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Antioxidant Activity of Syringic Acid Prevents Oxidative Stress in L-arginine–Induced Acute Pancreatitis: An Experimental Study on Rats

Oztekin Cikman[1], Omer Soylemez[2], Omer Faruk Ozkan[1], Hasan Ali Kiraz[3], Ilyas Sayar[4], Serkan Ademoglu[1], Seyithan Taysi[5], Muammer Karaayvaz[1]

[1]*Department of General Surgery, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey*

[2]*Department of General Surgery, Gaziantep 25 Aralık State Hospital, Gaziantep, Turkey*

[3]*Department of Anesthesiology and Reanimation, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey*

[4]*Department of Medical Pathology, Faculty of Medicine, Erzincan University, Erzincan, Turkey*

[5]*Department of Medical Biochemistry, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey*

The aim of this study was to investigate the possible protective role of antioxidant treatment with syringic acid (SA) on L-arginine–induced acute pancreatitis (AP) using biochemical and histopathologic approaches. A total of 30 rats were divided into 3 groups. The control group received normal saline intraperitoneally. The AP group was induced by 3.2 g/kg body weight L-arginine intraperitoneally, administered twice with an interval of 1 hour between administrations. The AP plus SA group, after having AP induced by 3.2 g/kg body weight L-arginine, was given SA (50 mg kg^{-1}) in 2 parts within 24 hours. The rats were killed, and pancreatic tissue was removed and used in biochemical and histopathologic examinations. Compared with the control group, the mean pancreatic tissue total oxidant status level, oxidative stress index, and lipid hydroperoxide levels were significantly increased in the AP group, being 30.97 ± 7.13 ($P < 0.05$), 1.76 ± 0.34 ($P < 0.0001$), and 19.18 ± 4.91 ($P < 0.01$), respectively. However, mean total antioxidant status and sulfhydryl group levels were significantly decreased in the AP group compared with the control group, being 1.765 ± 0.21 ($P < 0.0001$) and 0.21 ±

Corresponding author: Oztekin Cikman, Department of General Surgery, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey, 17100.
Tel.: +905334646577; Fax: +902862635957; E-mail: droztekin67@hotmail.com

0.04 ($P < 0.0001$), respectively. SA reduces oxidative stress markers and has antioxidant effects. It also augments antioxidant capacity in L-arginine–induced acute toxicity of pancreas in rats.

Key words: Acute pancreatitis – Syringic acid – Total oxidant status – Total antioxidant status

Acute pancreatitis (AP) is an acute inflammatory disorder of the pancreas with variable involvement of other regional tissues. The most common symptom of AP is acute abdominal pain. AP is a reversible inflammatory disorder that varies in severity, ranging from focal edema and fat necrosis to widespread hemorrhagic parenchymal necrosis. Approximately 80% of cases are attributed to either biliary tract disease or alcoholism.[1]

Reactive oxygen species (ROS), reactive nitrogen species (RNS), and other carbon-centered molecules are unstable chemicals generated in biologic systems under normal physiologic as well as pathophysiologic conditions. ROS include free radical intermediates, such as singlet oxygen superoxide anion ($O_2^{\bullet-}$), and hydroxyl radical ($OH^{\bullet-}$) as well as nonradical molecules, such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). RNS consist primarily of nitric oxide ($NO^{\cdot}$), peroxynitrite, and other nitrates, whereas carbon-centered molecules are rather complex in terms of their chemical structure and generally are produced in the xenobiotic metabolism.[2] Normally, there is a delicate balance between ROS and RNS production and tissue concentrations of antioxidants in the body. This balance is related to the rate of total antioxidant status (TAS) to total oxidant status (TOS), as determined by the oxidative stress index (OSI). ROS are produced both normally through the electron transfer chain system of the mitochondria and in excessive numbers in various conditions that increase energy (ATP) demand. The latter may include, among other factors, biologic factors and exposure to heat and certain chemicals and toxins.[3–5] ROS plays an important role in the pathogenesis of AP, and there is also a correlation between the production of ROS and the severity of AP. The detrimental effects of ROS and RNS are mediated by their direct actions on biomolecules, such as lipids, proteins, and DNA, and the activation of proinflammatory signal cascades, which subsequently lead to the activation of immune responses.[2]

The dietary plant polyphenolic compounds were shown to have beneficial effects in preventing oxidative stress, inhibiting the production of free radicals and the formation of lipid peroxidation. Scientific interest in phenolic compounds has been stimulated because of their anti-inflammatory, antimutagenic, and anticarcinogenic properties. They have antioxidant activity mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, free radical scavengers, metal chelators, and modulators of enzymatic activity, thereby preventing a lot of diseases, including diabetes mellitus, hypertension, atherosclerosis, and cancer.[6,7] Antioxidants are compounds that, even when present at much lower concentrations than those of oxidizable substrates, significantly delay (or even prevent) oxidation of oxidizable substrates.[8] Syringic acid (SA) is a natural phytochemical isolated from *Isatis indigotica* and *Radix isatidis*. SA exhibits multipharmacologic properties, such as strong antioxidant, antiproliferative, antiendotoxic, antimicrobial, anti-inflammatory, and anticancer effects.[7,8]

To our knowledge, there is no experimental study that investigates the effects of SA supplementation on L-arginine–induced AP in rats. For this reason, we aimed to investigate the protective effect of SA against L-arginine–induced acute toxicity of pancreas.

Materials and Methods

After receiving ethics consent approval from the Animal Research Ethical Committee of Canakkale Onsekiz Mart University Medical School, 30 male adult Sprague-Dawley rats, weighing 250 to 300 g, were used in the study. The animal study was conducted at the Experimental Surgery, Research and Animal Laboratory of Canakkale Onsekiz Mart University Faculty of Medicine (Canakkale, Turkey). The experimental protocols were carried out according to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health, revised 1985). All rats were housed under standard laboratory conditions with a 12 hours light/12 hours dark cycle and were allowed to have *ad libitum* food and water before and after surgery. During the experimental procedure, the animals were individually placed in cages and kept at room

temperature (22°C). All surgical procedures were performed under sterile conditions.

Experimental protocol

Before the experimental procedure, all animals were weighed with an analytic balance, and body weights were recorded. Rats were anesthetized with intramuscular injections of ketamine HCl (50 mg/kg, Ketalar, Parke-Davis, Morris Plains, New Jersey) and xylazine (10 mg/kg, Rompun, Bayer, Istanbul, Turkey). A total of 30 Sprague-Dawley rats were assigned into 3 groups.

The control group received normal saline (0.9% NaCl) intraperitoneally. The AP group was induced by 3.2 g/kg body weight L-arginine (Sigma-Aldrich, Steinheim, Germany) intraperitoneally administered twice, with an interval of 1 hour between administrations, which has been shown previously to produce severe necrotizing AP. The AP plus SA group, after having AP induced by 3.2 g/kg body weight L-arginine, was given SA (50 mg kg^{-1}) in 2 parts during 24 hours interval. After 36 hours, all rats were anesthetized. Blood samples were collected via intracardiac route, and then the rats were killed. Then, the abdomen was opened. The duodenal loop with whole pancreas was harvested as a sample for histopathologic confirmation and grading of AP.

Experimental design

The rest of the specimens were stored at –80°C for biochemical examination. A piece of lung tissue (approximately 300 mg) was homogenized in 10 volumes of ice-cold phosphate buffer solution (50 mM/L, pH 7.0) using a homogenizer (Ultra-Turrax T8 dispersing homogenizer, IKA, Staufen, Germany). Then, the homogenate was centrifuged at 10,000*g* for 15 minutes at 4°C. The supernatant was stored at –80°C in aliquots.

Biochemical analysis

Measurement of total antioxidant capacity

TAS and TOS levels were measured using a colorimetric method that was introduced by Erel[9]. The results were expressed as millimolar Trolox equivalent per liter (mmol Trolox equivalent/gr protein) for TAS and micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/ gr protein) for TOS. The ratio of TOS to TAS was accepted as the OSI. For the calculation, the resulting unit of TAS was converted to μmol / gr protein, and the OSI value was calculated according to the following formula[10]:

OSI (arbitrary unit) = [TOS (μmol H_2O_2 equivalent/g protein)/TAS (μmol Trolox equivalent/g protein)] × 100.

Measurement of sulfhydryl and lipid hydroperoxide levels

The sulfhydryl (–SH) levels of the liver tissue were assayed according to Ellman's[11] method. The results are expressed as millimoles per gram of protein. Lipid hydroperoxide (LOOH) levels were measured with the ferrous ion oxidation–xylenol orange method as previously described.[12] The results are expressed as micromoles per gram of protein. The protein content was determined as described previously.[13]

Histopathologic examination

The pancreatic tissue samples were stored in 10% neutral formalin solution and embedded in paraffin wax. Pancreas tissue sections stained with hematoxylin-eosin were examined under light microscopy by a blinded pathologist. AP was documented in control and experimental groups.

Statistical analysis

All of the statistical analyses were performed using the Statistical Package for the Social Sciences 15.0 (SPSS Inc, Chicago, Illinois). Data were expressed as mean ± SD. Statistical analysis was undertaken using the 1-way ANOVA test. Differences among the groups were analyzed by the Kruskal-Wallis test. Dual comparisons among groups with significant values were evaluated with the Mann-Whitney *U* test. A value of $P < 0.05$ was accepted as statistically significant.

Results

As shown in Table 1, when compared with the control group, the mean pancreatic tissue TOS level, OSI, and LOOH level were significantly increased in the AP group, being 30.97 ± 7.13 ($P < 0.05$), 1.76 ± 0.34 ($P < 0.0001$), and 19.18 ± 4.91 ($P < 0.01$), respectively. However, mean TAS and –SH group levels were significantly decreased in the AP group compared with the control group, being 1.765 ± 0.21 ($P < 0.0001$) and 0.21 ± 0.04 ($P < 0.0001$), respectively.

Table 1 Mean ± SD of biochemical parameters measured in study

	Control group	AP group	AP plus SA group
TAC, nmol Trolox equivalent/mg protein	2.27 ± 0.19[a]	1.765± 0.21[a,b]	2.18 ± 0.25
TOS, nmol H_2O_2 equivalent/mg protein	24.33 ± 5.45	30.97 ± 7.13[c,d]	21.06 ± 5.24
OSI, arbitrary unit	1.07 ± 0.27	1.76 ± 0.34[a,b]	0.97 ± 0.27
LOOH, μmol/g protein	13.96 ± 2.14[d]	19.18 ± 4.91[d,e]	13.41 ± 3.32
−SH, μmol/g protein	0.33 ± 0.03	0.21 ± 0.04[b]	0.22 ± 0.06[b]

[a] $P < 0.001$ versus AP group.
[b] $P < 0.0001$ versus control group.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] $P < 0.01$.

A pathologist who was blinded regarding the samples confirmed microscopy of the specimens of pancreatic gland as AP in all experimental groups with leading to interstitial edema, neutrophil infiltration, acinar necrosis, and focal necrotic areas (Figs. 1 and 2).

Discussion

AP is characterized by a local inflammation of the pancreas, which may lead to a systemic response. In the severe forms of the disease the mortality rate is high (20%) because of multiple organ failure. Several mechanisms seem to be involved in the development of the local and systemic responses in AP, namely, proinflammatory cytokines, chemokines, and ROS, as well as neuronal and vascular responses. The role of ROS in the pathogenesis of AP has been the subject of numerous studies.[14–16] It was postulated that generation of ROS may play an important role in the progression of AP.[16]

Much evidence involves ROS/RNS in many physiologic functions, such as vascular tone regulation, oxygen sensing, and host defense mechanisms. But it should be emphasized that the electrophilicity of the ROS/RNS make them highly vulnerable to reaction with biomolecules, including lipids, proteins, and DNA, which can change the functionality of these molecules. Therefore, the cellular concentration of ROS/RNS should be firmly controlled to prevent such harmful effects.[2] The balance between ROS/RNS-generating enzymes and scavenger enzyme systems can be upset in a number of pathologic conditions, such as systemic lupus erythematosus, diabetes mellitus, otitis media, rheumatoid arthritis, Behçet disease,[17–21] endothelial dysfunction, atherosclerosis, hypertension,[22] degenerative diseases, inflammatory bowel disease, and pancreatitis.[2,23]

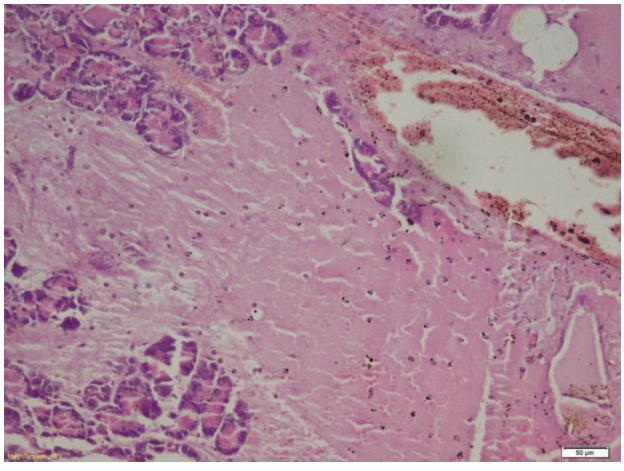

Fig. 1 Hematoxylin-eosin (×200) stained section showing the histopathologic appearance of pancreatitis with interlobular edema, leukocyte infiltration in the stroma, congestion, and acinar cell necrosis.

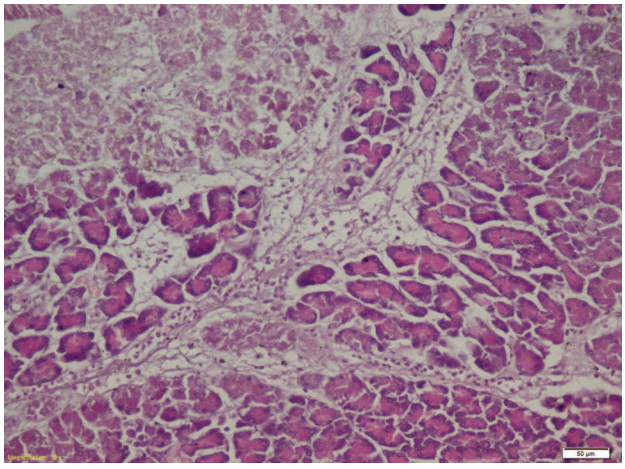

Fig. 2 Hematoxylin-eosin (×200) stained section showing mild leukocyte infiltration; acinar necrosis is seen around the significantly dilated interlobular areas of pancreas tissue.

Unchecked ROS/RNS generation leads to oxidative damage and activation of reactive signaling cascades, and thus ROS scavenging therapy has been proposed as a potential treatment for disorders related to excessive ROS/RNS generation.[2]

In the study we found that although pancreatic tissue TOS levels, OSI, and LOOH levels were significantly increased in the AP group compared with the control group, TAS and –SH group levels were significantly decreased. This is consistent with the hypothesis that AP may generate oxidative stress. Also, the TOS levels, OSI, and LOOH levels in this tissue were lower in AP rats that were administered SA. The results of the current study support the research hypothesis that the systemic administration of SA would reduce the oxidative damage in pancreatic tissue in L-arginine–induced acute toxicity of pancreas.

Following AP, lipids are one of the main targets for free radical damage. The instability of ROS and RNS predisposes them to react with essential cellular components. Polyunsaturated fatty acids, which are abundant in the plasma membrane and also in the mitochondrial membrane, are among the most vulnerable targets of the ROS/RNS.[2] They will induce lipid peroxidation by removing one hydrogen atom from polyunsaturated fatty acids and forming hydroperoxides. Therefore, perturbations in cellular fluidity and membrane integrity lead to disintegration of cells and necrotic cell death. As a result, subcellular structures released into the extracellular media will induce several inflammatory events and further worsen the ongoing damage.[1] The LOOH level—which is a well-known marker of oxidative stress formed from unsaturated phospholipids, glycolipids, and cholesterol through peroxidative reactions under oxidative stress—in the SA-treated AP group was found to be significantly lower compared with the L-arginine–induced AP group. These results suggest a protective effect against free radical–induced damage through inhibition of lipid peroxidation.

Under normal conditions, a delicate balance exists between the oxidant and antioxidant statuses of living organisms.[24] When evaluating the oxidative status, measuring just one of the oxidant or antioxidant parameters usually does not give proper information about the oxidative status of the organism. Hence, we measured both TOS and TAS in our study. In the study we found a significant reduction in TAS and –SH levels, and an increase in TOS levels, OSI, and LOOH levels in the pancreatic tissue of AP rats compared with the control group. The TOS levels, as another indicator of oxidative stress alteration, tended to increase after AP and to decrease after administration of SA. OSI is the ratio of TOS to TAS, and it has been suggested that this may reflect the state of oxidative status more accurately than when TAS and TOS are considered separately.[25] In the study, the OSI was significantly higher in the AP group compared with the control group. Supplementation with SA reduced the OSIs of AP rats to those of the control group, similar to the TOS values. The findings of the current study are in line with those of previous studies that have reported the antioxidant effects of SA. In addition, the finding that SA provides protective effects against AP-induced oxidative stress is consistent with previous reports on the protective effects of SA.[14,16,23]

Cells are equipped with several antioxidant defense systems, including total –SH groups, to detoxify the endogenous and exogenous oxidative challenges during aerobic metabolism or when they encounter stress-inducing agents.[26,27] Separate evaluation of the levels under imbalanced conditions or evaluation of these levels for detection of the protective effects of several substances on living organisms may not lead to a definitive conclusion when assessing oxidative balance or imbalance. However, these evaluations may provide an explanation for how oxidative stress–induced tissue damage or preventive effects occur.

It is now apparent that the future approach to treating AP-associated complications can consider either the use of SA or combinations of herbal plants having multipharmacologic activities. However, further studies are needed to understand more clearly the mechanism involved in modulating oxidative balance through the administration of SA.

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