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Research Paper

Ethanol consumption increases the expression of endothelial nitric oxide synthase, inducible nitric oxide synthase and metalloproteinases in the rat kidney

Luis F. Tirapelli[a], Alisson Martins-Oliveira[a], Marcelo E. Batalhão[b], Daniela P. Tirapelli[a], Evelin C. Carnio[b], José E. Tanus-Santos[a], Regina H. Queiroz[c], Claudia M. Padovan[d] and Carlos R. Tirapelli[b]

[a]Faculty of Medicine of Ribeirão Preto, [b]College of Nursing of Ribeirão Preto, [c]Faculty of Pharmaceutical Sciences of Ribeirão Preto, Department of Clinical, Toxicological, and Food Science Analysis, and [d]Faculty of Philosophy Science and Letters of Ribeirão Preto, Department of Psychology, University of São Paulo, SP, Brazil.

Keywords
ethanol; histopathological changes; kidney; metalloproteinase; nitric oxide

Correspondence
Carlos R. Tirapelli, Departamento de Enfermagem Psiquiátrica e Ciências Humanas, Laboratório de Farmacologia, Universidade de São Paulo, Escola de Enfermagem de Ribeirão Preto, Avenida Bandeirantes 3900, 14040-902, Ribeirão Preto, SP, Brazil.
E-mail: crtirapelli@eerp.usp.br

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Abstract

Objectives The effects of longterm ethanol consumption on the levels of nitric oxide (NO) and the expression of endothelial NO synthase (eNOS), inducible NO synthase (iNOS) and metalloproteinase-2 (MMP-2) were studied in rat kidney.

Methods Male Wistar rats were treated with 20% ethanol (v/v) for 6 weeks. Nitrite and nitrate generation was measured by chemiluminescence. Protein and mRNA levels of eNOS and iNOS were assessed by immunohistochemistry and quantitative real-time polymerase chain reaction, respectively. MMP-2 activity was determined by gelatin zymography. Histopathological changes in kidneys and indices of renal function (creatinine and urea) and tissue injury (mitochondrial respiration) were also investigated.

Results Chronic ethanol consumption did not alter malondialdehyde levels in the kidney. Ethanol consumption induced a significant increase in renal nitrite and nitrate levels. Treatment with ethanol increased mRNA expression of both eNOS and iNOS. Immunohistochemical assays showed increased immunostaining for eNOS and iNOS after treatment with ethanol. Kidneys from ethanol-treated rats showed increased activity of MMP-2. Histopathological investigation of kidneys from ethanol-treated animals revealed tubular necrosis. Indices of renal function and tissue injury were not altered in ethanol-treated rats.

Conclusions Ethanol consumption increased renal metalloproteinase expression/activity, which was accompanied by histopathological changes in the kidney and elevated NO generation. Since iNOS-derived NO and MMPs contribute to progressive renal injury, the increased levels of NO and MMPs observed in ethanol-treated rats might contribute to progressive renal damage.

Introduction

Longterm ethanol consumption has been associated with renal alterations, including tubular necrosis,[1] renal tubular dysfunction[2] and albuminuria.[3] However, the underlying pathophysiological mechanisms in the development of ethanol-induced nephropathy are not clear. Several studies strongly implicate the contribution of renal oxidative stress to the pathogenesis of nephropathy.[4,5] Oxidative stress has been demonstrated in the kidney in almost all types of nephropathy whatever the primary disease of its origin might be.[4] Ethanol induces oxidative stress in rat tissues such as the kidney,[5] which is associated with enhanced production of reactive oxygen species (ROS). In addition to ROS, nitric oxide (NO) is also important in the development of renal injury. NO is a free radical that is produced from L-arginine by the catalic action of NO synthase (NOS).[6] NOS is an enzyme that occurs in three major isoforms: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS).[7] A basal production of NO is necessary for maintaining the normal physiology of renal haemodynamics as well as adequate glomerular function. Transient generation of NO by eNOS is critical for mediation of vasorelaxation and protection against oxidative stress.[8] However, in addition to its physiological effects in the kidney, alterations in the NO pathway are understood to be an important contributor to

the pathophysiology of renal diseases. It has been reported that inhibition of NOS prevents hypoxic cellular damage in freshly prepared proximal tubules.[9] Moreover, oxidative stress resulted in increased immunodetectable iNOS, elevated NO release and nitrite production, and decreased cell viability, in epithelial BSC-1 cells.[10] In fact, sustained generation of NO by iNOS, depending on the cellular context, may turn on a broad spectrum of sequelae, from lipid peroxidation to DNA damage and pro-apoptotic effects.[11] Ethanol consumption increases iNOS expression and affects iNOS-derived NO in different tissues.[12–14]

Metalloproteinases (MMPs) are reported to be involved in the pathophysiology of many renal diseases.[15] MMPs are a family of zinc-dependent proteinases, which together have the capacity to break down all components of the extracellular matrix. MMP-2 induces the transformation of renal tubular epithelium to the myofibroblastic phenotype, a critical step heralding the development of renal interstitial fibrosis in many renal diseases.[16] Overexpression of MMP-2 in renal proximal tubular epithelial cells induces pathological changes that are characteristic of human chronic kidney disease.[17] In rodents, dysregulation of MMP-9 activity or expression has been demonstrated in proteinuric renal diseases[18] and antiglomerular basement membrane glomerulonephritis.[19] MMP-2 and MMP-9 expression can be modified by ethanol consumption. It has been reported that MMP-9 levels are elevated in the serum of alcohol abusers.[20] Moreover, longterm ethanol consumption upregulates MMP-2 activity in rat aorta.[21] However, whether ethanol is capable of inducing MMP expression in the kidney remains elusive.

Given the importance of NO and MMPs in the development of nephrotoxicity, in the present work, we studied the effect of longterm ethanol consumption on the levels of NO and the expression of eNOS, iNOS and MMPs in the rat kidney. Moreover, we investigated whether ethanol consumption induces renal dysfunction or histopathological changes in the kidney.

Materials and Methods

Experimental design

The rats were housed under standard laboratory conditions with free access to food and water. The housing conditions and experimental protocols were approved by the Animal Ethics Committee of the University of São Paulo, Brazil. Male Wistar rats (220–250 g, 50–70 days old) were randomly divided into two groups: control group and ethanol-treated group. Rats in the control group received water *ad libitum*, while rats in the ethanol-treated group received 20% (v/v) ethanol in their drinking water.[12] The ethanol-treated group was submitted to a brief and gradual period of adaptation: the animals received 5% ethanol in their drinking water in the first week, 10% in the second week and 20% in the third week (all values in volume ratios). At the end of the third week, the experimental stage began. The rats were treated for 6 weeks and then the kidneys were removed for biochemical and histological analysis.

Blood ethanol levels

Blood was collected from the inferior vena cava of anaesthetized rats (thiopental sodium, 0.4 mg/kg, i.p.) using heparinzed syringes, and the samples were analysed using a CG-17A gas chromatographer (Shimadzu, Kyoto, Japan) as previously described.[12,22] The results are expressed as mg ethanol/ml blood.

Biochemical parameters

Rats were anaesthetized (thiopental sodium, 0.4 mg/kg, i.p.) and blood samples were obtained from the inferior vena cava. The kidneys were removed, frozen in liquid nitrogen and stored at −80°C. Serum samples were used for the measurement of urea and creatinine levels, which were used as indicators of glomerular function. The levels of urea and creatinine were determined with an Abbott-Aeroset auto-analyzer (Chicago, IL, USA) using original kits.

Kidney mitochondria were prepared in 0.25 mol/l sucrose and 1 mmol/l ethylenediamine tetraacetic acid at pH 7.2 and 4°C using standard centrifugation procedures. Mitochondrial protein and mitochondrial respiratory function were measured as previously described.[22] Mitochondrial respiration was initiated by the addition of succinate (5 mmol/l final concentration) and oxidative phosphorylation by the addition of 200 mmol/l ADP. Oxygen consumption recordings allowed the calculation of V3 (rate of state 3 (ADP-stimulated) respiration), of V4 (rate of state 4 (non-ADP-stimulated) respiration), and of the respiratory control ratio (RCR = V3/V4). The oxygen uptake of V3 and V4 was expressed in nmol oxygen/min per mg mitochondrial protein. Mitochondrial swelling was determined in a hypotonic buffer by measuring the decrease in the absorbance at 540 nm, using a Beckman DU-640 spectrophotometer (Rockville, MD, USA).

Histopathological evaluation

For histology, tissue samples fixed in 10% buffered formalin were paraffin embedded for preparation of 5-μm sections that were stained with hematoxylin and eosin as previously described.[22] Using a binocular Zeiss microscope (model Axioskop 2 plus, Jena, Germany), the interstitial tubular damage was graded according to Goujon criteria,[23] which analyses six basic morphological patterns. The morphological changes were graded on a 5-point scale: 1, no abnormality; 2, mild lesions affecting 10% or less of kidney samples; 3, lesions affecting 25% of kidney samples; 4, lesions affecting

50% of kidney samples; and 5, lesion affecting 75% or more of kidney samples.

Assessment of lipid peroxide levels in the kidney

Lipid peroxide levels in renal homogenates were determined by measuring thiobarbituric acid reactive substances using a fluorometric method as previously described.[24] This method requires excitation at 515 nm and emission at 553 nm and uses 1,1,3,3-tetramethoxypropane as standard. The lipid peroxide levels were expressed in terms of malondialdehyde (MDA, nmol/ml). MDA results were normalized for protein concentration assessed with the Bradford technique.

Basal levels of nitrite and nitrate

Basal nitrite and nitrate levels were measured in supernatants from total kidney homogenates prepared under liquid N_2 as previously described.[25] In brief, aliquots of 5 μl were injected into a Sievers chemiluminescence analyzer (model 280, Boulder, CO, USA) and pelleted by centrifugation with VCl_3 and HCl (at 95°C) as reductants for nitrate and NaI, and acetic acid as reductants for nitrite. Results were normalized for protein concentration assessed with the Bradford technique. The results are expressed as μmol/l/mg protein.

Quantitative real-time polymerase chain reaction for eNOS and iNOS

For the quantitative analysis of the genes of interest, which consisted of eNOS (Rn02132634_s1) and iNOS (Rn00561646_m1), we used the commercially available system TaqMan Assays-on-Demand, which consists of oligonucleotides and probes (Applied Biosystems, Foster, CA, USA). The cDNA obtained was diluted 1 : 10 and 4.5 μl was used for each 10 μl of the real-time polymerase chain reaction mixture using the TaqMan Master Mix (Applied Biosystems). All reactions were carried out in duplicate and analysed with the 7500 Sequence Detection System apparatus (Applied Biosystems). Data were analysed using the ABI-7500 SDS software (Applied Biosystems). The total RNA absorbed was normalized on the basis of the Ct value for the GAPDH gene (Rn 01775763_m1). The variation of expression among samples was calculated by the $2^{-\Delta\Delta Ct}$ method, with the mean ΔCt value for a group of six samples from control rats being used as a calibrator.[25]

Imunohistochemistry

Paraffin-embedded kidney segments were stained by the avidin-biotinylated peroxidase complex method. Briefly, 4-μm sections (Reichert Jung 2040 microtome, Wetzlar, Germany) were cut and put through a deparaffinization protocol with xylene and ethanol. Endogenous peroxidase and biotin were blocked by immersing slides in 3% hydrogen peroxide. The following primary antibodies were incubated: iNOS (1126–1144; N7782; Sigma-Aldrich, St Louis, MO, USA) diluted 1 : 200, and eNOS (1185–1205; N3893; Sigma-Aldrich) diluted 1 : 200. The reactions were revealed using 0.2 mg/ml diaminobenzidine solution (10 mg tablets in 50 ml PBS, 0.01 M, pH 7.4; D5905; Sigma-Aldrich) and stained by Harris hematoxylin. In each slide, two fields were selected in areas with the higher concentration of positive cells or stained cells using 400× magnification. Positive and negative stained cells were counted. Results were expressed as percent of positive cells. The slides were analysed using a Leica microscope (model DM 5500 B, Wetzlar, Germany). The images were registered by a Leica digital camera DFC 290 (3MP) attached in the microscope, and filed by Leica QWin software.

Measurement of renal MMP-2 activity by gelatin zymography

Tissue extracts normalized for protein concentration were subjected to electrophoresis on 7% SDS-PAGE copolymerized with gelatin (1%) as the substrate as previously described.[26] Intergel analysis was possible after normalization of gelatinolytic activity with an internal standard (fetal bovine serum 2%). Drugs and reagents were purchased from Sigma-Aldrich. The pro- and active forms of MMP-2 were identified as bands at 72 and 64 kDa, respectively, which were inhibited by phenanthroline and not by other proteinase inhibitors, and were further identified by immunoprecipitation with specific antibodies.[26] Drugs and reagents were purchased from Sigma-Aldrich.

Net MMP activity in the renal homogenates was measured using a gelatinolytic activity kit (E12055; Molecular Probes, Eugene, OR, USA) as previously described.[26]

Statistical analysis

Data are presented as means ± SEM. Statistically significant differences were calculated by the Student's *t*-test (GraphPad Prism 3.0). $P < 0.05$ was considered as statistically significant. The morphological changes were graded on a 5-point scale,[23] and the results were expressed as the median. Comparison of these data was performed using the Mann-Whitney test (SPSS 17.0).

Results

Body mass

Before treatment, rats had a mean body mass of 234 ± 8g (control group, $n = 25$) and 240 ± 9g (ethanol-treated group, $n = 24$). After treatment for 6 weeks, rats in the ethanol-treated group showed a significant reduction in body mass (432 ± 16g) when compared with the control rats (515 ± 12g) ($P < 0.05$; Student's *t*-test).

Biochemical parameters and blood ethanol levels

Chronic ethanol consumption did not alter urea and creatinine levels. No differences were found in mitochondrial respiration between kidneys from control or ethanol-treated rats (Table 1). The blood ethanol level in the ethanol-treated rats ($n = 12$) was 1.3 ± 0.15 mg/ml (~28 mmol/l).

Table 1 Biochemical markers of kidney function in control and ethanol-treated rats

Biomarker	Control	Ethanol
Creatinine (mg/dl)	0.68 ± 0.04	0.64 ± 0.03
Urea (mg/dl)	45.9 ± 2.3	44.4 ± 3.9
State 3 respiration (nmol O_2/min per mg protein)	81.5 ± 6.5	80.6 ± 9.4
State 4 respiration (nmol O_2/min per mg protein)	26.3 ± 2.2	23.1 ± 3.7
Respiration control ratio (state 3 / state 4)	3.2 ± 0.3	3.7 ± 0.6
Mitochondrial swelling (Δ absorbance)	2.1 ± 0.20	2.5 ± 0.25

Values are means ± SEM of ten and eight animals for control and ethanol groups, respectively.

Histopathological evaluation

No histopathological alterations were observed in the kidneys from control rats (Figure 1a). In some animals, inflammatory foci close to venules were observed (Figure 1b). According to the Goujon scale,[23] the morphological patterns typical of proximal tubular damage in the five animals analysed in each group as well as its degree on a scale from 1 to 5 are presented in Table 2. The animals in the ethanol group showed changes, with scores of 1, 2 and 3 in various morphological parameters. The integrity of the brush border and tubular necrosis showed changes, with a score of 3 in some animals. Another change observed, but not assessed by the Goujon

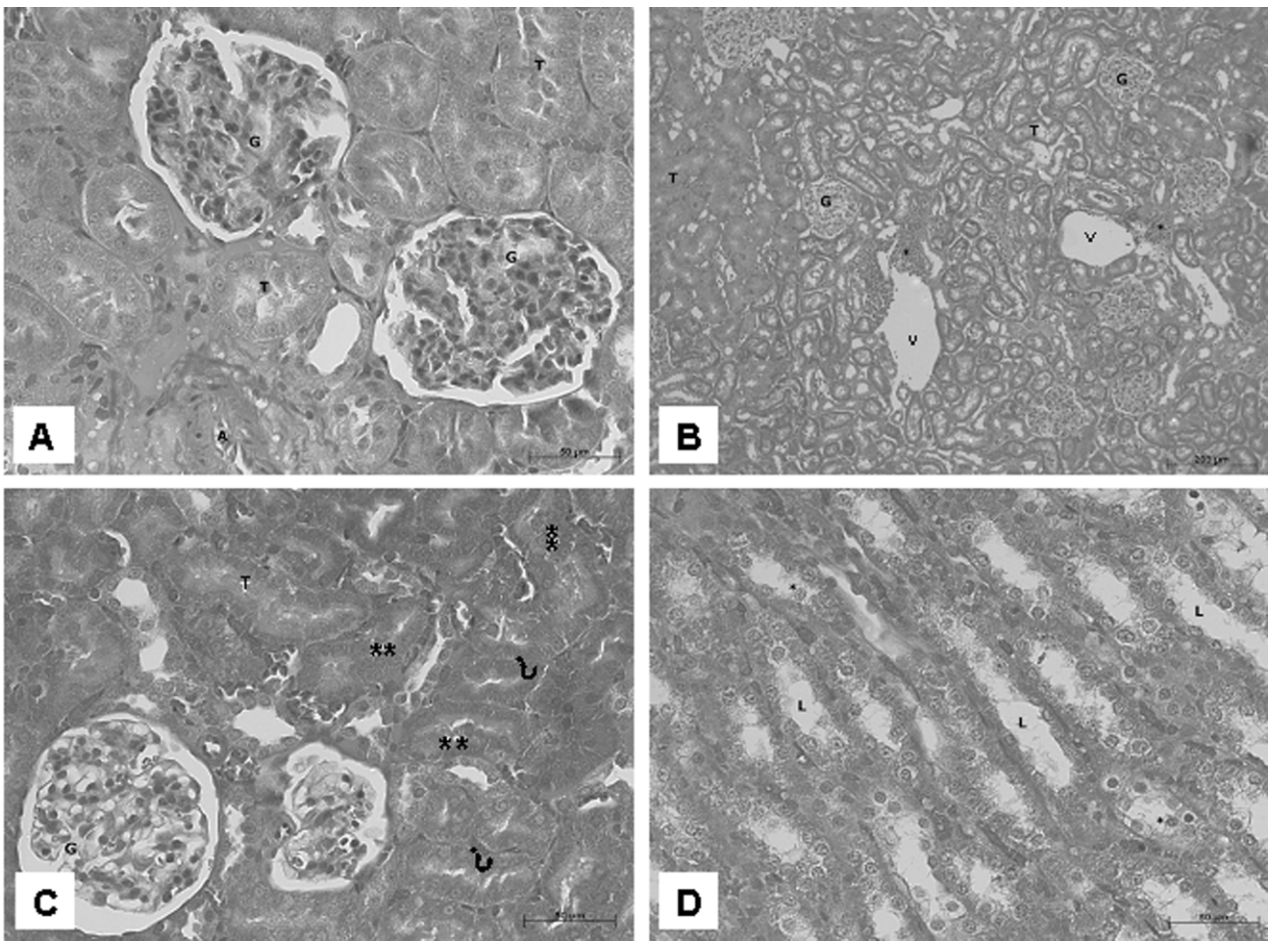

Figure 1 Photomicrograph of the renal cortex from control (a) (400×) and ethanol-treated rats (b, c and d). Presence of inflammatory focus (*) (b) (100×); loss of brushed border (→) and exfoliated cells (**) was observed in the lumen of proximal convoluted tubule in the cortex (c) (400×) and in the lumen of the collecting tubule of the medulla (d) (400×). A, arteriole; G, glomeruli; L, lumen of collecting tubule; T, proximal convoluted tubule; V, venule. Hematoxilin-eosin staining.

Table 2 Values of rating scales of morphological patterns in control and ethanol-treated rats according to Goujon criteria[23]

Morphological patterns	Control	Median	Ethanol	Median	*P* value
Apical cytoplasm vacuolization	1,1,1,1,1	1	1,2,1,2,2,	2 (1–2)	0.151
Tubular necrosis	1,1,1,1,1	1	2,1,2,3,2	2 (1–3)	0.032*
Tubular dilatation	1,1,1,1,1	1	1,2,1,2,2	2 (1–2)	0.151
Cell detachment	1,1,1,1,1	1	1,2,2,2,2	2 (1–2)	0.032*
Brush border integrity	1,1,1,1,1	1	2,2,2,3,3	2 (2–3)	0.008*
Denuded basement membrane	1,1,1,1,1	1	1,1,1,2,1	1 (1–2)	0.690

Scores are expressed as the median, $n = 5$ for each group. *Significantly different compared with the control group (Mann-Whitney test).

Table 3 Nitrate and nitrite levels and mRNA expression of eNOS and iNOS in kidneys from control and ethanol-treated rats

	Control	Ethanol
Nitrate (μmol/l per mg protein)	0.13 ± 0.01	0.35 ± 0.03*
Nitrite (μmol/l per mg protein)	0.06 ± 0.01	0.14 ± 0.01*
eNOS ($2^{-\Delta\Delta Ct}$)	0.35 ± 0.08	2.93 ± 0.85*
iNOS ($2^{-\Delta\Delta Ct}$)	1.94 ± 0.47	5.95 ± 1.76*

eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase. Values are means ± SEM of six animals for control and ethanol groups. *$P < 0.05$, significantly different compared with the control group (Student's *t*-test).

scale,[23] was the presence of the exfoliated cells in the lumen of collecting tubules of the renal medulla (Figure 1d).

Renal MDA levels

No difference in the MDA levels was found between tissues from control (0.23 ± 0.03 μmol/mg protein, $n = 6$) and ethanol-treated rats (0.19 ± 0.03 μmol/mg protein, $n = 6$).

Basal levels of nitrite and nitrate

Chronic ethanol consumption induced a significant increase in renal nitrite and nitrate levels (Table 3).

eNOS and iNOS mRNA levels

The results obtained by quantitative real-time polymerase chain reaction showed that treatment with ethanol increased renal mRNA for eNOS and iNOS (Table 3).

Imunohistochemistry for eNOS and iNOS

Immunohistochemical positive staining for the NOS isoforms (iNOS and eNOS) was found to be focally distributed throughout the renal cortex and medulla (Figure 2a). Ethanol consumption increased renal staining of both eNOS and iNOS (Figure 2b).

Renal MMP-2 activity

Figure 3a shows a representative zymogram of renal extracts showing pro- and activated MMP-2 bands. Kidneys from ethanol-treated rats showed increased activity of the active form of MMP-2 compared with control animals (Figure 3b). Similarly, ethanol consumption increased net MMP activity in the rat kidney (Figure 3c).

Discussion

Moderate ethanol consumption is generally considered to be over the range of 1–3 drinks/day,[27,28] giving rise to blood ethanol levels of approximately 5–25 mmol/l. In alcoholics, blood ethanol levels can reach 100 mmol/l.[29] Thus, the concentrations of ethanol found in our study (28 mmol/l) are physiologically relevant. In the present study, histopathological investigation revealed that ethanol consumption induces tubular necrosis. This result is in accordance with previous findings in humans, where longterm ethanol consumption induced renal alterations, including acute tubular necrosis.[1] In this study, the most prominent pathological abnormalities were observed in the renal cortex when compared with the medulla. Although ethanol consumption induces histopathological changes, we found that indices of renal function (creatinine and urea) and tissue injury (mitochondrial respiration) were not altered in ethanol-treated rats. A factor that might account for the lack of effect of ethanol on renal function in ethanol-treated animals is the period of treatment. In fact, there are reports of a positive correlation between the period of ethanol intake and the extent of tissue damage.[30] Thus, the period of exposure to ethanol could be an important factor in the development of renal dysfunction. The present data suggest that the deleterious effects of ethanol in the rat kidney could occur in an initial phase and that exposure of these animals to a longer period of ethanol treatment would aggravate the histopathological alterations and induced renal dysfunction. It is therefore relevant to study the time-course of the effects of chronic ethanol intake on histopathological kidney changes and renal function.

The histopathological alterations observed in the present study could be associated with excessive production of ROS induced by ethanol, as previously demonstrated in the kidney.[5] Rat kidney tissue contains alcohol dehydrogenase,[31] an enzyme that oxidizes ethanol to generate acetaldehyde. The oxidation of acetaldehyde by the acetaldehyde dehydrogenase generates ROS that are able to induce cell

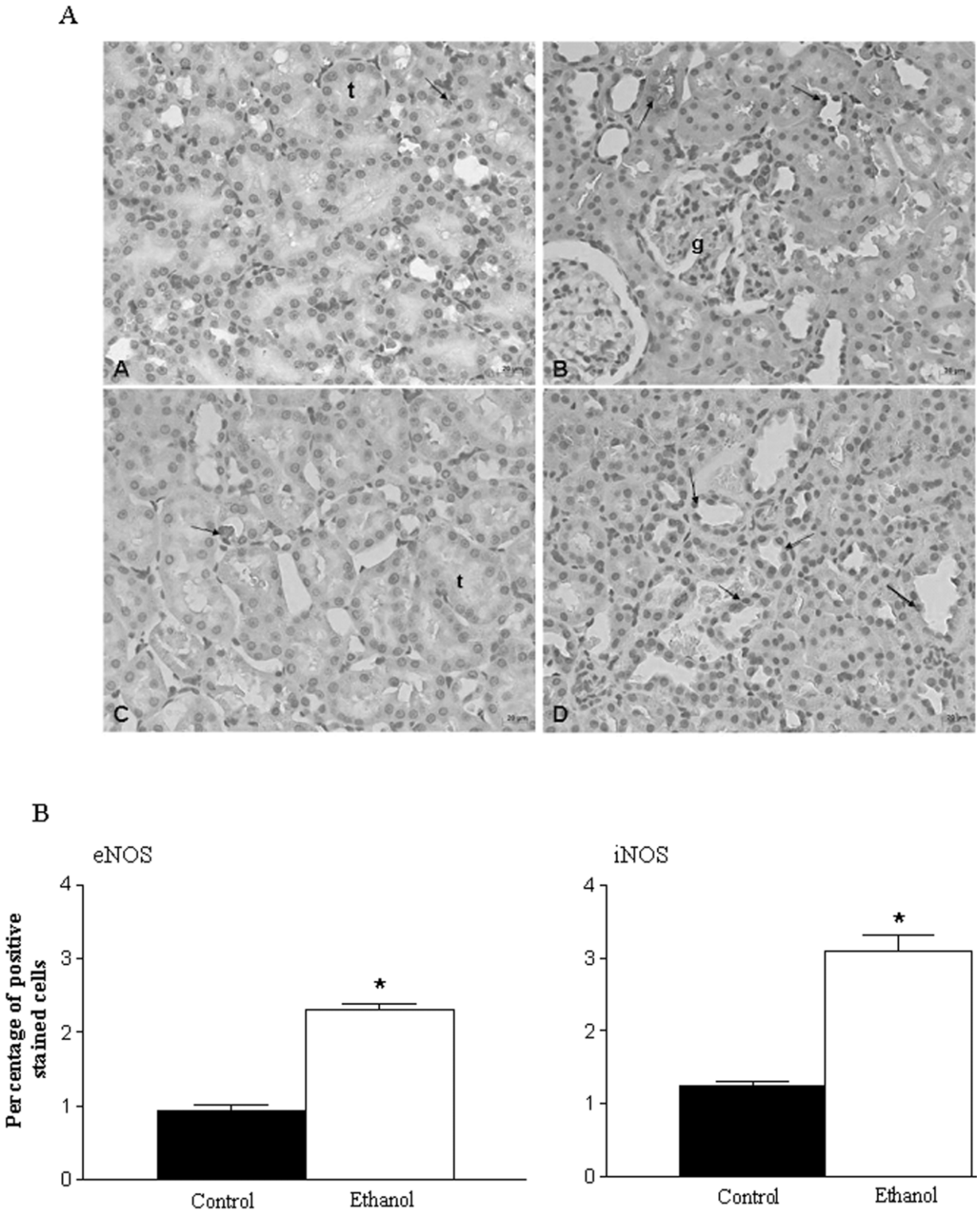

Figure 2 (a) Representative immunohistochemical photomicrographs of positive staining (arrows) for endothelial nitric oxide synthase antibody (A, control group; B, ethanol group) and inducible nitric oxide synthase (C, control group; D, ethanol group) in the kidney, showing labelling in the cytoplasm of renal tubule cells (400×). g, Glomeruli; t, lumen of the proximal convoluted tubules. (b) Bar graphs represent the percentage of positive stained cells for endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in kidneys from control and ethanol-treated rats. Values are means ± SEM of five animals for each group. *$P < 0.05$, significantly different compared with the control group (Student's *t*-test).

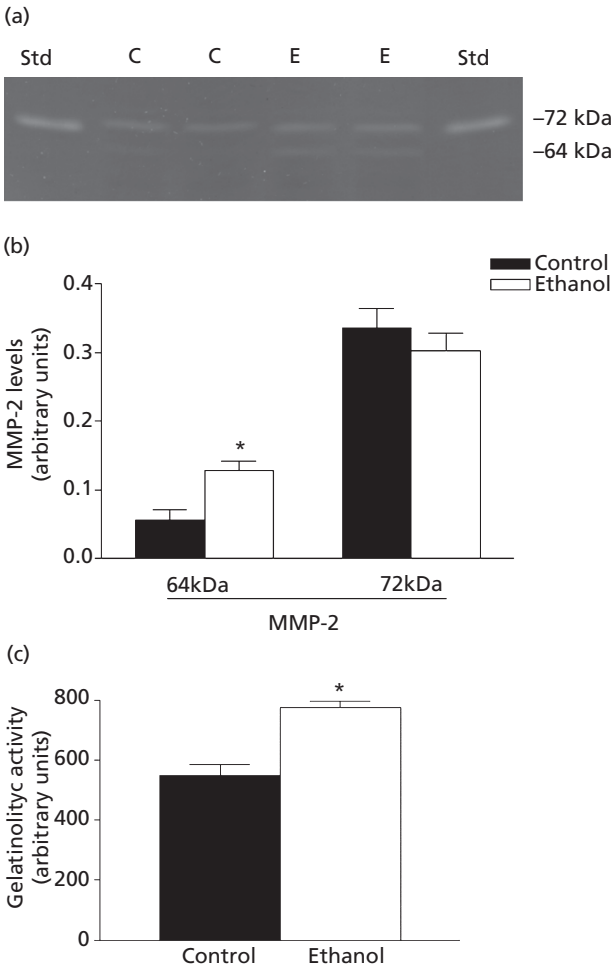

Figure 3 (a) Representative SDS-PAGE gelatin zymogram of renal samples. Molecular weights of MMP-2 bands were identified after electrophoresis on 7% SDS-PAGE. Std, internal standard, C, control, E, ethanol. (b) Effect of ethanol consumption on pro- (72 kDa) and active (64 kDa) forms of metalloproteinase-2 (MMP-2) in the rat kidney. (c) Effect of ethanol consumption on net activity of metalloproteinases. Values are means ± SEM of six to eight kidneys. $*P < 0.05$, significantly different compared with the control group (Student's *t*-test).

membrane damage.[32] The accumulation of ROS leads to damage in cellular components such as lipids and proteins, resulting in an increase in MDA levels. We found no alteration in the MDA levels in kidneys from ethanol-treated rats, suggesting that ethanol consumption did not induce ROS generation in our experimental model. Moreover, generation of ROS, which would lead to pathological lesions in the kidney, could be generated via infiltration of neutrophils.[33] However, in our study no polymorphonuclear cell infiltration was found.

Measurement of tissue nitrite and nitrate provides a reliable and quantitative estimate of NO formation *in vivo*, a free radical that is also important in the development of renal injury.[8] Our data show increased levels of both nitrite and nitrate in kidneys from ethanol-treated rats, further suggesting that chronic ethanol consumption increases the production of NO in this tissue. Our data corroborate previous findings that ethanol consumption increases NO levels in different tissues such as vascular endothelial cells[34] and aorta.[12] Involvement of NO in the pathogenesis of inflammation and some degenerative disorders has been linked to the generation of peroxynitrite ($ONOO^-$).[8] NO can interact with superoxide anion (O_2^-) to form $ONOO^-$, which is a powerful oxidant, causing a number of potentially dangerous reactions including lipid peroxidation, DNA cleavage and reduced vasodilating activity.[35] MDA, frequently used to show the involvement of free radicals in cell damage, is one of the final products of lipid peroxidation. However, as mentioned before, no alterations in MDA levels in the kidney from ethanol-treated rats were found, suggesting that ethanol consumption did not induce lipid peroxidation.

The mRNA expression of both eNOS and iNOS was increased by ethanol consumption. Moreover, immunohistochemical assays showed increased immunostaining for both NOS isoforms, indicating that ethanol consumption upregulates NOS expression at the pre-translational level. Nevertheless, the mechanisms by which ethanol upregulates NOS expression and whether it involves direct or indirect pathways are not yet clear. One possible explanation may involve the ethanol-induced enhancement in plasma levels of endotoxin. It was shown that endotoxemia upregulates the cardiac expression of iNOS in female rats treated with ethanol.[13] In fact, endotoxemia is associated with activation of eNOS and iNOS in the cardiovascular system[7] and it is described to play a role in the biological effects of ethanol.[36,37] Moreover, in response to lipopolysaccharide, young rats exposed to ethanol in the uterus showed increased splenic and iliac iNOS expression and activity.[38,39] Finally, it was recently reported that chronic ethanol consumption induced a 4-fold increase in plasma endotoxin along with significantly higher myocardial iNOS and eNOS expression.[14]

Increased expression of eNOS associated with ethanol consumption has been reported in cavernosal smooth muscle[25] and myocardium.[14] In cultured vascular endothelial cells ethanol increases the expression of eNOS and the production of NO.[34] The increased expression of eNOS could contribute to the increased levels of renal NO described here. Transient spike-like generation of NO, characteristic of eNOS activation, displays a renoprotective effect since it mediates vasorelaxation and protection against oxidative stress.[8] In fact, inhibition of eNOS is one of the hallmarks of developing endothelial cell dysfunction, which may accompany some forms of acute renal injury. On the other hand, sustained, high-output generation of NO by iNOS induces lipid peroxidation[11] and renal injury.[10,40] Ethanol consumption was previously reported to induce upregulation of iNOS in

several tissues such as female rat liver,[41] ovarian tissue,[42] aorta,[12,43] myocardium[14] and cavernosal smooth muscle.[25] The increase in renal NO bioavailability triggered by iNOS induction has been linked to renal dysfunction, since selective inhibition, depletion or deletion of iNOS is associated with renoprotective effects.[10,40] A possible link between increased NO production and renal dysfunction has been suggested.[8] Cytotoxicity of NO has been linked to the combined effects of reduced oxygen intermediates and the background cellular abundance of antioxidant enzymes.[8] Thus, the increased production of renal NO described here could account for the development of more severe histopathological changes and renal dysfunction. However, it is not possible to conclude from our data which NOS isoform is responsible for the increased levels of NO. Further studies are necessary to clarify this point.

Dysregulation of MMP activity has also been implicated in the pathophysiology of renal disease. MMPs are of significant biomedical interest because they have been implicated in many pathological processes characterized by dysregulated turnover of connective tissue matrices, such as occurs in nephropathy.[15] To the best of our knowledge, this is the first study demonstrating that ethanol consumption increases both MMP-2 activity and net MMP activity in the rat kidney. MMP-2 induces pathological changes in the kidney and for this reason it is associated with the development of renal disease.[16,17] The increased activity of MMPs described here could account for the histopathological changes observed and also for the future development of renal dysfunction. The mechanisms by which ethanol upregulates MMP-2 expression are not clear. Ethanol decreased the levels of TIMPs, a family of tissue inhibitors of MMPs,[44] resulting in increased MMP activity.[45] A role for NO in the downregulation of TIMP expression was previously suggested.[46] In the present study, the increased generation of NO in the kidney, could account for the increased activity of MMP-2.

Conclusions

A major new finding of the present study was that ethanol consumption increases renal MMP activity which was accompanied by histopathological changes in the kidney and elevated NO generation. Since iNOS-derived NO and MMPs contribute to renal injury, the increased levels of NO and MMP activity described in this study might contribute to progressive renal damage.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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