Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Effects of fructose-1,6-diphosphate on concentration of calcium and activities of sarcoplosnic Ca^{2+}-ATPase in cardiomyocytes of Adriamycin-treated rats

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Received Oct. 18, 2004; revision accepted Dec. 25, 2004

Abstract: Objective: To observe the effects of fructose-1,6-diphosphate (FDP) on serum levels of cardiac troponin I (cTnI) and creatine kinase-MB (CK-MB), as well as the concentration of calcium in cardiomyocytes (Myo[Ca^{2+}]) and activity of sarcoplosnic Ca^{2+}-ATPase ($SRCa^{2+}$-ATPase) in Adriamycin (ADR)-treated rats. Methods: Rats were intraperitoneally injected with ADR (2.5 mg/kg every other day for 6 times) and then with different dosages of FDP (every other day for twenty-one times). Bi-antibodies sandwich Enzyme linked immune absorption assay (ELISA) was performed to detect serum level of cTnI. CK-MB was detected by monoclonal antibody, Myo[Ca^{2+}] was detected by fluorescent spectrophotometry and the activity of $SRCa^{2+}$-ATPase was detected by inorganic phosphate method. Results: FDP (300, 600, 1200 mg/kg) significantly reduced the serum levels of cTnI and CK-MB, while at the same time decreased calcium concentration and increased $SRCa^{2+}$-ATPase activity in cardiomyocytes of ADR-treated rats (P<0.01). Conclusions: FDP might alleviate the cardiotoxic effects induced by ADR through decreasing calcium level as well as increasing $SRCa^{2+}$-ATPase activity in cardiomyocytes.

Keyword: Fructose-1,6-diphosphate, Adriamycin, Cardiomyocyte, Calcium, Sarcoplosnic reticulum Ca^{2+}-ATPase
doi:10.1631/jzus.2005.B0622 **Document code:** A **CLC number:** R331

INTRODUCTION

Although Adriamycin (ADR) has been widely used in tumor treatments since the 1960s, and is now considered as one of most potent antineoplastic agents, its clinical applications remain limited owing to a series of side-effects, of which the cardiotoxicity is thought to be a major and most destructive one. Maeda *et al*.(1998) found that the cardiomyocytes of rats were Ca^{2+}-overloaded and that the diastolic function could be remarkably damaged treatment with ADR, even if there were little changes in cardiac histology.

Studies have confirmed in recent years that fructose-1,6-diphosphate (FDP) might reduce the myocardial damages induced by ADR via regulating the activities of oxygen free radicals in cardiomyocytes (Maeda *et al*., 1998; Kang, 2003). Yet up to now there is no information on whether FDP could relieve the Ca^{2+}-overloaded state in ADR-treated cardiomyocytes. This study aims to determine the effects of FDP on serum levels of cardiac troponin I (cTnI) and creatine kinase-MB (CK-MB), as well as the concentration of calcium in cardiomyocytes (Myo[Ca^{2+}]) and activity of sarcoplosnic Ca^{2+}-ATPase ($SRCa^{2+}$-ATPase) in Adriamycin (ADR)-treated rats, so that knowledge on the mechanisms of FDP's effects against cardiotoxicity induced by ADR could be gained and the clinical applications of FDP can be further evaluated.

METHODS

Experimental animals

Wistar rats (n=40, 210~280 g, 20 males, 20 fe-

males) were supplied by Zhejiang Animal Experimental Center of China.

Drugs and agents

ADR was purchased from HAIHUA Pharmaceuticals of Zhejiang (catalog 020305) and FDP was supplied by HUAQI Pharmaceuticals of Beijing (catalog 020116). Fura-2/AM was purchased from Sigma Company Ltd. of American.

Groups and treatments

Rats were randomly divided into 5 groups, with 8 rats in each group. Rats in ADR group were intraperitoneally injected with ADR at dosage of 2.5 mg/kg every other day for 6 times, while in the ADR+FDP groups (I), (II), (III) they were intraperitoneally injected with ADR and then with FDP at dosage of 300, 600, 1200 mg/kg every other day for twenty-one times respectively. As a control group, rats were intraperitoneally injected with ADR and then with physiological saline following the same protocol. Twenty-four hours after treatments, rats were anesthetized by using mebumal sodium and then sacrificed; and the hearts were separated for detection of Myo[Ca^{2+}] level and activity of $SRCa^{2+}$-ATPase. Blood samples were collected from the tail vein to detect the serum level of cTnI and CK-MB.

The detection of serum level of cTnI and CK-MB

Bi-antibodies sandwich Enzyme linked immune absorption assay (ELISA) was applied to detect serum level of cardiac troponin I (cTnI). Creatine kinase-MB (CK-MB) was detected by immunoradiometric assay using monoclonal antibody.

The detection of Myo[Ca^{2+}]

The hearts of rats were moved to precooled Hank's solution (NaCl 8.006 g/L, KCl 0.4026 g/L, $MgCl_2$ 0.1.16 g/L, Hepes 2.3850 g/L, Glucose 1.800 g/L) for 5 min. Aorta cannula was separated on langendroff perfusion device for 5 min-perfusion of Hank's solution without calcium, then incubated in Hank's solution (containing 0.1% collagen enzyme) for 15 min, followed by a 5 min-perfusion of Hank's solution with low concentration of calcium. The hearts were treated at 37 °C and given continuous oxygen supply. Cardiomyocytes were then shaken in preheated and oxygen-supplied Hank's solution for 15 min and harvested to a concentration of 2×10^6 cells/L, while the concentration of calcium was adjusted to 1.2 mmol/L. Five μl Fura-2/AM solution at concentration of 1 mmol/L was added into the cell suspension, which was water bathed under 98% concentration of oxygen at 37 °C for 40 min, then washed with Hank's solution for three times followed by centrifugation (500 r/min, 1 min). Finally the cell suspension was adjusted to a concentration of 1.25×10^6 cells/ml for fluorometric quantitation. The fluorescence intensity (F) levels were measured by spectrophotometer (LC-240) with excitation and emission settings of 340 nm and 500 nm, respectively. The F_{max} and F_{min} were respectively detected after 0.1% Tritionx-100 and EDTA were added. The level of Myo[Ca^{2+}] was calculated using the formula as below: $[Ca^{2+}]_i=(F-F_{min})/(F_{max}-F)\times224$.

Detection of $SRCa^{2+}$-ATPase activity

The $SRCa^{2+}$-ATPase was prepared using the differential centrifugation method of Yang *et al*.(2002) Coomassie brilliant blue staining was used to quantify the concentration of $SRCa^{2+}$-ATPase and its activity was detected by inorganic phosphate method.

Statistical analysis

Data are expressed as the mean±standard error ($\bar{x}\pm s$). Statistical differences were determined by T test and linear correlation analysis.

RESULTS

Data and information on well-being of the rats

The animals showed no distress when injected with Adriamycin and/or fructose-1,6-diphosphate, their body weight decreased a little after the experiment, but there was no statistical significance ($P>0.05$).

Effect of FDP on levels of cTnI, CK-MB, Myo[Ca^{2+}] and activity of $SRCa^{2+}$-ATPase in myocardium of ADR-treated rats

The levels of cTnI, CK-MB and Myo[Ca^{2+}] in the ADR group were much higher, while the activity of $SRCa^{2+}$-ATPase decreased more significantly than those in the control group ($P<0.01$). When treated with FDP, the levels of cTnI, CK-MB and Myo[Ca^{2+}]

decreased and the activity of $SRCa^{2+}$-ATPase increased remarkably, showing statistical significance when compared with those in the ADR group ($P<0.01$, Table 1).

Correlation analysis of FDP with cTnI, CK-MB, Myo[Ca^{2+}] and $SRCa^{2+}$-ATPase

The effects of FDP on cTnI, CK-MB, Myo[Ca^{2+}] and $SRCa^{2+}$-ATPase were dose-dependent ($P<0.01$) (Table 2).

Correlation analysis of Myo[Ca^{2+}] with cTnI, CK-MB and $SRCa^{2+}$-ATPase

There was negative relationship between Myo[Ca^{2+}] and $SRCa^{2+}$-ATPsae ($P<0.01$), but positive relationship was found between Myo[Ca^{2+}] and cTnI or CK-MB ($P<0.01$, $P<0.05$, respectively) (Table 3).

DISCUSSION

The precise mechanisms of how ADR causes cardiotoxicity remains unclear even today, but evidences suggested free radicals play a pivotal role in the process (Yang *et al*., 2002). Increasing proofs indicated that calcium overload in myocardial cells could be closely correlated to ADR-induced cumulative cardiotoxitit (Li *et al*., 2002; Huang *et al*., 2003): ADR depolymerizes membrane phospholipids inlaid protein of myocardial cells, enhances the membrane permeability and increases the calcium influx; it also inhibits the activity of Na^+-K^+ ATPase, reduces the Na^+-K exchange and enhances the Na^+-Ca^{2+} exchange, therefore induces the calcium overload (Huang *et al*., 2003). At the same time, ADR down-regulates the calcium intake of sarcoplasmic reticulum, leading to an overload state of calcium in myocardial cells (Maeda *et al*., 1998).

Present studies showed that ADR might increase the serum levels of cTnI, CK-MB and Myo[Ca^{2+}]−a positive correlation was found between the latter and the former two−and decreased the activity of $SRCa^{2+}$-ATPase, whereas the effects of ADR was inhibited by FDP in a dose-dependant manner, suggesting that FDP could reduce the concentration of calcium and improve the activity of $SRCa^{2+}$-ATPase in myocardial cells, therefore alleviate the ADR-induced myocardial damages. The mechanisms are presumed to be as described below: (1) To enhance the energy supply of myocardial cells. When myocardial cells are injured because of anoxia, low-energy supply or other harmful factors, the extrinsic FDP may supply energies by producing substance such as ATP to participate in the myocardial metabolism (Zhou *et al*., 1999; Hua *et al*., 2003); (2) To reduce the reperfusion cardiomyopathy caused by oxygen free radicals by FDP's antioxidant effects (Maeda *et al*., 1998; Kang, 2003); (3) To relieve the calcium overload state. Although calcium is a key

Table 1 Effect of fructose-1,6-diphosphate (FDP) on levels of cTnI, CK-MB, Myo[Ca^{2+}] and activity of $SRCa^{2+}$-ATPase in myocardian of ADR-treated rats ($\bar{x} \pm s$, n=5)

Group	cTnI (ng/ml)	CK-MB (μg/L)	Myo[Ca^{2+}] (mmol/L)	$SRCa^{2+}$-ATPase (μmol pi/(mg protein·min))
Control	0.48±0.17	172±21.31	119±5.09	408±11.21
ADR (2.5 mg/kg)	5.03±2.26*	612±57.15*	289±12.17*	167±9.45*
ADR+FDP (I) (2.5 mg/kg, 300 mg/kg)	2.28±1.43#	431±32.27#	217±9.82#	221±11.13#
ADR+FDP (II) (2.5 mg/kg, 600 mg/kg)	1.31±0.94†	302±34.41†	173±10.06†	285±8.06†
ADR+FDP (III) (2.5 mg/kg, 1200 mg/kg)	0.73±0.24+	216±26.17†+	143±8.53+	368±12.53+

*$P<0.01$ vs control group; #$P<0.01$ vs ADR group; †$P<0.01$ vs ADR+FDP (I) group; +$P<0.01$ vs ADR+FDP (II) group; pi: inorganic phosphate

Table 2 Correlation analysis of FDP with cTnI, CK-MB, Myo[Ca^{2+}] and $SRCa^{2+}$-ATPase

	cTnI	CK-MB	Myo[Ca^{2+}]	$SRCa^{2+}$-ATPase
FDP	–0.913*	–0.874*	–0.925*	0.831*

*$P<0.01$ vs FDP

Table 3 Correlation analysis of Myo[Ca^{2+}] with cTnI, CK-MB and $SRCa^{2+}$-ATPase

	cTnI	CK-MB	$SRCa^{2+}$-ATPase
Myo[Ca^{2+}]	0.817*	0.632#	–0.819*

*$P<0.01$ vs cTnI and $SRCa^{2+}$-ATPase; #$P<0.05$ vs CK-MB

factor in mediating the excitement activities of myocardial cells, an extraordinary high concentration of calcium in myocardial cells will result in Ca^{2+}-overload and myocardial function failure. FDP enhances the synthesis of ATP and calcium transportation so that the concentrations of calcium in myocardial cells could be controlled (Hua *et al*., 2003). A study (Galzigna *et al*., 1989) revealed that besides its transmembrane activity, FDP might combined with the membrane of myocardial cells to inhibit the Ca^{2+} influx under conditions of anoxia or other myocardial injuries. The result is confirmed by Bickler and Kellecher (1992) who worked on cortex cells and astrocytes of rats by fluorescent probe technique.

In conclusion, we provide evidence that ADR leads to a higher serum levels of cTnI and CK-MB, which may in part be related with calcium overload in myocardial cells. FDP reduces the high level of calcium in myocardial cells, increases the activity of $SRCa^{2+}$-ATPase, and finally alleviate the myocardial injuries caused by ADR.

References

Bickler, P.E., Kellecher, J.A., 1992. Fructose-1,6-diphosphate stabilizes brain intracellular calcium during hypoxia in rats. *Stroke*, **23**:1617-1622.

Galzigna, L., Rizzoli, V., Bianchi, M., Rigobello, M.P., Scuri, R., 1989. Some effects of fructose-1,6-diphosphate on rat myocardial tissue related to amembeane-stabilixing action. *Cell Biochem Function*, **7**:91-96.

Hua, D., Zhuang, X., Ye, J., Wilson, D., Chiang, B., Chien, S., 2003. Using fructose-1,6-diphosphate during hypothermic rabbit-heart preservation: A high-energy phosphate study. *J Heart Lung Transplant*, **22**:574-582.

Huang, X.M., Zhu, W.H., Kang, M.L., 2003. Study on the effect of doxorubicin on expressions of genes encoding myocardiac sarcoplasmic reticulum Ca^{2+} transport myocardiac protection in rabbits. *J Zhejiang Univ Sci*, **4**:114-120.

Kang, Y.J., 2003. New understanding in cardiotoxicity. *Curr Opin Orag Discov Devel*, **6**:110-116.

Li, T., Danelisen, I., Singal, P.K., 2002. Early changes in myocardial antioxidant enzymes in rats treated with Adriamycin. *Mol Cell Biochem*, **232**:19-26.

Maeda, A., Honda, M., Kuramochi, T., Takabatake, T., 1998. Doxotabicin cardiotoxicity: Diastolic cardiac calcium handing in isolated cardiac myocytes. *Jpn Circ*, **62**:505-511.

Yang, G.M., Li, S.Q., Ye, S.Y., Li, J.L., Lin, S.X., 2002. The effects of fructose 1,6-diphosphate on tyrosine-nitro of cardiomyocytes in rats treated with Adriamycin. *Chin Pharmcol Bull*, **18**:161-165.

Zhou, Y., Wang, P., Lei, Z., He, H., Zhu, Z., 1999. Beneficial effects of fructose 1,6-diphosphate on hemorrahogic shock in rats. *Chin J Traumatol*, **15**:22-24.