Effect of 3,5-diiodothyropropionic Acid (DITPA) on Renal Ischemia/ Reperfusion Injury in a Mouse Model

Najah R. Hadi [1], Ph.D, FRCP, FACP; Fadhil G. Yousif [2], FACS, FRCS, MD; Ayad A. Hussein [1] B.Sc
[1]Department of pharmacology and therapeutics, College of Medicine, University of Kufa.
[2]Department of cardiovascular surgery, College of Medicine, University of Kufa.
Key words: renal, ischemia/ reperfusion, TH, DITPA.

الخلاصة
تمهيد: الفشل الكلوي الحاد من المشاكل السريرية المهمة مع معدلات أمراضية و وفيات عالية. احد الأسباب الأساسية للفشل الكلوي الحاد هو نقص الإرواء الدموي /أعادة الإرواء(ن/ا). يعتقد أن العملية الالتهابية والإجهاد ألتأكسدي من الأسباب الرئيسية المسببة لذلك ن/ا. DITPA هو مثيل صناعي للهرمون الدرقي بفعالية ايضية قليلة ولهو (بالإضافة إلى الهرمون الدرقي) أدلة على تأثيرات محسنة على ن/ا في القلب من خلال تعديل إرسال الإشارات الخلوية المستجيبة إلى أجهاد نقص الإرواء الدموي و هذا التعديل قد يكون مفيدا في الحماية من ضرر ن/ا الكلوي .
الأهداف: أن الهدف من الدراسة الحالية هو لتحديد تأثيرات DITPA على الإصابة الكلوية بنقص الإرواء الدموي /أعادة الإرواء والاعتلال الوظيفي الكلوي اللاحق في نموذج الفار.
طرق العمل: تم توزيع ثمانية عشر ذكرا بالغا من الفئران البيضاء السويسرية العرق توزيعا عشوائيا على ثلاث **مجاميع:**
- مجموعة ن/ا (٦ فئران): ٣٠ دقيقة من نقص الارواء الدموي الكلوي الثنائي الجانب ثم ٤٨ ساعة من اعادة الارواء.
- مجموعة التظاهر (٦ فئران):اي التظاهر بنقص الارواء حيث ان الفئران اخضعت لنفس طرق التخدير والعملية من دون احداث نقص الارواء .
- المجموعة المعالجة بDITPA: (٦ فئران) ن/ا + DITPA(٣,٧٥ملغ/كغم) بالحقن داخل غشاء البريتون نصف ساعة قبل نقص الإرواء و جرعة ثانية في بداية إعادة الإرواء.
في نهاية فترة اعادة الارواء تمت التضحية بالفئران ، تم أخذ نماذج الدم من القلب مباشرة لتحديد مستوى IL-18, IL-6 ، اليوريا و الكرياتينين في مصل الدم و ايضا تم نزع كلا الكليتين ،اخذت اليمنى ومزجت ثم تم تحديد معاييير الإجهاد التاكسدي (MDA & GSH) . اما الكلية اليسرى فقد عوملت بالفورمالين للفحص النسيجي.
النتائج: في نهاية الدراسة وجد ان مستوياتIL-6, IL-18 واليوريا والكرياتينين في مصل الدم وMDA في الكلية وحاصل نقاط عد التغيرات النسيجية كانت اعلى معنويا ($p< ٠.٠٥$)في مجموعة ن/ا من تلك في مجموعة التظاهر. مستوى GSH في الكلية نقص معنويا ($p< ٠.٠٥$)في مجموعة ن/ا عن ذلك في مجموعة التظاهر.DITPA لم يحرز تغيرات معنوية ($p> ٠.٠٥$) على مستويات كل معايير الدراسة عند المقارنة مع مجموعة ن/ا.
الاستنتاج: أظهرت نتائج الدراسة الحالية إن DITPA لم يقلل الضرر الكلوي الناتج من نقص الإرواء الدموي /أعادة الإرواء معنويا.

Abstract
Introduction: Acute renal failure (ARF) is an important clinical problem with a high mortality and morbidity. Ischemia/reperfusion (I/R) was considered one of the primary causes of ARF. Inflammatory process is thought to be the major mechanism that contribute to I/R injury.

There are evidences of the importance of thyroid hormone (TH) in the response of the myocardium to ischemic stress and cardiac remodeling following myocardial infarction. 3,5-diiodothyropropionic acid (DITPA) is a synthetic TH analogue with a low metabolic activity. Also DITPA have evidences of improving effects on I/R in heart through modulation of cellular signaling in response to ischemic stress and this modulation may be beneficial in protection from renal I/R damage.

Objectives: The objective of present study was to assess the effects of DITPA on renal I/R injury and the resulted kidney dysfunction in a mouse model.

Materials and Methods: A total of 18 Adult males of Swiss albino mice were randomized to three groups: I/R group (n=6), mice underwent 30 minute bilateral renal ischemia and 48 hr reperfusion. Sham group (n=6), mice underwent same anesthetic and surgical procedures except for ischemia induction. DITPA treated group: (n=6), I/R + DITPA (3.75 mg/kg) by intraperitoneal injection.
After the end of reperfusion phase mice were sacrificed, blood samples were collected directly from the heart for determination of serum IL-18, IL-6, urea and Creatinine. Both kidney were excised, the right one homogenized for oxidative stress parameters (MDA and GSH) measurements and the left kidney fixed in formalin for histological examination.

Results: Serum IL-18, IL-6, urea and Creatinine, kidney MDA levels and scores of histopathological changes were significantly ($P< 0.05$) elevated in I/R group as compared with that of sham group. Kidney GSH level was significantly ($P < 0.05$) decreased in I/R group as compared with that of sham group. DITPA caused non-significant ($P>0.05$) changes in levels of all study parameters as compared with that of I/R group.
Conclusion: The results of the present study show that DITPA insignificantly ameliorated kidney damage resulted from I/R.

Introduction

Acute renal failure (ARF) is an important clinical problem with a high mortality and morbidity. It affects 5% of hospitalized patients and has a mortality rate of approximately 50%[1]. One of the primary causes of ARF is ischemia/reperfusion (I/R), a drop in blood flow leading to inadequate supply of oxygen and nutrients to renal tissue which can be caused by, amongst others, surgery, organ transplantation and shock[1].

In injury due to ischemia, neutrophil stimulation with accompanying oxygen radical-mediated injury is the main event that leads to injury; under ischemic conditions, reduced oxygen supply leads to enhanced neutrophil adherence to tubular endothelial cells[2-4]due to increased surface expression of adhesion molecules on tubular endothelial cells[2,4,5]. This ultimately results, on reperfusion, in diapedesis of neutrophils and their oxidative burst, which results in oxygen radical production[6,7]. So in addition to the direct cytotoxic effects of hypoxia, renal I/R induces an inflammatory reaction within the renal parenchyma[8] by causing renal synthesis of pro-inflammatory cytokines such as Interleukin (IL)-1, IL-6, IL-18, and tumor necrosis factor (TNF)-α[9-12].

Studies in animal and cell-based models give evidences that acute or chronic pretreatment with TH before I/R can protect from I/R injury[13]. The protective effect of acute T_3 pretreatment was shown in an ex vivo canine heart preparation and in another study a model of rat heart in which cardiac work and cardiac efficiency were found to increase after no flow global I/R[14,15] and similarly, long-term pretreatment with l-thyroxine for 2 weeks or T_3 for 10 days was also shown to improve postischaemic recovery of function in isolated rat hearts subjected to zero-flow global I/R[16,17].

This protective effect may occur by attenuating the I/R-induced activation of the pro-apoptotic p38 Mitogen-activated protein kinases (MAPKs) by either negative

regulation of Protein kinase C (PKCδ) or by decreased phosphorylation due to reduced ATP availability (due to reduced glycogen content which occurs during ischemia in hyperthyroid hearts) because stress kinases cannot be phosphorylated by upstream kinases without ATP[18]. Heat shock proteins (HSP)70 overexpression negatively regulates I/R-induced c-jun N-terminal kinases (JNKs) activation. Phosphorylation of HSP27 has a protective effect by stabilizing cytoskeleton and/or inhibiting apoptosis[18]. The mechanism that leads to overexpression of HSPs may be likely due to the direct TH genomic effect or a secondary effect due to mild oxidative stress[18].

3,5-diiodothyropropionic acid (DITPA) is a synthetic TH-related compound with low metabolic activity which binds both nuclear TR isoforms with decreased affinity (Kd, 10^{-7} M)[19]. Also DITPA binds integrin αVß3 through which it mediate angiogenesis[20]. Administration of DITPA after myocardial infarction in rabbits improved post-infarct recovery function[21]. Also In a rabbit post-infarction model, use of DITPA prevented abnormal sarcoplasmic reticulum Calsium transport and abnormal contractile function associated with myocardial infarction[22].

Based on these encouraging results about the effect of DITPA in experimental models of I/R in heart, in this study the effect of DITPA on I/R in kidney was studied.

Methods

A total of 18 adult male of Swiss Albino mice weighing 30-35 g, were purchased from Animal Resource Center, the Institute of embryo research and treatment of infertility, Al-Nahrain University. Animals were housed in the animal house of College of medicine/Al Kufa Univercity in a temperature-controlled (24 ± 2 °C) room with alternating 12-h light/12-h dark cycles and were allowed free access to water and diet until the start of experiments. After one week of acclimatization the mice were randomized into three groups (6 mice in each group) as follow:

Ischemia/reperfusion group: Mice underwent ischemia for 30 minute then reperfusion for 48 hours.

Sham group: Mice received 0.1 N NaOH; mice underwent the same anesthetic and surgical procedures (for an identical period of time for ischemia and reperfusion) except for ischemia induction .

DITPA treated group: Mice received DITPA 3.75 mg/kg intraperitoneal injection 30 min before the induction of ischemia, and the same dose was repeated just before reperfusion period. Solution of DITPA was prepared immediately before use by dissolving the powder in 0.1 N NaOH and diluting with 0.9% saline (pH 9)[23].

Induction of I/R

Animals were intraperitoneally anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine[24]. According to **Sharyo *et al* 2009**[25], after anesthesia, an abdominal incision was made and the renal pedicles were dissected bilaterally. A vascular clamp (Biotechno, Germany) was placed on each renal pedicle for 30 min. After clamps were released, the incision was closed in two layers with 2-0 sutures and mice were returned back to their cages and left for 48 hr for reperfusion. Sham operations were conducted using the same procedure without placing a clamp on each renal pedicle. After the end of reperfusion phase mice were sacrificed using over dosing of anesthesia, blood samples were taken directly from the heart ,both kidneys were excised, the right one homogenized then kept in deep freeze at – 80 °C for oxidative stress measurement and the left kidney fixed in 10% neutral buffered formalin for histological examination.

Inflammatory and kidney function markers

At 48 hr after ischemia induction, (at the end of reperfusion period), from each mouse about 1.5 ml of blood was collected from the heart. The blood samples were allowed to clot at 37^{o}C and centrifuged at 3000 rpm for 15 min; Sera were removed, and analyzed for determination of serum IL-18 (using ELISA kits of MBL, Japan), IL-6 (using ELISA kits of Immunotech, France), urea (using kit of BioMěrieux®sa , France) and Creatinine (using kit of Spinreact, Spain).

Histopathological evaluation

Renal sections were examined by light microscopy and scored according to a semiquantitative scale designed according to **Asaga *et al* (2008)**[26] to evaluate the severity of renal damage. This done by a pathologist who was unaware of the treatment conditions. The slide section divided into 10 intersections. A score from 0 to 3 was given for each tubular profile involving each intersection : 0 = normal histology; 1 = tubular cell swelling, brush border loss, nuclear condensation, with less than one-third of the tubular profile showing nuclear loss; 2 = same as for score 1, but greater than one-third and less than two-thirds of the tubular profile showing nuclear loss; and 3 = greater than two-thirds of the tubular profile showing nuclear loss. Then the total score for each kidney (section) was calculated by the summation of the all 10 scores for the all 10 intersections with a maximum Score of 30. According to the total severity score the kidney injury was Classified to normal (0), mild (1-10), moderate (11-20) and severe (21-30).

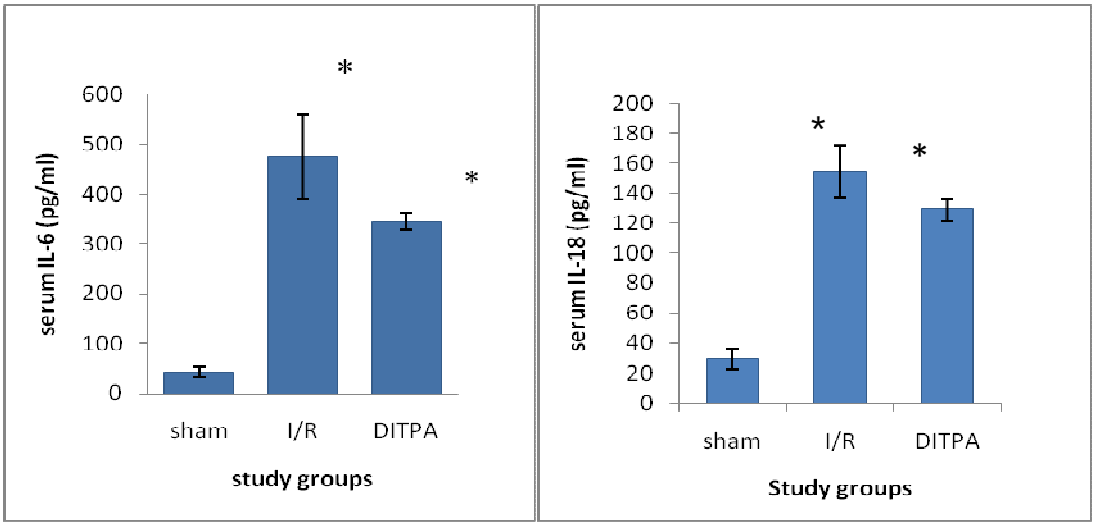
Statistical analysis

Statistical analyses were performed using SPSS 12.0 for windows.lnc. An expert statistical advice was sought for tests used. Data of quantitative variables were normally distributed (except for histopathological changes) and thus were expressed as mean ± SEM. Analysis of Variance (ANOVA) was used for the multiple comparisons among all groups followed by post-hoc tests using LSD method. For the histopathological renal changes the Mann-Whitney U was used to assess the statistical significance of difference between 2 groups in total severity score. In all tests, $P< 0.05$ was considered to be statistically significant.

Results

Effects on inflammatory parameters

I/R caused significant ($P< 0.05$) elevation in serum IL-18 and IL-6 levels as compared with that of sham group. DITPA insignificantly inhibited the elevated levels observed in I/R group.

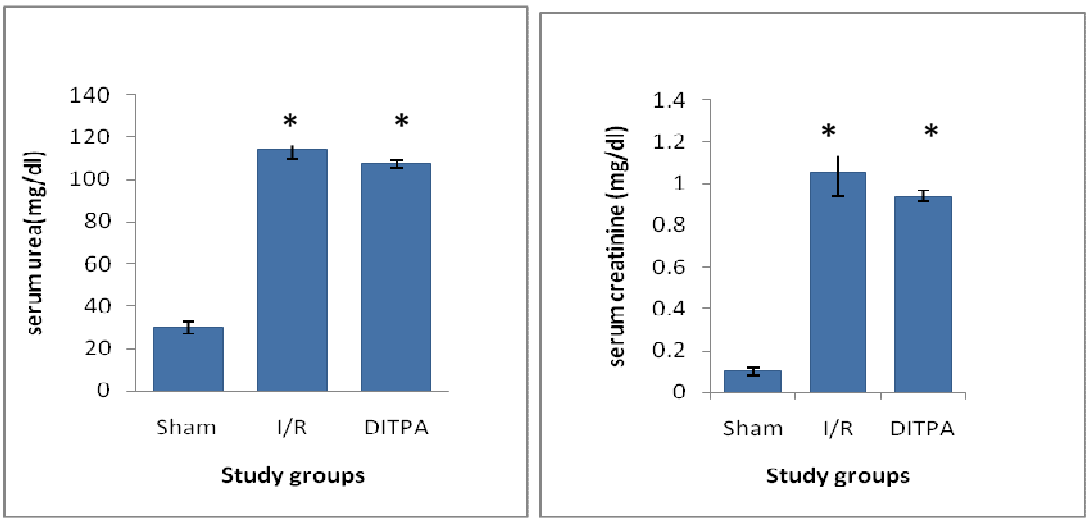


A B

Figure (1): Error bar charts show the difference in mean ±SEM values of serum IL-6 (A) and IL-18 (B) levels (pg/ml) in the three experimental groups at 48hr after surgical operation (N=6 in each group), * p<0.05 when compared with sham group, ** p<0.05 when compared with I/R group

Effects on kidney function parameters

I/R caused significant (P< 0.05) elevation in serum urea and creatinine levels as compared with that of sham group. DITPA insignificantly inhibited the elevated levels observed in I/R group.

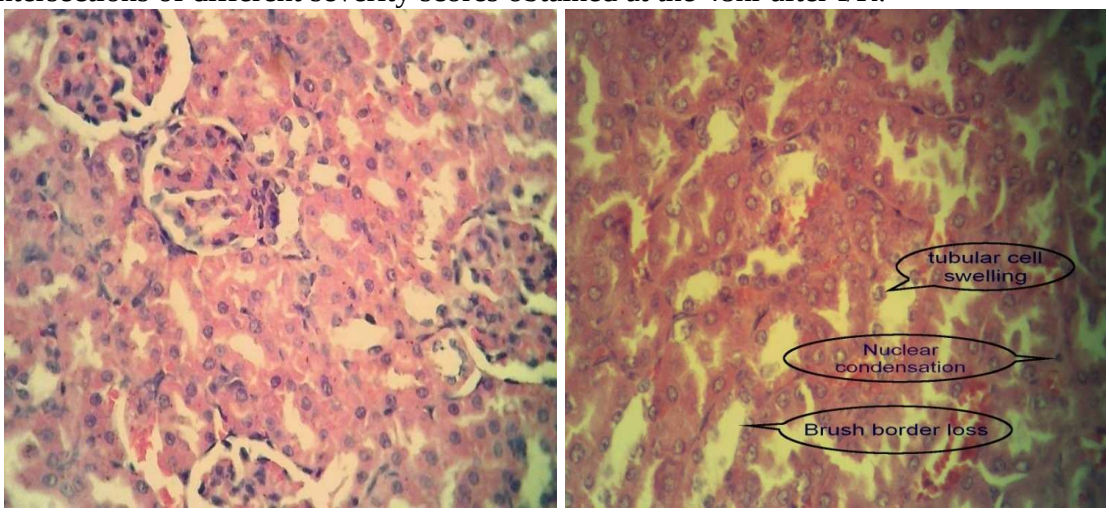


A B

Figure (2): Error bar charts show the difference in mean±SEM values of serum urea (A) and creatinine (B) levels (pg/ml) in the three experimental groups at 48hr after surgical operation (N=6 in each group), * p<0.05 when compared with sham group,
**** p<0.05 when compared with I/R group**

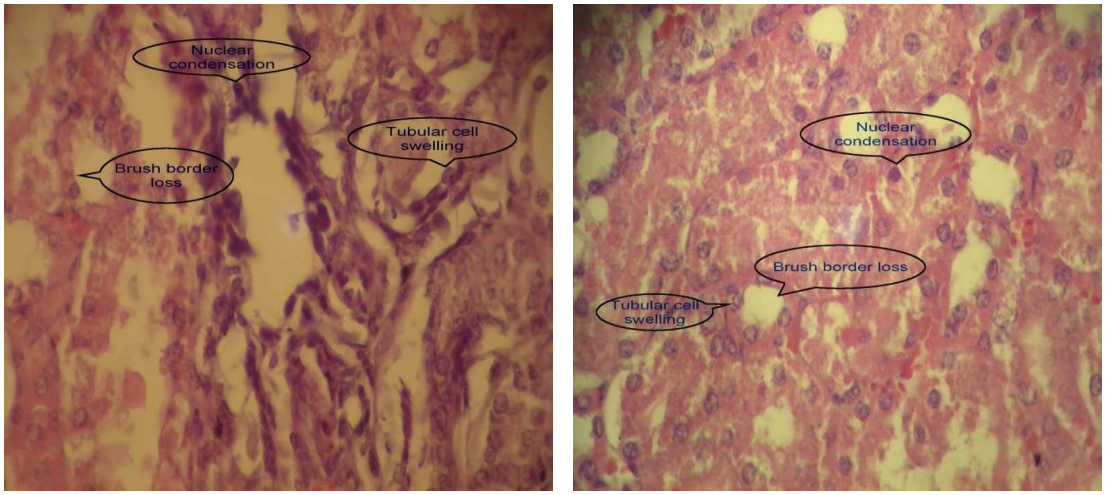
Effects on kidney parenchyma

Histopathological changes resulted at the end of the study were graded as normal, mild, moderate and severe injury according to the severity scores (0-3) for all intersections of each slide section and the fallowing photomicrographs will show intersections of different severity scores obtained at the 48hr after I/R:



A B

Figure (3): A; Photomicrograph of a kidney intersection shows score 0 (no abnormality), B; score 1 (tubular cell swelling, brush border loss, nuclear condensation, with up to one third of the tubular profile showing nuclear loss).Stained with haematoxylin and Eosin (X 400).



A B

Figure (4): A; Photomicrograph of a kidney intersection shows score 2 (tubular cell swelling, brush border loss, nuclear condensation, with greater than one-third and less than two-thirds of the tubular profile showing nuclear loss). B; score 3 (tubular cell swelling, brush border loss, nuclear condensation, with greater than two-thirds of the tubular profile showing nuclear loss). Stained with haematoxylin and Eosin (X 400).

Groups finding

-Sham group :

Slide sections of kidneys of 4 mice (66.6 %) in this group show normal grading (architecture) while 2 mice (33.4%) show mild grading. Mean of total severity scores of the sections of this group was 0.83 which is less than 1 so this group grade was considered as normal.

-I/R group :

Slide sections of kidneys of 4 mice (66.6 %) in this group show severe grading while 2 mice (33.4 %) show moderate grading. Mean of total severity scores of the sections of this group was 21.33 which is more than 20 scores so the grade of this group was considered as severe . This score was significantly higher than that of Sham group(P=0.003) .

-MK-886 group :

Slide sections of kidneys of 3 mice (50 %) in this group show mild grading while 2 mice (33.4%) show moderate grading and only one mouse (16.6%) show normal grading. Mean of total severity scores of the sections of this group was 8.66 which is more than 1score and less than 10 scores so the grade of this group was considered as mild . This score was significantly higher than that of Sham group (P=0.02) and significantly lower than that of I/R group (p=0.004).

Table (1):

The differences in histopathological grading of kidney changes among the three study groups at 48hr after surgical operation.

Histopathological changes grading	Sham		I/R		DITPA	
	N	%	N	%	N	%
Normal	4	66.6	0	0	0	0
Mild	2	33.4	0	0	1	16.6
Moderate	0	0	2	66.6	3	50
Severe	0	0	4	33.4	2	33.4
Total	6	100	6	100	6	100
Grade of the group	**Normal**		**Severe**		**Moderate**	

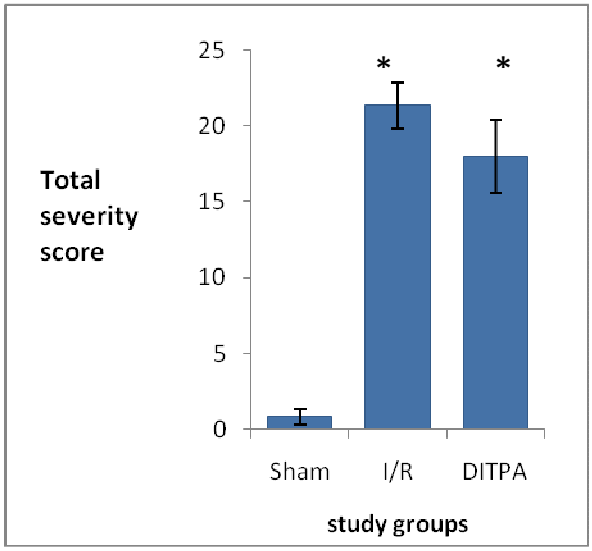


Figure (5): Error bar chart shows the difference in mean ±SEM values of total severity scores in the three experimental groups at 48hr after surgical operation (N=6 in each group).

* p<0.05 when compared with sham group

** p<0.05 when compared with I/R group

Discussion

To the best of our knowledge, this is the first study that used DITPA in model of renal I/R but TH (whether by acute T_3 pretreatment or by long-term pretreatment with l-thyroxine)[15,16] and also DITPA[21]were used in many models of I/R in several organs other than kidney and observed protective and/or improving action.

In the heart, when myocardial ischemia was severe and induced irreversible damage, the improved post infarction function recovery by THs or DITPA may be mainly by their angiogenic action[20,27] which may have no role to play in reversible renal I/R model when inflammation play the major role in inducing injury and may explain why DITPA failed to exert protective effect in the reversible I/R model in this study

Other possible mechanisms that underlie TH-induced cardioprotection are that Complex intracellular signalling underlies the cellular response to stress and the balance between pro-death and pro-survival pathways seem to determine cellular fate after an ischaemic insult. MAPK p38 and JNKs, which plays a key role in inducing cell death in response to stressful stimuli JNK kinases are thought as a pro-death signaling which was inhibited due to decreased phosphorylation due to reduced ATP availability (due to reduced glycogen content which occurs during ischemia in hyperthyroid hearts)[18]. Furthermore, heat shock proteins are found to be important cardioprotective end-effector molecules in the pro-survival intracellular signalling[18]. In the present study, DITPA which has low metabolic activity so no enough effect on ATP availability and so no inhibition of pro-death signals that required to protect the kidney.

For DITPA, administration of DITPA after myocardial infarction in rabbits improved post-infarct recovery function[21]. Also In a rabbit post-infarction model, use of DITPA prevented abnormal sarcoplasmic reticulum Calcium transport (that mediate necrosis and permanent injury) in myocardial infarction[22]. So DITPA may not interfere with reversible I/R model in which inflammation rather than calcium dyshomeostasis have major role to play to cause tissue damage**s.**

In an organ other than heart, **Fernández *et al*** (2006)[28]showed that the use of T_3 in I/R in liver (1 hr ischemia and 20 hr reperfusion) show that T_3 administration causing transient oxidative stress in the liver (occurred within a period of 36 hours of T_3 treatment) exerts significant protection against I/R injury, a novel preconditioning maneuver that is related to a gain of liver cell signaling functions represented by recovery of NF-κB and STAT3 DNA binding (NF-κB and STAT3 activation) and acute-phase response, which are lost during I/R. These responses may protect the liver against I/R-induced oxidative stress by re-establishing redox homeostasis[28]. In the present study, DITPA administration was only 30 min prior to ischemia induction and this may be short time course to exert preconditioning effect on oxidative stress similar to that induced in hepatic I/R injury in which T_3 administration was 36 hr prior to ischemia induction[28] and this may give suggestion for plane future in studying DITPA in I/R model

References

1. Hoste EA, Schurgers M. Epidemiology of acute kidney injury: how big is the problem? Crit Care Med 2008; 36:S146-S151.

2. Milhoan KA, Lane TA, Bloor CM. Hypoxia induces endothelial cells to increase their adherence for neutrophils: role of PAF. Am J Physiol 1992;263:H956–H962.

3. Lucchesi BR. Role of neutrophils in ischemic heart disease: pathophysiologic role in myocardial ischemia and coronary artery reperfusion. Cardiovasc Clin 1987; 18:35–48.

4. Goldman G, Welbourn R, Klausner JM, Valeri CR, Shepro D, Hechtman HB. Thromboxane mediates diapedesis after ischemia by activation of neutrophil adhesion receptors interacting with basally expressed intercellular adhesion molecule-1. Circ Res 1991; 68:1013–1019.

5. Jerome SN, Dore M, Paulson JC, Smith CW, Korthuis RJ. P-selectin and ICAM-1-dependent adherence reactions: role in the genesis of postischemic no-reflow. Am J Physiol 1994; 266:H1316–H1321.

6. Paterson IS, Klausner JM, Goldman G, Kobzik L, Welbourn R, Valeri CR, et al. Thromboxane mediates the ischemia -induced neutrophil oxidative burst. Surgery 1989; 106:224–229.

7. Lucchesi BR. Myocardial ischemia, reperfusion, and free radical injury.Am J Cardiol 1990; 65:141–231.

8. Bonventre JV, Zuk A. Ischemic acute renal failure: an inflammatory disease? Kidney Int. 2004 ;66: 480—485.

9. **Takada M, Nadeau KC, Shaw GD, Marquette KA, Tilney NL.** The cytokine-adhesion molecule cascade in ischemia/reperfusion injury of the rat kidney. Inhibition by a soluble P-selectin ligand, J. Clin. Invest. 1997;99: 2682—2690.

10. Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR. Early kidney TNF-alpha expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion. Am. J. Physiol. 1999;277: R922—R929.

11. Daemen MA, van't Veer C, Wolfs TG, Buurman WA . Ischemia/reperfusion-induced IFN-gamma up-regulation: Involvement of IL-12 and IL-18. J Immunol 1999;162 : 5506 –5510 .

12. Burne-TaneyMJ , Kofler J, Yokota N, Weisfeldt M, Traystman RJ, Rabb H . Acute renal failure after whole body ischemia is characterized by inflammation and T cell mediated injury. Am. J. Physiol.: Renal. Physiol. 2003;285: F87—F94.

13. Pantos C, Malliopoulou V, Varonos D, Cokkinos DV Thyroid hormone and phenotypes of cardioprotection, Basic Res. Cardiol. 2004;99: 101–120.

14. Klemperer JD, Zelano J, Helm RE, Berman K, Ojamaa K, Klein I, et al . Triiodothyronine improves left ventricular function without oxygen wasting effects after global hypothermic ischaemia, J. Thorac. Cardiovasc. Surg. 1995;109(3): 457–465.

15. Liu Q, Clanachan AS, Lopaschuk GD . Acute effects of triiodothyronine on glucose and fatty acid metabolism during reperfusion of ischaemic rat hearts, Am. J. Physiol. 1998;275(3):E392–E399.

16. Buser PT, Wikman-Coffelt J, Wu ST, Derugin N, Parmley WW, Higgins CB . Postischaemic recovery of mechanical performance and energy metabolism in the presence of left ventricular hypertrophy: a 31P-MRS study, Circ. Res.1990;66(3): 735–746.

17. Pantos C, Malliopoulou V, Mourouzis I, Karamanoli E, Paizis I, Steimberg N, et al. Long-term thyroxine administration protects the heart in a similar pattern as ischaemic preconditioning. Thyroid. 2002;12: 325–329.

18. **Pantos C, Mourouzis I, Cokkinos DV.** Myocardial ischemia-basic concepts, stress signalling in myocardial ischemia. In: D.V. Cokkinos, C. Pantos, G. Heusch and H. Taegtmeyer, Editors, Myocardial Ischemia; from Mechanisms to Therapeutic Potentials, Springer, New York, NY 10013, USA (2006), pp. 29–52.

19. Morkin E, Ladenson P, Goldman S, Adamson C 2004 Thyroid hormone analogs for treatment of hypercholesterolemia and heart failure: past, present and future prospects. J Mol Cell Cardiol 37:1137–1146.

20. Mousa SA, O'Connor L, Davis FB, Davis PJ 2006 Proangiogenesis action of the thyroid hormone analog 3,5-diiodothyropropionic acid (DITPA) is initiated at the cell surface and is integrin mediated. Endocrinology 147:1602–1607.

21. Morkin E, Pennock GD, Spooner PH, Bahl JJ, Goldman S, Clinical and experimental studies on the use of 3,5- diiodothyropropionic acid, a thyroid hormone analogue, in heart failure, Thyroid. 2002;12(6): 527–533.

22. Pennock GD, Spooner PH, Summers CE, Litwin SE. Prevention of Abnormal Sarcoplasmic Reticulum Calcium Transport and Protein Expression in Post-infarction Heart Failure Using 3,5-Diiodothyropropionic Acid (DITPA) 2000;32(11): 1939-1953.

23. Niranjan M, Cynthia A, Kevin G, Scott K, James H, Joseph JB . Regulation of Gene Expression in Rats With Heart Failure Treated With the Thyroid Hormone Analog 3,5-Diiodothyropropionic Acid (DITPA) and the Combination of DITPA and Captopril Journal of cardiovascularPharmacology. 2007;50(5):526-534.

24. Feitoza CQ, Gonçalves GM, Semedo P, Cenedeze MA, Pinheiro H, Beraldo FC , Inhibition of COX 1 and 2 prior to Renal Ischemia/Reperfusion Injury Decreases the Development of Fibrosis. mol med 2008;14(11-12)724-730.

25. Sharyo S, Kumagai K, Yokota-Ikeda N, Ito K, Ikeda M . Amelioration of Renal Ischemia–Reperfusion Injury by Inhibitionof IL-6 Production in the Poloxamer 407–Induced Mouse Modelof Hyperlipidemia. J Pharmacol Sci 2009;110:47 – 54.

26. Asaga T, Ueki M, Chujo K, Taie S . JTE-607, an Inflammatory Cytokine Synthesis Inhibitor, Attenuates Ischemia/Reperfusion-Induced Renal Injury by Reducing Neutrophil Activation in Rats J. Bio sci. Bio eng. 2008;106(1): 22–26.

27. Davis PJ, Davis FB, Cody V 2005 Membrane receptors mediating thyroid hormone action. Trends Endocrinol Metab 16:429–435

28. Fernández V, Castillo I, Tapia G, Romanque P, Uribe-Echevarría S, Uribe M, et al. Thyroid hormone preconditioning: protection against ischemia-reperfusion liver injury in the rat. Hepatology. 2007 ;45(1):170-7.