Preconditioning with thyroid hormone (3,5,3-triiodothyronine) prevents renal ischemia-reperfusion injury in mice

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Background. *3,5,3-triiodothyronine (T3) was found to decrease ischemia-reperfusion (I/R) injury of liver and myocardium in animal models when preconditioned 48 hours in advance of the I/R injury. The purpose of this study was to evaluate the effects of T3 preconditioning on renal I/R injury with different time intervals and to determine the changes in antioxidants, apoptosis, and nitric oxide synthase (NOS) in each condition.*
Methods. *In male C57BL/6 mice, renal I/R injury was induced by temporary ligation of the bilateral renal pedicles for 45 minutes followed by a reperfusion period for 24 hours. Preconditioning with intraperitoneal injection of T3 was performed 24 or 6 hours before or at the time of I/R injury.*
Results. *From the histologic examination, tubular injury was decreased in mice preconditioned with T3 6 hours before I/R injury. The levels of proinflammatory cytokines were decreased with T3 preconditioning, either 6 hours or at the time of I/R injury. The levels of glutathione were increased in all treatment groups. Expressions of neuronal NOS were increased when preconditioned 6 hours before or at the time of I/R injury. The number of apoptotic tubular epithelial cell evaluated by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay was decreased when preconditioned immediately before I/R injury.*
Conclusion. *Preconditioning with T3 6 hours or immediately before I/R injury had a protective effect on renal I/R injury. The changes of NOS and antiapoptosis, other than well-known antioxidative properties, may play a role in the effect of short-term preconditioning. (Surgery 2014;155:554-61.)*

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

Tolerance to I/R injury can be achieved by preconditioning.[3] Preconditioning induces an adaptational response to brief ischemia that protects against subsequent prolonged ischemic insults.[4] One of the methods used for preconditioning is the administration of 3,5,3-triiodothyronine (T3). Preconditioning with T3 has been reported to decrease I/R injury in the cardiovascular system and liver.[5-7] In the kidneys, preconditioning of rats 24 hours before I/R injury decreased proteinuria and increased the levels of antioxidative enzymes.[8]

In previous literature, the protective mechanism of T3 preconditioning was limited to antioxidation, even though various other mechanisms might be involved. In addition, the effect of T3 preconditioning in a short period of time before I/R injury is not well understood. In a clinical situation, it would take less than 12 hours from the selection of a recipient to performing the transplantation; thus, the effect of short-term T3-preconditiong becomes very relevant and important. We hypothesized that T3 preconditioning within short intervals of 6 hours or immediately before I/R injury could prevent I/R injury. We also thought that the mechanisms other than antioxidation could affect the results.

The aims of this study were to evaluate the protective effects of T3 preconditioning on renal I/R injury at lesser intervals of time (≤6 hours) and

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to determine the changes in antioxidants, apoptosis, and nitric oxide synthase (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 for 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 23−26 g were housed at the experimental animal center affiliated with Seoul National University on a 12-hour light and dark cycle with free access to water and rat chow. T3 (Sigma Chemicals, St. Louis, MO) was prepared with previously described methods.[7,9] Animals received a single dose of T3 (0.1 mg/kg body weight) or equivalent volumes of normal saline intraperitoneally (IP). The injection of T3 was scheduled either 24 or 6 hours before the operation or at the time of the operation. Mice were anesthetized with an IP injection of zoletil (0.8 mL/kg) and xylazine (0.4 mL/kg). Renal warm I/R injury was induced by temporary ligation of the renal pedicles bilaterally for 45 minutes followed by a reperfusion period of 24 hours.[2] During the procedures, temperature loss was controlled with a temperature-regulated table. After the 24-hour reperfusion period, bilateral nephrectomy was performed. We referred to the previous reports about the phamarcokintic data of the thyroid hormone in mice.[9]

Mice were divided randomly into six experimental groups as follows: (1) control-sham (n = 7): mice received IP normal saline (0.9% Nacl) and underwent a laparotomy without induction of renal I/R injury; (2) control with I/R injury (n = 7): mice received normal saline IP and underwent operative manipulation inducing I/R injury; (3) T3 sham (n = 7): mice received T3 IP and underwent a laparotomy without induction of renal I/R injury; (4) T3 (24 hours) with I/R injury (n = 7): mice received T3 IP 24 hours before undergoing operative manipulation inducing I/R injury; (5) T3 (6 hours) with I/R injury (n = 6), mice received T3 IP 6 hours before undergoing operative manipulation inducing I/R injury; and (6) T3 (0 hour) with I/R injury (n = 6), mice received T3 IP just before inducing undergoing operative manipulation inducing I/R injury.

Histologic examination. The renal tissues were fixed in 10% phosphate-buffered formalin for 24 hours, embedded in paraffin, and sectioned at 5 μm. The sections were deparaffinized, hydrated, and stained with hematoxylin-eosin. Stained tissues were scored according to the degree of tubulointerstitial injury, such as tubular necrosis, tubular dilatation, or cellular edema and the degree of inflammatory cell infiltration.[9] A greater 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. Preliminary work showed that change in inflammatory cell infiltration was not present at 24 hours.

Measurement of activities of antioxidants. Antioxidative activity was evaluated by the assessment of superoxide dismutase (SOD) and reduced glutathione (GSH) concentration. Assays were performed using the SOD and GSH assay kits (Cayman, Ann Arbor, MI).

Real-time quantitative polymerase chain reaction (RQ-PCR) to detect proinflammatory markers (tumor necrosis factor-α [TNF-α], interleukin [IL]-6, and *macrophage inflammatory protein 1α* [MIP-1α]) and NOS. Total RNA was extracted from the frozen kidney tissue stored −80°C with TRIzol reagent (Invitrogen, Carlsbad, CA). A mixture of total RNA, primer, and 10 mM dNTPs was made and added to the cDNA synthesis mix: 10X RT-buffer, 0.1 M dithiothreitol, 40 U RNaseOUT, and 200 U Superscript III RT (all supplied by Invitrogen). The mixture was incubated at 25°C for 10 minutes for annealing, 50°C for 50 minutes for cDNA synthesis, and at 85°C for 5 minutes for termination of the reaction. RNA was removed by adding 1 μL of RNase H and incubated at 37°C for 20 minutes. The PCR products were reacted with TagMan Universal PCR Master Mix, and primer (Applied Biosystems, Foster cityCity, CA) 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 Biosystems).[10-13]

Terminal deoxynucleotidyl transferase-mediated nicked labeling (TUNEL) staining. Renal tissues were fixed in 10% phosphate-buffered formalin, embedded in paraffin, and sectioned at 5 μm. To detect apoptosis, TUNEL was performed. Twenty high-power (400x) fields were selected randomly, and the percentages of the numbers of TUNEL-positive nuclei to the numbers of total cell nuclei were counted.

Statistical analyses. Continuous variables are presented as median with range and were compared with the Kruskal-Wallis and Mann-Whitney U tests. A P value of <.05 was considered statistically

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