

Short Report

The Mechanism of Preventive Effect of Captopril on Renal Ischemia Reperfusion Injury is Independent of ATP Dependent Potassium Channels

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ABSTRACT

Background: Renal ischemia reperfusion (IR) injury has been a major source of concern during the past decades and angiotensin converting enzyme (ACE) inhibitors have been successfully used to prevent this injury. There have been some controversial reports about the involvement of K_{ATP} channels in the mechanism of action of ACE inhibitors. In this study, we examined the effect of K_{ATP} channel blocker (Glibenclamide) on preventive effect of captopril on renal IR injury. **Methods:** Male sprague-dawley rats were pretreated with glibenclamide (1, 5 and 25 mg/kg) and/or captopril (5 mg/kg). They were anesthetized using ketamine (50 mg/kg) and xylazine (10 mg/kg). The left flank was incised and the left renal artery was clamped for 30 minutes. After that, the kidney was reperfused for 2 hours and then the animal was killed. The Right and left kidneys were removed and evaluated for microscopic damage. **Results:** Captopril reduced renal IR injury while glibenclamide by itself caused no change. Glibenclamide did not change the preventive effect of captopril. **Conclusion:** It seems that the preventive effect of captopril is not directly mediated by K_{ATP} channels and further attention should be paid to other receptor-mediated angiotensin II effects. *Iran. Biomed. J. 12 (4): 241-245, 2008*

Keywords: Angiotensin converting enzyme (ACE) inhibitors, Angiotensin II, Captopril, Glibenclamide, K_{ATP} channels

INTRODUCTION

Reperfusion is known to produce ischemic and hypoxic lesions *in vivo* and *in vitro* in different organs [1]. Several factors have been implicated in the pathophysiological changes of ischemia reperfusion (IR) injury [2]. There has been great interest in the role of renin angiotensin system and in particular angiotensin II (Ang II) in cardiovascular and renal physiology and pathology. Until the last decade, this interest was directed only towards the haemodynamic effect of angiotensin as a vasoactive peptide [3]. The discovery of angiotensin converting enzyme (ACE) inhibitors added a new dimension to our understanding of non-hemodynamic effects of Ang II [4-6]. Captopril, a ACE inhibitor, has been successfully used in

protecting against IR induced renal injury.

In general, Ang II stimulation facilitates the protein kinase C (PKC) activation. The involvement of PKC pathway for the regulation of K_{ATP} , however, remained controversial [7-11]. K_{ATP} Channels [12] activate and serve as metaboelectrical sensors, thereby regulate the duration of action potential, Ca^{2+} influx, and myocardial contractility [13]. The activation of the channels minimizes the infarct size in several animal models [14]. K_{ATP} channel regulation of vasoactivity in vascular beds, including the renal medulla, has been examined by testing the ability of glibenclamide to lower blood flow. Gardiner and colleagues [15] found that glibenclamide infusion into rats induced renal vasoconstriction. In the kidney, a role for modulation of arteriolar tone by K_{ATP} channels has

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been repeatedly observed [16, 17]. There have been some controversial reports about the involvement of K_{ATP} channels in the mechanism of action of ACE inhibitors. Some believe that ACE inhibitors' mechanism of action is mediated by K_{ATP} channels [18] while others disagree with this concept [19]. In this study, we examined the effect of captopril on renal IR injury in the presence and absence of glibenclamide (K_{ATP} channel blocker).

MATERIALS AND METHODS

Animals. Male sprague-dawley rats weighting 150 to 200 grams with free access to food and water, were divided into 9 groups each containing 7 rats (Table 1). Animals were pretreated with intraperitoneal glibenclamide (1, 5 and 25 mg/kg) 2 hours before surgical procedure and/or subcutaneous captopril (5 mg/kg) one hour before surgical procedure. We measured blood pressure in all groups before ischemia.

Surgical procedure. The surgical procedure was based on two kidneys one clamped (2K1C) model. Animals were anesthetized using xylazine (10 mg/kg) and ketamine hydrochloride (50 mg/kg) intraperitoneally. In sham operation group, the left flank was incised and then closed without any further manipulation. In other groups, the left flank was incised and the left renal artery was exposed. Then, it was clamped using an arterial clamp (Ischemic phase). The skin was temporarily closed and animal was kept in a suitable warm environment. After 30 minutes of ischemia, the arterial clamp was removed and the skin was closed (Reperfusion

phase). Two hours later, animal was killed and both kidneys were removed and fixed in 10% formalin.

Study groups. Animals were randomly assigned to one of the following groups of 7 rats each. Group I was the control group (sham operated). Group II received normal saline by the subcutaneous route 2 hours before the occlusion of renal artery. Group III received 5 mg/kg captopril, groups IV, V and VI received glibenclamide 1, 5 and 25 mg/kg, respectively before IR and groups VII, VIII and IX received glibenclamide 1, 5 and 25 mg/kg, respectively before pretreatment with captopril 5 mg/kg and then IR was induced.

Pathological evaluation. The samples were stained using H and E technique and evaluated for microscopic damage. The left kidney was compared to the right kidney (control) and reperfusion injury was graded based on its severity as follow: 0, No damage seen; 1, Necrosis of individual cells; 2, Necrosis of all adjacent proximal convoluted tubules, with survival of surrounding tubules; 3, Necrosis confined to the distal third of the proximal convoluted tubules with a band of necrosis extending into the inner cortex; 4, Necrosis affecting all the three segments of the proximal convoluted tubules [20].

Drugs. Captopril was purchased from Quimica Sintetica, S.A., Spain. Glibenclamide was purchased from Tehran-Darou Pharm. Co., Iran. Xylazine and Ketamine hydrochloride were purchased from Alfason-Woerden, Holland.

Table1. Grading the effects of different doses of captopril and glibenclamide on ischemia reperfusion-induced renal injury in rats.

Group	Treatment	Grading the Histological severity				
		0	1	2	3	4
I	Saline + SO	7	0	0	0	0
II	Saline + IR ^S	0	0	0	7	0
III	Capto 5 mg/kg + IR ^S	0	5	2	0	0
IV	Gli 1mg/kg + IR ^{NS}	0	0	1	6	0
V	Gli 5mg/kg + IR ^{NS}	0	0	1	6	0
VI	Gli 25mg/kg + IR ^{NS}	0	0	0	6	1
VII	Gli 1mg/kg + Capto 5mg/kg + IR ^{NS}	0	6	1	0	0
VIII	Gli 5mg/kg + Capto 5mg/kg + IR ^{NS}	0	5	2	0	0
IX	Gli 25mg/kg + Capto 5mg/kg + IR ^{NS}	0	6	1	0	0

SO, sham operation; IR, ischemia reperfusion; Capto, captopril; Gli, glibenclamide; S, significant difference; NS, non-significant difference. Group II was compared to group I. Groups III, IV, V, VI were compared to group II. Groups VII, VIII, IX were compared to group III.

Statistical evaluation. Mann-Whitney mean rank test was used to assess the significance of differences between treated groups. Differences among groups were considered significant at P value of ≤ 0.05 . Calculations were performed using the SPSS 14 statistical package.

RESULTS

Subcutaneous injection of saline and subsequent sham operation caused no renal injury. Saline and subsequent ischemia and reperfusion caused renal injury with pathologic grades of 3 and 4. Captopril (5 mg/kg) reduced the IR injury to grade 1 and this reduction was statistically significant ($P < 0.05$) compared to IR group. Glibenclamide itself (1, 5 and 25 mg/kg) could not change the grading of renal injury, while pretreatment with glibenclamide (1, 5 and 25 mg/kg) caused no change in protective effect of captopril (Table 1). Indeed, we have not seen any change in blood pressure of studied rats.

DISCUSSION

In this study, we found out that IR caused severe renal injury and captopril was able to prevent this damage. Ang II is a potent vasoconstrictor, known to activate channels that depolarize vascular smooth muscle and allow Ca^{2+} entry. Its effects in reducing the activity of K^+ channels should enhance its action in causing membrane depolarization, and so contribute to its vasoconstrictor action, since voltage-activated Ca^{2+} channels, and so Ca^{2+} entry are highly sensitive to small changes in membrane potential. Further, it is clear that the activity of such channels contributes to resting blood flow in a number of vascular beds including those of the coronary, mesenteric, skeletal muscle and renal circulations, since glibenclamide increases resistance to blood flow in those tissues. Since K_{ATP} channel activity is increased by a number of vasodilators and by metabolic stress such as hypoxia, the potential for inhibition by Ang II to reduce K^+ conductance should also be increased under such conditions. Thus, the action of Ang II in inhibiting K_{ATP} channels via activation of PKC described here may make an important contribution to the vasoconstrictor action of the peptide [18, 21].

Antagonization of Ang II action by ACE inhibitors improves the symptoms of congestive heart failure and reduces reperfusion injury and arrhythmias [22-

24]. Using the rat model, Linz et al demonstrated that ACE inhibitors reduce both ischemic areas at risk and zones of infarcted muscle. The inhibitory pathway of K_{ATP} may be one of several actions of Ang II and in part account for the cardioprotective and renoprotective actions of ACE inhibitors [25-27].

There have been some controversial reports that ACE inhibitors' mechanism of action is/isn't mediated by K_{ATP} channels [18, 19]. The results of this study showed that the protective effect of captopril (ACE inhibitor) is not affected by glibenclamide (K_{ATP} channel blocker). One might propose that the protective effect of captopril is not mediated by K_{ATP} channels but some data suggest that there might be some sort of relationship between these two. Sargent et al have indicated that SH containing ACE inhibitors (captopril like) show an effect on K_{ATP} channel open probability in vascular smooth muscle but Koppel *et al.* [19] showed that there is little or no effect on K_{ATP} channels due to the presence of SH branch. This protective effect of captopril did not change in the presence of glibenclamide (inhibitor K_{ATP} channel). From our experiments, we conclude that the postulated opening of K_{ATP} channels by SH-group-containing ACE inhibitors contributes to the vasodilation of vascular/microvascular kidney caused by ACE inhibitors, and that SH groups have no influence upon K_{ATP} channels of vascular/microvascular kidney. Also, our recent study showed that ACE inhibitor was able to attenuate kidney IR injury and this preventive effect may be independent of Ang II AT1 receptors [28]. It seems logical to expect some kind of relationship between ACE inhibitors and K_{ATP} channels. Our study indicates that no direct relationship exists between these two but further exploration focused on other mechanisms of Ang II receptor-mediated function might be the answer to this mystery.

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REFERENCES

1. Cio, H., Kodak, Y., McElroy, P., Craig, L., Housini, I. and Star, R.A. (1997) α -melanocyte-stimulating hormone protects against renal injury after ischemia

- in mice and rats. *J. Clin. Invest.* 99:1165-1172.
2. Maxwell, S.R.J. and Lip, G.Y.H. (1997) Reperfusion injury: a review of the pathophysiology, clinical manifestation and therapeutic options. *Int. J. Cardiol.* 58: 95-117.
 3. Adam, A. and Raij, L. (2000) Nitric oxide-angiotensin II axis in renal and cardiovascular injury. *J. Nephrol.* 13: 211-220.
 4. Wolf, G. (1999) Angiotensin II: a pivotal factor in the progression of renal diseases. *Nephrol. Dial. Transplant.* 14 Suppl 1: 42-44.
 5. Maschio, G., Alberti, D., Janin, G., Locatelli, F., Mann, J.F., Motolese, M., Ponticelli, C., Ritz, E. and Zucchelli, P. (1996) Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The angiotensin-converting-enzyme inhibition in progressive renal insufficiency study group. *N. Engl. J. Med.* 334: 939-945.
 6. Wolf, G. and Ziyadeh, F.N. (1997) The role of angiotensin II in diabetic nephropathy: emphasis on nonhemodynamic mechanisms. *Am. J. Kidney. Dis.* 29: 153-163.
 7. Wang, Y.G. and Lipsius, S.L. (1995) Acetylcholine activates a glibenclamide-sensitive K^+ current in cat atrial myocytes. *Am. J. Physiol.* 268: H1322-H1334.
 8. Light, P.E., Allen, B.G., Walsh, M.P. and French, R.J. (1995) Regulation of adenosine triphosphate-sensitive potassium channels from rabbit ventricular myocytes by protein kinase C and type 2A protein phosphatase. *Biochemistry* 34: 7252-7257.
 9. Baker, K.M., Singer, H.A. and Aceto, J.F. (1989) Angiotensin II receptor-mediated stimulation of cytosolic-free calcium and inositol phosphates in chick myocytes. *J. Pharmacol. Exp. Ther.* 251: 578-585.
 10. Hu, K., Duan, D., Li, G.R. and Nattel, S. (1996) Protein kinase C activates ATP-sensitive K^+ current in human and rabbit ventricular myocytes. *Circ. Res.* 78: 492-498.
 11. Light, P.E., Sabir, A.A., Allen, B.G., Walsh, M.P. and French, R.J. (1996) Protein kinase C-induced changes in the stoichiometry of ATP binding activate cardiac ATP-sensitive K^+ channels: a possible mechanistic link to ischemic preconditioning. *Circ. Res.* 79: 399-406.
 12. Noma, A. (1983) ATP-regulated K^+ channels in cardiac muscle. *Nature* 305:147-148.
 13. Nabika, T., Velletri, P.A. and Lovenberg, W. (1985) Increase in cytosolic calcium and phosphoinositide metabolism induced by angiotensin II and [Arg] vasopressin in vascular smooth muscle cells. *J. Biol. Chem.* 260: 4661-4670.
 14. Gross, G.J. and Auchanpach, J.A. (1992) Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ. Res.* 70: 223-233.
 15. Gardiner, S.M., Kemp, P.A., March, J.E., Fallgren, B. and Bennett, T. (1996) Effects of glibenclamide on the regional haemodynamic actions of -trinitro and its influence on responses to vasodilators in conscious rats. *Br. J. Pharmacol.* 117: 507-515.
 16. Sadowski, J., Kompanowska-Jezierska, E., Dobrowolski, L., Walkowska, A. and Badzyna, B. (1997) Simultaneous recording of tissue ion content and blood flow in rat renal medulla: evidence on interdependence. *Am. J. Physiol. Renal. Physiol.* 273: F658-F662.
 17. Cao, C., Lee-Kwon, W., Silldorff, E.P. and Pallone, T.L. (2005) K_{ATP} channel conductance of descending vasa recta pericytes. *Am. J. Physiol. Renal. Physiol.* 289: F1235-F1245.
 18. Hayabuchi, Y., Davies, N.W. and Standen, N.B. (2001) Angiotensin II inhibits rat arterial K_{ATP} channels by inhibiting steady-state protein kinase A activity and activating protein kinase C. *J. Physiol.* 530: 193-205.
 19. Koppel, H., Gasser, R., Klein, W. and Holzmann, S. (1996) ACE inhibitors with SH groups have no effect of ATP-dependent K channels--a dissertation. *Acta. Med. Austriaca.* 23: 109-112.
 20. Jablonski, P., Howden, B.O., Rae, D.A., Birrell, C.S., Marshall, V.C. and Tange, A.J. (1983) Experimental model for assessment of renal recovery from warm ischemia. *Transplantation* 35: 198-204.
 21. Kubo, M., Quayle, J.M. and Standen, N.B. (1997) Angiotensin II inhibition of ATP-sensitive K^+ currents in rat arterial smooth muscle cells through protein kinase C. *J. Physiol.* 503: 489-496.
 22. Thous, D.W., Baker, K.M., Ayers, C.R., Vaughan, E.D. and Carley, R.M. (1980) Acute and chronic effect of captopril in hypertensive patients. *Hypertension* 2: 567-575.
 23. Elfellah, M.S. and Oglive, R.I. (1985) Effect of vasodilator drugs on coronary occlusion and reperfusion arrhythmias in anaesthetized dogs. *J. Cardiovasc. Pharmacol.* 7: 826-832.
 24. Van Gilst, W.H., De Graeff, P.A., Wesseling, H. and de Langen, C.D.J. (1986) Reduction of reperfusion arrhythmias in the ischemic isolated rat heart by angiotensin converting enzyme inhibitors: a comparison of captopril, enalapril and HOE 498. *J. Cardiovasc. Pharmacol.* 8: 722-728.
 25. Hirakata, H., Found-Tarazi, F.M., Bumpus, F.M., Khosla, M. and Healy, B. (1990) Angiotensins and the failing heart: enhanced positive inotropic response to angiotensin-I in cardiomyopathic hamster heart in the presence of captopril. *Circ. Res.* 66: 891-899.
 26. Urata, H., Kinoshita, A., Misono, K.S., Bumpus, F.M. and Fusain, A. (1990) Identification of a highly specific chymase as the major angiotensin-II-forming enzyme in the human heart. *J. Biol. Chem.* 265: 22348-22357.
 27. Yatsu, T., Aoki, M., Uchida, W. and Inagaki, O.

- (2005) Comparison between YM099 and captopril in rats with renal mass reduction-induced progressive renal disease. *Biol. Pharm. Bull.* 28: 367-369.
28. Pazoki-Toroudi, H.R., Hesami, A., Vahidi, S., Sahebjam, F., Seifi, B. and Djahanguiri, B. (2003) The preventive effect of captopril or enalapril on reperfusion injury of the kidney of rats is independent of angiotensin II AT1 receptors. *Fundam. Clin. Pharmacol.* 17 (5): 595-598.