Nicotine & Tobacco Research, 2017, 859–864
doi:10.1093/ntr/ntw198
Original investigation
Received February 1, 2016; Editorial Decision July 20, 2016; Accepted July 26, 2016

Original investigation

Protective Effect of Nicotine on Sepsis-Induced Oxidative Multiorgan Damage: Role of Neutrophils

Zarife N. Özdemir-Kumral MSc[1], Dilek Özbeyli PhD[1], Ahmet F. Özdemir MD[1], Buğra M. Karaaslan MD[1], Kübra Kaytaz MD[1], Mustafa F. Kara MD[1], Olgu E. Tok MSc[2], Feriha Ercan PhD[2], Berrak Ç. Yeğen MD[1]

[1]Department of Physiology, Marmara University School of Medicine, Istanbul, Turkey; [2]Department of Histology & Embryology, Marmara University School of Medicine, Istanbul, Turkey

Corresponding Author: Berrak Ç. Yegen, MD, Department of Physiology, Marmara University School of Medicine, Basibüyük Mah. Maltepe Basibüyük Yolu No. 9/1, Maltepe, 34854 Istanbul, Turkey. Telephone: 90 0-216-421 2222/1688; Fax: 90-0-216-414 4731; E-mail: byegen@marmara.edu.tr

Abstract

Introduction: Despite its adverse health consequences, tobacco smoking is associated with lower incidence of several neurodegenerative and inflammatory diseases. The present study is aimed to show the effects of nicotine, major tobacco constituent, on five organs targeted by sepsis.
Methods: Male Wistar albino rats received tap water with (5 mg/kg) or without nicotine for 14 days. Under ketamine anesthesia, sepsis (n = 50) was induced by ligation and puncture of the cecum, while sham group (n = 8) had only laparotomy. In other rats, nicotine drink was withdrawn for 5 days before sepsis induction, while in acute nicotine group, rats were injected with nicotine (30 mg/kg, i.p.) before sepsis, but had no oral intake. Rats were decapitated 24 hours after surgery to obtain lung, liver, ileum, heart, and kidney tissues to determine malondialdehyde (MDA) and glutathione (GSH) levels and myeloperoxidase (MPO) activities. Data were analyzed by one-way analysis of variance and Tukey multiple comparison tests or Student's t test.
Results: Chronic nicotine administration or its withdrawal reduced lipid peroxidation and MPO activity and prevented GSH depletion with some varying results in different target tissues. Nicotine injection prior to sepsis depressed MPO activity in all tissues and reduced MDA levels except for the lung, while GSH levels were elevated only in the hepatic and ileal tissues. Histologically observed injury was ameliorated by all nicotine treatments at varying degrees.
Conclusions: The findings of the present study indicate that long-term nicotine administration reduces sepsis-induced oxidative damage in several tissues, which appears to involve inhibition of neutrophil activity in the inflamed tissues.
Implications: Nicotine administration or its withdrawal reduced lipid peroxidation and neutrophil content and prevented GSH depletion with some varying results in different target tissues. A single injection prior to sepsis induction depressed MPO activity in all the tissues and reduced all tissue MDA levels except for the lung. When nicotine was withdrawn for 5 days, its inhibitory effect on MPO activity was still present in all the tissues except for the liver. Microscopically an improved inflammatory response was observed in all the tissues of rats that have received different nicotine pretreatment regimens.

Introduction

Cigarette smoking is proved to precipitate the progression of many diseases by impairing the functions of several tissues.[1,2] Although the adverse health consequences of tobacco smoking are extensively described, epidemiological studies have also revealed a lower incidence of several neurodegenerative and inflammatory diseases in smoking patients.[3,4] Both the adverse and beneficial effects of smoking were attributed to nicotine, which is a natural alkaloid in *Nicotiana tabacum* known to suppress the immune system.[5–8] In certain diseases, such as osteoarthritis[9] or ulcerative colitis,[10] nicotine delays the onset of the disease or reduces its severity or facilitates the healing process in several tissues.[11–13]

Despite the improvements in the management of intensive care, sepsis is still a life-threatening problem with a high incidence of multiorgan failure and consequently a high mortality rate.[14] In contrast to the general belief that trauma patients who were using nicotine for long-term would have a higher incidence of sepsis, smoking status was found not to play a significant role in the outcome of patients with trauma, and unexpectedly incidence of sepsis or organ failure was not higher in severely injured smokers.[15] On the other hand, experimental and clinical studies have revealed that oxidative stress causing an exaggerated inflammatory response has a crucial impact on the outcome of sepsis,[16,17] suggesting that additional therapeutic strategies capable of controlling the oxidative inflammatory response may be of benefit in the management of sepsis.[18] Accordingly, nicotine was shown to improve survival in experimental endotoxin peritonitis via its cholinergic agonistic effect,[19] which may be attributed to its inhibitory action on the production of proinflammatory cytokines.[20,21] Regarding the conflicting results that associate the nicotine use with the outcomes of sepsis, the present study was designed to elucidate the effect of acute or chronic nicotine use and its withdrawal on oxidative damage of sepsis-targeted multiple organs.

Materials and Methods

Animals

Male Wistar albino rats (300–350 g, 10 weeks old), supplied by the Marmara University (MU) Animal Center (DEHAMER), were housed in a humidity- (65%–70%) and temperature-controlled room (22°C ± 2°C) with standardized light/dark (12/12 hours) cycles. Rats were fed with standard rat pellets and tap water ad libitum. All experimental protocols were approved by the MU Animal Care and Use Committee.

Experimental Design and Surgery

Before commencing the surgery for sepsis, rats received either tap water or tap water containing nicotine (nicotine bitartrate, 5 mg/kg; Sigma, St. Louis, MO) for 14 days (Figure 1). The dose is equal to the average nicotine uptake (5 mg/kg; 30 cigarettes/day) of a moderate-to-heavy cigarette smoker.[22] In a subgroup of rats, nicotine was withdrawn and replaced with tap water on the ninth day, and tap water was continued for the following 5 days. Another group of rats that had tap water ingestion were administered intraperitoneally (i.p.) a single injection of nicotine (30 mg/kg) before the surgery, while all the other rats were injected with saline. The rationale for the nicotine dose was based on a previous study, in which the antioxidant effect of nicotine on acetic acid-induced gastric ulcer was investigated.[11]

Under ketamine (100 mg/kg; Pfizer, İstanbul) and chlorpromazine (3 mg/kg; Eczacıbaşı, İstanbul) combined anesthesia, surgery for cecal ligation and puncture (CLP) procedure was performed to induce sepsis (n = 50). Immediately before CLP or sham operation, i.p. saline or nicotine was injected (Figure 1). Following a midline laparotomy, a ligature was put around the cecal segment and three random punctures were made on the cecum, while the sham-operated control group (n = 8) underwent a laparotomy without any ligatures. During the surgery, rats were kept on a heating pad. Following the closure of the abdominal incision, rats were returned to their home cages and were resuscitated with saline (3 mL/100 g body weight) subcutaneously. The rats were deprived of food but had free access to water for the following 24 hours until they were decapitated. Lung, heart, kidney, liver, and ileum tissues were obtained for the determination of malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activities, and for histological evaluation.

Measurement of MDA and GSH Levels

Using a tissue homogenizer (IKA, T10 basic Ultra-Turrax, Handheld Homogenizer, Germany), tissue samples were homogenized in ice-cold trichloroacetic acid (1 g tissue + 9 mL 10% trichloroacetic acid; Sigma). Homogenized tissue samples were then centrifuged (Universal 16R Centrifuge, Hettich, Germany) at 2000g for 15 minutes at 4°C. The supernatant was removed and re-centrifuged at 41 400g for 8 minutes. Thiobarbituric acid reactive substance formation was measured according to previously described method for the evaluation of the MDA levels as indicators of lipid peroxidation.[23] Lipid peroxidation, given in terms of MDA equivalents using an extinction coefficient of $1.56 \times 105\,M^{-1}\,cm^{-1}$, was expressed as nmol MDA/g tissue.

GSH measurements were performed using a modification of the Ellman procedure.[24] Briefly, the homogenates were centrifuged

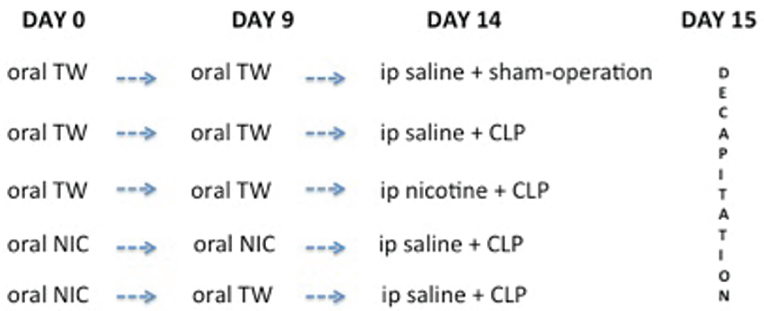

Figure 1. Representative scheme showing the procedure followed in the methods for the chronic oral intake of TW/NIC or i.p. NIC injection before the induction of sepsis with CLP. CLP, cecal ligation and puncture; NIC, nicotine; TW, tap water.

at 2000*g* for 10 minutes to obtain a 0.75 mL of supernatant, and this supernatant was added to 1 mL of 0.3 mol/L $Na_2HPO_4 \cdot 2H_2O$ solution. Afterwards, 0.2 mL of 5,5′-Dithiobis(2-nitrobenzoic acid) (Sigma) was added (0.4 mg/mL) in 1% sodium citrate (Sigma), and immediately the absorbance was measured at 412 nm. Calculation of GSH levels was made using an extinction coefficient of $1.36 \times 105\ M^{-1}\ cm^{-1}$. The results are expressed in µmol GSH/g tissue.

Measurement of MPO Activity

Activity of tissue MPO, an enzyme that is found predominantly in the azurophilic granules of polymorphonuclear leukocytes, is used as an indication of tissue neutrophil content, because tissue MPO activity correlates with the number of polymorphonuclear leukocytes determined histochemically in inflamed tissues.[25] The method used for the determination of tissue MPO was previously described.[26] Tissue samples (0.2–0.3 g) were homogenized in 10 volumes of ice-cold potassium phosphate buffer (50 mM K_2HPO_4, pH 6.0) containing hexadecyltrimethylammonium bromide (0.5%, wt/vol; Sigma). The homogenate was centrifuged at 41 400*g* for 10 minutes at 4°C. Following the removal of the supernatant, the pellet was used. The pellet was then re-homogenized with an equivalent volume of K_2HPO_4 (50 mM) containing (0.5%; wt/vol) hexadecyltrimethylammonium bromide and 10 mM ethylenediaminetetraacetic acid (Sigma). H_2O_2-dependent oxidation of o-dianizidine dihydrochloride (Sigma) was measured to assess MPO activity, where one unit of enzyme activity was defined as the amount of MPO present per gram of tissue weight that caused a change in absorbance of $1.0\ min^{-1}$ at 460 nm and 37°C.

Histopathological Evaluation

For light microscopic investigations, tissue specimens from the lung, heart, kidney, liver, and ileum were fixed in 10% formaldehyde and processed routinely for embedding in paraffin. Tissue sections (4 µm) were stained with hematoxylin and eosin (H&E) for general histopathological evaluation. All tissue sections were examined under a photomicroscope (Olympus BX51, Tokyo, Japan) for the evaluation of histopathological changes by an experienced histologist who was unaware of the treatment conditions. The histological score of the organ was calculated as the sum of the scores (0–3) given for each criterion, using the semiquantitative scale outlined in Table 1.[27,28]

Table 1. Criteria for the Microscopic Scoring of Tissue Damage

Tissue	Appearance
Lung	• Alveolar structural disturbance • Inflammatory cell infiltration • Vascular congestion and interstitial edema
Heart	• Inflammatory cell infiltration • Degeneration of muscle fibers
Kidney	• Degeneration of Bowman space and glomeruli • Degeneration of proximal and distal tubules • Vascular congestion, interstitial edema, inflammatory cell infiltration
Liver	• Dilation and vacuolization of hepatocytes • Vascular congestion and dilation of sinusoids • Kupffer cell infiltration
Ileum	• Degeneration of surface and crypt epithelium • Degeneration of villus structure • Inflammatory cell infiltration

Scores for each criterion: 0—none; 1—mild; 2—moderate; 3—severe. At least five microscopic areas were examined to score each specimen.

The maximum score calculated for lung, liver, kidney, and ileum was 9, while the maximum score that could be given was 6 for the cardiac tissue.

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA). Data were analyzed by one-way analysis of variance followed by post hoc Tukey multiple comparison test. To compare the difference between two means, Student's *t* test was used after analyzing the data for normal distribution. All data were presented as mean ± SE and *p* values were regarded as significant when it was lower than .05.

Results

In comparison to sham-operated rats presenting with 100% survival rate (8/8), septic rats that have received either tap water (8/11; 73%) or single nicotine administration (11/14; 79%) showed a reduction in survival at the 24th hour following the induction of CLP. However, the rats that had chronic intake of nicotine had a 100% survival (11/11), while the survival rate was lower (11/14; 79%) in the rats that had a 5-day nicotine withdrawal following its chronic intake.

As an end product of lipid peroxidation, MDA was measured in all tissues to assess the level of oxidative tissue injury. In all of the tissues, MDA levels were significantly elevated due to sepsis as compared to sham-operated control group ($p < .05$–.001; Supplementary Figure 1). Either given orally for 14 days, stopped for the last 5 days or injected before sepsis, nicotine at all routes significantly decreased the ileal MDA levels ($p < .01$–.001; Figure 2). Additionally, single nicotine injection decreased hepatic ($p < .05$), cardiac, and renal ($p < .01$) MDA levels, while pulmonary and hepatic MDA levels were depressed by long-term oral administration of nicotine ($p < .01$; Supplementary Figure 1).

As a marker of neutrophil content of the tissue, MPO activity in all tissues was found to be significantly higher in the nontreated CLP group than that of the control group ($p < .01$–.001; Figure 2 and Supplementary Figure 2). All three nicotine treatment regimens significantly decreased the CLP-induced elevations in MPO activity of the heart, lung, and ileum tissues ($p < .05$–.001; Figure 2 and Supplementary Figure 2). Sepsis-induced elevations in the MPO activity of the liver were depressed by injection ($p < .01$) or chronic oral administration ($p < .001$) of nicotine, while its injection or withdrawal for 5 days reduced renal MPO activity ($p < .01$).

GSH, measured as an intracellular antioxidant, was found to be significantly lower in all the tissues of nontreated CLP groups as compared to those of sham-operated control group ($p < .05$–.001; Figure 2 and Supplementary Figure 3), while chronic treatment with oral nicotine reversed the reductions in GSH in all the tissues ($p < .05$–.001), except for the lung that demonstrated an additional reduction ($p < .05$). On the other hand, nicotine withdrawal or its single dose injection also prevented CLP-induced GSH depletion in both the liver ($p < .01$) and ileum ($p < .05$; Figure 2 and Supplementary Figure 3).

Light microscopic evaluation by H&E staining of sham-operated control groups (Supplementary Figures 4 and 5) revealed regular morphological organization of lung and liver parenchyma, cardiac muscle, ileal mucosa, glomeruli, and tubules. Lungs of the nontreated sepsis group demonstrated severe alveolar disturbance, vascular congestion, and inflammatory cell infiltration, which were replaced with moderate

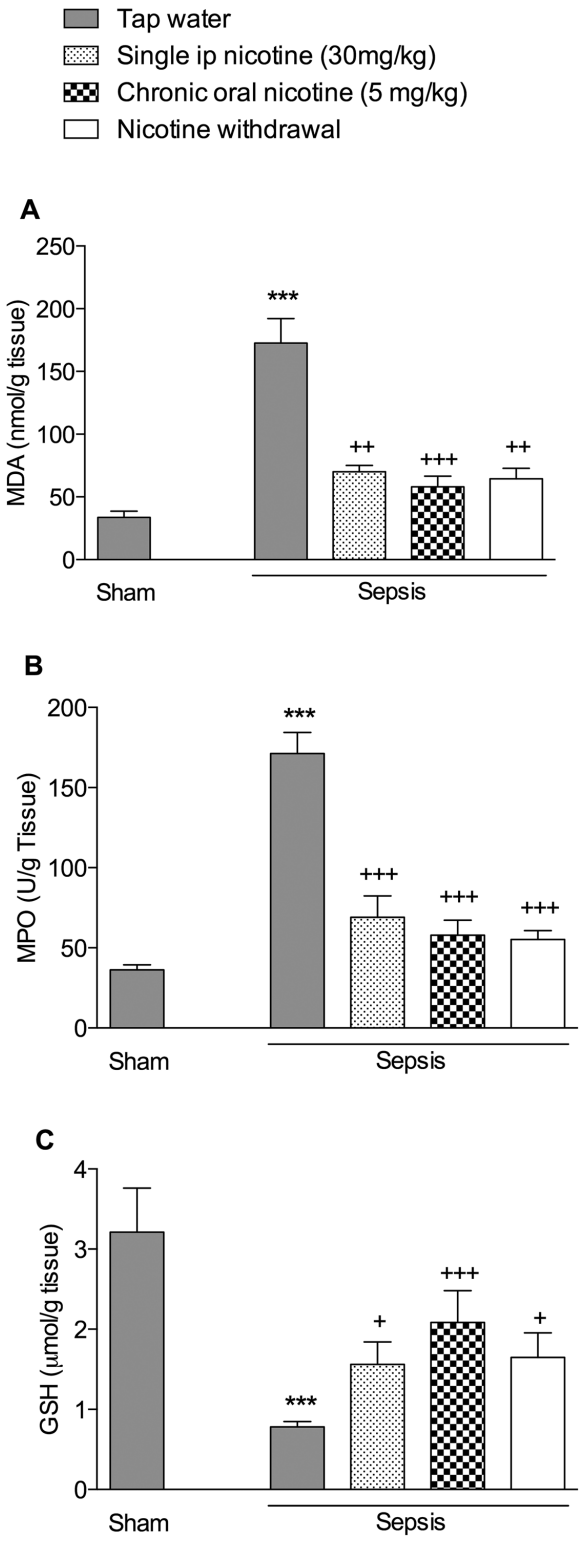

Figure 2. (A) MDA, (B) MPO activity, and (C) GSH in the ileum of sham-operated or sepsis-induced (cecal ligation puncture [CLP]) groups that have received tap water, single dose nicotine or chronic oral nicotine or had nicotine withdrawal. Each group consists of 6–11 rats. Data were analyzed by one-way analysis of variance followed by post hoc Tukey multiple comparison test. To compare the difference between two means, Student's *t* test was used after analyzing the data for normal distribution. Values are represented as mean ± SEM. $^{***}p < .001$ vs. sham group; $^{+}p < .05$, $^{++}p < .01$, $^{+++}p < .001$ vs. tap water CLP group. GSH, glutathione; MDA, malondialdehyde; MPO, myeloperoxidase.

degrees of disturbance, congestion, and inflammatory cell infiltration when the rats were treated with oral intake or single injection of nicotine, showing lower microscopic damage scores. Nontreated CLP group showed a high degree of hepatocyte degeneration with vacuole formation and dilatation in sinusoids, but the degeneration and sinusoidal dilatation were relieved when nicotine was given either orally or intraperitoneally. Disorganization of cardiac muscle fibers with vascular congestion, detected in nontreated CLP group, was almost reversed by chronic oral or single dose injection of nicotine, but the microscopic scores were not significantly changed. The ileal tissue of nontreated sepsis group showed severe desquamation of apical epithelial cells and inflammatory cell infiltration in lamina propria. Injection of nicotine reduced only infiltration of inflammatory cells, while its oral intake decreased both desquamation of apical epithelial cells and the infiltration of inflammatory cells of the ileal mucosa, while the microscopic damage scores were not different among the groups. Kidneys of nontreated sepsis group showed marked degeneration of renal glomeruli and tubules with vasocongestion in the parenchyma. In both groups with chronic intake or single injection, tubular and glomerular degeneration were reduced presenting a better morphology with moderate congestion and mild degeneration in the glomerular and tubular cells; however, these changes were not reflected in the microscopic scores. In the nicotine withdrawal group, all the parameters indicating degeneration or inflammation were apparently reduced, but the impact of nicotine treatment was not as efficient as those of the other treatment regimens.

Discussion

The present data revealed that acute or chronic nicotine administration, with some varying results in different target tissues, generally reduced lipid peroxidation and MPO activity and prevented GSH depletion. Except for the renal tissue, chronic nicotine administration was shown to be protective in all the tissues as assessed by reductions in MDA levels and MPO activities with respect to saline-treated sepsis group. Including the renal tissue, antioxidant GSH was maintained in the chronic nicotine-treated groups, while pulmonary GSH was further depleted. Similarly, a single injection prior to sepsis induction significantly depressed MPO activity in all the tissues and reduced all tissue MDA levels except for the lung, while GSH levels were elevated only in the hepatic and ileal tissues. Following a 5-day withdrawal, the inhibitory effect of nicotine on neutrophil content was still present in all the tissues except for the liver, while GSH levels in the liver and ileum were raised. Reduction in sepsis-elevated MDA levels was observed only in the cardiac and ileal tissues of the nicotine-withdrawn group. Although microscopic damage scores of the tissues were reduced significantly only in the lung tissue, a tendency for lower damage scores was observed in all the tissues of rats that have received different nicotine treatment regimens.

A great proportion of critically ill patients develop systemic inflammatory response syndrome and sepsis.[29,30] Chronic tobacco users present with a lower survival rate due to an increased risk of acute lung injury and adult respiratory distress syndrome.[31,32] In addition, smokers were claimed to undergo acute nicotine withdrawal following traumatic events that require intensive care, leading to a higher incidence of inflammatory complications with multiple organ dysfunction; but a retrospective cohort of trauma patients showed that nicotine withdrawal during the hospitalization period did not alter the incidence of complications in the trauma victims.[15] The cellular and molecular mechanisms involved in the pathogenesis of sepsis

were significantly clarified in recent years, which involve the role of oxidative injury.[33] The results of our study support the contribution of the oxidative and antioxidant mechanisms in sepsis-induced multiple organ damage, while the ameliorative impact of nicotine on sepsis-induced oxidative organ damage is demonstrated. Experimental and clinical studies have suggested that the effect of nicotine, the major constituent of tobacco, was not detrimental as cigarette smoking. Although cigarette smoking is defined as a global public health threat responsible of several diseases that can be partially prevented by quitting, smokers were proven to be less prone to some chronic inflammatory diseases.[9] We have previously shown that nicotine did not aggravate gastric injury in rats induced with gastric ulcer, while nicotine withdrawal has attenuated gastric mucosal injury.[11]

Since the life-threatening multiple organ failure in sepsis is well known to progress by an imbalance between reactive oxygen species and antioxidant enzymes, several agents having an antioxidant action have been screened for their protective effects on the organs of rats or mice submitted to sepsis. Accordingly, alpha-lipoic acid, rosmarinic acid, beta glucan, ghrelin, and melatonin were proven to be effective in protecting several tissues, including the kidney, heart, liver, and lung tissues of septic rats by reducing MPO activity and lipid peroxidation.[27,34–37] Similar to anti-inflammatory effects of the aforementioned agents, the present findings verify that chronic nicotine ingestion or its acute administration alleviates multiorgan injury in septic rats. In contrast to studies that have demonstrated increased testicular lipid peroxidation and decreased antioxidant levels in the serum and testis of nicotine-treated rats,[38,39] nicotine was shown to attenuate oxidative organ damage in several other models. Administration of oral nicotine in mice or rats induced with colitis has resulted in decreased disease severity, histologic damage scores, and reduced production of proinflammatory cytokines.[40,41] Moreover, electrical stimulation of the vagus nerve was also shown to depress production of proinflammatory cytokines and mortality during sepsis.[42] Similarly, nicotine pretreatment has significantly attenuated the severity of lipopolysaccharide-induced acute lung injury in mice and reduced mortality via the suppression of leukocyte infiltration and proinflammatory cytokine production.[43,44] Moreover, the cardioprotective[45] and neuroprotective[46,47] effects of nicotine were also attributed to its anti-inflammatory action via the inhibition of proinflammatory cytokine production. Nicotine, administered as an acetylcholine receptor agonist, was shown to reduce mortality in sepsis and septic peritonitis[19,48] via the cholinergic anti-inflammatory pathway by suppressing the inflammatory response through the release of acetylcholine from the vagus nerve.[49] The effects of acute/chronic nicotine administration on systemic inflammation were studied in mice induced with CLP or systemically administered with lipopolysaccharide.[50] In contrast to our findings, they have shown that long-term nicotine administration in either model of inflammation had no effect on survival. However, in that study the chronically infused daily dose of nicotine in mice was about sixfolds greater than the dose we have orally administered to rats. In contrast to the protective effect of our single injection of nicotine, Steiner et al.[50] have shown that acute administration of nicotine by a 3-day infusion reduced the survival in sepsis. Wang et al.[19] have shown that nicotine diminished the clinical symptoms of sepsis, such as hypothermia, diarrhea, and body weight loss via an anti-inflammatory pathway. Indicating the role of the anti-inflammatory mechanism in a murine model of viral myocarditis, Li-Sha et al.[51] have recently reported that nicotine significantly increased survival rate and decreased myocardial inflammation by suppressing inflammatory cytokines at the 14th day of virus inoculation. In contrast to our results obtained in rats with acute CLP sepsis, elevated lipid peroxidation and depleted antioxidant capacity following viral myocarditis were not changed by nicotine treatment, which may be explained by differences in sepsis model, species, and timing of examination following inflammation. Based on the aforementioned studies showing the protective effects of cholinergic stimulation by pharmacological or electrical methods in sepsis, the present study extends these findings, and measured parameters of oxidative stress in the present study demonstrated a significant improvement when CLP groups were treated with nicotine. Thus, to our knowledge, this is the first report verifying that the mechanism underlying the amelioration of CLP-induced sepsis by nicotine treatment depends on the balance between the oxidant (as measured by MPO and MDA levels) and antioxidant (eg, GSH) systems. Moreover, the results suggest that even the single dose of nicotine, similar to its long-term administration, is efficient in protecting the tissues against oxidative damage. Thus, nicotine may be advantageous in regulating systemic inflammation by balancing the oxidant and antioxidant systems and inhibiting the MPO activity. Although it was speculated that nicotine withdrawal may have a destructive effect on systemic inflammation,[52] our results have shown that chronic intake of nicotine maintains its anti-inflammatory effect despite its withdrawal for a 5-day period.

In conclusion, the findings of the current study suggest that nicotine intake prior to sepsis had no worsening effect, but contrarily ameliorated oxidative injury of sepsis- targeted organs with varying degrees. Differences in the kinetics and biodistribution of orally or intraperitoneally administered nicotine may be responsible of varying changes on MDA, MPO, and GSH levels of the sepsis-targeted tissues. Moreover, these organs are expected to have varying degrees of vulnerability in sepsis, making their response to nicotine be different. Despite the health risks of toxic and/or carcinogenic chemicals in tobacco,[5] nicotine appears to have beneficial effects in systemic inflammatory response. Clinical studies that include the supplementation of nicotine are required to further elucidate the therapeutical potential of nicotine in sepsis.

Supplementary Material

Supplementary Figures 1–5 can be found online at http://www.ntr.oxfordjournals.org

Declaration of Interests

None declared.

References

1. Danson SJ, Rowland C, Rowe R, et al. The relationship between smoking and quality of life in advanced lung cancer patients: a prospective longitudinal study. *Support Care Cancer* 2016;24(4):1507–1516.
2. Doll R, Peto R. Mortality in relation to smoking: 20 years' observations on male British doctors. *Br Med J*. 1976;2(6051):1525–1536.
3. Fratiglioni L, Wang HX. Smoking and Parkinson's and Alzheimer's disease: review of the epidemiological studies. *Behav Brain Res*. 2000;113(1–2):117–120.
4. Manthorpe R, Benoni C, Jacobsson L, et al. Lower frequency of focal lip sialadenitis (focus score) in smoking patients. Can tobacco diminish the salivary gland involvement as judged by histological examination and anti-SSA/Ro and anti-SSB/La antibodies in Sjögren's syndrome? *Ann Rheum Dis*. 2000;59(1):54–60.

5. Sopori M. Effects of cigarette smoke on the immune system. *Nat Rev Immunol*. 2002;2(5):372–377.
6. Rezonzew G, Chumley P, Feng W, Hua P, Siegal GP, Jaimes EA. Nicotine exposure and the progression of chronic kidney disease: role of the α7-nicotinic acetylcholine receptor. *Am J Physiol Renal Physiol*. 2012;303(2):F304–F312.
7. Bruin JE, Petre MA, Lehman MA, et al. Maternal nicotine exposure increases oxidative stress in the offspring. *Free Radic Biol Med*. 2008;44(11):1919–1925.
8. Bryden DW, Burton AC, Barnett BR, et al. Prenatal nicotine exposure impairs executive control signals in medial prefrontal cortex. *Neuropsychopharmacology*. 2016;41(3):716–725.
9. Felson DT, Zhang Y. Smoking and osteoarthritis: a review of the evidence and its implications. *Osteoarthritis Cartilage*. 2015;23(3):331–333.
10. Lunney PC, Leong RW. Review article: ulcerative colitis, smoking and nicotine therapy. *Aliment Pharmacol Ther*. 2012;36(11–12):997–1008.
11. Deniz M, Sahin HH, Tekin S, et al. Nicotine withdrawal alleviates acetic acid-induced gastric injury in rats. *Environ Toxicol Pharmacol*. 2009;27(2):200–205.
12. Hastrup SG, Chen X, Bechtold JE, et al. Effect of nicotine and tobacco administration method on the mechanical properties of healing bone following closed fracture. *J Orthop Res*. 2010;28(9):1235–1239.
13. Lee MY, Chen L, Toborek M. Nicotine attenuates iNOS expression and contributes to neuroprotection in a compressive model of spinal cord injury. *J Neurosci Res*. 2009;87(4):937–947.
14. Remick DG. Pathophysiology of sepsis. *Am J Pathol*. 2007; 170(5):1435–1444.
15. Ferro TN, Goslar PW, Romanovsky AA, Petersen SR. Smoking in trauma patients: the effects on the incidence of sepsis, respiratory failure, organ failure, and mortality. *J Trauma*. 2010;69(2):308–312.
16. Boveris A, Alvarez S, Navarro A. The role of mitochondrial nitric oxide synthase in inflammation and septic shock. *Free Radic Biol Med*. 2002;33(9):1186–1193.
17. Takahashi T, Suzuki T, Yamasaki A, Tsukiji T, Hirakawa M, Akagi R. Heat shock response in a rat model of septic multiple organ dysfunction syndrome. *Nihon Yakurigaku Zasshi*. 1999;114(5):295–302.
18. Boomer JS, Green JM, Hotchkiss RS. The changing immune system in sepsis: is individualized immuno-modulatory therapy the answer? *Virulence*. 2014;5(1):45–56.
19. Wang H, Liao H, Ochani M, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med*. 2004;10(11):1216–1221.
20. Zhou G, Hu S, Lv Y, Song Q, Zou X, Sheng Z. Carbachol alleviates rat cytokine release and organ dysfunction induced by lipopolysaccharide. *J Trauma*. 2011;71(1):157–162.
21. Reale M, Di BM, Di NM, et al. Nicotinic receptor activation negatively modulates pro-inflammatory cytokine production in multiple sclerosis patients. *Int Immunopharmacol*. 2015;29:152–157.
22. Boulos DN, Loffredo CA, El Setouhy M, Abdel-Aziz F, Israel E, Mohamed MK. Nondaily, light daily, and moderate-to-heavy cigarette smokers in a rural area of Egypt: a population-based survey. *Nicotine Tob Res*. 2009;11(2):134–138.
23. Casini AF, Ferrali M, Pompella A, Maellaro E, Comporti M. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene-intoxicated mice. *Am J Pathol*. 1986;123(3):520–531.
24. Aykaç G, Uysal M, Yalçin AS, Koçak-Toker N, Sivas A, Oz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. *Toxicology*. 1985;36(1):71–76.
25. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol*. 1982;78(3):206–209.
26. Hillegass LM, Griswold DE, Brickson B, Albrightson-Winslow C. Assessment of myeloperoxidase activity in whole rat kidney. *J Pharmacol Methods*. 1990;24(4):285–295.
27. Sener G, Toklu H, Kapucu C, et al. Melatonin protects against oxidative organ injury in a rat model of sepsis. *Surg Today*. 2005;35(1):52–59.
28. Demling R, LaLonde C, Knox J, Youn YK, Zhu D, Daryani R. Fluid resuscitation with deferoxamine prevents systemic burn-induced oxidant injury. *J Trauma*. 1991;31:538–543.
29. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med*. 2001;29(7):1303–1310.
30. Brun-Buisson C, Doyon F, Carlet J, et al. Incidence, risk factors, and outcome of severe sepsis and septic shock in adults. A multicenter prospective study in intensive care units. French ICU Group for Severe Sepsis. *JAMA*. 1995;274(12):968–974.
31. Montgomery AB, Stager MA, Carrico CJ, Hudson LD. Causes of mortality in patients with the adult respiratory distress syndrome. *Am Rev Respir Dis*. 1985;132(3):485–489.
32. Fowler AA, Hamman RF, Good JT, et al. Adult respiratory distress syndrome: risk with common predispositions. *Ann Intern Med*. 1983;98(5 Pt 1):593–597.
33. Ivády B, Béres BJ, Szabó D. Recent advances in sepsis research: novel biomarkers and therapeutic targets. *Curr Med Chem*. 2011;18(21):3211–3225.
34. Petronilho F, Florentino D, Danielski LG, et al. Alpha-lipoic acid attenuates oxidative damage in organs after sepsis. *Inflammation*. 2016;39(1):357–365.
35. Bacanli M, Aydin S, Taner G, et al. Does rosmarinic acid treatment have protective role against sepsis-induced oxidative damage in Wistar albino rats? *Hum Exp Toxicol*. 2016;35(8):877–886.
36. Sener G, Toklu H, Ercan F, Erkanli G. Protective effect of beta-glucan against oxidative organ injury in a rat model of sepsis. *Int Immunopharmacol*. 2005;5(9):1387–1396.
37. Khowailed A, Younan SM, Ashour H, Kamel AE, Sharawy N. Effects of ghrelin on sepsis-induced acute kidney injury: one step forward. *Clin Exp Nephrol*. 2015;19(3):419–426.
38. Oyeyipo IP, Raji Y, Bolarinwa AF. Nicotine alters serum antioxidant profile in male albino rats. *N Am J Med Sci*. 2014;6(4):168–171.
39. Oyeyipo IP, Raji Y, Bolarinwa AF. Antioxidant profile changes in reproductive tissues of rats treated with nicotine. *J Hum Reprod Sci*. 2014;7(1):41–46.
40. AlSharari SD, Akbarali HI, Abdullah RA, et al. Novel insights on the effect of nicotine in a murine colitis model. *J Pharmacol Exp Ther*. 2013;344(1):207–217.
41. Sykes AP, Brampton C, Klee S, Chander CL, Whelan C, Parsons ME. An investigation into the effect and mechanisms of action of nicotine in inflammatory bowel disease. *Inflamm Res*. 2000;49(7):311–319.
42. Villegas-Bastida A, Torres-Rosas R, Arriaga-Pizano LA, et al. Electrical stimulation at the ST36 acupoint protects against sepsis lethality and reduces serum TNF levels through vagus nerve- and catecholamine-dependent mechanisms. *Evid Based Complement Alternat Med*. 2014;2014:451674.
43. Ni YF, Tian F, Lu ZF, et al. Protective effect of nicotine on lipopolysaccharide-induced acute lung injury in mice. *Respiration*. 2011;81(1):39–46.
44. Mabley J, Gordon S, Pacher P. Nicotine exerts an anti-inflammatory effect in a murine model of acute lung injury. *Inflammation*. 2011;34(4):231–237.
45. Li-Sha G, Jing-Lin Z, Guang-Yi C, Li L, De-Pu Z, Yue-Chun L. Dose-dependent protective effect of nicotine in a murine model of viral myocarditis induced by coxsackievirus B3. *Sci Rep*. 2015;5:15895.
46. Park HJ, Lee PH, Ahn YW, et al. Neuroprotective effect of nicotine on dopaminergic neurons by anti-inflammatory action. *Eur J Neurosci*. 2007;26(1):79–89.
47. Ravikumar R, Flora G, Geddes JW, Hennig B, Toborek M. Nicotine attenuates oxidative stress, activation of redox-regulated transcription factors and induction of proinflammatory genes in compressive spinal cord trauma. *Brain Res Mol Brain Res*. 2004;124(2):188–198.
48. van Westerloo DJ, Giebelen IA, Florquin S, et al. The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *J Infect Dis*. 2005;191(12):2138–2148.
49. Andersson J. The inflammatory reflex–introduction. *J Intern Med*. 2005;257(2):122–125.
50. Steiner AA, Oliveira DL, Roberts JL, Petersen SR, Romanovsky AA. Nicotine administration and withdrawal affect survival in systemic inflammation models. *J Appl Physiol (1985)*. 2008;105(4):1028–1034.
51. Li-Sha G, Jing-Lin Z, Li L, et al. Nicotine inhibits the production of pro-inflammatory cytokines of mice infected with coxsackievirus B3. *Life Sci*. 2016;148:9–16.
52. Matthay MA, Ware LB. Can nicotine treat sepsis? *Nat Med*. 2004;10(11):1161–1162.