Moderate alcohol intake has no impact on acute and chronic progressive anti-thy1 glomerulonephritis

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NUTRITION PLAYS a major role in human health and disease. Modifying food and fluid intake was probably one of the first approaches humankind used to prevent, influence, or treat its various diseases. For renal disorders, beneficial effects of dietary modifications have been documented, mainly in experimental settings, for the intake of calories, proteins, certain amino acids, lipids, minerals, and vitamins (2, 3, 22). In cardiovascular disease, positive effects of moderate alcohol intake have been documented in numerous experimental and human studies (12, 20, 21, 36), whereas only little is known about its action on the course of kidney disorders.

As for cardiovascular disease, pathological expansion of extracellular matrix proteins is a hallmark of acute and chronic renal disease (4, 17). Glomerular and tubulointerstitial matrix accumulation result from an increase in the production of matrix proteins such as fibronectin, biglycan, and collagens; a decrease of matrix protein degradation by increased production of protease inhibitors such as plasminogen activator inhibitor (PAI)-1; and an overexpression of matrix-binding integrins on the cell surface (4, 25). Overproduction of the cytokine transforming growth factor (TGF)-β has been identified as a key feature of tissue fibrosis wherever it occurs. While in acute renal disease TGF-β overexpression and matrix deposition are transient and reversible, chronic renal disease is characterized by ongoing tissue injury resulting in persisting TGF-β overproduction and progressive renal fibrosis and insufficiency (4). This concept is reflected exemplarily in the rat model of anti-thy1 glomerulonephritis. In animals with two kidneys, anti-thy1 antibody injection leads to acute and reversible mesangio proliferative glomerulonephritis (1), whereas in uninephrectomized rats, injection of anti-thy1 antibody results in persisting glomerulosclerosis and progressive tubulointerstitial fibrosis (23), which may be related to a continuous hyperfiltration injury of the remaining kidney.

Because cardiovascular and kidney disorders share a number of similarities at the cellular and molecular level, we hypothesized that moderate alcohol intake may limit TGF-β overexpression and matrix expansion in renal disease. To test this hypothesis, we administered 40 ml beer/day to rats with acute or chronic progressive anti-thy1 glomerulonephritis. In acute anti-thy1 glomerulonephritis, alcohol actions on the initial mesangial cell injury and subsequent matrix expansion were determined (day 1 and day 7 after antibody injection, respectively). In chronic anti-thy1 glomerulonephritis, ethanol’s effects on renal function,
glomerular sclerosis, and tubulointerstitial fibrosis were analyzed 15 wk after disease induction.

METHODS

Materials

Unless otherwise indicated, materials, chemicals, or culture media were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Animals

Male Wistar rats (180–250 g) obtained from Charles River (Sulzfeld, Germany) were fed a normal protein diet (22.5% protein, Altromin, Lage, Germany) for at least 3 days before the start of the experiment to allow equilibration. Animal care and treatment were in conformity with the guidelines of the American Physiological Society and approved by local authorities. Animals were housed in a constant-temperature room with a 12:12-h light-dark cycle. Body weight was determined at the beginning and end of each experiment. Food, beer, and water intakes were monitored daily.

Induction of Acute and Chronic Progressive Anti-Thy1 Glomerulonephritis

Acute anti-thy1 glomerulonephritis was induced by tail vein injection of the monoclonal antibody OX-7 (1 mg/kg body wt in PBS) as previously described (29). For chronic progressive glomerulonephritis, one kidney was surgically removed and the monoclonal antibody mAb1–22–3 (4 mg/kg body wt in PBS) was intravenously injected 3 days later. In the kidney, OX-7 and mAb1–22–3 antibodies bind to a thy1-like antigen (although to different epitopes) on the surface of mesangial cells and cause complement- and nitric oxide (NO)-dependent cell lysis (1, 23, 24). Control animals were injected with equal volumes of PBS only.

Production of OX-7 and mAb 1–22–3

OX-7 and mAb 1–22–3 were produced from hybridoma cell lines as previously described (29). The antibodies were diluted in PBS (pH 7.4) and stored at −70°C until use.

Moderate Alcohol Consumption

A moderate alcohol intake was achieved by supplying 40 ml beer (4.9% ethanol, 23 bitter units, expressing the bitterness of the beer’s taste) per day and rat. The beer was provided every late afternoon, and in general the animals started drinking immediately. In addition, the animals had free access to tap water. This approach results in a daily intake of 2 ml ethanol/animal, which, for rats, constitutes moderate alcohol intake (9).

Experimental Design

In protocol 1, the action of moderate alcohol intake on early mesangial cell lysis (protocol 1A, injury phase, day 1) and on the subsequent matrix expansion (protocol 1B, matrix expansion phase, day 7) was analyzed in rats following the induction of acute anti-thy1 glomerulonephritis. In protocol 2, the effect of moderate alcohol consumption was investigated in rats with chronic progressive anti-thy1 glomerulonephritis (progression from acute glomerular to chronic tubulointerstitial fibrosis) 15 wk after disease induction.

In experiments 1B and 2, the histological grading of renal matrix accumulation was paralleled by protein measurements of the key fibrosis mediator and marker TGF-β. In addition, renal expression of the matrix protein fibronectin was measured as an indicator of matrix protein production. The protease inhibitor PAI-1 was used as a sensitive marker of the matrix-degrading system. TGF-β1, fibronectin, and PAI-1 expression were measured at the protein level in the supernatant of cultured glomeruli or minced cortical tissue harvested from individual animals. The interaction of ethanol with inducible NO production was tested in vivo in protocol 1A (NO-mediated mesangial cell damage) and in vitro in protocols 1B and 2 (stimulation of cultured glomeruli of cortical tissue with LPS).

Protocol 1A

Effect of moderate alcohol intake on the injury phase of acute anti-thy1 glomerulonephritis (day 1 after antibody injection). Five days before antibody injection, Wistar rats were assigned to the following groups: 1) PBS-injected controls (control; n = 4); 2) anti-thy1 antibody-injected animals, no treatment (aGN; n = 8); and anti-thy1 antibody-injected rats plus moderate alcohol intake (aGN + C2H5OH; n = 8).

One day after antibody injection, the histological degree of mesangial cell lysis as well as the release of basal and LPS-stimulated nitrite production of cultured glomeruli were analyzed. At this point, mesangial cell lysis is complete and inducible glomerular NO production is markedly increased (28).

Protocol 1B

Effect of moderate alcohol intake on the matrix expansion phase of anti-thy1 glomerulonephritis (day 7 after antibody injection). One day after antibody injection, when the mesangial cell lysis had occurred and the fibrotic response had started (28), Wistar rats were assigned to the following groups: 1) control (n = 4); 2) aGN (n = 8); and 3) aGN + C2H5OH (n = 8).

Seven days after disease induction, histological glomerular matrix accumulation and production of nitrite, TGF-β1, fibronectin, and PAI-1 of cultured glomeruli were determined. In acute anti-thy1 glomerulonephritis, the fibrotic response peaks 7 days after antibody injection and provides a large “therapeutic window” between normal and disease levels (29).

Protocol 2

Effect of moderate alcohol intake on progression from acute to chronic progressive anti-thy1 glomerulonephritis (15 wk after antibody injection). Five weeks after uninephrectomy and antibody injection, Wistar rats were treated as follows: 1) uninephrectomized, PBS-injected controls (control; n = 4); 2) uninephrectomized, anti-thy1 antibody-injected animals, no treatment (cGN; n = 10); and 3) uninephrectomized, anti-thy1 antibody-injected rats plus moderate alcohol intake (cGN + C2H5OH; n = 10).

Fifteen weeks after induction of chronic anti-thy1 glomerulonephritis, parameters of renal function [glomerular filtration rate (GFR), serum creatinine, and blood urea nitrogen (BUN)] and indexes of glomerular and tubulointerstitial matrix accumulation (histological matrix score, glomerular and cortical protein expression of TGF-β1, fibronectin, and PAI-1) were determined. In addition, basal and LPS-stimulated nitrite production were assessed in cultured glomerular and cortical tissue.

Measurement of Systolic Blood Pressure and Albuminuria

In animals with chronic anti-thy1 glomerulonephritis, systolic blood pressure was measured 2 days before death in
conscious animals by the tail-cuff method as previously de-
scribed (29). Because acute anti-thy1 glomerulonephritis has
been shown to be normotensive (29), blood pressure was not
measured in this model. In both acute and chronic anti-thy1
glomerulonephritis, a 24-h urine was collected from each rat
the day before death, using metabolic cages. Albuminuria
was measured using a microplate technique and a rabbit
anti-rat albumin peroxidase-conjugated antibody (19). Albu-
munuria is expressed as milligrams of protein per 24 hours.

Death
At the end of each experiment, the animals were anesthe-
tized with ether. After a midline abdominal incision, 5–
10 ml blood were drawn from the abdominal aorta and the kidneys
were subsequently perfused with 30 ml ice-cold PBS. For
histological examination, cortical tissue was fixed in 10%
neutral buffered formalin.

Measurement of Renal Function and Serum
Ethanol Concentrations
Serum and urine creatinine, BUN, and serum ethanol
concentrations were measured spectrophotometrically in en-
zyme-based assays. GFR was calculated on the basis of se-
rum and urinary creatinine concentration and the corre-
sponding urinary volume.

Production of TGF-β1, Fibronectin, and PAI-1 by Glomeruli
or Cortical Tissue in Culture
In acute and chronic progressive anti-thy1 glomerulone-
phritis, glomeruli from individual rats were isolated by a
graded sieving technique (150-, 125-, 106-, and 75-μm mesh
metal sieves) as described previously (29). In chronic anti-
thy1 animals, a piece of cortical tissue was weighed and
minced extensively with a razor blade. Glomeruli or cortical
tissue was suspended in DMEM supplemented with 0.1 U/ml
insulin, 100 U/ml penicillin, and 100 μg/ml streptomycin. For
stimulation of inducible NO synthase (iNOS), 10 μg LPS/ml
from Escherichia coli (serotype 0127:B8) per milliliter were
added. Glomeruli were cultured in a density of 2,000/ml for
48 h and minced cortical tissue at a density of 10 mg/ml,
respectively. After 48-h incubation at 37°C and 5% CO₂,
supernatants were harvested and stored at −70°C until
analysis of TGF-β1, fibronectin, PAI-1, or nitrite content.

Light Microscopy
All microscopic examinations were performed in a blinded
fashion. Three-micrometer sections of paraffin-embedded tis-
sue were stained with periodic acid-Schiff (PAS). For calcu-
lation of mesangial cell lysis, the number of the remaining
cell nuclei was counted in 30 glomeruli of 80- to 100-
diameter from each animal. Glomerular matrix expansion
was rated as described above. Tubulointerstitial matrix deposition was as-
sayed in 20 randomly selected cortical areas per sample
observed at 250 magnification using the following scale:
0 = normal, 1 = lesions involving <10% of cortical area, 2 =
involving 10–30%, 3 = involving 31–50%, and 4 = involving
>50%, respectively. The individual renal fibrosis score was
derived by adding the mean glomerular and tubulointersti-
tial matrix index of each animal.
Measurement of TGF-β1, Fibronectin, and PAI-1

TGF-β1 content of culture supernatant was measured after acid activation using a commercially available ELISA kit (TGF-β1 Duoset, R&D Systems, Wiesbaden, Germany) according to the manufacturer’s instructions. Fibronectin and PAI-1 levels were measured with modified inhibitory enzyme-linked immunoassays (ELISA) according to published methods (31). Three samples from each rat were analyzed.

Measurement of Nitrite

Nitrite is a stable end-product of NO and served as an indicator of endogenous NO synthesis (25). Nitrite levels in culture supernatant were measured by the Griess reaction (13). Briefly, 100 μl of sample were mixed with 100 μl Griess reagent [0.05% N-(1-naphthyl) ethylene diamine dihydrochloride, 0.5% sulfanilamide in 45% glacial acetic acid] in 96-well plates. After 10-min incubation in the dark, absorbance was read at 546 nm in an automated plate reader (MRX II, Dynex Technologies, Frankfurt/Main, Germany). Standard samples were prepared with sodium nitrite.

Statistical Analysis

Data are expressed as means ± SE. Statistical analysis between the groups was performed by one-way ANOVA and subsequent t-test with Bonferroni correction for multiple comparison. A P value <0.05 was considered significant.

RESULTS

Body Weight and Ethanol Intake

In protocols 1A and 1B, there were no significant differences in body weight gain between the groups investigated. In protocol 2, the body weight in week 15 was significantly lower in both groups of nephritic rats.
(cGN: 515 ± 11 g, cGN + C2H5OH: 509 ± 14 g) compared with the normal controls (574 ± 12 g, P < 0.05). This finding is probably a reflection of chronic renal disease and insufficiency in these two groups. In all three experiments, animals drank the 40 ml beer (4.9% ethanol) provided each day. This corresponds to a daily ethanol intake of 1.96 ml/rat, which, in the acute anti-thy1 animals, resulted in an approximate ethanol intake of 8–10 ml/kg body wt and, in the chronic anti-thy1 rats, of 4–6 ml/kg body wt according to their weight gain over time, respectively. Because the term moderate alcohol intake covers a range of alcohol intakes and the 40 ml provided each day are close to the usual daily drinking volume of the rats, the amount of beer was not increased in protocol 2. In both protocols, ethanol concentrations in the blood taken the morning before death were below a detection limit of 0.02 per thousand. This confirms that the alcohol intake actually achieved was moderate.

Protocol 1A

Effect of moderate alcohol intake on the injury phase of acute anti-thy1 glomerulonephritis. Compared with the normal control animals, injection of anti-thy1 antibody resulted in a significantly reduced glomerular cell number (60.5 ± 1.8 vs. 45.8 ± 1.0, P < 0.001; Fig. 1) as well as basal (1.1 ± 0.2 vs. 9.2 ± 1.5 nmol nitrite/ml, P < 0.001; Fig. 2A) and LPS-stimulated glomerular NO production (3.5 ± 0.9 vs. 43.8 ± 3.9 nmol/ml, P < 0.001; Fig. 2B), indicating the level of iNOS expression. Compared with the nephritic animals, the 6-day alcohol administration showed no significant action on disease activity [glomerular cell number: 46.3 ± 1.5, basal and LPS-stimulated glomerular NO production: 8.0 ± 1.2 and 40.7 ± 4.3 nmol nitrite/ml, respectively, all P = not significant (NS) vs. aGN; Figs. 1 and 2].

Protocol 1B

Effect of moderate alcohol intake on the matrix expansion phase of acute anti-thy1 glomerulonephritis. Seven days after injection of anti-thy1 antibody, disease was characterized by a significant increase in albuminuria (46.8 ± 12.3 mg/24 h; Fig. 3), histological matrix accumulation (matrix score 2.9 ± 0.1), and glomerular production of TGF-β1 (673 ± 53 pg/ml), fibronectin (5,747 ± 338 ng/ml), and PAI-1 (1,095 ± 103 ng/ml, P < 0.001 vs. aGN for all parameters; Fig. 4, Figs. 5A, 5B, 5C, 5D).
A-D). Providing a 6-day moderate alcohol supply did not significantly limit albuminuria (46.1 ± 9.6 mg/24 h) or reduce the fibrotic response (matrix score 3.0 ± 0.2, TGF-β1 680 ± 110 pg/ml, fibronectin 5,730 ± 735 ng/ml, PAI-1 1,253 ± 92 ng/ml, all P = NS vs. aGN; Figs. 3 and 4). Inducible NO production of glomeruli in culture was not different between nephritic rats with and without alcohol feeding (aGN: 3.9 ± 1.0 nmol nitrite/ml vs. aGN + C2H5OH 4.4 ± 1.0 nmol nitrite/ml, P = NS).

Taken together, the results of protocol 1 show that moderate alcohol consumption does not limit mesangial cell injury or subsequent glomerular TGF-β overexpression and matrix accumulation in acute anti-thy1 glomerulonephritis.

Protocol 2

Effect of moderate alcohol intake on the progression from acute to chronic progressive anti-thy1 glomerulonephritis. As depicted in Fig. 5, chronic anti-thy1 glomerulonephritis shows glomerular sclerosis and a progress of the matrix expansion into the tubulointerstitial space. Compared with the nonnephritic controls, fibrotic disease in chronic anti-thy1 glomerulonephritis was characterized by moderately elevated blood pressure (124 ± 2 vs. 138 ± 7 mmHg, P < 0.01), persisting high albuminuria (39 ± 11 vs. 128 ± 31 mg/24 h; Fig. 6), increased serum creatinine (0.5 ± 0.1 vs. 1.0 ± 0.2 mg/dl; Fig. 7A) and BUN (57 ± 4 vs. 102 ± 25 mg/dl; Fig. 7B), and reduced GFR (2.3 ± 0.1 vs. 1.3 ± 0.3 ml/min; Fig. 7C; all parameters P < 0.05 vs. control).

Fig. 6. Effect of moderate alcohol intake (+C2H5OH) on albumin excretion 15 wk after induction of chronic anti-thy1 glomerulonephritis (cGN). Moderate alcohol supply was started 5 wk after disease induction and continued for 10 wk. Normal control animals (control) received a PBS injection. Urine was collected for 24 h using metabolic cages (#P < 0.05 vs. cGN).

Fig. 7. Effect of moderate alcohol intake (+C2H5OH) on renal function 15 wk after induction of chronic anti-thy1 glomerulonephritis (cGN). Moderate alcohol supply was started 5 wk after disease induction and continued for 10 wk. Normal control animals (control) received a PBS injection. Serum creatinine (A), blood urea nitrogen (BUN; B), and glomerular filtration rate (GFR; C) are shown (#P < 0.05 vs. cGN).
The renal matrix score was markedly increased (control 1.2 ± 0.3 vs. cGN 3.5 ± 0.6, \( P < 0.05 \); Fig. 5). In addition, nephritic rats showed significantly higher glomerular expression of TGF-\( \beta \) (79 ± 17 vs. 12,632 ± 2,905 pg/ml; Fig. 8A), fibronectin (6,442 ± 413 vs. 12,632 ± 2,905 ng/ml; Fig. 8B), and PAI-1 (194 ± 14 vs. 430 ± 60 ng/ml; Fig. 8C; \( P < 0.05 \) vs. cGN for all parameters), respectively. At the tubulointerstitial level, protein expression was increased significantly for TGF-\( \beta \) (37 ± 3 vs. 157 ± 35 pg/ml; Fig. 9A), fibronectin (5,514 ± 485 vs. 9,909 ± 1,356 ng/ml; Fig. 9B), and PAI-1 (443 ± 33 vs. 855 ± 139 ng/ml; Fig. 9C; \( P < 0.05 \) for all parameters).

In chronic anti-thy1 glomerulonephritis, moderate alcohol intake had no significant influence on disease severity as shown for albuminuria (125 ± 23 mg/day); renal function (creatinine 0.9 ± 0.3 mg/dl, BUN 104 ± 23 mg/dl, GFR 1.5 ± 0.3 ml/min); histological renal matrix accumulation (renal matrix score 3.6 ± 0.6), glomerular (TGF-\( \beta \) 175 ± 32 pg/ml, fibronectin 12,098 ± 2,506 ng/ml, PAI-1 453 ± 68 ng/ml); and cortical matrix protein expression (TGF-\( \beta \) 139 ± 28 pg/ml, fibronectin 10,593 ± 1,274 ng/ml, PAI-1 819 ± 80 ng/ml) (Figs. 5–9; all \( P = \text{NS} \) vs. cGN). Systolic blood pressure was slightly but not significantly lower in alcohol-fed animals (134 ± 3 mmHg) compared with untreated disease controls (138 ± 7 mmHg). In vitro stimulation with LPS did not result in significantly different inducible NO production of cultured glomeruli (cGN: 0.7 ± 0.2 nmol nitrite/ml vs. cGN + C\(_2\)H\(_5\)OH 0.9 ± 0.3 nmol nitrite/ml) or cortical tissue (cGN: 3.8 ± 0.4 nmol nitrite/ml vs. cGN + C\(_2\)H\(_5\)OH 3.5 ± 1.2 nmol nitrite/ml).

Thus the data gathered in chronic anti-thy1 glomerulonephritis are consistent with the results in acute anti-thy1 animals. Taken together, both protocols show that moderate alcohol consumption neither limits nor aggravates matrix expansion and iNOS expression in the model of acute and chronic anti-thy1 glomerulofibrosis.

**DISCUSSION**

Although alcohol consumption is common among the general population, only little is known about its effect on the course of renal disease. This contrasts with cardiovascular disorders, where several epidemiological human studies have found that moderate alcohol intake, ranging from one to two drinks per day, is associated with a lower risk of coronary heart disease, ischemic stroke, dementia, and total mortality, especially in elderly men and women (12, 20, 21, 32). In experimental studies, furthermore, moderate ethanol intake reduces blood pressure and subsequent renal...
vascular injury in spontaneously hypertensive rats (36). In a rabbit model of vascular balloon injury, moderate alcohol intake has been found to limit neointimal hyperplasia in a pressure-independent manner involving less local chemokine expression (10).

To extend these findings to renal disease, the present study employed the rat model of anti-thy1 glomerulonephritis to test in vivo the hypothesis that moderate alcohol consumption protects from acute and chronic renal matrix accumulation and renal insufficiency. It must be emphasized that experimental circumstances were varied in many ways to allow detection of even small protective effects. These variations included 1) the use of acute anti-thy1 glomerulonephritis, a model in which glomerular matrix expansion is fast and marked and, as recently shown, even small anti-fibrotic actions can be detected (29); 2) the use of chronic progressive anti-thy1 glomerulonephritis, in which fibrosis slowly progresses from the glomerulus into the tubulointerstitium and little beneficial actions mount up to a detectable level over time (23); 3) determination of the key fibrosis mediator TGF-β1, which, as shown recently, is a valid and sensitive marker of renal matrix expansion (26, 29); 4) measurement of the matrix protein fibronectin and the protease inhibitor PAI-1 to allow detection of potential anti-fibrotic actions independent of TGF-β; and 5) analysis of renal function and urinary protein excretion. However, in neither of the two models nor on any parameter was moderate alcohol intake associated with a beneficial effect. Because the mechanisms of matrix expansion in various renal diseases are rather common, the findings of this study may be relevant for fibrotic renal disease in general. However, the present study does not exclude potential benefits of moderate alcohol intake in other renal models or human kidney disease.

The precise mechanism of how alcohol intake influences cardiovascular disease is not fully understood. Several pathways have been proposed and may be involved. Epidemiological studies suggest that moderate alcohol consumption influences cardiovascular risk factors, primarily blood pressure, but also plasma cholesterol and triglyceride levels, platelet function, and fibrinolytic parameters, thereby preventing initiation and progression of atherosclerosis (12, 20, 21). At the molecular and cellular level, ethanol’s actions have been associated with the inhibition of proliferation of many cell types (5, 6, 11, 18), suppressed postprandial vascular smooth muscle cell hypertrophy, a downregulation of PAI-1 expression (15), increased expression of vascular endothelial growth factor and subsequent angiogenesis (16), and stimulation of endothelial NO production and action (35, 37). Taken together, most of these mechanisms involved in the beneficial actions of moderate alcohol intake converge in effects that would limit tissue matrix protein production and accumula-

Fig. 9. Effect of moderate alcohol intake (+C₂H₅OH) on cortical TGF-β₁ (A), fibronectin (B), and PAI-1 production (C) 15 wk after induction of chronic anti-thy1 glomerulonephritis (cGN). Moderate alcohol supply was started 5 wk after disease induction and continued for 10 wk. Normal control animals (controls) were injected with PBS. Renal cortical tissue was extensively minced and cultured at a density of 10 mg/ml for 48 h (#P < 0.05 vs. cGN).
tion. For the present study, we therefore decided to test the effect of moderate alcohol intake on renal matrix expansion as a common downstream pathway rather than on an upstream “surrogate” parameter. Because matrix accumulation in cardiovascular and in kidney disease shares many similarities (4), the lack of any benefit of moderate alcohol intake in renal fibrosis is surprising. The reason for this important difference is not clear. One explanation could be that endothelial dysfunction, in which many protective effects of alcohol converge as well, may be less important for the course and progression of fibrotic renal disease.

In addition to matrix expansion, the present study analyzed the effect of moderate alcohol intake on renal inductive NO production for the reason that ethanol has been found to inhibit iNOS in several cell types in vitro (14, 33, 34). Induction of iNOS is a key injurious stimulus in several models of renal disease, including the injury phase of acute anti-thy1 glomerulonephritis, acute tubular necrosis, renal transplant rejection, and lupus nephritis of the MRL/lpr mouse strain (27). As a consequence of tissue injury, inducible NO production results in subsequently increased matrix expansion, as shown in acute anti-thy1 glomerulonephritis and chronic MRL/lpr lupus nephritis (28, 30). In the present study, moderate alcohol intake showed no effect on the in vivo induction of iNOS in the injury phase of anti-thy1 glomerulonephritis or on its in vitro activation in cultured glomerular and cortical tissue from fibrotic anti-thy1 animals. This solid finding is highly consistent with the results on renal matrix expansion in acute and chronic anti-thy1 glomerulonephritis.

Other than the amount of alcohol, some studies have suggested that there might be an association between the type of alcoholic beverage and prevention of cardiovascular disease. A strong case has been made for what is called the “French paradox.” This term refers to the fact that the cardiovascular mortality rate in France is just approximately half of that of other Western countries, although the prevalence of risk factors is not very different (12). This phenomenon has been related to the high consumption of red wine in France. Red wine contains polyphenol compounds, which are strong antioxidants and may mediate protective actions (12, 21). Furthermore, a recent study showed that red wine directly reduces vascular endothelin expression (7), which is a key growth factor in atherosclerosis. In the present study, beer was used to supply alcohol to rats with fibrotic renal disease. Beer contains high amounts of flavonoids, which are strong antioxidants as well (8, 12). However, it has to be pointed out that a possible superiority of one type of alcoholic beverage has never been investigated systematically, and most cohort studies do not support an association between the preferred kind of drink and the prevention of cardiovascular disease (12). Thus, in addition to the moderate alcohol intake achieved in this study, the choice of beer as an alcoholic beverage is probably of less importance for the interpretation of its results.

In conclusion, moderate alcohol intake does not influence renal matrix expansion in anti-thy1 models of acute and chronic progressive glomerulofibrosis. The effect of alcohol intake in various doses, in other renal models and human kidney disease warrants further investigation.

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