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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

The effect of sulforaphane on the levels of serum cystatin-c in acetaminophen-induced nephrotoxicity in rats

Sülforafanın ratlarda asetaminofenin-indüklediği nefrotoksisitede sistatin-c düzeylerine etkisi

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ABSTRACT

Objective: The exposure of living creatures to drugs and chemicals often results in toxicity of liver and kidney. Drugs constitute an important and big part of the community and hospital-acquired kidney diseases. In this study, we investigated the effect of sulforaphane (SFN) on the levels of cystatin-C and lipid peroxidation on acetaminophen (APAP)- induced nephrotoxicity in rats.

Methods: Thirty-six Sprague-Dawley rats were separated equally into four experimental groups: control group, SFN group, APAP group, and APAP + SFN group. In the experimental treatment groups APAP was administered oral gavage at 1 g/kg 3 h after SFN treatment in last day and, in the APAP + SFN group, SFN was administered oral gavage at a dose of 500 µg/kg exactly for three days. Rats were euthanized and sacrificed 24 h after APAP administration.

Results: APAP administration showed to significant increase in serum BUN, creatinine, urea and LDH concentrations as compared to the control datas indicating the induction of severe nephrotoxicity ($p<0.001$). SFN treatment significantly decreased the cystatin-C levels and lipid peroxidation compared to APAP group ($p<0.05$).

Conclusion: The present study demonstrate that the attachment of SFN to the nephrotoxicity treatment protocol will be beneficial and further studies should be conducted for cystatin C which plays an important role in kidney toxicity and disease to be routinized as a biomarker.

Key words: Acetaminophen, Cystatin-C, Lipid peroxidation, Nephrotoxicity

ÖZET

Amaç: Canlıların kimyasallara ve ilaçlara maruziyeti sıklıkla karaciğer ve böbrek toksisitesi ile sonuçlanır. Toplum ve hastane kaynaklı böbrek hastalıklarının önemli ve büyük bir kısmını ilaçlar oluşturmaktadır. Çalışmamızda, asetaminofen (APAP) ile ratlarda indüklenen nefrotoksisitede sülforafanın (SFN) sistatin-C düzeyleri ve lipid peroksidasyonu üzerine olan etkilerinin araştırılması amaçlanmıştır.

Yöntemler: 36 Sprague-Dawley sıçan her bir grup eşit hayvan içerecek şekilde dört gruba ayrıldı. Gruplarımız, kontrol grubu, SFN grubu, APAP grubu ve APAP+SFN grubundan oluşmaktadır. SFN ve APAP+SFN grubundaki sıçanlara tedavi için üç gün süresince 500 µg/kg dozunda SFN oral gavaj yoluyla verildi. APAP ve APAP+SFN grubundaki sıçanlara hepatotoksisite oluşturmak için APAP sıcak salin içerisinde çözüldü ve çalışmanın son gününde 1g/kg olacak şekilde tek doz oral gavaj yoluyla uygulandı. APAP+SFN grubunda, APAP uygulaması SFN uygulamasından üç saat sonra yapıldı. APAP uygulamasından 24 saat sonra sıçanlar sakrifiye edildi.

Bulgular: APAP uygulamasının serum üre, kreatinin, LDH ve BUN düzeylerini kontrol grubuna göre anlamlı derecede arttığı gözlemlenmiştir ($p<0.001$). SFN uygulamasının sistatin C düzeylerini ve lipid peroksidasyonunu APAP grubuna göre azalttığı gözlemlenmiştir ($p<0.05$).

Sonuç: Sülforafanın, asetaminofen kaynaklı nefrotoksisitede tedavi protokolüne eklenmesinin yarar sağlayacağını düşünmekteyiz. Böbrek hastalıklarında önemli bir rol oynayan sistatin C'nin bir biyomarker olarak rutine girebilmesi için çeşitli çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Asetaminofen, Sistatin C, Lipid peroksidasyonu, Nefrotoksisite

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INTRODUCTION

The exposure of living creatures to drugs and chemicals often results in toxicity. Drugs constitute an important part of the community and hospital-acquired kidney diseases. Kidneys are dynamic organs, and some of their biochemical and physiological properties make them more vulnerable to ischemic or toxic damage. The dangerous factors that cause susceptibility to the injury are present in most people with the occurrence of drug-induced nephrotoxicity. These are the patients, renal and drug-specific risk factors. Nephrotoxicity may affect all parts of the kidney, and this may result in much clinical renal pathology, containing acute or chronic renal failure, tubulopathy and proteinuria [1,2].

Acetaminophen (APAP) is a highly reliable drug in therapeutic doses which is commonly used all over the world and also is analgesic and antipyretic agent causing serious damage to the liver and kidney as a result of unconscious use since it is easily and quickly accessible [3,4]. Although the nephrotoxicity resulting from APAP overdose is less compared to hepatotoxicity formation, acute renal damage may develop instantaneously in the absence of liver damage. It was reported in the studies carried out that oxidative stress and lipid peroxidation play an important role in the development of renal injury induced by APAP [5]. Nowadays, the practical markers that are most commonly used for monitoring of renal functions are serum creatinine, urea and BUN levels. Level of serum creatinine is a more sensitive and specific indicator of the kidney function compared to urea. However, the measurement of serum creatinine level is thrilled by various variables such as sex, age and the amount of muscle tissue. Besides, it was observed in many researches that serum creatinine values did not change at a remarkable level at the early stage during which the renal functions begin to deteriorate [6,7]. Therefore, searching for simple and reliable new markers alternative to creatinine is still going on in faster and more accurate assessment of renal damage. Among these markers, the most promising for use in the clinic as a renal marker is cystatin-C [8].

Cystatin-C that takes part in various biological and pathological events is a low molecular weight inhibitor of cysteine proteases, and its main catabolism area is the kidney. It is filtered freely from the kidneys by glomerular filtration and reabsorbed by the proximal tubule cells, and metabolized here [9,10]. Serum cystatin-C levels have no tubular secretion and are not affected by variables such as age and gender [11]. Cystatin-C is claimed to play a role in defense against viral and bacterial infections as well as it may be a potent regulator in the inflammatory processes [12]. Today, the investigation of new protective molecules without adverse effects against the toxic and ischemic damages of drugs on the kidney and other organs is carried out around the world [13]. In addition, it has been found that some antioxidants have protective effects against renal damage induced by the use of excessive dose APAP [14,40]. Numerous traditional herbal products are used to treat renal diseases through legal or illegal ways [2,13]. Sulforaphane (SFN), a special phytochemical, found in vegetables such as broccoli, Brussels sprouts, cauliflower and cabbage is a compound having high antioxidant properties. It has been reported in the studies carried out in recent years that sulforaphane is safe and tolerable and has anti-inflammatory, anti-tumor, liver and kidney protective effects [15,16,17].

This study was made to detect protective effect of sulforaphane in APAP-induced nephrotoxicity in rats, with emphasis on lipid peroxidation and cystatin-C trying to elucidate the mechanism by which sulforaphane may execute its protective effect.

METHODS

Chemicals

APAP (CAS Number: 103-90-2) and SFN (CAS Number: 4478-93-7) were purchased from Sigma Chemical Co (St. Louis, MO, Germany).

Animals

Animal Experiments Local Ethics Committee of Atatürk University authorized the experimental procedure described in this study. Thirty-six Sprague Dawley male rats, which were selected at random, weigh 200-250 gr, 6-8 weeks old, were used in the present study. The rats were taken nourishment with standard diet and tap water in private rooms, which were provided at a temperature 22 ± 2 ºC and relative humidity of 50-60%, with a 12-hour light/dark cycle. The study was conducted in Atatürk Uni-

versity Medical and Experimental Application and Research Center (ATADEM) laboratories in accordance with the decision of Animal Experiments Local Ethics Committee of Atatürk University dated 30.04.2014 and numbered 2014/63.

Experimental Groups

The rats were divided into 4 groups each containing 9 animals. The treatment protocol was as follows:

Group 1 (Control), which called as healthy control, received tap water and standard diet for 3 days.

Group 2 (APAP) which called as renal damage group, received tap water and standard diet for 3 days and was administered with a single dose of APAP (1 g/kg body weight) on the 3th day [18].

Group 3 (SFN), which called as SFN control, received tap water and standard diet for three days and was administered with SFN (500 µg/kg body weight) for three days [19].

Group 4 (APAP+SFN) was treated oral gavage with SFN (500 µg/kg body weight) for three days and a single-dose administration of APAP (1 g/kg body weight) was performed three hours later than SFN administration on the 3th day.

Administration of APAP and SFN were given to the animals by oral gavage method. At the end of the experiment, the rats were anesthetized by intraperitoneal injection of a mix of xylazine (12 mg/kg) and ketamine hydrochloride (60 mg/kg) and sacrificed 24 hours after the last administration. Samples of blood were taken from each rat through intracardiac application and centrifuged for 10 minutes at 3500 rpm to obtain a clear serum, which was stored at -80°C. Serum samples were used to determine the lactate dehydrogenase (LDH) activity and creatinine, urea, blood urea nitrogen (BUN), cystatin-C levels. The kidneys of each animal were instantly taken and the tissues were washed using ice-cold saline solution. Kidney tissues were homogenized (10% w/v), separately, in ice-cold 0.1 M phosphate buffer (pH 7.4). The kidney tissues were used for the determination of MDA and total protein.

Biochemical Analysis

Levels of serum BUN, creatinine, urea and LDH activity were determined by enzymatic kinetic method in Roche Cobas C501 auto analyzer through using its original kit. Results of BUN, creatinine and urea were stated as mg/dL and result of LDH activity was stated as U/L. Based on the method defined by Ohkawa et al. [20], the liver MDA levels were measured with 532 nm wavelength spectrophotometric method. The MDA results were stated as nmol/mg protein. Tissue protein analysis was carried out with the method, which was defined by Lowry et al [21]. The serum cystatin-C levels were determined by using of Enzyme-Linked Immunosorbent Assay (ELISA) method, with Rat ELISA kit (SUNRED, Shangai Yehua Biological Technology Co., Ltd., Shanghai, China) (Catolog No: 201-11-0146) with the working principle suggested by the producer. The cystatin-C results were stated as mg/L.

Statistical Analysis

All data analyses were performed by using Statistical Package for the Social Sciences (SPSS) for Windows 19.0 package program (SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov test was done to evaluate the distribution of variables. The normally distributed datas were compared across the groups by using one-way analysis of variance (ANOVA) with the Tukey method and the Student-Newman-Keuls method multiple comparisons. The descriptive statistics were demonstrated by n (i.e., the sample size) and by the mean and standard deviation for continuous variables. A $p<0.05$ was accepted as significant.

RESULTS

Table 1 shows that APAP-induced nephrotoxicity resulted in a significant increase in BUN, creatinine, urea and LDH concentrations as compared to the control datas showing the induction of severe renal failure ($p<0.001$). Administration of SFN significantly restored the normal values of serum creatinine, urea, BUN and LDH ($p<0.001$). The tissue MDA levels in the APAP administered group was significantly higher ($p<0.05$) than the control group. Administration of SFN at dose of 500 µg/kg, prior to the APAP administration decreased the MDA levels significantly compared to APAP group ($p<0.05$) (Fig 1). The serum cystatin-C levels were significantly increased in APAP group as compared to the control group ($p<0.05$). SFN treatment significantly reduced the cystatin-C levels compared to APAP group ($p<0.05$) (Fig 2).

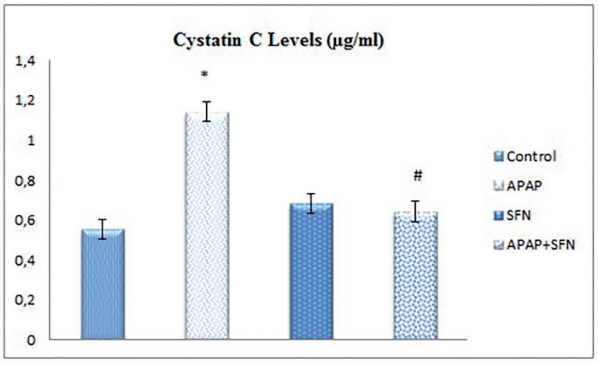

Figure 1. Serum cystatin C levels in rats

*, Significantly different when compared with control group, (p<0.05)

#, Significantly different when compared with APAP group, (p<0.05)

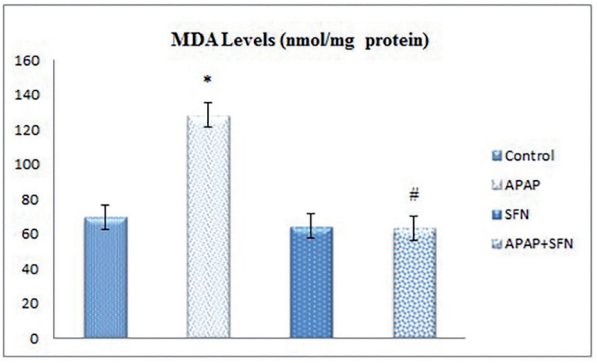

Figure 2. Kidney MDA levels in rats

*, Significantly different when compared with control group, (p<0.05)

#, Significantly different when compared with APAP group, (p<0.05)

Table 1. The levels of BUN, creatinin, urea and LDH in serum of rats

	Control	APAP	SFN	APAP+SFN
BUN (mg/dL)	19.61±1.02	63.67±3.88[a]	17.43±2.12[d]	19.37±2.96[b,d]
Creatinin (mg/dL)	0.97±0.04	3.68±0.10[a]	0.90±0.03[d]	1.41±0.07[c,e]
Urea (mg/dL)	42.04±2.23	124.33±4.23[a]	37.28±8.31[d]	41.42±6.35[b,d]
LDH(U/L)	150.68±35.28	492.33±42.22[a]	217.72±26.13[d]	288.33±31.64[c,e]

Data were given as mean ±SD.

BUN: Blood Urea Nitrogen, LDH: Lactate dehydrogenase, APAP: Acetaminophene, SFN: Sulforaphane

a Significantly different when compared with control group, (p<0.001).

b Significantly different when compared with APAP group, (p<0.001).

c Significantly different when compared with APAP group, (p<0.05).

d Significantly different when compared with control group, (p>0.05)

e Significantly different when compared with control group, (p<0.05).

DISCUSSION

Our kidneys are our sensitive organs having a weak defense against many substances with nephrotoxic potential. Occasionally, while a prescribed drug may have nephrotoxic property, damage may also occur by foods [1]. APAP is in the first rank among drugs that cause poisoning around the world and commonly causes liver and kidney damages when taken in overdose [22]. Taking the necessary precautions by making an accurate evaluation of kidney functions before impairment begins is extremely important in terms of mortality [23]. In the present study worked in rats, a single dose of APAP resulted in important renal failure as indicated by biochemical variations including increased serum activity of kidney markers such as cystatin C, BUN, urea, creatinine and LDH, and lipid peroxidation.

APAP toxic dose is frequently associated to many metabolic disorders including BUN, serum urea and creatinine imbalance. Increased levels of serum urea, creatinine and BUN are marker for APAP-induced renal damage [22,26]. In their study in which damage was formed in the kidney by administering 1gr/kg acetaminophen, Cekmen et al. [25] observed that levels of urea and creatinine were increased in the APAP group compared to the control group. In another study conducted by Fouad et al. [5], it was reported that creatinine and BUN levels significantly increased in the rats to which 2.5g/kg single dose APAP was administered compared to

the control group. The biotransformation of xenobiotics occurs in the kidney proximal tubules; damage occurred in the proximal tubule cells is known to cause a change in BUN, creatinine and urea levels [26, 27]. APAP-induced nephrotoxicity results from the toxic effects of its extremely reactive middle metabolite. This toxic metabolite is N-acetyl-para-amino-benzoquinoneimine (NAPQI), which arylates proteins in the S3 segment of the proximal tubule, initiating cell death of renal tubular cells. APAP and the other drugs-induced nephrotoxicity is often associated with highly increases in serum creatinine, urea and BUN levels [27,30]. In our study results that are compatible with the literature, it was observed that the kidney damage that developed in the acetaminophen administered group increased the BUN, urea, creatinine and LDH levels, besides, these values that increased in the sulforaphane administered group were reduced to the level of the control group. According to biochemical results of our study that SFN was also effective at protecting the kidneys against APAP overdose-induced acute nephrotoxicity in rats, and that this protective effect helps to improve of renal function.

Nephrotoxicity can occur when body is exposed to harmful substances. In the literature, a few therapeutic plants were researched for their nephroprotective activities [29]. Scientists are investigating for the research of protective plants that would ensure high protection to the liver, kidney as well as other organs and actually a few or no side effects would be applied during their function in the body. Many traditional herbs are used in various countries during drugs or chemical toxins induced renal damage [22,28].

Lipid peroxidation is one of the major mechanisms, which cause APAP-induced tissue damage and comes out of depending on the free oxygen radicals. The formation of free radicals leads to increasing destruction of unsaturated fatty acids in acetaminophen toxicity and causes damage to the cell. Lipid peroxidation was reported to have a major role in the loss of cell function in tissues under oxidative stress [26,31]. In their study in which kidney damage was formed by administering orally 750 mg/kg APAP for 7 days, Abdul Hamid et al. [32] reported that a significant increase was observed in the MDA level in plasma and renal tissues in the group with APAP administration compared to the control group. Aycan et al. [33] reported in their study that APAP administered group significantly increased MDA levels compared to the control group. In our study results that are compatible with the literature, it is observed that renal MDA levels significantly increased in the acetaminophen-administered group. Sulforaphane is one of the natural antioxidants affecting the major cellular defense systems. Yoon et al. [16] also reported a renal protective effect of SFN against the injury induced by ischemia and reperfusion. They reported that the protective effect of SFN was associated with the increased of GSH levels and decreased lipid peroxidation in rats [16]. Guerrero-Beltrán et al. [34] reported in their study that sulforaphane significantly inhibited the lipid peroxidation, and that SFN exhibited its renal protective effect by inhibiting oxidative stress. Also in this study in parallel with the literature results, it was observed that sulforaphane played a protective role against the nephrotoxicity induced by APAP and that the renal MDA levels of APAP+SFN group significantly decreased compared to the group creating APAP toxicity. In the presence of SFN, tissue damage caused by APAP was fairly restricted in terms of decrease MDA levels in the kidney, which was also given by data referring to the antioxidant activity of SFN.

Numerous studies have been carried out to protect the growing elderly population around the world, those treated or those using more than one drug for various problems from drug-induced nephrotoxicity, or to reduce their exposure to it [1]. Early detection of nephrotoxicity is very important in the case of withdrawal of the agent causing this disorder or dose adjustment is applied as it is reversible. It was determined in various studies that serum cystatin-C measurement was more sensitive in renal function disorders compared to serum creatinine, and that cystatin-C values began to rise even in moderate GFR declines [23,35]. In a study carried out by Herget-Rosenthal et al. [36] in intensive care patient with high risk of developing acute kidney damage, it was reported that serum cystatin-C levels detected the acute kidney damage 1.5 days earlier compared to serum creatinine level. In a study carried out by Si et al. [37], they formed an acute renal damage in rats with experimental ischemia-reper-

fusion and determined the cystatin-C levels. They reported that cystatin-C levels significantly showed rapid growth compared to the control group [37].

In our study results that are compatible with the literature, it was observed that the kidney damage that developed in the APAP administered group significantly increased the cystatin-C levels, besides, these values that increased in the SFN administered group were reduced to the level of the control group. Shokeir et al. [38] showed that SFN improved the kidney functions, improved redox state in kidney tissues and SFN had a powerful antioxidant effect on kidney. Negrette-Guzmán et al. [39] showed that SFN administration was able to decrease the renal dysfunction, which was evident by the reduction in the changes of renal damage characterized by rising in BUN, urea and creatinine blood levels. This protective effect of SFN would be associated with prevention of lipid peroxidation/or, mainly, activating of antioxidant effect. No other study that examined the effect of SFN on cystatin-C levels in the nephrotoxicity induced by APAP has been encountered in the literature. In the present study, we demonstrated that cyctatin-C inhibition by SFN administration protected kidney against APAP-induced nephrotoxicity. Brguljan et al. [8] proposed that cystatin-C protects towards proteolytic tissue damage by cysteine proteases released during normal processes. It may used as a marker for damage tissues. According to recent studies, determination of cystatin-C levels could conduce to the diagnosis and treatment of various diseases and it could become an important marker in clinical diagnosis [8].

The results of our study showed that SFN administration significantly inhibited to APAP overdose-induced acute renal damage in rats. The antioxidant effect, reduced cystatin-C production, and blocked of lipid peroxidation are the factors to the renal protective effect of SFN. It is expected that the preventive and protective role of SFN will be studied in supplementary experimental researches. Today, further studies will be required to detect SFN maximum protective effects, the included various research and the way it acts on various disease types. In the near future, SFN may be found useful as protective agent against drug-induced nephrotoxicity.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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