Ankara Ecz. Fak. Derg.
39 (1) 35-42, 2010

J. Fac. Pharm, Ankara
39 (1) 35-42, 2010

Original article

EFFECT OF CYCLOOXYGENASE-2 INHIBITION ON NICOTINE-INDUCED OXIDATIVE STRESS

NİKOTİNİN İNDÜKLEDİĞİ OKSİDATİF STRES ÜZERİNE SİKLOOKSİJENAZ-2 İNHİBİSYONUNUN ETKİSİ

Songül ÜNÜVAR, Göknur AKTAY*

İnönü University, Faculty of Pharmacy, Department of Pharmacology, 44280 Malatya, TURKEY

ABSTRACT

We examined the tissue thiobarbituric acid reactive substances (TBARS), non-protein thiol (NP-SH) and total thiol (T-SH) group levels in order to deduce the role of cyclooxygenase-2 (COX-2) pathway on nicotine-induced oxidative tissue damage. Wistar Albino male rats were divided into three groups: Group I; 0.9% saline (ip), Group II; nicotine ditartarate (1.5 mg/kg, ip), Grup III; celecoxib (15 mg/kg, ip)+nicotine ditartarate (1.5 mg/kg ip). At the end of the 7th day, liver, lung, kidney, heart and brain tissues were removed. Nicotine treatment significantly increased TBARS, NP-SH and T-SH levels in all tissues. However, celecoxib treatment prior the nicotine injection, significantly decreased the TBARS levels and T-SH contents in all tissues in addition to NP-SH content in kidney, liver, lung and brain compared to nicotine group. Nicotine treatment caused excessive production of free radicals and evoked the antioxidant molecules. However, inhibition of cyclooxygenase-2 selectively prevented the nicotine-induced oxidative tissue damage dramatically. We concluded that, cyclooxygenase-2 pathway may be a notable mechanism of the nicotine toxicity.

Key words: *Antioxidant activity, Cyclooxygenase-2, Lipid peroxidation, Nicotine toxicity, Oxidative stress.*

ÖZET

Siklooksijenz-2 (COX-2) yolağının nikotinle indüklenen oksidatif stres üzerindeki rolünü anlamak için doku tiyobarbitürik asit (TBARS), proteinsiz tiyol grupları (NP-SH) ve total tiyol gruplarını (T-SH) inceledik. Wistar Albino erkek ratlar üç gruba ayrıldı: Grup I; serum fizyolojik (ip), Grup II; nikotin (1.5 mg/kg, ip), Grup III; selekoksib (15 mg/kg, ip)+nikotin ditartarat (1.5 mg/kg, ip). Yedinci günün sonunda

karaciğer, akciğer, böbrek, kalp ve beyin dokuları çıkarıldı. Nikotin, tüm dokularda TBARS, NP-SH ve T-SH düzeylerinde artışa neden oldu. Oysa, nikotin enjeksiyonundan önce selekoksib uygulaması, nikotin grubuna göre böbrek, karaciğer, akciğer ve beyin dokusunda NP-SH düzeylerinin yanısıra tüm dokularda TBARS ve T-SH düzeylerini anlamlı derecede azalttı. Nikotin uygulaması, fazla miktarda serbest radikal oluşumuna neden olarak antioksidan molekülleri uyarmaktadır. Bununla birlikte, siklooksijenaz-2'nin seçici olarak inhibisyonu nikotine bağlı oksidatif hasarı belirgin derecede önlemektedir. Siklooksijenaz-2 yolağının nikotin toksisitesinde dikkate değer bir mekanizma olabileceği sonucuna vardık.

Anahtar kelimeler: *Antioksidan aktivite, Siklooksijenaz-2, Lipit peroksidasyon, Nikotin toksisitesi, oksidatif stress.*

***Corresponding author**

INTRODUCTION

It is known that nicotine, a major toxic component of cigarette smoke, induces oxidative stress both *in vitro* and *in vivo*. (1,2). Oxidative stress can lead to DNA damage, cellular degeneration, neoplastic transformation, tumorigenesis and an increase in metastatic capability (3-5). It is generally believed that oxidative stress is associated with depletion antioxidant system parameters with increased lipid peroxidation and increased ROS scavenging enzymes activities (1,6,7). There are some evidences that nicotine treatment may play a dual role in oxidative stress, depending on differences in the dosage of the drug used and its acting mechanisms. Nicotine possesses both prooxidant and antioxidant properties that depend both on its concentration and on the underlying cellular oxidative status. It has been shown that, a high dose of nicotine can induce neurotoxicity and stimulate oxidative stress, while reasonably low concentrations might act as an antioxidant and have a considerable neuroprotective effect (6-9). The increased generation of ROS by nicotine can produce a condition of oxidative stress which has been suggested to play a major role in the pathogenesis of several smoking-related diseases such as cancer, cardiovascular and oral diseases (10). Cyclooxygenase, is a rate-limiting enzyme for the synthesis of prostaglandins from arachidonic acids. COX-2, one of the key enzymes mediating inflammatory responses, is inducible by tumor promoters, growth factors, and cytokines. The selective inhibition of COX-2 has been suggested as a potential strategy for preventing cancers in colon, breast, prostate, and lung (5,11-15). Thus, the present study was planned to elucidate if concomitant administration of celecoxib with nicotine can ameliorate toxic effects of nicotine in terms of biochemical variables indicative of oxidative stress. This effect can be blocked by celecoxib, suggesting interaction of nicotine and COX-2 pathways.

MATERIALS AND METHODS

Chemicals and instruments

The chemical reagents used for antioxidative activity in the current study were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Spectrophotometric analysis was performed using a Shimadzu UW 1240.

Animals

Locally bred Wistar Albino male rats (250-300 g, n:18) were purchased from the animal breeding laboratories of Inonu University (Malatya, Turkey). The animals were fed a standard pellet diet and water *ad libitum* in a temperature-controlled room. The food was withdrawn 16 hours before the experiment though water was allowed *ad libitum.* Rats used in the present study were cared for in accordance with the directory of the Inonu University Animal Care Unit, which applies the guidelines of the National Institutes of Health on laboratory animal welfare. Procedures concerning animals and their care were conducted in conformity with international laws and policies and animal studies accepted by Inonu University Ethical Council (02.06.2005/prot. no. 20).

Eighteen rats were equally divided into three groups and treated as below for 7 days:

Group I Control (n:5); 0.9% Saline (vehicle) ip.

Group II Nicotine ditartarate (n:7); 1.5 mg/kg ip.

Group III Celecoxib (n:6); 15 mg/kg ip., an hour ago 1.5 mg/kg ip nicotine ditartarate injection.

We have chosen the dose of nicotine according to the literature available (8). All treatments were maintained daily for 7 days. The experiment was terminated at the end of 7 days and all animals were sacrificed under ether anesthesia. Tissues were immediately removed and washed with ice-cold (4 °C) saline, labeled and stored at -40 °C until homogenization. Liver, brain, kidney, heart and lung tissues were used for determination of the parameters such as TBARS, NP-SH and T-SH.

Determination of TBARS levels

The method of Ohkawa *et al* (16) as modified by Jamall and Smith (17) was used to determine TBARS in tissue samples. The method is based on the formation of a red chromophore which absorbs at 532 nm, following the reaction of thiobarbituric acid (TBA) with malondialdehyde and other breakdown products of peroxidized lipids. 1,1,3,3,-tetraethoxypropan (TEP) was used as standard for calibration of the curve. Stock solution was provided at 800 nmol/ml concentration. Then standard solutions were prepared at 25, 50, 100, 200 and 400 nmol/ml concentrations. Results were expressed as nmol TBARS/g wet weight.

Determination of NP-SH (GSH) levels

NP-SH levels were determined according to the methods employed by Sedlak and Lindsay (18). Reduced glutathione (GSH) was used as standard for calibration of the curve. Stock solution was provided at 2.10^{-4} µmol/ml concentration. Standard solutions were prepared at 0.125, 0.25, 0.5, 1.0 and $2.0x10^{-4}$ µmol/ml concentrations. Results were expressed as µmol/g wet weight.

Determination of T-SH levels

T-SH levels were determined according to the methods employed by Sedlak and Lindsay (18). GSH was used as standard for calibration of the curve. Stock solution was provided at 2.10^{-4} µmol/ml concentration. Standard solutions were prepared at 0.125, 0.25, 0.5, 1.0 and $2.0x10^{-4}$ µmol/ml concentrations. Results were expressed as µmol/g wet weight.

Statistical analysis

Statistical calculations were done by using INSTAT. Results were expressed as means±standard deviation (SD). Differences between groups were examined using one-way analysis of variance (ANOVA) followed by Tukey's test.

RESULTS AND DISCUSSION

The effect of nicotine on different organs has not been completely elucidated to date. It was illustrated that iminium metabolite of nicotine via electron transfer with redox cycling might be involved in the production of radical entities. Free radicals are widely involved in the intracellular processes low levels of free radicals appear to participate in the basic metabolic processes whereas high concentrations are associated with oxidative insult (19,20). Nicotine can disrupt the mitochodrial respiratory chain leading to the increased generation of superoxide anions and hydrogen peroxide (21,22). Malondialdehyde, a commonly used biomarker of lipid peroxidation, belongs to the group of aldehydes arising mainly from lipid peroxidation in the body. However, usually measured levels of TBARS in tissues can be considered an indirect index of oxidative injuries associated with lipid peroxidation (23).

As shown in Table 1, nicotine increased the TBARS levels significantly and nicotine plus celecoxib treatment caused extremely significant decrease in TBARS levels in all tissues ($p<0.001$). The TBARS results of our study suggest that acute nicotine treatment (at the dose of 1.5 mg/kg) can increase the oxidative tissue damage in all tissues. This might be due to the excessive production of free radicals. Although it is hard to identify which organ is the target for nicotine, we can say that nicotine affects all tissues unfavorably.

Table 1. TBARS levels (nmol/g wet wt) of liver, lung, kidney, brain and heart tissues.
Results are mean values ± SD; a: compared to control; b: compared to nicotine; *** p<0.001

Groups	Liver	Lung	Kidney	Brain	Heart
Control	305.9±26.8	148.5±12.1	213.2±11.6	230.91±3.3	357.31±5.2
Nicotine[a]	548.35±9.5***	235.34±1.2***	294.11±8.9***	412.82±1.4***	408.62±1.9***
Nicotine+Celecoxib[b]	427.72±0.9***	105.41±0.0***	237.78±.9***	248.81±0.8***	307.61±7.0***

In addition, statistically significant increases were also observed in NP-SH levels in tissues in nicotine treated group as seen in Table 2. Glutathione, (L-γ-glutamyl-L-cysteinylglycine), a tripeptide is the predominant defense against free radicals in different tissues of the body and intracellular GSH levels modulate cell responses of oxidative stress. Majority of intracellular NP-SH is GSH and it can react directly with reactive oxygen species and electrophilic metabolites. Glutathione also protects essential thiol groups from oxidation and serves as a substrate for several enzymes and plays numerous important physiological roles in many biological processes, such as protein and DNA synthesis, transport, catabolism, and metabolism. The altered level of the GSH concentration in the body is responsible for some diseases, such as arteriosclerosis, occlusive vascular, leukemia, diabetes, AIDS and cataracts (24).

Table 2. GSH levels (µmol/g wet wt) of liver, lung, kidney, brain and heart tissues.
Results are mean values ± SD; a: compared to control; b: compared to nicotine; ** p<0.01; *** p<0.001.

Groups	Liver	Lung	Kidney	Brain	Heart
Control	6.9±.04	6.1±.02	6.5±.03	6.1±.03	6.2±.03
Nicotine[a]	7.4±.06***	6.6±.01***	7.1±.02***	6.3±.01***	6.5±.02***
Nicotine+Celecoxib[b]	7.1±.06**	6.5±.02**	6.7±.02***	6.2±.02***	6.3±.02***

Table 3.T-SH levels (µmol/g wet wt) of liver, lung, kidney, brain and heart tissues.
Results are mean values ± SD; a: compared to control; b: compared to nicotine; ** p<0.01; *** p<0.001.

Groups	Liver	Lung	Kidney	Brain	Heart
Control	7.7±.03	6.8±.02	7.4±.02	6.8±.02	7.0±.02
Nicotine[a]	9.1±.09***	7.5±.02***a	7.9±.02***a	7.1±.02***	7.2±.02***
Nicotine+Celecoxib[b]	7.8±.03**	7.3±.02**	7.5±.01***	6.9±.01***	7.1±.02***

Increased NP-SH and T-SH levels (Table 3.) with nicotine treatment may be related with the tissue -SH groups feedback system and may depend on the cellular defence system attack. However, in some studies, chronic nicotine treatment consumes antioxidant defence system such as GSH and

the other thiols. Glutathione homeostasis is a highly complex process, which is predominantly regulated by the liver, lung and kidney (24-27).

Celecoxib plus nicotine treatment caused extremely significant decreases in the TBARS levels in all tissues compared to nicotine group. In addition, treatment with the combination of nicotine and celecoxib drastically decreased NP-SH and T-SH content in all tissues compared to nicotine group. It is well known that lung and liver are highly susceptible to free radical generation. The lung also depends on the liver for its release of GSH into the plasma. The kidney, which is the site for elimination and retention of reactive metabolites, may also be influenced by nicotine and its metabolites (2,8,23). We suspected that this enhancement can be an acute response towards oxidative stress.

Since several reports suggest that the nicotine treatment can elevate COX-2 expression in some types of cells (4,5,8), we made a novel hypothesis that the inhibition of COX-2 might modulate the oxidative stress burden in animals. Measured lipid peroxidation content in tissues raises a possibility that nicotine-mediated stress can be alleviated by COX-2 inhibitor. Consequently, it has been shown that nicotine increases oxidative stress by induced COX-2 pathway (4, 25). The results of our investigation suggest that selective inhibition of COX-2 may be an important protective mechanism for the nicotine-induced tissue damage.

The emerging role of COX-2 in various diseases (27,28) and the present findings further support the therapeutic potential of celecoxib as a protectant in the treatment of oxidative cellular injury. As a conclusion; selective COX-2 inhibitors can be potent antioxidants and hence may have useful properties as an antioxidant supplement, capable of preventing tissue damage caused by oxidative stress.

Acknowledgement: This study was supported by Inonu University Scientific Research Council (Project No: 2005-80).

REFERENCES

1. Baker RR, Massey ED, Smith G, An overview of the effects of tobacco ingredients on smoke chemistry and toxicity, Food and Chemical Toxicology, 42, 53-83, 2004.
2. Zevin S, Gourlay SG, Benowitz NL, Clinical pharmacology of nicotine, Clinics in Dermatology,16, 557-64, 1998.
3. Fujimoto Y, Uno E, Sakuma S, Effects of reactive oxygen and nitrogen species on cyclooxygenase-1 and -2 activities, Prostaglandins, Leukotrienes & Essential Fatty Acids (PLEFA), 71, 335-340, 2004.
4. Chang YC, Tsai CH, Yang SH, Liu CM, Chou MY, Induction of cyclooxygenase-2 mRNA and protein expression in human gingival fibroblasts stimulated with nicotine, Journal of Periodontal Research, 38, 496-501, 2003.

5.Simone RD, Ajmone-Cat MA, Carnevale D, Minghetti L, Activation of alfa-7 nicotinic acetylcholine receptor by nicotine selectively up-regulated cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures, Journal of Neuroinflammation, 2, 1-10, 2005.

6.Shin VY, Cho CH, Nicotine and gastric cancer, Alcohol, 35, 259-264, 2005.

7. Newman MB, Arendash GW, Shytle RD, Bickford PC, Tighe T, Sanberg PR, Nicotine's oxidative and antioxidant properties in CNS, Life Sciences, 71, 2807-2820, 2002.

8. Tu´ neza I, Montilla P, Drucker-Colı´n R, Effect of nicotine on 3-nitropropionic acid-induced oxidative stress in synaptosomes, European Journal of Pharmacology, 504, 169-175, 2004.

9.Slotkin TA, MacKillop EA, Ryde IT, Seidler FJ, Ameliorating the developmental neurotoxicity of chlorpyrifos: A mechanisms-based approach in PC12 cells, Environmental Health Perspectives, 115(9), 1306-1313, 2007.

10.Therriault MJ, Proulx LI, Castonguay A, Bissonnette EY, Immunomodulatory effects of the tobacco-specific carcinogen, NNK, on alveolar macrophages, Clinical & Experimental Immunology, 132, 232-238, 2003.

11.Mohan S, Epstein JB, Carsinogenesis and cyclooxygenase: the potential role of COX-2 inhibition in upper aerodigestive tract cancer, Oral Oncology, 39, 537-546, 2003.

12. Cathcart MC, O'Byrne KJ, Reynolds JV, O'Sullivan J, Pidgeon GP, COX-derived prostanoid pathways in gastrointestinal cancer development and progression: Novel targets for prevention and intervention, Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1825(1), 49-63, 2012.

13.Im JW, Kim HK, Kim ND, Choi JS, Yu BP, Yang HS, Chung HY, Activation of cyclooxygenases by H_2O_2 and t-butylhydroperoxide in aged rat lung, Biotechnology Letters, 26, 1665-1669, 2004.

14. Tiano HF, Loftin CD, Akunda J, Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis, Cancer Research, 62, 3395-3401, 2002.

15.Romano M, Claria J, Cyclooxygenase-2 and 5-lipoxygenase converging functions on cell proliferation and tumor angiogenesis: implications for cancer therapy, The FASEB Journal, 17, 1986-1995, 2003

16.Ohkawa H, Ohishi N, Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Analytical Biochemistry, 95, 351-358, 1979.

17.Jamall IS, Smith JC, Effects of cadmium on glutathione peroxidase, superoxide dismutase and lipid peroxidation in the rat heart: A possible mechanism of cadmium cardiotoxicity, Toxicology and Applied Pharmacology, 80, 33-42, 1985.

18.Sedlak J, Lindsay RH, Estimation of total protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent, Analytical Biochemistry, 25, 192-205, 1968.

19.Jain A, Dwivedi N, Bhargava R, Flora SJS, Silymarin and naringenin protects nicotine induced oxidative stress in young rats, Oxidants and Antioxidants in Medical Science, 1(1), 41-49, 2012.

20.Shin VY, Wu WK, Ye YN, Nicotine promotes gastric tumor growth and neovascularization by activating extracellular signal-regulated kinase and cyclooxygenase-2, Carcinogenesis, 25, 2487-2495, 2004.

21.Şener G, Şehirli AÖ, İpçi Y, Çetinel Ş, Taurine treatment protects against chronic nicotine-induced oxidative changes, The Journal of Clinical Pharmacology, 19, 155-64, 2005.

22.Kim DH, Suh YS, Mun KC, Tissue levels of malondialdehyde after passive smoke exposure of rats for a 24-week period, Nicotine & Tobacco Research, 6, 1039-1042, 2004.

23.Husain K, Scott BR, Reddy SK, Somani SM, Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system, Alcohol, 25, 89-97, 2001.

24.Mitozo PA, Souza LF, Loch-Neckel G, Flesch S, Maris AF, Figueiredo CF, Santos ARS, Farina M, Dafre AL, A study of the relative importance of the peroxiredoxin-, catalase-, and glutathione-dependent systems in neural peroxide metabolism, Free Radical Biology and Medicine, 51, 69-77, 2011.

25.Kara E, Var A, Vatansever S, Cilaker S, Kaya Y, Coşkun T, Effects of rofecoxib, a selective cyclooxygenase-2 inhibitor on endothelial dysfunction, lipid peroxidation, and hepatocyte morphology in rats with sepsis-induced liver damage, Current Therapeutic Research, 65, 278-291, 2004.

26.DeLeve LD, Kaplowitz N, Glutathione metabolism and its role in hepatotoxicity, Pharmacology & Therapeutics, 52, 287-305, 1991.

27.Aksenov MY, Makesbery WR, Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease, Neuroscience Letters, 302, 141-145, 2001.

28.Strauss IK, Antiinflammatory and neuroprotective actions of COX2 inhibitors in the injured brain, Brain, Behavior, and Immunity, 22, 285-298, 2008.

Received: 12.09.2012
Accepted: 13.12.2012