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Protective effect of thyroid hormone (3,5,3-triiodothyronine)on renal ischemia-reperfusion injury in mouse model

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Abstract

Introduction Preconditioning is a strategy to prevent ischemia-reperfusion (I/R) injury by causing transient ischemia or increases in oxygen demand, thus resulting in protective actions in tissues and cells. 3,5,3-triiodothyronine(T3) was found to reduce cardiac or hepatic I/R injury in animal models when preconditioned 48 hours in advance. The purpose of this study was to evaluate the protective effects of T3 preconditioning on renal I/R injury with different interval of time, including a short period of time before I/R injury.

Methods In male C57BL/6 mice, renal warm I/R injury was induced by temporary ligation of bilateral renal pedicle for 45 minutes followed by reperfusion period of 24 hours. Preconditioning with T3 was performed 24 or 6 hours before or at the time of I/R injury. Histologic tubular injury, expressions of proinflammatory markers, activities of antioxidative enzymes, and expressions of nitric oxide synthase (NOS) were evaluated.

Results In histologic exam, tubular injury was significantly reduced in mice preconditioned with T3 6 hours before I/R injury. The levels of proinflammatory cytokines in renal tissue were decreased with T3-preconditioning 6 hours before or at the time of I/R injury. The levels of

glutathione (GSH) were definitely increased in all treatment groups. The activities of superoxide dismutase (SOD) did not show statistically significant changes, but had an increased tendency in preconditioning 24 or 6 hours before I/R injury. Expressions of neuronal NOS (nNOS) was significantly increased in all treatment groups, especially in mice preconditioned with T3 6 hours before or at the time of I/R injury. However, inducible NOS (iNOS) and endothelial (eNOS) were significantly decreased when mice were preconditioned with T3 6 hours before or at the time of I/R injury.

Conclusions Preconditioning with T3 in a short interval of time before I/R injury had a significant protective effect on renal warm I/R injury. It may be an applicable therapeutic protocol for deceased donor kidney transplantation in clinical practice.

Key words Renal ischemia-reperfusion injury, 3,5,3-triiodothyronine, preconditioning

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Introduction

Ischemia-reperfusion (I/R) injury is commonly encountered problem in organ transplantation. Especially, in deceased donor kidney transplantation, the time for organ procurement and transport is inevitable, and the longer ischemia time is related to I/R injury of grafts[1]. I/R injury is associated with delayed graft function and acute rejection, therefore, it eventually adversely affects the long term graft survival[2].

I/R injury is a complex pathologic process involving intracellular and extracelluar processes that result in metabolic, inflammatory and thrombotic changes[1]. It includes both direct cellular damage caused by ischemic insult and delayed damage due to activation of inflammatory pathway[3]. Neutrophil stimulation with oxygen radical-mediated injury is the main event during ischemia, and on reperfusion, production of oxygen radial caused by extravasation of neutrophil and their oxidative burst is the main mechanism of damage[4]. The pathogenesis of I/R injury, also, involves activation of the apoptosis gene, disorder of mitochondria function, and calcium overload[5]. Renal I/R injury is a result of a series of events, including changes in vascular tone, enhanced vascular permeability, structural change in renal tubules and

accumulation of activated neutrophils[6]. Many efforts had been made to reduce I/R injury by blocking the processes.

Ischemic preconditioning is an adaptational response of briefly ischemic tissues which serves to protect against subsequent prolonged ischemic insults and reperfusion injury[7,8]. Remote ischemic preconditioning was proved to induce multi-organ protection against myocardial I/R injury in mice by upregulating interleukin-10[9]. Remote ischemic preconditioning, which meant transient repeated arm ischemia with a blood-pressure cuff, was found to prevent contrast medium-induced acute kidney injury[10]. Remote ischemic preconditioning by inducing hind-limb ischemia, improved hepatic mitochondrial oxygenation and reduced acidosis in rabbit hepatic I/R injury model[11].

Pharmacologic preconditioning is also possible with a various materials. Pretransplant administration of rabbit anti-rat thymocyte immunoglobulin limited tissue injury and tubular apoptosis during I/R injury in rat kidney[12]. Darbepoetin alfa and carbamylated nonerythropoietic derivative of erythropoietin prevented renal graft dysfunction, tubular oxidative stress, and apoptosis after I/R injury in rat kidney[13]. Rutin, one of the flavonoid, was also found to have protective effect on I/R injury in rat kidney[14]. Carbon monoxide

ameliorated renal cold I/R injury by increasing of vascular endothelial growth factor by activation of hypoxia-inducible factor[14].

One of the attempts was the use of 3,5,3-triiodothyronine (T3) which was a kind of thyroid hormone. It increases metabolism and induces catabolism in the body. By activating the cellular respiration in mitochondria and cytochrome P450, reactive oxygen species (ROS) is produced by T3[15,16]. Although T3 seems to adversely affect I/R injury, preconditioning with T3 works differently. Inducing a transient oxidative stress before I/R injury results in protective effects by promoting expression of antioxidants[15,17].

Many studies with animal models showed that thyroid hormone could reduce I/R injury in tissues or organs. In cardiovascular system, preconditioning with thyroid hormone was proved to limit apoptosis and improve postischemic functional recovery[18-20]. Fernandez et al[15] reported that T3 injection 48 hours before hepatic I/R injury resulted in decreased elevation of liver enzyme, GSH depletion, and protein oxidation. In a research with isolated rabbit renal tubular cells, thyroxine reduced the free oxygen radical levels and cellular structure destruction[21]. Ferreyra et al[22] reported that preconditioning of rat 24 hours before renal I/R injury reduced proteinurina and increased the

levels of antioxidative enzymes.

Administration of T3 had been found to increase nitric oxide (NO). There was a study showed that T3 stimulated production of NO synthase (NOS) isoforms within 30 minutes in rat vascular smooth muscle cell[23]. Acute and chronic hyperthyroidism were proved to enhance the endothelium-dependent relaxation mediated by NO and endothelium-derived hyperpolarizing factor[16,24]. Recently, it is demonstrated that NO-related action of T3 was mediated by both genomic and non-genomic pathways[25].

In previous literatures, the protective mechanism of T3-preconditioning was antioxidation, and information about the role of NO was limited. In addition, the effect of T3-preconditioning in a short period of time before I/R injury was not understood well. In clinical situation, it would take less than 12 hours from selection of the recipient to performing the transplant, thus the effect of short-term T3-preconditiong is important.

The aim of this study was to evaluate the protective effects of T3-preconditioning on renal I/R injury at different intervals of time including 6 hours before or at the time of injury. Another aim was to determine the changes of antioxidants and NOS in each condition.

Materials and methods

This study was approved by the Seoul National University Hospital Institutional Animal Care and Use Committee (11-0110). The animals in this study were cared in accordance with the guidelines of the 'Guide for the Care and Use of Laboratory Animals' published by the National Institutes of Health (NIH Publication No.85-23, revised 1996)

Animals and experimental design

Male C57BL/6 mice weighing 23-26g were housed at the experimental animal center affiliated to Seoul National University on 12-hour light and dark cycle with free access to water and rat chow. T3 (Sigma Chemicals, St. Louis, Mo, USA) were prepared with previously described methods[17,19]. Animals received a single dose of T3 (0.1mg/kg body weight) or equivalent volumes of normal saline intraperitoneally. The injection of T3 was scheduled either 24 or 6 hours before operation or at the time of operation. Mice were anesthetized with intraperitoneal injection of zoletil (0.8ml/kg) and xylazine (0.4ml/kg). Renal warm I/R injury was induced by temporary ligation of bilateral renal pedicles for 45 minutes followed by reperfusion period of 24 hours. After 24-hour-reperfusion period, bilateral nephrectomy was done and one quarter of

the right kidney was immediately fixed in 10% phosphate-buffered formalin. The rest kidneys were frozen in liquid nitrogen and then transferred to a -80℃ freezer.

Mice were randomly divided into six experimental groups as follows. (A) Control-sham (n=7): mice received the injection of normal saline and underwent laparotomy without induction of renal I/R injury, (B) Control with I/R injury (n=7): mice received the injection of normal saline and underwent surgical manipulation inducing I/R injury, (C) T3-sham (n=7): mice received T3 and underwent laparotomy without induction of renal I/R injury, (D) T3 (24 hours) with I/R injury (n=7): mice received T3 24 hours before surgery and underwent surgical manipulation inducing I/R injury, (E) T3 (6 hours) with I/R injury (n=6): mice received T3 6 hours before surgery and underwent surgical manipulation inducing I/R injury, and (F) T3 (0 hour) with I/R injury (n=6): mice received T3 just before inducing ischemia (Fig. 1.)

Histologic examination

The renal tissues were fixed in 10% phosphate-buffered formalin for 24 hours, embedded in paraffin and sectioned at 5μm. The sections were deparaffinized, hydrated and stained with hematoxylin-eosin.

Stained tissues were scored according to the scale to evaluate the degree of tubulointerstitial injury such as tubular necrosis, tubular dilatation, or cellular edema and the degree of inflammatory cell infiltration. The findings were graded according to the percentage of the injured tubules[17]. High score represents more severely damaged tubules: 0, normal kidney; 1, minimal necrosis (<10% involvement); 2, mild necrosis (10-25% involvement); 3, moderate necrosis (25-50% involvement); and 4, severe necrosis (>50% involvement). All slides were reviewed blindly by one pathologist.

Measurement of activities of antioxidants

Antioxidative activity was evaluated by assessment of superoxide dismutase (SOD) and reduced glutathionine (GSH) concentration. Assay was performed using the SOD and GSH assay kit (Cayman, Ann Arbor, MI, USA). The GSH assay kit (Cayman, Ann Arbor, MI, USA) was based on DTNB (5,5'-dithio-*bis*-2-nitrobenzoic acid) cycle reaction. The stored kidney tissue was rinsed with PBS solution to remove any red blood cells and clots. It was homogenized in cold buffer and centrifuged at 10,000 x g for 15 minutes at 4℃. After removal of the supernatant, it was stored at -20℃. To perform the assay,

standard sample and tissue homogenate were placed in each well on the plate. Assay cocktail including reconstituted DTNB were added to the sample and incubated in the dark on an orbital shaker. The absorbance in the wells was measured at 405-414 nm using plate reader at 5 minutes intervals for 30 minutes. The SOD assay utilized a terrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. For SOD assay, the stored kidney tissue was rinsed with a PBS solution to remove any red blood cells and clots. It was homogenized in cold HEPES buffer and centrifuged at 15,000 x g for 5 minutes at 4℃. After removal the supernatant, it was stored at -80℃. The reaction was initiated by adding 20μl of diluted xanthine oxidase to sample in each well. After incubation on a shaker for 20 minutes at room temperature, the absorbance was read at 440-460 nm using plate reader.

RQ-PCR for proinflammatory markers(TNF-α, IL-6, and MIP-1α) and NOS

Total RNA was extracted from frozen kidney tissue stored -80℃ with TRIzol reagent(Invitrogen, Carlsbad, CA, USA). The mixture of total RNA, primer, and 10Mm dNTP were made and it added to cDNA

synthesis Mix: 10XRT-buffer, 0.1M DTT, RNaseOUT™, Superscript™ III RT (all supplied by Invitrogen). The mixture was incubated at 25 ℃ during 10 minutes for annealing, 50 ℃ during 50 minutes for cDNA synthesis, and at 85 ℃ during 5 minutes for terminating the reaction. RNA was removed by adding 1μml RNAase H and incubated in 37℃ for 20 minutes. PCR products were reacted with TagMan Universal PCR Master Mix, primer (Applied Biosystem, Foster city, CA, USA) and detected by ABI PRISM 7700. GAPDH was used as a positive control for cDNA integrity. The specific primers were based on previously published sequences (Applied Biosystem, Foster city, CA, USA)[26-29]. Relative differences in expression between the groups were expressed using cycle time values. The Ct values of the target genes were first normalized with GAPDH of the same sample, and then the relative differences between control and treatment groups were calculated and presented as relative increases, setting the control as 100%[30]. Data were analyzed using the comparative Ct($2^{-\Delta\Delta Ct}$) method.

Statistical Analyses

Continuous variables are presented as means ± SEM or SD and were compared using Kruskal-Wallis test and the Mann-Whitney U tests. A *P*

value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS 18.0 software (SPSS Inc, Chicago, IL).

Results

T3-preconditioning attenuated the tubular injury in histologic results

When the animals were treated with T3, the degree of tubular injury was reduced, regardless of the time intervals before I/R injury (Fig.2B-D). Injection of T3 6 hours before I/R injury had a statistically significant effect on preservation of histologic tubular structures compared to control (0.83 ± 0.307 vs. 2.14 ± 0.404, P= 0.035) (Fig.2E). The renal tissues were less damaged in the mice preconditioned with T3 24hours before I/R injury or treated with T3 at the time of I/R injury than control group. Even though the histologic score was numerically lower than control, the difference was not statistically significant.

T3-preconditioning decreased the mRNA expressions of proinflammatory cytokines

The levels of proinflammatory cytokines were decreased in mice preconditioned 6 hours before or at the time of I/R injury (Fig.4A-C). The expressions of mRNA of TNF-α and MIP-1α in mice preconditioned with T3 24 hours before I/R injury were similar to control group. The level of mRNA expression of IL-6 was higher in

mice preconditioned with T3 24 hours before I/R injury compared to control group. When the mice were preconditioned with T3 6 hours before I/R injury, the mRNA expressions of TNF-α, IL-6, and MIP-1α were statistically significantly decreased than control group (TNF-α: 1.17 ± 0.732 vs. 4.22 ± 0.197, $P < 0.05$, IL-6: 9.27 ± 0.729 vs. 18.04 ± 1.485, $P < 0.05$, MIP-1α: 1.77 ± 0.040 vs. 2.88 ± 0.211, $P < 0.05$). The animals treated with T3 just before I/R injury showed lower levels of TNF-α, IL-6, and MIP-1α than control group (TNF-α: 1.09 ± 0.443 vs. 4.22 ± 0.197, $P < 0.05$, IL-6: 7.35 ± 0.386 vs. 18.04 ± 1.485, $P < 0.05$, MIP-1α: 1.08 ± 0.080 vs. 2.88 ± 0.211, $P < 0.05$).

T3-preconditioning promoted activities of antioxidative enzymes

The level of SOD in renal tissue was increased in group preconditioned with T3 24 hours before I/R injury than control (Fig.3A). In this group, there was an obvious tendency to enhance the expression of SOD, but the difference did not reach the statistical significance. The effect was disappeared in animals treated with T3 at comparably short intervals before injury. The level of GSH was depleted in I/R injury group without T3 treatment (Fig.3B). The level of GSH was found to be preserved in mice treated with T3, regardless of

the injection time. All treatment groups showed higher levels of GSH and the differences were statistically significant (24-hour: 15.78 ± 0.891 vs. 6.46 ± 0.510, 6-hour: 15.01 ± 0.575 vs. 6.46 ± 0.510, 0-hour: 14.92 ± 0.693 vs. 6.46 ± 0.510, $P = 0.001$ for all).

T3-preconditioning increased the mRNA expression of nNOS

The expressions of mRNA of iNOS, eNOS, and nNOS in renal tissue were not changed in animal receiving I/R injury compared to sham-operated group (Fig. 5A-B). Treatment with T3 decreased the mRNA expressions of iNOS and eNOS. However, they were influenced by the time interval between T3 injection and I/R injury. When T3 was given 24 hours in advance, neither iNOS nor eNOS was not changed. In mice preconditioned with T3 6 hours before I/R injury, the mRNA expressions of iNOS and eNOS were statistically significantly decreased (iNOS: 0.16 ± 0.006 vs. 1.14 ± 0.016, $P < 0.05$, eNOS: 0.29 ± 0.003 vs. 1.31 ± 0.015, $P < 0.05$). The effect was also preserved in animal injected T3 at the time of I/R injury (iNOS: 0.17 ± 0.007 vs. 1.14 ± 0.016, $P < 0.05$, eNOS: 0.28 ± 0.002 vs. 1.31 ± 0.015, $P < 0.05$). The result was opposite with mRNA expression of nNOS. The expression of nNOS was increased in all treatment groups regardless of

injection timing. The levels were much higher in the mice preconditioned with T3 6 hours before or at the time of I/R injury than mice preconditioned 24 hours before I/R injury.

Discussion

I/R injury is caused by direct ischemic damage and delayed anoxic injury after reperfusion[3]. Generation of ROS is thought to be the critical factors for reperfusion injury. Direct ischemic damage is evitable in many condition, therefore reducing the reperfusion injury caused by oxidative stress has been a main target of treatment[3,4,31]. Recently, ischemic preconditioning is an important strategy to reduce I/R injury. Actually it is a concept of prevention by creating protective environments by inducing reversible and transient oxidative stress. Preconditioning with variable materials, such as erythropoietin or ethanol, were determined to have protective effect in animal models[3,32]. Preconditioning with hyperbaric oxygen or remote hind limb preconditioning was another strategies for prevention of I/R injury[5,8,31]. However, they seemed to be difficult to apply to the clinical situation.

Several studies showed that treatment with T3 could reduce renal I/R injury in animal models[21,22]. Recent study demonstrated that preconditioning with T3 48 hours before warm ischemia had a protective effect on renal I/R injury in rat and it was mediated by heme oxygenase overexpression[17]. Antioxidative effect was regarded as the main mechanism of T3-preconditioning in previous literatures. The

other functions of T3-preconditioning, such as increased NOS activities or antiapoptosis were limited. Furthermore, most of the studies on kidney used T3 far before I/R injury. Allocation of organs and performing the operation usually proceeded within 12 hours in deceased donor transplantation. Previous studies showed the protective effect of T3 in animals preconditioned 48 hours before I/R injury and the results are not relevant to critical situation.

In this study the animals were treated with T3 at different times before I/R injury, including 24, 6 hours before or at the time of ischemia. The degree of tubulointerstitial injury was evaluated at the end of 24-hour reperfusion. Tubular necrosis, tubular dilatation, or cellular edema was mainly examined. Although the degree of inflammatory cell infiltration was evaluated, it was not definite and the histologic results represented about the tubular injury mainly. The changes of inflammation were indirectly evaluated with expressions of proinflammatory cytokines in renal tissues.

Preconditioning with T3 in a short interval of time before I/R injury could reduce the mRNA expression of proinflammatory cytokines in renal tissues. We had hypothesized that injection of T3 caused the transient increases in oxygen demand and inflammation, then, it could

induce anti-inflammatory response as a protection. The effects related to pro- or anti-inflammatory cytokines seem to be an acute and temporary reaction, not maintain longer than a day. And the inflammatory changes in I/R injury is also observed in early reperfusion period. Freitas et al[2] demonstrated that mRNA expressions of proinflammatory cytokines were higher after 5-hour reperfusion than 24-hour in a mouse warm I/R injury model. In a study to determine the TNF bioactivity in different periods of ischemia and reperfusion in rat, TNF protein expression and bioactivity were peaked following 2 hours reperfusion than any longer reperfusion period[33]. In this study, 24-hour-preconditioning was thought to be too long to induce optimal environments at the time of I/R injury in term of attenuating inflammation. Preconditioning 6 hours before or at the time of I/R injury was effectively reduced the inflammation in early phase of reperfusion. Measurements of the expressions of TNF-α, IL-6 and MIP-1, along with anti-inflammatory cytokines, after reperfusion for 4-6 hours could be helpful to determine the mechanism more exactly.

There was a clear tendency to increase the activity of SOD when mice were preconditioned 24 hours or 6 hours before I/R injury, however, it did not reach the statistical difference. In mice treated with T3 at the

time of ischemia, the concentration of SOD was lower than control group. It might take time to increase the activity of SOD and less than 6 hours was not enough. When the antioxidative effect was assessed by measuring levels of GSH in renal tissue, the effects were maintained significant in mice preconditioned with T3, regardless of injection timing. We measured the GSH levels indirectly by detection of glutathione reductase. The enzymatic levels of glutathione reductase were elevated in all treatment groups, which meant the antioxidative state was made even though the T3 was acutely administrated at the time of I/R injury. The results are meaningful as it determined the antioxidative reaction created by T3 in a short period of time. Further study to measure and calculate the ratio of GSH to oxidative glutathione (GSSH) is required.

The effect of thyroid hormone on cardiovascular system is well known. Thyroid hormone induced hyperdynamic circulation and decreased the vascular resistance[16,34]. Local release of vasodilators and direct actions on arteriolar smooth muscle tone were found to be the underlying mechanisms[35-38]. Increased production of NO was observed in tissues primarily related to blood pressure control[39]. Bussemaker et al[35] reported that acute and chronic hyperthyroidism enhanced

endothelium-dependent relaxation of isolated renal artery of rat mediated by endothelial-derived hyperpolarizing factor (EDHF) and NO. T3-induced NO production was also observed in vascular myocytes with expressive participation of iNOS and nNOS[23].

The correlations between NO and I/R injury were found in several studies. In a study with liver biopsy at retrieval and implantation, reduced NO bioavailability and eNOS protein were thought to contribute to early reperfusion injury[30]. Inhaled NO had been found to accelerate restoration of liver function in patients underwent orthotopic liver transplantation[40]. In patients with sustained acute kidney injury after cadaveric renal transplantation showed diminished NO generation and loss of macular densa neuronal NOS than control group[41]. Little is known about the changes of NO bioactivities after T3-preconditioning in renal I/R injury.

In this study, the results of eNOS and iNOS were opposite to the changes of nNOS. Three NOS isoforms are widely distributed in organs related to blood pressure control and in the normal kidney[16]. Inducible NOS was known to synthesize excess NO in inflammatory conditions and could have deleterious effects in late phase of reperfusion[1,42]. In contrast, endogenous NO were produced by constitutive NOS (cNOS)

and appeared to have protective effect on I/R injury[43]. In ischemia, initial high production of NO caused depletion of local L-arginine concentration around cNOS and disarrangement of cNOS, which resulted in production of superoxide radicals(O_2^-)[44]. Antioxidative effect of T3-preconditioning could prevent dysfucntional cNOS in early reperfusion period. Although nNOS is known to be less active in kidney and liver than brain and muscle tissue, the presence of nNOS in macula densa of human renal tissue was proved with inmmunofluorescnece staining[41]. Further studies to determine the differences in western blot or immunoflouroresence staining of nNOS between groups or the response with specific nNOS inhibitor are warranted to clarify the effect of nNOS in renal I/R injury.

NO is known to regulate vascular tone and also have anti-inflammatory and antithrombotic properties by inhibiting NF-Kb and platelet aggregation[1,30]. Anti-inflammatory effect, lowered apoptosis, and vasodilatory ability of the renal vasculature were suggested as the contributing factors associated with NO[30,40,41]. An investigation to directly determine whether NO was increased after T3-preconditioning and, furthermore to find the specific function of T3-induced NO production are worth studying.

The results suggested that preconditioning with T3 6 hours before I/R injury reduced the renal I/R injury in mice. Acute administration of T3 also could induce a protective state to I/R injury in a short period of time. The tubular damage in histologic exam was significantly attenuated in mice preconditioned 6 hours before I/R injury. The expressions of proinflammatory cytokines were significantly reduced in mice preconditioned 6 hours before or at the time of I/R injury. The well-known antioxidative effect of T3-preconditioning was preserved in mice preconditioned 6 hours before or at the time of I/R injury when evaluated with GSH. The activity of SOD was also increased in 6-hour-preconditining group. The expression of nNOS was significantly enhanced in mice preconditioned 6 hours before or at the time of I/R injury. Although the results could not consistently reach the statistically significant differences, the protective tendency is meaningful and can be a basis for further studies. It is also encouraging results for clinical applications. In most cases, it would take less than 12 hours from selection of the recipient to performing the transplant. The results of short-term preconditioning are important in clinical situation.

Most of previous literatures had focused on the effect related to diminishing oxidative stress. I/R injury is complex process including

inflammatory, oxidative and thrombotic component, therefore various pathways associated should be blocked and adjusted. In this study, the antioxidative effect was preserved when the mice were preconditioned at short intervals of time. Additionally, enhancement of nNOS had been found in mice preconditioning in short periods of time, even though the underlying mechanism with increased nNOS was not determined. Increased activities of NOS and NO can affect I/R injury in various ways, therefore it is worth pursuing the specific processes mediated by NO in order to develop novel treatments.

There were several limitations in this study. The surgical manipulation to induce renal I/R injury in this study was ligation of both renal artery and vein simultaneously. It was possible that venous occlusion could occur in some mice. It was an animal model of warm I/R injury, so the outcomes could be slightly different from exact cold I/R injury in renal transplantation models. With the results of this study, further experiments in an animal model of renal transplantation are required. This study just focused on the changes of NOS and the specific effects or functions of them were not known. Further understanding of the specific pathways and related molecules in different reperfusion period will lead to discovery of new treatments to prevent renal I/R injury.

Conclusion

In conclusion, preconditioning with T3 in a short interval of time before I/R injury had a significant protective effect on renal warm I/R injury. Antioxidative effect decreased expression of proinflammatory cytokines may play a role in short-term preconditioning of T3. It may be an applicable therapeutic protocol for deceased donor kidney transplantation in clinical practice. Further studies to determine the specific functions of NOS and acute phase change in I/R injury are warranted.

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Fig 1. Experimental protocol for T3 preconditioning.

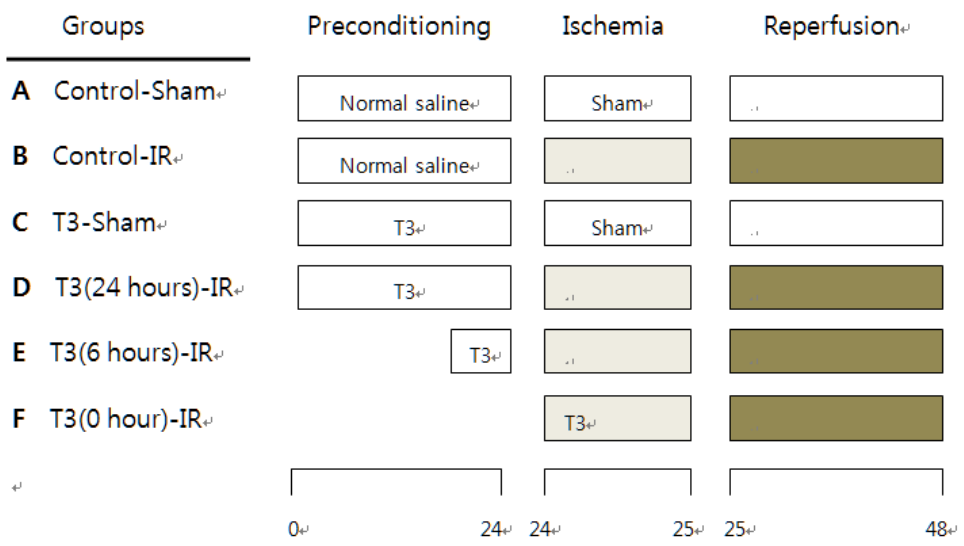
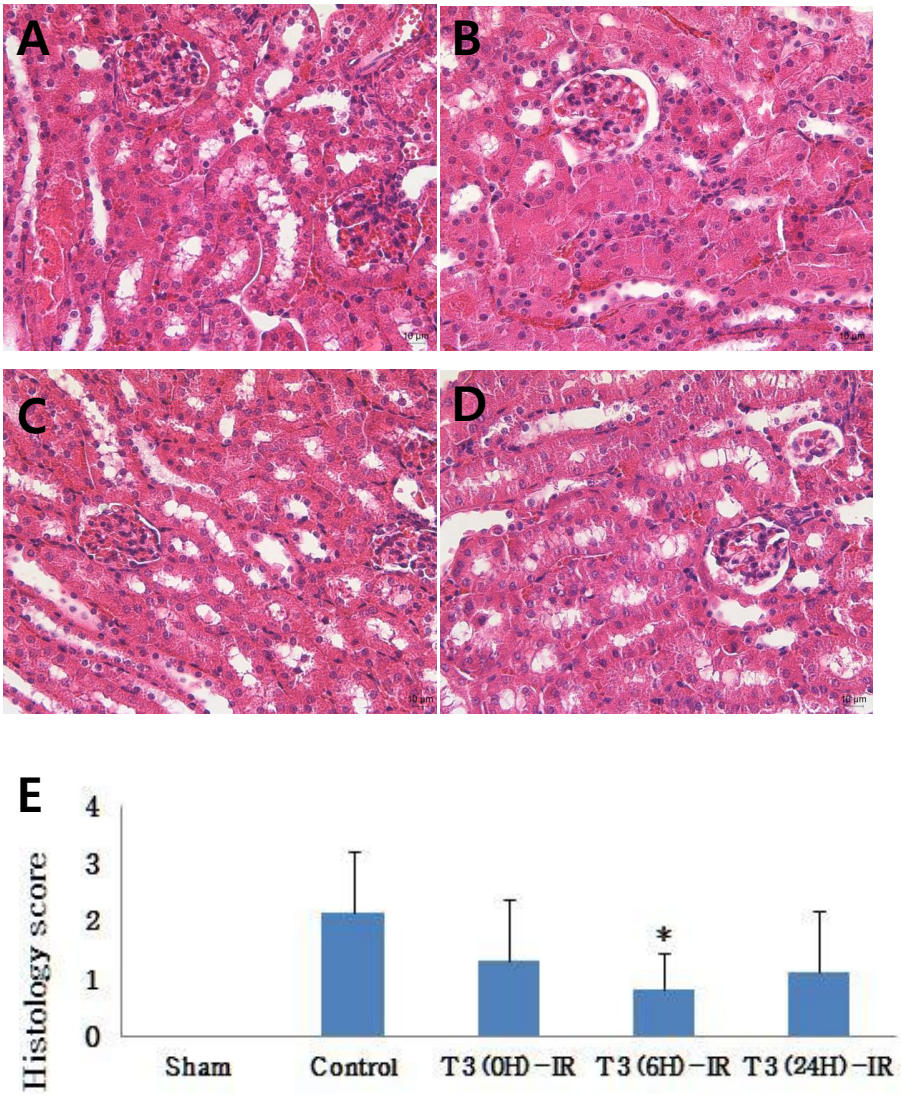


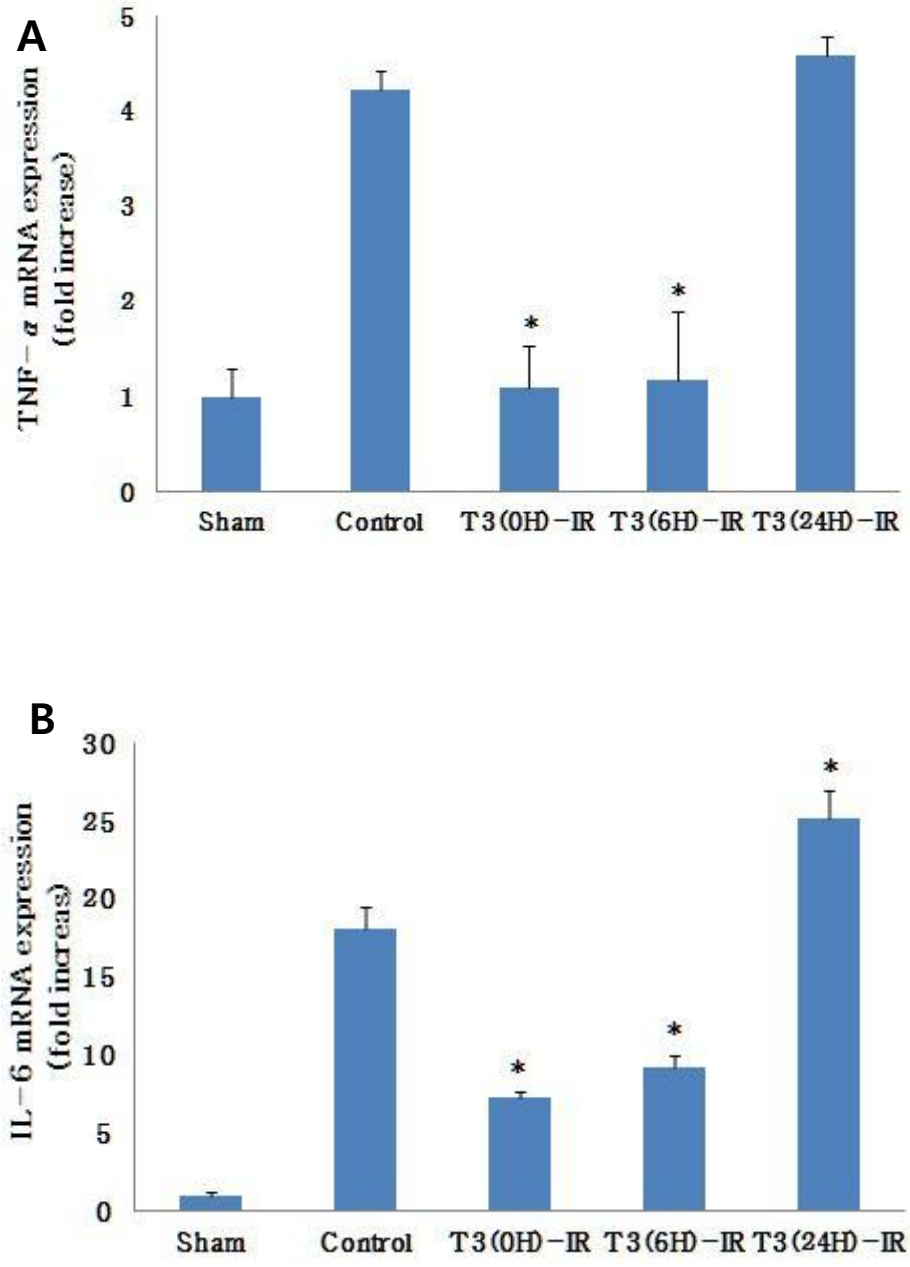
Fig 2. Preconditioning with T3 attenuated renal tubular injury.

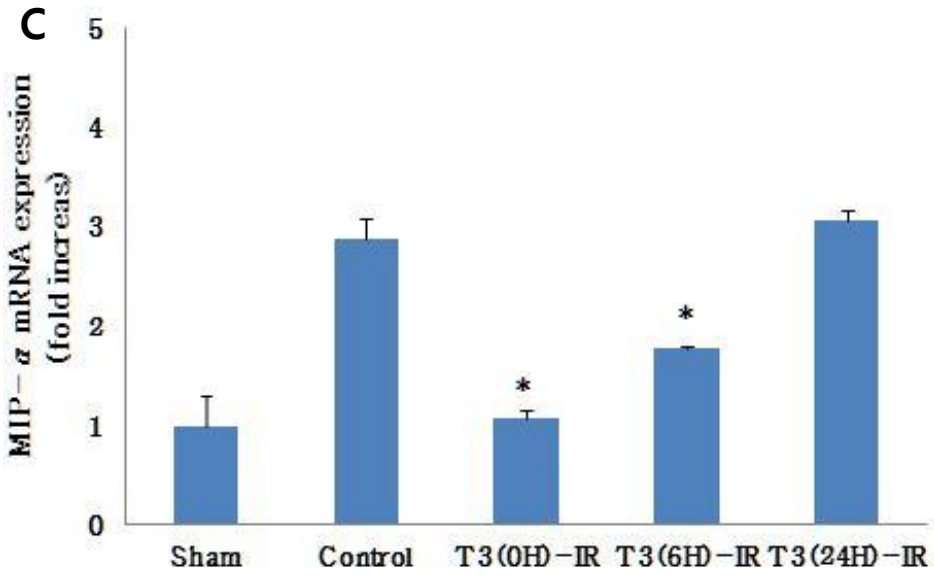


Histologic changes in the kidney from mice of the various treatment groups observed 24 hours after renal I/R injury. Representative slides of each group. N=6 or 7 for each group. (A) Severe tubular injury in control group. (B) Mild tubular injury in preconditioning 24 hours

before I/R injury. (C) Minimal tubular injury in preconditioning 6 hours before, and (D) at the time of I/R injury. Kidney tissues were fixed, embedded, sectioned, and stained with hematoxylin and eosin. Original magnification x 400. Histologic changes included tubular necrosis, tubular dilatation, inflammatory cell infiltration or cellular edema. (E) Tubular injury scores are expressed as mean±SD. $^{*}P$ <0.05 versus control.

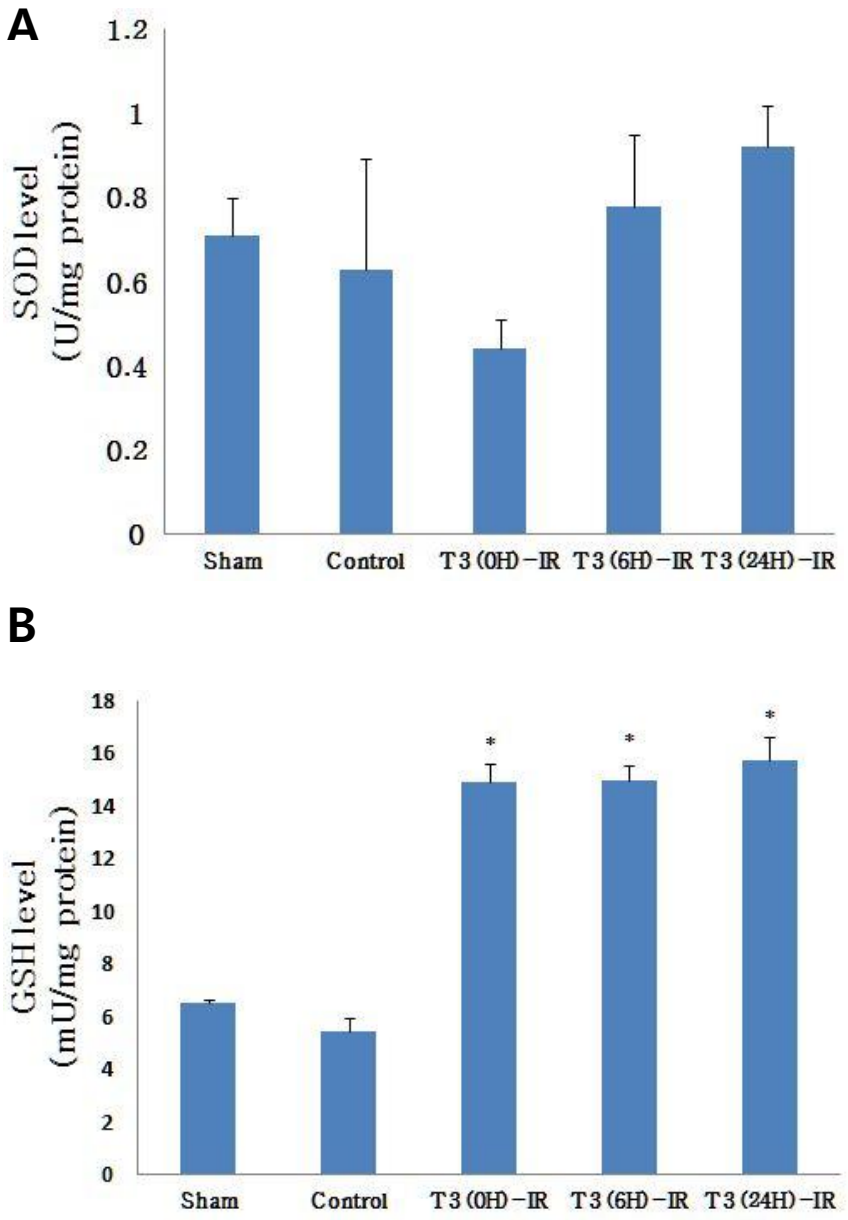
Fig 3. Preconditioning with T3 decreased the mRNA expressions of proinflammatory cytokines.





The mRNA expression of (A) TNF-α (B) IL-6, and (C) MIP-1α in renal tissue evaluated via RQ-PCR at the end of 24-hour-reperfusion. Values shown are expressed as the mean±SEM. N=6 or 7 for each group. $^{*}P$ <0.05 versus control.

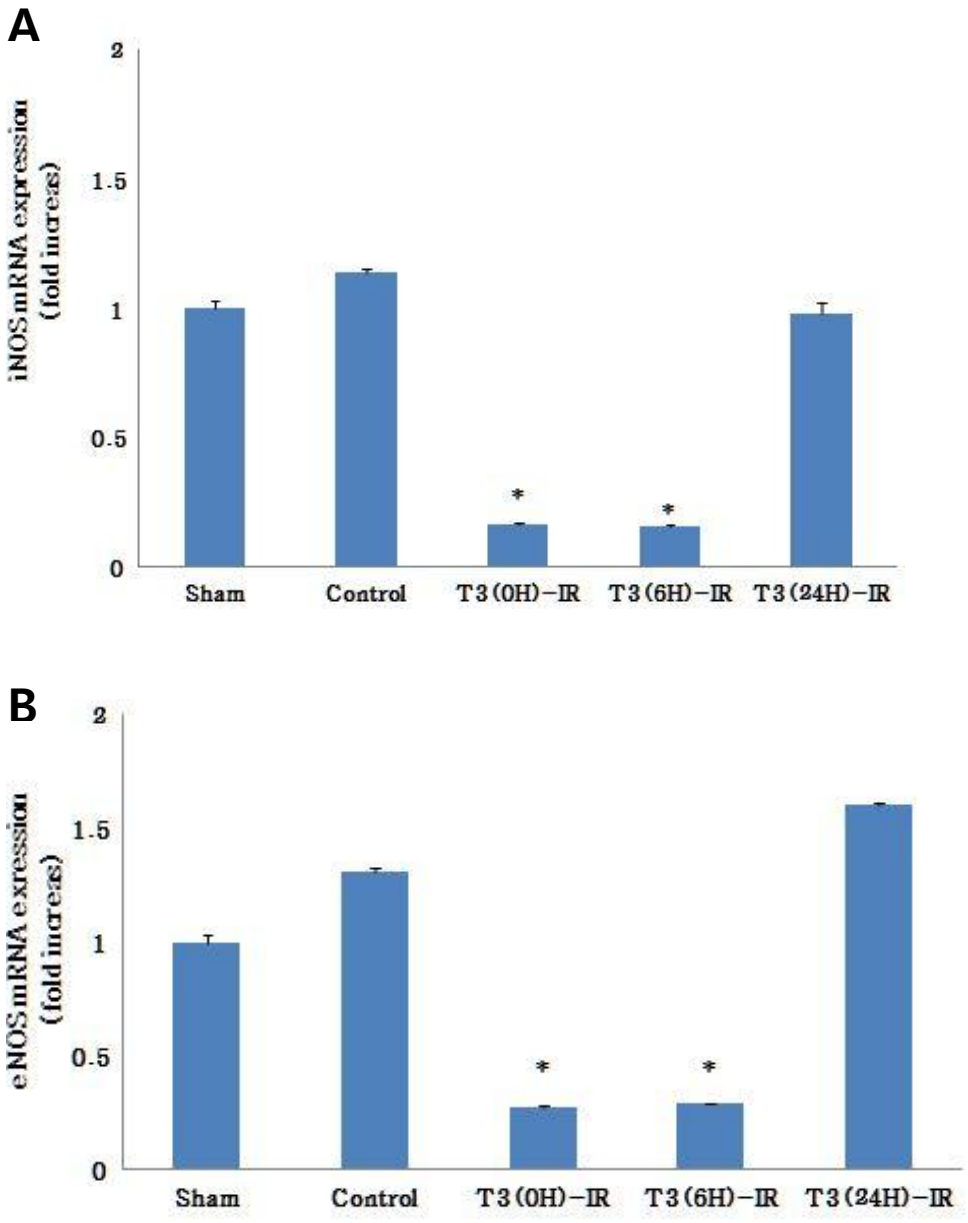
Fig 4. Preconditioning with T3 promoted the activities of antioxidative enzymes.

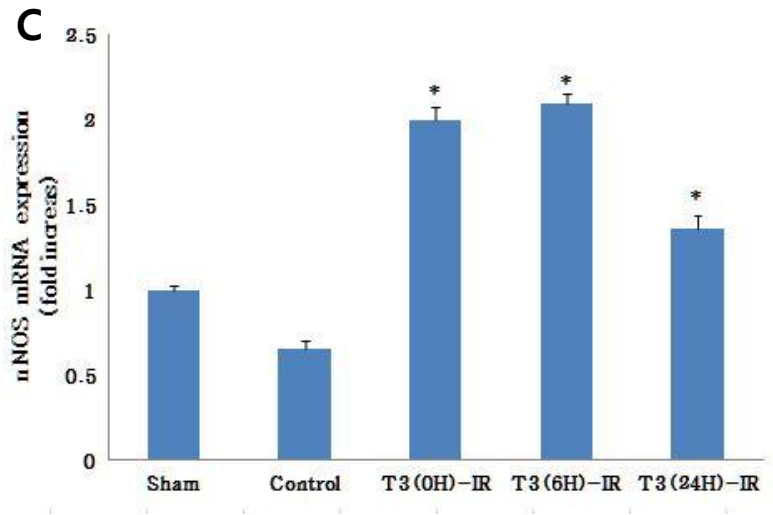


(A) SOD activities and (B) GSH levels in renal tissue from mice of the

various treatment groups observed 24 hours after renal I/R injury. Values shown are expressed as the mean±SEM. N=6 or 7 for each group. $^{*}P$ <0.05 versus control.

Fig 5. Preconditioning with T3 increased the mRNA expression of nNOS.





The mRNA expression of (A) iNOS (B) eNOS, and (C)nNOS in renal tissue evaluated via RQ-PCR at the end of 24-hour-reperfusion. Values shown are expressed as the mean±SEM. N=6 or 7 for each group. $^{*}P$ <0.05 versus control.

국문초록

마우스 신장 허혈-재관류 손상에서 갑상선 호르몬 (3,5,3-triiodothyronine)의 보호효과

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김 서 민

배경: 전처치(preconditioning)은 허혈-재관류 손상을 예방하기 위해 사용되는 방법으로 일시적인 허혈을 유발하거나 산소 요구도를 높여서 조직이나 세포 내의 방어적인 변화를 유도하는 것이다. 심장 또는 간의 허혈-재관류 손상 동물 모델에서 3,5,3-triiodothyronine을 48시간 이전 전처치할 경우, 허혈-재관류 손상을 감소시키는

것으로 알려져 있다. 이 연구의 목적은 마우스에서 시점을 다르게 하여 3,5,3−triiodothyronine을 전처치 할 경우 신장 허혈−재관류 손상에 대한 보호 효과를 평가하고 각각의 경우 관련 요인을 분석하는 것이다.

방법: 수컷 C57BL/6 마우스에서 양측 신장의 신문을 일시적으로 45분 간 결찰한 후 결찰을 풀고 24시간 동안 재관류 시켜 신장 허혈−재관류 손상을 유도하였다. 조직학적 신세관의 손상 정도, 항산화 효소의 활성화, 항염증 인자와 산화 질소(nitric oxide)의 발현 정도를 평가하였다.

결과: 허혈−재관류 손상 6시간 이전 3,5,3−triiodothyronine을 전처치한 경우 조직학적인 신세관 손상이 통계적으로 유의하게 감소하였다. 염증성 싸이토카인은 3,5,3−triiodothyronine을 6시간 이전 또는 허혈−재관류 손상 직전에 전처치한 경우 감소하는 결과를 보였다. 과산화물제거효소(superoxide dismutase)는 24시간 그리고 6시간 이전 3,5,3−triiodothyronine로 전처치한 경우 증가하는 경향을 보였으나 통계적인 유의성은 없었다. 글루타티온 (glutathione)의 경우 3,5,3−triiodothyronine을 주입한 군에서는 시간에

관계 없이 모두 통계적으로 유의하게 증가하는 경향을 보였다. 산화 질소 합성효소의 발현은 3,5,3−triiodothyronine을 6시간 이전 또는 혀혈−재관류 손상 유발 직전 전처치한 경우, neuronal NOS는 유의하게 증가하고 inducible NOS와 endothelial NOS는 유의하게 감소하는 경향을 보였다.

결론: 허혈−재관류 손상 이전 짧은 시간 이내에 3,5,3−triiodothyronine을 전처치한 경우 허혈−재관류 손상에 대한 의미 있는 보호 효과를 나타냈다. 이는 뇌사자 신장 이식에서 적용 가능한 치료가 될 수 있을 것으로 생각한다.

중요단어: 신장 허혈−재관류 손상, 3,5,3−triiodothyronine, 전처치

학번: 2010−21782