP, creatine (Cr), inorganic phosphorus (Pi).[2,3] Prolonged periods of anoxia and ischemia lead to the arrest also of glycolysis which is the only metabolic cycle able to supply a certain amount of ATP during oxygen deprivation. This is due to the toxic effect of lactate and protons which act as potent inhibitor of both 6-phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase.[4,5]

In tissues , such as the myocardium, this sequence of events is more evident because of the continuous needs of energy for the contractile activity. In such tissues, the marked increase of tissue hypoxanthine, xanthine, inosine, AMP and lactate, induced by ischemia, is concomitantly mirrored by a decrease of nicotinic coenzymes, pyruvate and of glycogen.[2,6,7] This decrease is particularly important since it may seriously compromize the resynthesis of high-energy phosphates, thereby jeopardizing, at least in part, the effectiveness of reperfusion.[8–11]

The possibility that the generation of oxygen radicals could be strictly related to the degree of high-energy phosphates depletion, and hence to the duration of oxygen deprivation has been hypothesized by several authors.[12,13] In effect, irreversible conversion of xanthine dehydrogenase (XDH) into xanthine oxidase (XO), which is considered one of the main sources of superoxide anions, appears to be dependent on the time of anoxia or ischemia.[14–16] Although $O_2^{\cdot-}$ itself does not represent the most dangerous radical species, it is considered the starting point for the hypothesized sequence of reactions[17] leading to possible peroxidation phenomena and therefore to reperfusion-induced tissue injuries.

The role of the ATP decrease in driving the proteolytic-induced transformation of XDH into XO could be focused in the imbalance of Ca^{2+} homeostasis which would result in an increase of cytosolic free Ca^{2+} (due to the release from intracellular stores and the increase of the intake from the extracellular environment)[18–21] and in a subsequent activation of not yet identified Ca^{2+}-dependent proteases responsible for cleavage of the native XDH.[22–24] Despite some contradictory results,[25,26] recent observations confirmed that the transformation of XDH into XO occurs in the isolated rat heart as a function of myocardial reperfusion.[27,28] Hence, ATP depletion appears to be involved not only in the conversion of XDH into XO but also in the supply of a considerable amount of substrate for XO during reperfusion. Therefore, reperfusion is often characterized by a worsening of tissue damage. In effect, although sometime questioned because of the unspecific analytical method used (i.e., the thiobarbituric acid reaction), several reports showed a marked increase of lipid peroxidation in reperfused tissues.[27,29,30] Moreover, release of cytosolic enzymes, as markers of tissue necrosis, rather limited during anoxia, is conversely clearly evident during oxygen readmission.[27] These reperfusion-associated damages have been related to the production of oxygen radicals which have been clearly revealed by ESR spectroscopy.[31–33]

PHARMACOLOGICAL STRATEGIES TO REDUCE INJURIES TO REPERFUSED TISSUES

In the light of the above described phenomena, most of the preclinical studies carried

out in the last decade, have been addressed to the development of possible pharmacological interventions capable of reducing free radicals injuries to reperfused tissues.

Since in post-ischemic tissues oxygen free radicals have been indicated as a possible source of molecular derangement, and XO has been suggested as the enzyme responsible for their production, synthetic and naturally occurring free radical scavengers and XO inhibitors have been tested for a possible protective activity of the reperfused tissues. The rationale for their application is found, in the case of free radical scavengers, in their ability to transform oxygen radicals in less reactive molecules. Several papers have been published concerning the beneficial effects of exogenous superoxide dismutase alone or associated with catalase,[34–38] and of vitamin E[39,40] in different experimental models of ischemia and reperfusion. The effectiveness of scavenger enzymes, due to their inability to cross the cell membrane, might be explained if the main localization of XO were inside the epithelial cells, so that most of the oxygen radicals could be released in the extracellular milieu.[15,37,41] A different situation exists in the case of XO inhibitors such as allopurinol. Thus, beside the numerous evidence concerning its beneficial effects during reperfusion, the mechanism of action of allopurinol (and its analogs) has been related to its permeation into the cell and to its subsequent competition with XO substrates, being thereby independent to the *in vivo* localization of the enzyme.[42–44] In addition, a direct scavenging activity towards hydroxyl radical has been demonstrated.[17]

However, all the above mentioned compounds act after the ischemia-induced metabolic changes have taken place. Hence, none of them avoids the eventual irreversible transformation of XDH into XO or inhibits adenine nucleotides loss, thus resulting not completely effective for the normalization of energy metabolism. At this purpose, data obtained from this, as well as from other laboratories, indicating a positive effect of exogenous fructose-1,6-bisphosphate (F-1,6-P_2) to reduce reperfusion injuries and free radical damages in different experimental models, are the purpose of the present review.

EXOGENOUS FRUCTOSE-1,6-BISPHOSPHATE AS AN AGENT TO REDUCE REPERFUSION TISSUE INJURY

Protection Against Free Radicals and Oxygen Radicals Toxicity

In the general evaluation of F-1,6-P_2 pharmacological activity it is worth mentioning the dose-related inhibitory ability of F-1,6-P_2 on chemiluminescence response and superoxide production of phorbol esters-stimulated human polymorphonuclear leucocytes (Figures 1 and 2).[45] In these experiments the increasing cellular ATP concentration was correlated to the progressive decrease of both chemiluminescence emission and superoxide generation as a function of preincubation of the cells with increasing concentrations of F-1,6-P_2 in the suspending medium. In this cell system, ATP is considered a negative allosteric effector of the enzyme responsible for O_2^- production, i.e., the membrane bound NADPH–oxidase complex. It was also demonstrated that inhibition of O_2^- production by F-1,6-P_2 is mediated by its effects on cell metabolism rather than on superoxide anions themselves since in a cell-free O_2^- generating system, such as the xanthine–xanthine oxidase one, F-1,6-P_2 did not show any activity on the SOD-inhibitable cytochrome-*c* reduction.

These data were confirmed by other experiments which showed a normal phagocyting capacity of PNML stimulated by opsonized latex particles.[46]

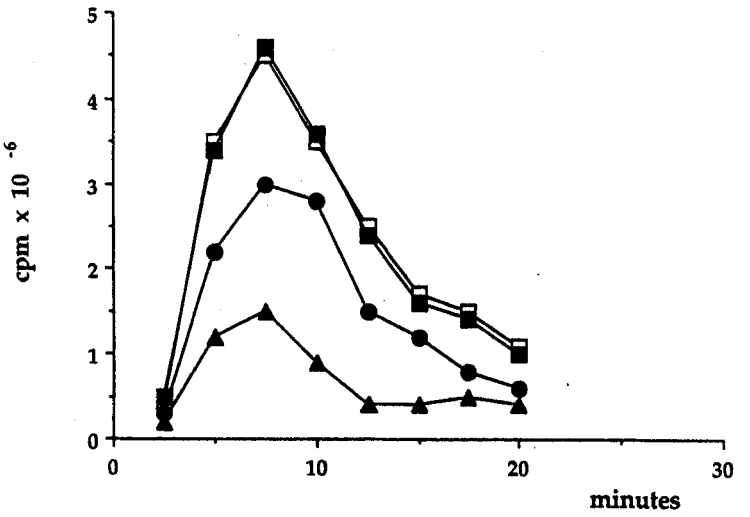

FIGURE 1 Inhibitory effect of increasing concentrations of F-1,6-P_2 on the chemiluminescence response of human neutrophils stimulated *in vitro* with phorbol esters. Cells (2×10^5/ml) were challenged with either 0.1 μg/ml PMA alone (□) or with 0.1 μg/ml PMA in the presence of F-1,6-P_2 0.2 (■), 1 (●) or 5 mM (▲). The luminol amplified chemiluminescence does not allow to discriminate among the various oxgyen radical species produced by stimulated neutrophils during oxygen burst.

Since it has been shown that F-1,6-P_2 induces a reduction of histamine release from isolated rat mast cells stimulated with the anticancer anthracycline doxorubicin (DXR)[47] it seemed reasonable to test the effect of F-1,6-P_2 administration on cardiotoxicity induced by subchronic DXR injection in mice.[48] This model was chosen as an example of xenobiotic toxicity whose mechanism is in part due to the generation of free radical species. In these experiments it was demonstrated that the daily i.p. injection of 750 mg/kg b.w. F-1,6-P_2 to mice treated three times per week (for up to

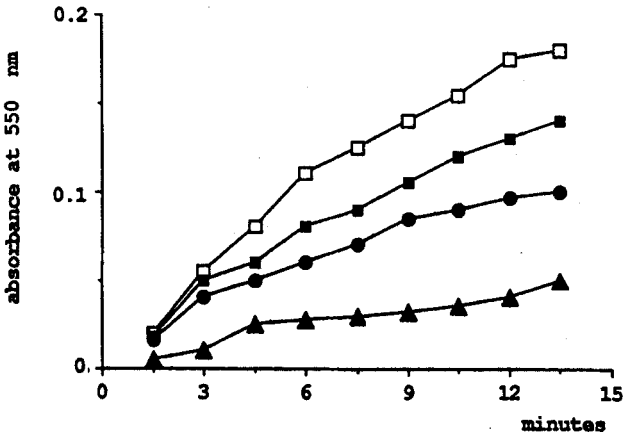

FIGURE 2 Inhibition of $O_2^{\cdot-}$ production of PMA-stimulated human neutrophils (□) induced by F-1,6-P_2 0.2 (■), 1 (●) and 5 mM (▲). Superoxide generation was monitored on 10^6 cells/ml (both control and F-1,6-P_2 incubated cells) following the reduction of cytochrome *c*.

TABLE I

Protective effect of F-1,6-P_2 on alterations induced by subchronic DXR administration of myocardial parameters related to the redox state of the tissue. All animals received DXR 2 mg/kg body weight i.p. twice a week for 1, 2, 3 and 4 weeks. One group received F-1,6-P_2 i.p. 750 mg/kg/day while the control group received equal volumes of 0.15 M NaCl. F-1,6-P_2 or NaCl were injected 20 min before DXR administration. Animals were sacrified 3 days after the last dose of DXR

Treatment group	Weeks of treatment	Total lipid peroxidation (nmol MDA/g protein)	Catalase (10^{-3} × U/g protein)
DXR + NaCl	0 (N = 20)	27.86[a]	4.91
		(7.56)	(2.97)
	1 (N = 10)	47.83	9.45
		(5.37)	(3.62)
	2 (N = 10)	54.29	8.89
		(5.01)	(4.37)
	3 (N = 10)	83.90	12.50
		(23.79)	(2.25)
	4 (N = 10)	88.64	13.38
		(9.52)	(1.67)
DXR + F-1,6-P_2	0 (N = 20)	27.86	4.91
		(7.56)	(2.97)
	1 (N = 10)	33.53[b]	7.71
		(9.05)	(3.63)
	2 (N = 10)	36.44[c]	8.60
		(4.72)	(3.43)
	3 (N = 10)	37.32[c]	6.85[c]
		(15.69)	(1.56)
	4 (N = 10)	43.82[c]	7.97[c]
		(12.12)	(2.11)

[a]Mean; S.D. in parentheses.
[b]Significantly different from group treated with DXR + NaCl ($p < 0.01$).
[c]Significantly different from group treated with DXR + NaCl ($p < 0.001$).
Abbreviations used are: DXR = doxorubicin; MDA = malondialdehyde.

4 weeks) with 2 mg/kg b.w. DXR, inhibits the increase of lipid peroxidation and the increase of catalase (determined in heart tissue homogenates) observed on a group of mice treated with the same DXR administration protocol and daily injected with an equal volume of 0.9% NaCl instead of F-1,6-P_2 (Table I). The protective effect of F-1,6-P_2 was also evident either on total LDH activity or on the relative composition of the LDH isoenzymes determined in plasma at various time intervals.

In addition, other studies on DXR cardiotoxicity outlined that: (i) very low doses of F-1,6-P_2 (dose ranging 0.01–0.1 mM) ameliorates acute ECG changes induced by DXR in isolated perfused rat hearts;[49] (ii) pretreatment with 375 and 750 mg/kg b.w. F-1,6-P_2 prevents ECG changes induced by i.v. acute toxicity of 10 mg/kg b.w. DXR in the anesthetized rat;[50] (iii) daily injection of 600 mg/kg b.w. F-1,6-P_2 significantly improves hemodynamic parameters of isolated hearts and reduces the severity of myocardial hystological damage of rats chronically treated with 3 mg/kg b.w. DXR thrice a week for six weeks.[50]

Another experimental model of free radical toxicity in the rat (i.e. the acute CCl_4 hepatotoxicity) showed that the concomitant i.p. injection of 2 g/kg b.w. F-1,6-P_2 and 2.5 ml/kg b.w. CCl_4, reduced the hepatic biochemical alterations observed in control

rats treated with the chemical noxa alone.[51] In particular, it was found that F-1,6-P_2 protects the enzymes of the polyamine metabolism (ornithine decarboxylase, S-adenosyl methionine decarboxylase and spermidine N-acetyltransferase) and reduces tissue necrosis as evidenced by a decrease of plasma aspartate aminotransferase and alanine aminotransferase. These protective effects were correlated to intracellular ATP and F-1,6-P_2, whose concentrations strongly decreased in rats treated with CCl_4 alone and were significantly different in F-1,6-P_2 + CCl_4-treated rats. Moreover, stimulation of glycolysis in rats injected only with F-1,6-P_2 was indicated by a significant increase in both hepatic ATP and F-1,6-P_2 in comparison with control untreated rats.[51]

F-1,6-P_2 in Ischemic and Reperfused Brain

The experimental model to evaluate the protective effects of F-1,6-P_2 in brain tissue has been based on the evidence that F-1,6-P_2-supplemented primary cultures of rat cerebral astrocytes showed a marked reduction of hypoxic cell injury induced by 18 h incubation in the presence of 95% N_2 + 5% CO_2.[52] The maximal protective effect, expressed in terms of inhibition of LDH release in the cell medium, cell edema and disruption, was achieved with 6 mM F-1,6-P_2 but was observed only with the simultaneous presence of glucose in the buffer. In this study it was also demonstrated that normoxic astrocytes can take up ^{14}C-F-1,6-P_2.

Data obtained in ischemic-hypoxic and reperfused rabbit brain *in vivo*, indicate that F-1,6-P_2 accelerates the time of recovery of electrical activity, decreases hystological neuronal lesions, and reduces the post-ischemic mortality.[53]

Although metabolic parameters were not determined, the mechanism of F-1,6-P_2 protection in the reperfused brain was attributed to its ability to increase ATP through a stimulation of glycolysis. However, notwithstanding these data evidenced a beneficial effect of exogenous i.v. infusion of F-1,6-P_2, the capacity of this drug to cross the blood brain barrier should be better clarified.

F-1,6-P_2 in Ischemic and Reperfused Heart

Due to the great clinical relevance of ischemic heart disease, most of the studies on reperfusion tissue injuries have been carried out on the myocardium using different experimental models. Therefore, a significant number of papers on F-1,6-P_2 as a potential protective drug to reduce both ischemic and reperfusion damages concerns the heart as selected organ.

Whole animal. Several studies were conducted on the whole animal by inducing experimental ischemia either by occluding one major branch of the left coronary artery[54,55] or by mechanically reducing the blood flow to a preselected value.[56] In experiments on left coronary artery (LCA) occlusions, 1.25 mg/kg/min of F-1,6-P_2 administered to dogs 45 min after the onset of ischemia for the following 4 h of oxygen deprivation (total amount of F-1,6-P_2 infused: 6 to 9 g) induced an increase in left ventricular end dyastolic pressure (LVEDP) and cardiac output, a reduction of the ischemia associated ECG abnormalities, and a significant protection of ATP and CrP concentrations in comparison to controls which have been receiving an equal amount of glucose.[54] In other experiments,[55] dogs were subjected to one or two hours of LCA occlusion and subsequently treated, 5 min prior to reperfusion, either with an i.v.

bolus of 75 mg/kg b.w. F-1,6-P_2 followed by a constant influsion of the drug for 2 h (for a total amount of 16–19 g of F-1,6-P_2), or with an equal amount of glucose in 0.9% NaCl. A significant amelioration of hemodynamic data was evidenced in the F-1,6-P_2-treated dogs (increased values of LVEDP, cardiac output, arterial pressure, and contractility) which also showed a marked reduction in the infarct size. In addition, of the 22 dogs in each group, in the F-1,6-P_2 group 17 survived whilst in the control group only 7 survived.[55]

A study reporting no effects of F-1,6-P_2 administration in myocardial ischemia in the dog has also been published, although the very low doses of the drug infused should explain this negative result.[56]

Isolated heart. A direct effect of F-1,6-P_2 on myocardial function and metabolism were demonstrated in studies on the isolated rabbit or rat heart using different models of ischemia/anoxia and reperfusion. Isolated rabbit hearts subjected to 40 min of cold cardioplegic arrest (3 min perfusion with a 4°C buffer containing 20 meq/l K^+, repeated once every 5 min) followed by normothermic reperfusion showed that exogenous F-1,6-P_2 (1.5 mM) administered prior to, and after cold cardioplegia prevented the ventricular wall rigidity induced by ischemic arrest, improved contractility both before and after cardioplegia, reduced the perfusion pressure and the time to recovery of myocardial contractile activity.[57]

In case of isolated rat heart, we focused our attention on the effect of F-1,6-P_2 on heart metabolism[6] and on oxygen free radical injuries[27] of anoxic and reperfused heart. We subjected a group of control hearts to 20 min of substrate-free anoxic perfusion followed by 20 min reperfusion with 11 mM glucose.[6] Two other groups of hearts received either 5 mM F-1,6-P_2 or 5 mM fructose throughout the various perfusion conditions (i.e., during preperfusion and reperfusion the above mentioned compounds were present together with glucose, whilst they were the only possible substrate during anoxia). Table II summarizes the values referring to phosphorylated compounds and to glycogen of the myocardium determined at the end of each perfusion step in each group of hearts. Protection of high energy phosphates during anoxia was observed only in 5 mM F-1,6-P_2-treated hearts which, after reperfusion, showed a normalization of most of the parameters considered. In addition, glycogen concentration was preserved in F-1,6-P_2-treated hearts after anoxia, being almost normalized after reperfusion. Differences among F-1,6-P_2-treated hearts and the two other groups were recorded also for lactate and pyruvate output determined in the perfusate throughout the whole duration of the experiments. It is of particular interest that the total amount of lactate produced either during preperfusion, anoxia, or reperfusion resulted significantly higher in F-1,6-P_2-treated hearts in comparison with the two other groups of hearts.

The same experimental model was adopted to verify whether F-1,6-P_2 could exert some beneficial effect on oxygen free radical damages induced by anoxia and reperfusion. In this study,[27] oxygen derived free radical insult was evaluated by determining at the end of each perfusion step, thiobarbituric acid reactive substances (TBARS) and XDH/XO ratio in tissue homogenates of control hearts (receiving 11 mM glucose during preperfusion and reperfusion, and no substrate during 20 min of anoxia) and of F-1,6-P_2-treated hearts (receiving 5 mM F-1,6-P_2 + 11 mM glucose during preperfusion and reperfusion, and 5 mM F-1,6-P_2 alone during anoxia). In addition, high-energy metabolite concentrations and release of LDH in the perfusate of both groups of hearts were recorded.

TABLE II

Effect of fructose-1,6-bisphosphate on the concentrations of phosphorylated compounds in isolated rat heart subjected to anoxic perfusion and normoxic reperfusion

	STEP	ATP	ADP	AMP	CrP	Cr	Glycogen
Control hearts	1	24.80	7.53	2.30	32.68	21.35	112.36
		(2.05)	(0.83)	(0.42)	(1.46)	(1.97)	(6.64)
	2	6.04	8.11	8.00	5.02	38.12	4.12
		(1.69)	(1.75)	(1.49)	(1.94)	(3.56)	(1.43)
	3	8.33	7.69	4.12	13.56	32.45	54.33
		(1.33)	(1.07)	(0.58)	(3.94)	(4.17)	(5.01)
5 mM Fructose	1	22.14	7.94	2.21	32.66	20.39	107.16
		(1.79)	(1.21)	(0.36)	(2.18)	(2.32)	(5.27)
	2	5.14	7.78	7.61	5.39	37.96	6.03
		(1.03)	(1.87)	(0.91)	(1.95)	(2.24)	(1.31)
	3	8.19	8.18	4.06	12.70	36.42	48.09
		(1.88)	(0.95)	(0.57)	(4.43)	(2.85)	(4.43)
5 mM F-1,6-P_2	1	24.77	7.92	2.16	33.41	22.15	109.66
		(2.00)	(1.51)	(0.23)	(2.67)	(2.51)	(4.13)
	2	11.78[b]	7.61	3.95[c]	12.00[c]	29.15[a]	33.69[c]
		(2.26)	(1.66)	(0.81)	(3.01)	(2.54)	(1.98)
	3	21.02[c]	7.11	2.36[b]	29.16[c]	21.87[a]	82.09[c]
		(3.61)	(1.95)	(0.81)	(4.13)	(2.50)	(5.30)

Values are expressed as μmole/g d.w. and represent the mean (S.D.) of 6 to 10 hearts.
Step 1 corresponds to 10 min preperfusion; Step 2 corresponds to 20 min glucose-free anoxic perfusion; Step 3 corresponds to 20 min normoxic reperfusion. Fructose and F-1,6-P_2-treated hearts received one of the two compounds throughout the whole duration of perfusion (i.e. also during anoxia)

[a] Significantly different from the corresponding step in the two other groups ($p < 0.05$).
[b] Significantly different from the corresponding step in the two other groups ($p < 0.01$).
[c] Significantly different from the corresponding step in the two other groups ($p < 0.001$).
Abbreviations used are: CrP = creatine phosphate; Cr = creatine.

These data, summarized in Table III, indicate that: (i) the changes in the XDH/XO ratio is associated to peroxidative damage of cell membranes (increase of tissue TBARS) and tissue necrosis (increase of LDH released in the perfusate); (ii) 5 mM exogenous F-1,6-P_2 inhibits XDH transformation into XO (as confirmed by the constant value of the XDH/XO ratio in this group of heart), avoids increase of TBARS, and significantly reduces the release of tissue LDH.

From the data referring to the energy state of the myocardium, it may be hypothesized that the reduction of oxygen radical injury, induced by F-1,6-P_2, is secondary to its effects on energy metabolism. Therefore, a higher ATP tissue concentration would result in an inhibition of XO formation and, if XO had however been produced, in a reduction of purine concentrations to be oxidized by the eventual presence of any XO.

These results, on the basis of the experimental model appropriately chosen (no other metabolizable substrate present during anoxia out of F-1,6-P_2 or fructose), seem to indicate that intact F-1,6-P_2 might represent a favourable substrate to the heart for its energy requirements (either in normoxic or in anoxic conditions), also in consideration of the complete absence of effects induced by fructose, whose maintenance (during anoxia) and recovery (during reoxigenation) would produce a decrease of oxygen radicals-induced tissue injury. Furthermore, a general indication confirming

TABLE III
Effect of 5 mM F-1,6-P_2 on the energy state, lipid peroxidation, XDH/XO ratio and LDH release of anoxic and reperfused rat heart

	STEP	ATP μmole/g d.w.	CrP μmole/g d.w.	Phosphorylation potential $[ATP]_f/[ADP]_f \times [Pi]$ ($10^3 \times M^{-1}$)	TBARS nmole MDA/g d.w.	XDH/XO	LDH U/min
Control hearts	1	23.19 (1.97)	32.08 (1.52)	72.57 (10.33)	97.25 (3.89)	2.38 (0.84)	0.055 (0.011)
	2	4.76 (1.52)	6.32 (2.00)	5.05 (0.72)	120.42 (12.77)	0.46 (0.18)	0.098 (0.023)
	3 #	9.03 (0.98)	12.85 (2.35)	23.14 (3.40)	152.66 (6.85)	0.25 (0.06)	0.277 (0.062)
F-1,6-P_2 Treated hearts	1	23.61 (2.03)	32.81 (3.03)	69.86 (9.27)	100.33 (12.78)	2.30 (0.88)	0.057 (0.009)
	2	11.54 (1.78)	12.14 (2.45)	18.36 (2.00)	112.56 (10.09)	2.16 (1.05)	0.077 (0.012)
	3	20.32 (2.28)	28.79 (5.17)	57.07 (11.88)	101.68 (8.98)	2.27 (1.17)	0.109 (0.035)

Values are mean (S.D.) of 6 different hearts per each step, both for control and F-1,6-P_2-treated hearts. Step 1 corresponds to 10 min preperfusion; Step 2 corresponds to 20 min glucose-free anoxic perfusion; Step 3 corresponds to 20 min normoxic reperfusion. F-1,6-P_2-treated hearts received 5 μM F-1,6-P_2 throughout the whole duration of perfusion (i.e. also during anoxia).

[a] Significantly different from the corresponding step of control hearts ($p < 0.05$).

[b] Significantly different from the corresponding step of control hearts ($p < 0.01$).

[c] Significantly different from the corresponding step of control hearts ($p < 0.001$).

Abbreviations used are: CrP = creatine phosphate; TBARS = Thiobarbituric acid reaction substances.

the beneficial effects of an increased glycolytic activity in ischemic tissues is supported by these data.[58–62]

Clinical studies of F-1,6-P_2 in Patients suffering from Myocardial Ischemia

Although carried out in a limited number of patients, clinical trials on F-1,6-P_2 effects in myocardial ischemia gave encouraging results, thereby confirming the data obtained in preclinical studies. A trial conducted in patients suffering from acute myocardial ischemia, reported a significant amelioration of cardiac index and left ventricular stoke work index after administration of 250 mg/Kg i.v. F-1,6-P_2 in comparison with saline infusion.[63] Other papers concern the effects of F-1,6-P_2 in chronic ischemic heart disease, as evaluated by monitoring hemodynamic and electrocardiographic parameters using different experimental protocols.[64–69] In general, all these clinical observations indicate an improvement of myocardial performances following i.v. administration of F-1,6-P_2.

CONCLUSIONS

All the available data concerning the effect of exogenous F-1,6-P_2 as a protective drug to ischemic and reperfused tissues demonstrate, in our opinion, two major points: (i) F-1,6-P_2 is able to reduce damages related to the experimental ischemia and reperfusion; (ii) clinical trials in patients suffering from ischemic heart desease indicate an effectiveness of F-1,6-P_2 also in human beings.

A general comment on preclinical data published at present time is that F-1,6-P_2 seems to elicit its protective effects in ischemia and reperfusion independently to the tissue involved and to the experimental model adopted. A common feature is the positive influence of the drug on energy metabolism which secondarily affects macroscopic parameters (such as survival, infarct size, functional properties of the organ involved, etc.).

The encouraging results obtained in clinical trials are a great stimulus to undertake new studies to better characterize F-1,6-P_2 effects in ischemic heart disease. In this respect, it might be interesting to evaluate the effects of a constant F-1,6-P_2 infusion in patients suffering from acute myocardial ischemia during fibrinolytic therapy.

Albeit data from several studies seem to indicate that intact F-1,6-P_2 can enter the cell, being subsequently metabolized through glycolysis,[26,70,71] direct and conclusive observations which confirm this hypothesis have been only recently demonstrated. We have in fact evidenced that exogenous F-1,6-P_2 (5 mM) induces a 30% decrease of ^{3}H-5-glucose consumption in isolated normoxic rat heart strongly supporting its direct metabolization as an alternative fuel.[72]

To better focus the mechanism of F-1,6-P_2 in reducing ischemia and reperfusion damages, it is worth mentioning that we have recently evaluated its direct effect on the energy metabolism (as determined by NMR), on mitochondrial oxidative metabolism (as determined by reflectance spectrofluorometry) and we also evidenced a Ca^{2+}-binding activity of F-1,6-P_2 which partly influences myocardial performances of isolated normoxic rat heart.[73] We have also recently reported a reduction of oxygen consumption and a concomitant increase of myocardial efficiency, in a dose-related fashion, following infusion of various concentrations of F-1,6-P_2 in the isolated working rat heart.[74] These properties might be important in partially explaining its

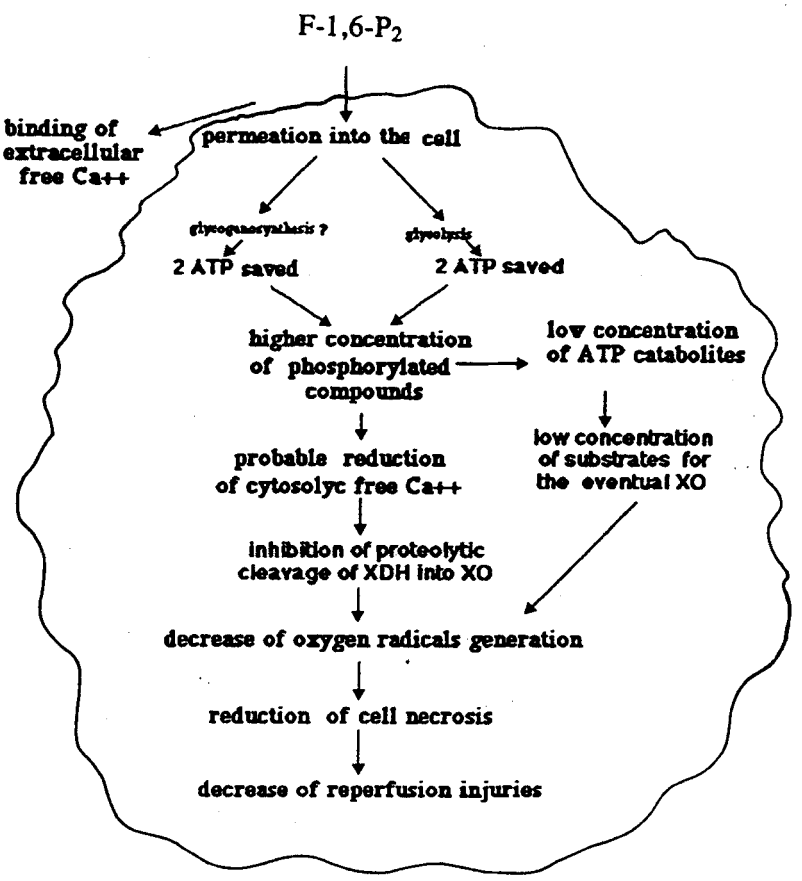

FIGURE 3 Possible scheme for F-1,6-P_2 beneficial activity in ischemic and reperfused tissues. Inhibition of ATP degradation should be a consequence of F-1,6-P_2 permeation into the cells. F-1,6-P_2 should then result as an energy saving substrate by the fact that two molecules of ATP are saved through its glycolytical metabolization, and two other ATP molecules may be saved during glycogenosynthesis taking F-1,6-P_2 as the starting point. The overall protection of ischemia-induced molecular damages exerted by F-1,6-P_2 might be completed by extracellular binding of free Ca^{2+} which should contribute to inhibit the proteolytic transformation of XDH into XO and the onset of the calcium paradox. Therefore, the general result should be a protection of high-energy metabolism of non-ischemic areas of ischemic tissues which should reduce reperfusion related risks.

beneficial effects in ischemia and reperfusion (possible inhibition of: onset of the Ca^{2+} paradox, and of transformation of XDH into XO, and possible improvement of myocardial functional recovery due to increase of high-energy compounds and myocardial efficiency).

We have recently carried out experiments in the isolated normoxic rat heart perfused either with [U-^{14}C]F-1,6-P_2 or with [U-^{14}C]fructose in order to evaluate the eventual uptake of F-1,6-P_2 by the myocytes. On the basis of the values of $^{14}CO_2$ and ^{14}C-lactate, which resulted about 10 times higher in the F-1,6-P_2-perfused hearts, we determined that 0.89 μmole/min/g d.w. of F-1,6-P_2 are oxidized to CO_2 and 6.19 μmol/min/g d.w. are transformed to lactate.[75]

From these data, that clearly indicate the permeation into the cells of a given amount of F-1,6-P_2 in its unalterated form, we may propose the scheme reported in Figure 3 representing a probable mechanism for F-1,6-P_2 protection towards ischemia and reperfusion damages.

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