THE INHIBITORY EFFECT OF QUERCETIN ON LONG-TERM ALCOHOL CONSUMPTION-INDUCED PRO-MATRIX METALLOPROTEINASE-9 LEVELS IN PLASMA AND LIVER OF RATS

Ahmet Kahraman PhD, Hamdullah Çakar, Tülay Köken MD

Afyon Kocatepe University, The School of Medicine, Department of Biochemistry, Afyonkarahisar

ABSTRACT

Objective: *Long-term alcohol consumption has been suggested to have detrimental effects on liver, such as inflammation, fibrosis and oxidative injury. Matrix metalloproteinases (MMPs), non-invasive marker of fibrosis, play an important role in degradation of extracellular matrix proteins. Tumor necrosis factor-alpha (TNF-α) is also a key proinflammatory cytokine that acts to provoke inflammation, proliferation, and tumorigenesis. Our objective was to investigate the inhibitory effects of quercetin, a polyphenol, on long-term ethanol consumption-induced levels of proMMP-9 (pMMP-9) and TNF-α.*

Material and Method: *Twenty eight rats were randomly divided into four groups: Control group (C), ethanol treatment group (EtOH) (1 ml/day, 80%, for 30 days, intragastrically (i.g.), quercetin treatment group (Q), (100 mg/kg-body wt. once in every three days, for 30 days) i.g. and ethanol plus quercetin treatment group (EtOH+Q) (1 ml/day, 80% of ethanol and 100 mg/kg-body wt. of quercetin once in every 3 days, for 30 days) i.g.*

Results: *Administration of ethanol for one mounth significantly increased the plasma aspartate aminotransferase (AST) activity, as a common biomarker for alcoholic injury ($p<0.05$). The plasma and liver pMMP-9 and liver TNF-α levels were significantly higher in ethanol group than control group ($p<0.05$, 0.05, 0.01; respectively). Quercetin treatment significantly decreased the plasma and liver pMMP and liver TNF-α levels-induced by ethanol, ($p<0.05$, 0.05, $p<0.01$; respectively). Furthermore, treatment of rats with quercetin prevented ethanol induced AST activity increases ($p<0.01$).*

Conclusion: *It seems reasonable to expect that administration of quercetin would relieve ethanol-induced hepatic tissue damage, and inflammation and provide a protective effect against liver fibrosis.*

Key Words: *pMMP-9, TNF-alpha, quercetin.* ***Nobel Med 2012; 8(2): 111-115***

SIÇANLARIN KARACİĞER VE PLAZMASINDA UZUN SÜRELİ ALKOL TÜKETİMİNİN İNDÜKLEDİĞİ PRO-MATRİKS METALLOPROTEİNAZ-9 SEVİYELERİ ÜZERİNDE QUERCETİNİN İNHİBİTÖR ETKİSİ

ÖZET

Amaç: Uzun süreli alkol tüketiminin karaciğer üzerinde inflamasyon, fibrozis ve oksidatif hasar gibi zararlı etkilere neden olduğu ileri sürülmektedir. Fibrozisin non-invaziv bir belirteci olan Matriks metalloproteinazlar (MMPs) ekstrasellüler matriks proteinlerinin yıkılmasında önemli bir rol oynarlar. Tümör nekroz faktör-alfa (TNF-α) da inflamasyon, proliferasyon ve tümörogenezisi provoke eden anahtar inflamatuvar bir sitokindir. Amacımız bir polifenol olan quercetin'in uzun süreli etanol kullanımının indüklediği proMMP-9 (pMMP-9) ve TNF-α seviyeleri üzerinde inhibitör etkisini araştırmaktır.

Materyal ve Metod: Yirmi sekiz sıçan rasgele 4 gruba ayrıldı: kontrol grubu (C), etanol verilen grup (EtOH) (1 ml/gün, 30 gün boyunca, %80 intragastrik olarak (i.g.); quercetin verilen grup (Q) (100 mg/kg rat, 3 günde 1 kez, 30 gün boyunca) i.g. ve etanol+quercetin verilen grup (EtOH+Q) (1 ml/gün, %80 etanol, 3 günde bir ve 100 mg quercetin/kg sıçan, 30 gün boyunca) i.g.

Bulgular: Etanol verilmesi alkolik hasarın genel bir biomarkerı olarak plazma AST aktivitesini artırdı ($p<0,05$). Etanol grubu plazma ve karaciğer pMMP ve karaciğer TNF-α seviyeleri kontrol gurubundan anlamlı olarak daha yüksekti (sırasıyla $p<0,05$, 0,05, 0,01). Quercetin verilmesi plazma ve karaciğer pMMP ve karaciğer TNF-α seviyelerini anlamlı olarak azalttı ($p<0,05$, 0,05, 0,01; sırasıyla). Ayrıca, sıçanlara quercetin verilmesi alkole bağımlı AST aktivitelerindeki artışa engel oldu ($p<0,01$).

Sonuç: Sonuç olarak, quercetin verilmesinin etanolun indüklediği hepatik doku hasarını ve inflamasyonu azaltmaya katkıda bulunabileceği ve karaciğer fibrozisine karşı koruyucu bir etki sağlayabileceği söylenebilir.

Anahtar Kelimeler: pMMP-9, TNF-alfa, quercetin.
Nobel Med 2012; 8(2): 111-115

INTRODUCTION

Consumption of alcoholic beverages among adults and adolescents has increased significantly during recent years. It has been estimated that almost one million Turk drink alcoholic beverages on regular basis or unsafe drinking. As a result, most of them suffer from alcohol-related diseases, such as neuron-behavioral disorders, liver, pancreas, heart-related diseases, inflammatory and immune disorders.

Matrix metalloproteinases (MMPs) are a family of at least 24 zinc-dependent endopeptidases that are involved mainly in the degradation of the extracellular matrix (ECM). According to their substrate specificity and structural features, MMPs are classified into five major groups: gelatinase A and B (MMP2, MMP9; respectively), stromelysins (MMP3, MMP10, MMP11), elastases (MMP12, MMP7), collagenases (MMP1, MMP8, MMP13, MMP18), and membrane-type matrix metalloproteinases (MT1-MMP, MT2-MMP, MT3-MMP, MT4-MMP). MMPs selectively degrade the components of ECM such as collagen IV, V, gelatin, elastin, and fibronectin.[1,2] They are known to play important roles in maintaining normal physiologic conditions, including tissue remodeling, wound healing, embryonic development. However, excessive expression and activation of MMPs are implicated in many pathologic processes including, *Helicobacter pylori* infected and ethanol-induced gastric ulcers, some chronic inflammatory conditions such as rheumatoid arthritis, vascular remodeling and inflammatory skin diseases.[1,3,4] MMP-9 (gelatinase B) degrades type IV collagen constituting the major structural component of the basement membrane and ECM, and the activity of MMP-9 has been used to assess fibrogenesis as one of non-invasive serum markers.[5-7]

Alcohol can stimulate hepatic stellate cells to proliferate and produce various profibrotic cytokines, which in turn result in hepatic fibrosis. Long-term ethanol consumption has previously been shown to increase circulating and liver levels of MMP-9.[8] Reactive oxygen and nitrogen species-regulate by ethanol regulate MMP activity, and disrupt the balance of MMP activation and inactivation.[2,8] MMP-9 is considered to be an inflammatory protein and is regulated through NFκB-mediated transcriptional control.[2,9]

MMP-9 can be stimulated by the tumor necrosis factor-α (TNF-α), epidermal growth factor or phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), through activation of different intracellular-signaling pathways.[1,2,4,9] Their activity is regulated on several levels, including gene expression, zymogen-enzyme activation, and natural inhibition by Tissue Inhibitors of Matrix Metalloproteinases (TIMPs).[2] →

Current evidences indicate that matrix degradation is essential for liver repair reactions, such as hepatic fibrogenesis, which is characterized by an increase and altered deposition of newly formed ECM components.[10-12] The deposition of ECM is induced by an increase of ECM synthesis but is fundamentally the result of an imbalance between enhanced matrix synthesis and diminished or modified matrix breakdown.[11-13] Earlier studies indicated that in fibrotic liver collagenolytic activity was decreased together with raised TIMP-1 and TIMP-2 levels. This point is believed to result in a net inhibition of ECM breakdown and an accumulation of fibrillar collagens.[14,15]

Quercetin (3,5,7,3',4'-penthydroxy flavone) is an important constituent of the flavonoids and is found in many fruits and vegetables, as well as olive oil, red wine, onion, bean, tea etc. Quercetin's various pharmacological activities, such as free radical scavenging, atherosclerosis, or chronic inflammation preventing effects, have been demonstrated.[16-18] Our previous studies reported that quercetin had the antiperoxidative, antioxidant and antihistaminic effects on acute ethanol-induced gastric mucosal lesions.[19] Furthermore, it has been known that quercetin inhibits TNF-α-induced upregulation of MMP-9.[1] Previous studies reported that quercetin had the ability to inhibit lipopolysaccharide-induced inflammation in mouse macrophages and TPA-induced MMP-9 expression in glioma cells.[20,21] Lin et al. showed that quercetin significantly suppressed MMP-9 gene expression via blocking the proteinkinase C (PKC)d/extracellular signal-regulated kinase (ERK)/AP-1-signaling pathway, and consequently reductions in migration and invasion of human breast carcinoma cells.[4]

Our study is designed to investigate the effect of quercetin on circulating and liver levels of the pMMP-9 induced by long-term ethanol consumption. Thus, it is to research if quercetin has protective effect against ethanol-induced liver fibrosis and liver injury.

MATERIAL and METHOD

This study was given approved by the Local Animal Ethics Committee. Interventions concerning animals were performed in Afyon Kocatepe University according to the Guide for the Care and Use of the Laboratory Animals. In this experimental study, 28 male Sprague-Dawley rats (adult) weighing 280-320 g were used. They were housed for one month in a cage at a temperature of 25±3°C with a fixed 12 h of light-dark cycle for month. They were fed with a standard rat chaw (Oğuzlar Yem, Eskişehir, Turkey) and allowed to drink water ad libitum, but they were deprived of food for 12 h before the experiments.

Twenty-eight rats were randomly divided into 4 groups (7 in each group); the control group (C) received physiologic saline (PS), intragastrically (i.g.) (2 ml/day). Ethanol treatment group (EtOH) received ethanol, (1 ml/day, 80% v/v) and PS (1 ml/day) i.g. Quercetin treatment group (Q); received quercetin (100 mg/kg-body wt.) once in every 3 days and PS (1 ml/day) i.g. Ethanol+Quercetin treatment group (EtOH+Q) received quercetin (100 mg/kg-body wt.) once in every 3 days i.g. 2 h before EtOH administration (1ml, 80% v/v). Quercetin was suspended in PS.

The treatment continued to 30 days. 24 hours after the last injection, rats were anesthetized by ketamin (100 mg/kg-body wt. i.p.). The liver tissues were quickly removed and washed with physiological saline and stored at -20°C until use. They were homogenized in 1:10 (w:v) with 0,02 M phosphate buffer (pH 7.0) for pMMP-9 with a Ultra Turrax homogenizer (T25, Janke and Kunkel). Homogenates were centrifuged at 5000 rpm for 10 min. The supernatants were received as specimens. Blood samples were drawn with a heparinized syringe by cardiac puncture and were collected in heparinized tubes. Plasma was obtained by centrifugation at 3000 rpm for 10 minutes at +4^{0}C and stored at -20 °C until the analyses.

Ethanol was obtained from MERCK (E.Merck, Daimstadt Germany). Quercetin dihidrat, and all other reagents were purchased from Sigma Chemical Co. (St. Louis, USA).

Liver and plasma pMMP-9 levels were measured by using the method of quantitative sandwich enzyme immunoassay with commercial kit from R&D systems (R&D systems Inc. 614 McKinley Place NE Minneapolis, MN 55413, USA). TNF-α levels were measured by using the method of quantitative sandwich enzyme immunoassay with commercial kit from Biosource (BioSource Europe S.A. Rue de 1'Industrie, 8 B-1400 Nivelles, Belgium). Plasma AST activities were determined with a Hitachi 912 autoanalyzer (Manhaime, Germany), using commercial kits (Manhaime, Germany) obtained from Roche Diagnostics (Istanbul, Turkey). Liver and plasma pMMP-9 levels were expressed as pg/g protein and pg/dl, respectively. Liver TNF-α levels were expressed as pg/mg protein. Plasma AST activities were expressed as IU/L. The protein levels were determined by the Fluka kit (Protein quantification kit-Rapid, Busch/Schweiz), spectrophotometrically.→

Table 1: Plasma and liver pMMP, TNF-α levels and plasma AST activities

The analyzed parameters	Control group (Mean±SD) (n=7)	EtOH group (Mean±SD) (n=7)	Q group (Mean±SD) (n=7)	EtOH+Q group (Mean±SD) (n=7)
Plasma MMP-9 (pg/dl)	64±17	97±23[a]	54±17	61±12[c]
Liver MMP-9 (pg/g protein)	5.2±1.2	7.3±1.3[a]	5.0±1.4	5.4±0.8[c]
Liver TNF-α (pg/mg protein)	43±8	62±8[b]	38±9	45±4[c]
Plasma AST (U/L)	107±24	150±38[a]	98±28	100±18[d]

a: EtOH vs control ($p<0.05$), b: EtOH vs control ($p<0.01$), c: EtOH vs EtOH+Q ($p<0.05$), d: EtOH vs EtOH+Q ($p<0.01$).

Statistical Analysis

The results were decoded after being reported and expressed as the mean ± standard deviation (SD). Statistical analysis of the data were done with the Kruskal-Wallis and Mann-Whitney U tests. p value <0.05 was considered significant.

RESULTS

The plasma aspartate aminotransferase (AST) activity is a common biomarker for alcohol consumption. Intragastric administration of ethanol for one month significantly increased the plasma AST activities compared to control group ($p<0.05$) (Table1). The plasma and liver pMMP-9 levels were significantly higher in ethanol group than control group ($p<0.05$) (Table 1). Treatment of rats with quercetin prevented ethanol-induced increases in AST activities (Table 1). As shown in Table 1, quercetin treatment significantly decreased the liver and plasma pMMP levels ($p<0.05$). In addition, quercetin significantly decreased the TNF-α levels which were increased by ethanol ($p<0.05$).

DISCUSSION

The present study shows that quercetin treatment significantly decreases MMP-9, TNF-α, and AST levels which were increased by ethanol. Quercetin protects from ethanol-induced liver injury, as reflected by increased AST activity, and inhibits inflammation that is judged by significantly increased TNF-α and pMMP-9 levels.

In recent years, the biological and pharmacological properties of quercetin have received great attention in the scientific community. This is mainly stemmed from the focus that quercetin could exhibit a broad spectrum of biological activities, for instance, antibacterial, antifungal, anti-viral, immunostimulatory, anti-inflammatory, anti-allergic, anti-carcinogenic, hepatoprotective and anti-oxidative and anti-fibrotic activity.[16-24] The biological activity of flavonoids are expected to be dependent on the chemical structure, particularly the number of phenolic hydroxyl groups.[25]

The data presented in this paper indicate that ethanol-induced liver damage is accompanied by significant increases in hepatic and circulating pMMP-9 levels and liver TNF-α levels, a key proinflammatory cytokine. The present study has also shown that quercetin relieves ethanol-induced MMP-9, TNF-α, and AST elevation.

Earlier studies have demonstrated that reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$) and lipid peroxidation play an important role in the pathogenesis of acute gastric damages induced by ethanol.[26-28] ROS provoke severe changes at cellular level leading to cell death because of their extreme reactivity.

Several studies have shown that ROS can activate positively several different MMPs.[2,29] MMP-9 and TNF-α is considered as inflammatory proteins and are regulated through NFκB-mediated transcriptional control.[2,9] Evidence has been provided that TNF-α, which is regulated by NFκB-mediated transcriptional control, induces the production of MMP-9.[1,2] Furthermore, present evidence indicates that matrix degradation is essential for liver repair reactions, such as hepatic fibrogenesis, which is characterized by an increased and altered deposition of newly formed ECM components.[10-12] Long-term ethanol consumption resulting in hyper-matrix degradation has an increased level of MMP-9 activity for liver repair reactions.

Some investigations indicate that ethanol-associated matrix degradation and fibrosis may involve oxidative stress.[11-13] The decreased MMP and TNF-α levels could be due to antioxidant and anti-oxidative effects of quercetin. Furthermore, Vijayababu et al. reported quercetin was a potent inhibitor of MMP-2 and MMP-9 expressions involved in MAP kinase signaling pathways.[23]

CONCLUSION

In conclusion, our study documents that quercetin not only protects from ethanol-induced liver injury, as reflected by increased AST activity, but also inhibits inflammation that is judged by significantly increased TNF-α and pMMP-9 levels, one of non-invasive serum markers of fibrosis. To reveal a mechanistic basis for quercetin's ability to protect against ethanol-induced liver fibrosis which involves in MMP-9 activity, additional studies are required.

C **CORRESPONDING AUTHOR:** Ahmet Kahraman MD Afyon Kocatepe University, The School of Medicine, Department of Biochemistry, 03100, Afyonkarahisar ahmetkah@aku.edu.tr
✓ **DELIVERING DATE:** 21 / 05 / 2010 • **ACCEPTED DATE:** 10 / 08 / 2010

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• *This study was supported by the Scientific Investigation Foundation of Afyon Kocatepe University (031.TIP.05).*