Cerebral oxidative stress induces spatial working memory dysfunction in uremic mice: neuroprotective effect of tempol

Kiichiro Fujisaki1, Kazuhiko Tsuruya1,2, Mayumi Yamato3, Jiro Toyonaga1, Hideko Noguchi1, Toshiaki Nakano1, Masatomo Taniguchi1, Masanori Tokumoto4, Hideki Hirakata5 and Takanari Kitazono1

1Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan, 2Department of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan, 3Department REDOX Medicinal Science, Faculty of Pharmaceutical Sciences, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan, 4Department of Medicine, Fukuoka Dental College, Sawara-ku, Fukuoka 814-0193, Japan and 5Division of Nephrology and Dialysis Center, Japanese Red Cross Fukuoka Hospital, Minami-ku, Fukuoka 815-8555, Japan

Correspondence and offprint requests to: Kazuhiko Tsuruya; E-mail: tsuruya@intmed2.med.kyushu-u.ac.jp

ABSTRACT

Background. Chronic kidney disease (CKD) is frequently associated with uremic encephalopathy and cognitive impairment. Recent studies have demonstrated that cerebral oxidative stress contributes to cognitive dysfunction. Although oxidative stress has been reported to increase in the uremic rat brain, the relationship between increased oxidative stress and cognitive impairment in uremia is unclear. In the present study, the effects of tempol (TMP), an antioxidant drug, were investigated in uremic mice.

Methods. CKD was induced in male C57BL/6 mice (n = 8) by left nephrectomy and 2/3 electrocoagulation of the right renal cortex. Working memory performance was tested by the radial arm water maze test. We have prepared two protocols (‘time course study’ and ‘treatment study’). First, we examined the working memory test and histological examination of mouse brains after 4 and 8 weeks. Next, we investigated the effect of TMP (3 mM) against uremia-induced neurodegeneration and oxidative stress in the mouse brain.

Results. Eight weeks after CKD induction, vehicle-treated mice made significantly more errors than sham-operated control mice, while TMP improved working memory performance in CKD mice. CKD was associated with accumulation of 8-hydroxy-2′-deoxyguanosine in the hippocampal neuronal cells, but not in TMP-treated CKD mice. Increased numbers of pyknotic neuronal cells were observed in the hippocampus of CKD mice at 8 weeks, but pyknotic neuronal cell numbers were decreased under the influence of TMP in uremic mice.

Conclusions. The present study provided evidence that uremia is associated with spatial working memory dysfunction in mice and that treatment with TMP protects against cerebral oxidative stress and improves cognitive dysfunction in uremic mice, suggesting their potential usefulness for the treatment of cognitive dysfunction in uremia.

Keywords: chronic kidney disease, cognitive dysfunction, oxidative stress, uremia

INTRODUCTION

Uremic encephalopathy presents with diverse symptoms including headache, visual abnormalities, tremor, asterixis, multifocal myoclonus, chorea, seizure, clouding of consciousness, delirium and coma [1]. Several studies have suggested that the frequency of cognitive disturbance is higher than that previously suspected and can be detected even in moderate chronic renal insufficiency [2–4]. In addition, clinical studies have demonstrated a high risk for dementia and cognitive impairment in patients with chronic kidney disease (CKD) and those undergoing hemodialysis [3, 5–7].

The association between oxidative stress and cognitive dysfunction has previously been shown. Row et al. [8] reported...
that intermittent hypoxia caused increased oxidative stress and deficits in spatial learning in rats. Oxidative stress is also implicated in neurodegenerative diseases with memory impairment such as Alzheimer’s disease [9]. The hippocampus is considered important for learning and memory function. Moreover, hippocampal volume can be used to detect cognitive dysfunction and identify elderly people at risk for Alzheimer’s disease [10]. However, the presence of hippocampal injury is unclear in human CKD. Deng et al. [11] reported that chronic renal failure (CRF) resulted in oxidative stress and increased tyrosine nitration in the cerebral cortex. Furthermore, antioxidant therapy alleviated CRF-induced oxidative stress and mitigated tyrosine nitration in rats with CRF. We have also focused on oxidative stress in the development of cognitive dysfunction in uremia and the effect of antioxidant drugs against uremia-induced cognitive dysfunction. Uremia is associated with depressed superoxide dismutase (SOD) levels and elevated NAD(P)H oxidase expression. This was shown by favorable responses to the administration of tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl; TMP), a nitroxide compound with SOD-mimicking activity [12]. Thus, in the present study, we hypothesized that TMP provides protection against chronic uremia-induced cognitive dysfunction through its antioxidative and neuroprotective effects on hippocampal neurons.

To verify this hypothesis, we developed a stable mouse model of uremic CKD and measured cognitive dysfunction using the radial arm water maze (RAWM) test (Figure 1). We undertook protocols to (i) determine the association of uremia-induced cognitive dysfunction with oxidative stress in uremic mice (Protocol 1) and (ii) examine the effect of antioxidants against uremia-induced cognitive dysfunction in uremic mice (Protocol 2).

MATERIAL AND METHODS

Preparation of mice

The present study was conducted in C57BL/6J male mice (8 weeks old; CLEA, Tokyo, Japan). All animal study protocols were reviewed and approved by the Kyushu University Animal Care Committee at the Center for Animal Care Facility. All experiments were conducted according to the National Institute of Health Guideline for the Care and Use of Laboratory Animals. Mice were kept at the Experimental Animal Center of Kyushu University Medical Institution and housed in groups of four or five per cage in a temperature-controlled room (23–25°C). All mice had free access to food and water during the experiments. Mice were sacrificed under anesthesia.

Induction of CKD in mice

CKD was induced in 8-week-old C57BL/6J male mice using a one-step procedure involving electrocoagulation of the surface of a surgically exposed right kidney and left nephrectomy. Details of this method have been reported previously [13, 14]. Briefly, electrocoagulation of the entire surface of the right kidney except for a 2-mm rim of renal tissue around the hilum was followed by left nephrectomy. All mice were subjected to surgery under controlled ether anesthesia, with a surgical approach through small bilateral flank incisions, leaving the intestines and upper abdominal content undisturbed. Renal electrocoagulation was performed using a foot-operated single point cautery angled at 30° (PROG DS3-M; Muromachi Kikai Co., Tokyo, Japan). The right kidney was freed from the perirenal fat and adrenal gland prior to electrocoagulation and special care was taken not to injure the right ureter. After electrocoagulation, the kidney was placed into the renal fossa and completely covered with tissue from the abdominal wall. A left nephrectomy was performed under identical operating conditions. After each surgical procedure, the incisions were closed in layers with clips applied to the skin. The skin-to-skin operative time did not exceed 5 min. The control mice received a sham operation that included decapsulation of both kidneys. Special care was taken to avoid damage to the adrenals. All surgical procedures were performed by a single investigator, and the choice of treatment (TMP or vehicle water) was determined randomly.

Experimental protocols

Time course study—Protocol 1. Eight-week-old C57BL/6J male mice (n = 32) were randomly divided into two groups. All mice were allowed to drink water freely. One group was a sham-operated control group (n = 16), and the other group was CKD-operated group. After surgery on both kidneys, the 32 mice were divided into four groups: sham-operated mice observed for 4 weeks (Cont-4W: n = 8); sham-operated mice observed for 8 weeks (Cont-8W: n = 8); remnant kidney mice observed for 4 weeks (CKD-4W: n = 8) and remnant kidney
mice for 8 weeks (CKD-8W: n = 8). All mice were sacrificed under ether anesthesia at 4 or 8 weeks after surgery.

**Treatment study—Protocol 2.** Eight-week-old C57BL/6j male mice (n = 32) were randomly subdivided into the following four groups (n = 8 for each group): sham-operated mice with drinking vehicle water (Cont-Veh); sham-operated mice with drinking 3 mM TMP solution (Sigma, St Louis, MO) (Cont-TMP); CKD-operated mice with drinking vehicle water (CKD-Veh) and CKD-operated mice with drinking 3 mM TMP solution (CKD-TMP). The effective concentration of TMP was determined by our preliminary study and a previous study [15]. TMP solution was administered in an opaque container and was changed every two days. All mice were sacrificed on day 56 (week 8).

**Biochemical parameters**

Blood samples were drawn from mice before sacrifice. Mice were fully anesthetized and held by the back. Then, blood samples were collected via tail vein, and samples for measurement of serum creatinine (Scr) and blood urea nitrogen (BUN) were immediately frozen at −80°C until measurement. Measurements of Scr and BUN were performed at SRL Inc. (Tokyo, Japan). Hematocrit (Ht) was determined by centrifugation of microcapillary tubes at 10 000 rpm for 5 min.

**RAWM test for spatial working memory assessment**

For the RAWM test, an apparatus was constructed as previously described [16, 17]. The RAWM is a radial arm maze placed in a pool of water (Figure 1). In this experiment, the RAWM consisted of a circular pool measuring 1 m in diameter with 6 19-cm-wide arms radiating out from an open central area, with a submerged escape platform located at the end of one arm. Visual spatial cues including a light were placed on the wall of the testing room. The escape platform was placed on a different arm each day (the platform location does not change over one day), forcing the mice to use their memory to solve the task. A semi-random sequence from four of the remaining four arms was then selected as starting points for each day's four 'acquisition' trials (trial 1–4: T1–4). For any given acquisition trial, the animal was placed into the designated start arm facing the common circular swim area. For the ensuing 1-min trial, the animal was allowed to swim into arms, with each non-goal arm selection (i.e. error; swimming into an arm that did not contain the submerged platform) resulting in the mouse being returned (across the surface of the water) back to that trial's start arm to continue the trial. If a mouse entered the goal arm, but could not locate the submerged platform in that arm, an error was scored and the animal was returned to that trial’s start arm to continue the trial (i.e. a ‘win-stay strategy’). After the fourth trial, the mouse was placed in a cage for 30 min and then returned to the maze and administered the fifth 'memory retention' trial (T5) to assess short-term memory retention. T5 trial was then performed, wherein the start arm was the same as for the T4 trial. The number of errors in the fifth trial on the fifth day was used as the result of the RAWM test for each mouse.

**Open field activity testing**

To test activity and exploratory behavior, each mouse was placed in an open gray cylindrical box, consisting of a circular pool measuring 50 cm in diameter, which has lines (four horizontal and four vertical) to demarcate 16 squares. The total number of line crossings in a single 5-min trial was recorded [16].

**Histological examination of mouse brains**

Perfusion of the hippocampus was performed using a previously reported method with some modification [18]. Briefly, mice were perfused intracardially with a solution containing 4% paraformaldehyde (w/v) in 0.1 mol/L phosphate buffer, pH 7.3–7.5. The brain was dissected and then placed in the same fixative solution for 6 h at 4–5°C. Brains were then dehydrated in ethanol, transferred to 1:1 (v/v) ethanol–xylene, cleared in toluene and embedded in Paraplast (Fischer Scientific, Park Lane, PA). Serial sections (4 μm thick) were mounted on gelatin-coated glass slides. Paraplast was removed using toluene (2 × 10 min), and sections were passed through graded ethanol, washed with distilled water and colored with hematoxylin and eosin (H&E). To determine neuronal density, neuronal nuclei were counted in the 4-μm-thick sections to avoid double counting of neurons.

**Enumeration of degenerated neuronal cells**

H&E-stained sections of the mouse brain were examined by light microscopy (Eclipse E800M model; Nikon, Tokyo, Japan). The investigator was blinded to the groups and counted pyknotic cells in eight ×400 fields in the hippocampus region from each section (four sections per mouse) [19]. The number of pyknotic cells was presented per section (total counts in eight fields per section).

**Immunohistochemical evaluation of hippocampus lesions for oxidative stress**

All specimens were fixed in 10% formalin and routinely processed for paraffin. Formalin-fixed, paraffin-embedded tissue sections were serially cut at 4-μm and mounted on amipropyltriethoxysilane-coated glass slides. Sections were deparaffined in xylene and dehydrated through an ethanol series. Oxidative stress was examined by immunohistochemical staining for anti-8-hydroxy-2’-deoxyguanosine (8-OHdG). Brain sections from experimental mice were immunostained with anti-8-OHdG mouse monoclonal antibody (1:100, Japan Institute for the Control of Aging, Shizuoka, Japan) as described previously [19, 20]. For 8-OHdG staining, the glass slides were treated with 10 μg/mL proteinase K and 4N HCl. Sections were then pre-treated in 0.3% H₂O₂ in methanol for 30 min to inactivate endogenous peroxidase, preincubated with 5% skim milk to reduce nonspecific binding and incubated overnight at 4°C with anti-8-OHdG antibodies. After washing, the sections were incubated with biotinylated secondary antibodies for 1 h at room temperature followed by horseradish peroxidase (HRP)-conjugated streptavidin (100 μg/mL; Nichirei, Tokyo, Japan) for 30 min. HRP was visualized by reaction with 3,3-diaminobenzidine tetrahydrochloride (Nichirei). Staining for 8-OHdG was analyzed from digitalized images using Image
J 1.43 imaging software (National Institute of Mental Health, Bethesda, MD) [21]. We selected four representative hippocampal regions from each mouse and produced image files. The image files were opened in gray scale mode. The plot area, mean density and plot number corresponding to the number of positively stained cells were then determined in the hippocampus using the ‘Analyze Particles’ command after setting an appropriate threshold.

Statistical analysis

All data are expressed as mean ± SEM in the text and figures. Data were analyzed and compared among groups by one-way ANOVA. When a statistically significant effect was found, post hoc analysis (Dunnett method) was performed to detect the difference between the groups. A value of *P < 0.05 was considered statistically significant. Statistical comparisons were conducted using the StatView v5.0 program (Abacus Concept, Berkeley, CA).

RESULTS

Protocol 1

Characteristics of control and CKD mice. Table 1 shows time course of body weight (BW), BUN, SCr and Ht in normal control mice and uremic mice. SCr and BUN concentrations in the CKD-4W and CKD-8W mice were significantly higher than in the control mice (P < 0.05, respectively). There was no significant difference in all parameters between CKD-4W and CKD-8W mice. In addition, Ht and BW were significantly lower in CKD mice compared with control mice. These results indicate that experimental uremic mice are an appropriate model for the study of CKD.

Impaired spatial working memory in the RAWM test in uremic mice 8 weeks after induction of CKD. To determine the association of uremia with the spatial working memory dysfunction, we examined mice using the RAWM test (Figure 1). We trained mice for four days (training days) and then conducted the spatial working memory test on the fifth day. On memory retention test (T5), the numbers of errors of CKD-4W mice were comparable to those of Cont-4W mice, while CKD-8W mice showed significantly more errors than the Cont-8W mice, suggesting that CKD-8W mice showed impaired spatial working memory, probably due to uremia (Figure 2A). Activity and exploratory behavioral studies were performed using an open field activity test to determine whether uremia-induced cognitive dysfunction was associated with a decline in activity or physical abnormalities. We measured the total number of lines crossed in a 5-min period after RAWM test. There was no significant difference in the number of crossed blocks among all groups. The CKD-8W mice did not exhibit a decrease in open field activity or physical performance compared with the control mice (Figure 2B).

Increased number of pyknotic neuronal cells in the hippocampus of uremic mice. To determine whether CKD was associated with neuronal degeneration, we examined the histological changes of neuronal cells using H&E-stained brain sections from all mice (Figure 3). At ×400 magnification, neuronal cells with a condensed and darkly stained cell body and nuclei were considered pyknotic neuronal cells (Figure 3B). The number of pyknotic neuronal cells in the cornu ammonis 3 (CA3) region of the hippocampus showed no difference between the control mice and CKD-4W mice but was significantly higher in the CKD-8W mice compared with the control mice (Figure 3).

Generation of oxidative DNA damage in the hippocampus of uremic mice. To determine whether CKD was associated with oxidative DNA damage in the pyknotic changes of neuronal cell nuclei in the hippocampus, we examined the accumulation of 8-OHdG in the hippocampus by immunohistochemistry. Strong 8-OHdG immunoreactivity was detected in the nuclei of hippocampal CA3 neurons in the CKD-8W mice (Figure 5A). In comparison, nuclei of hippocampal neurons of the control mice and CKD-4W mice exhibited weak immunoreactivity for 8-OHdG, and the numbers of positive cells were significantly lower in the control and CKD-4W mice than CKD-8W mice. (Figure 5B, P < 0.05).

Protocol 2

Characteristics of CKD-Veh and TMP-treated mice. SCr and BUN concentrations in the CKD-Veh and CKD-TMP mice were significantly higher than in the control mice at 8 weeks after operation (P < 0.05, respectively, Table 2). In addition, Ht and BW were significantly lower in all CKD mice compared with the control mice. All parameters were comparable between the CKD-Veh and CKD-TMP mice.

Table 1. Characteristics of control and CKD mice for Protocol 1

<table>
<thead>
<tr>
<th></th>
<th>Cont-4W (n = 8)</th>
<th>Cont-8W (n = 8)</th>
<th>CKD-4W (n = 8)</th>
<th>CKD-8W (n = 8)</th>
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<tr>
<td></td>
<td>Sham operation</td>
<td></td>
<td>Nx+2/3EC</td>
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<tr>
<td>BW (g)</td>
<td>26.8 (26.2–29.2)</td>
<td>28.4 (26.4–29.8)</td>
<td>25.1* (23.5–28.5)</td>
<td>25.8** (24.4–26.1)</td>
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<tr>
<td>BUN (mg/dL)</td>
<td>33.2 (30.2–39.0)</td>
<td>29.0 (22.4–37.8)</td>
<td>72.9* (58.0–102.0)</td>
<td>78.4** (60.8–80.8)</td>
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<tr>
<td>SCr (mg/dL)</td>
<td>0.09 (0.08–0.12)</td>
<td>0.09 (0.06–0.15)</td>
<td>0.26* (0.12–0.48)</td>
<td>0.28** (0.16–0.45)</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>50.8 (48.5–52.0)</td>
<td>51.0 (50.0–53.0)</td>
<td>42.0* (40.0–46.5)</td>
<td>42.3** (38.5–44.1)</td>
</tr>
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</table>

Data are medians with ranges. Cont-4W, sham-operated control mice observed for 4 weeks; Cont-8W, sham-operated control mice observed for 8 weeks; CKD-4W, remnant kidney mice observed for 4 weeks; CKD-8W, remnant kidney mice observed for 8 weeks; CKD, chronic kidney disease; Nx+2/3EC, left nephrectomy and 2/3 electrocoagulation of the right renal cortex; BW, body weight; BUN, blood urea nitrogen; SCr, serum creatinine; Ht, hematocrit.

*P < 0.05 versus Cont-4W mice; **P < 0.05 versus Cont-8W mice.
Prevention of uremia-induced spatial working memory dysfunction by treatment with TMP. To determine the effect of TMP against the spatial working memory dysfunction induced by uremia, we administered the RAWM test to mice (Figure 1). During the memory retention test (T5), the CKD-Veh mice exhibited more errors compared with the control mice, and the treatment with TMP significantly decreased the number of errors to a level similar in control mice (Figure 6).

Prevention of uremia-induced pyknotic changes in neuronal cells by TMP treatment. To determine whether TMP ameliorated neuronal degeneration induced by CKD, we examined the histological changes of neuronal cells using H&E-stained brain sections from all mice (Figure 7A). The number of pyknotic neuronal cells in the CA3 region of the hippocampus was significantly higher in the CKD-Veh mice compared with the control mice. Treatment with TMP protected against the effects of CKD, as there was no significant difference in the number of pyknotic neuronal cells among the control and CKD-TMP mice (Figure 7B).

TMP treatment prevented generation of oxidative DNA damage. To examine the effect of TMP against oxidative DNA damage, we performed immunohistochemistry for 8-OHdG (Figure 8A). Strong 8-OHdG immunoreactivity was detected in the nuclei of hippocampal neurons in the CKD-Veh mice. In comparison, nuclei of hippocampal neurons from the CKD-TMP mice exhibited weak immunoreactivity for 8-OHdG (Figure 8B, P < 0.05).

**DISCUSSION**

The present study provided evidence that uremia induces spatial working memory dysfunction in mice and that oxidative stress is associated with cognitive impairment in the...
uremic mouse model. To our knowledge, this is the first report to show that an antioxidant prevents spatial working memory dysfunction through inhibition of cerebral oxidative stress in uremic mice.

During uremia, the accumulation of neurotoxic metabolites, hormonal disturbances and an imbalance in excitatory and inhibitory neurotransmitters may be important in the pathogenesis of uremic encephalopathy [1, 22]. In addition
cognitive impairment in models of Alzheimer's disease is particularly appropriate for examining this important initial change in cognitive function. The water maze test is considered a useful tool for assessing spatial memory in mice. The number of errors in CKD-TMP mice is significantly lower than in CKD-Veh mice. The ends of the box represent the upper and lower quartiles; thus, the box spans the interquartile range. The median is marked by a vertical line inside the box. The two lines outside the box that extend to the highest and lowest observations represent the whiskers. *P < 0.05 versus control mice.

The present study demonstrated that in the RAWM test, uremic mice in the CKD-8W mice could not reach the escape platform without more errors than the control mice, indicating uremia-induced spatial working memory dysfunction (Figure 2A). In this regard, one may argue that impairment in the RAWM test in uremic mice was attributable to generalized neuronal depression typically seen in uremia. However, the open field test did not demonstrate decreased physical performance or activity in these mice, indicating that these results are valid (Figure 2B). Overall, this study provided clear evidence that chronic uremia caused cognitive dysfunction in the CKD mouse model. There are limitations to the open field test. It does not completely exclude physical impairment and uremia-induced autonomic neuropathy and is influenced by several factors including general activity, anxiety and exploration [26]. In addition, anemia can impair cognitive function and motor/movement functions [27].

In the present study, histopathological analysis revealed the presence of significant differences in the structure of hippocampal CA3 neurons in mice with chronic uremia compared with control mice (Figure 4). Pyknotic neurons were observed in the hippocampus of CKD-8W mice. Liu et al. [19] reported that severe ischemic acute kidney injury induced similar neuropathological changes. Thus, neuropathological lesions of the hippocampus may cause hippocampal dysfunction. The present results also demonstrated that the hippocampus was the only area of the brain where large numbers of pyknotic neurons were found in CKD-8W mice, and only low numbers of pyknotic neurons were noted in other areas of the brain such as the cerebral cortex.

Among various products of oxidative DNA damage, 8-OHdG is the most important due to its abundance and mutagenicity. Immunostaining for 8-OHdG showed significant differences in the structure of hippocampal CA3 neurons in mice with chronic uremia compared with control mice (Figure 4). Among various products of oxidative DNA damage, 8-OHdG is the most important due to its abundance and mutagenicity. Immunostaining for 8-OHdG showed significant differences in the structure of hippocampal CA3 neurons in mice with chronic uremia compared with control mice (Figure 4). Among various products of oxidative DNA damage, 8-OHdG is the most important due to its abundance and mutagenicity. Immunostaining for 8-OHdG showed significant differences in the structure of hippocampal CA3 neurons in mice with chronic uremia compared with control mice (Figure 4). Among various products of oxidative DNA damage, 8-OHdG is the most important due to its abundance and mutagenicity. Immunostaining for 8-OHdG showed significant differences in the structure of hippocampal CA3 neurons in mice with chronic uremia compared with control mice (Figure 4). Among various products of oxidative DNA damage, 8-OHdG is the most important due to its abundance and mutagenicity. Immunostaining for 8-OHdG showed significant differences in the structure of hippocampal CA3 neurons in mice with chronic uremia compared with control mice (Figure 4).
cognitive function. A previous study reported that oxidative stress in the diabetic rat brain caused cognitive dysfunction and that treatment with an antioxidant agent improved cognitive dysfunction [29].

TMP is a membrane-permeable radical scavenger and readily crosses the blood–brain barrier [30, 31]. In the present study, TMP was used to confirm that cognitive dysfunction in uremia is induced through oxidative stress, as TMP prevented cognitive dysfunction.
dysfunction through inhibition of oxidative DNA damage in the hippocampus without improvement of renal function. Previous studies demonstrated that TMP decreased oxidative damage in cerebral mitochondria by targeting upstream peroxynitrite-derived free radicals, which would be expected to ameliorate Ca$^{2+}$ overload, reduce calpain-mediated proteolysis and decrease neurodegeneration in traumatic brain damage [32]. Thus, our results may be explained by these mechanisms.

Asymmetric dimethylarginine (ADMA) derives from methylation of arginine residues in proteins. Hydrolysis of methylated proteins releases ADMA, which competitively inhibits NOS. Patients with end-stage renal disease have high levels of the endogenous NOS inhibitor ADMA. Dimethylamine dimethylaminohydrolase, the main enzyme responsible for degrading ADMA can be functionally impaired by oxidative stress [33]. Torre et al. [34] reported that NOS inhibition with L-NAME decreased spatial memory after common carotid artery occlusion. ROS cause excitotoxicity by facilitating glutamate release, which activates NMDA and non-NMDA receptors. This results in highly increased intracellular Ca$^{2+}$ levels, activation of neuronal NO synthases, formation of peroxynitrite, protein nitration and mitochondrial damage, culminating in neuronal injury [12]. Taken together, our results suggest that TMP, an antioxidative agent, ameliorated cognitive dysfunction in uremic mice through the inhibition of oxidative stress. The neural and cognitive mechanisms of the symptom-reducing and/or recovery-promoting effects of an antioxidative agent in the uremic brain are currently unclear. The striking improvements observed in both behavioral and pathological outcomes following long-term antioxidative agent treatment warrant further analysis.

In conclusion, the present study demonstrated that inhibition of oxidative stress by TMP treatment provided significant protection against uremia-induced cognitive dysfunction. Although the precise mechanisms of the symptom-reducing and/or recovery-promoting effects remain unclear, the striking improvement of behavioral and pathological outcomes following TMP treatment has increased our understanding of the pathogenesis of uremic encephalopathy. Further investigation is required to elucidate the precise mechanisms and develop effective therapies for cognitive dysfunction in patients with CKD.

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**CONFLICT OF INTEREST**

Part of this study was presented at the 41st and 42nd Annual Meetings of the American Society of Nephrology 30 (Philadelphia, USA, 2008; San Diego, USA, 2009).


**REFERENCES**


_Cerebral oxidative stress in uremic encephalopathy_ 537
Role of PCSK9 and IDOL in the pathogenesis of acquired LDL receptor deficiency and hypercholesterolemia in nephrotic syndrome

Shuman Liu and Nosratola D. Vaziri

Division of Nephrology and Hypertension, Department of Medicine, University of California, Irvine, CA, USA

Correspondence and offprint requests to: Nosratola D. Vaziri; E-mail ndvaziri@uci.edu

ABSTRACT

Background. Nephrotic syndrome (NS) leads to elevation of serum total and LDL cholesterol. This is largely due to impaired LDL clearance, which is caused by hepatic LDL receptor (LDLR) deficiency despite normal LDLR mRNA expression, pointing to a post-transcriptional process. The mechanism(s) by which NS causes LDLR deficiency is not known. By promoting degradation of LDLR, Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) and inducible degrader of the LDL receptor (IDOL) play a major role in post-translational regulation of LDLR. We, therefore, tested the hypothesis that LDLR deficiency despite its normal gene expression in NS may be due to upregulation of hepatic PCSK9 and IDOL.

Methods. LDLR, IDOL and PCSK9 expressions and nuclear translocation of liver X receptor (LXR) that regulates IDOL expression were determined in the liver of rats with puromycin-induced NS and control (CTL) rats.

Results. Compared with the CTLs, the NS rats showed marked elevation of serum total and LDL cholesterol and a significant reduction in hepatic LDLR protein expression. This was accompanied by marked upregulation of hepatic PCSK9 and IDOL expressions and heightened LXR activation.

Conclusions. LDLR deficiency, hypercholesterolemia and elevated plasma LDL in NS are associated with upregulation of PCSK9 and IDOL. Interventions targeting these pathways may be effective in the management of hypercholesterolemia and the associated cardiovascular and other complications of NS.

Keywords: atherosclerosis, lipid disorders, liver X receptor, lipid disorders, proteinuria