EFFECTS OF EXTRACORPOREAL SHOCK-WAVE LITHOTRIPSY DIRECTED AT THE PAROTID GLAND ON OXIDATIVE STRESS PARAMETERS AND SOME TRACE ELEMENT LEVELS IN FACIAL NERVE OF RATS

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ABSTRACT: *Introduction:* This study was designed to assess the effect of extracorporeal shock-wave lithotripsy (ESWL) exposure of the parotid gland on oxidative stress and some trace element levels in the facial nerves of rats. *Methods:* Twelve male Wistar albino rats were divided into two groups, each consisting of 6 animals. The rats in the first group served as controls. The left parotid glands of animals in the second group were treated with 1000 18-kV shock waves while anesthetized with ketamine. The animals in both groups were euthanized 72 h after the ESWL treatment, and the right facial nerve was harvested for determination of oxidant/antioxidant status and trace element levels. *Results:* Lipid peroxidation product malondialdehyde (MDA) and antioxidant glutathione (GSH) levels increased, and the activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), decreased in the facial nerves of ESWL-treated rats. The levels of iron, lead, manganese, and cobalt increased, and magnesium, cadmium, and copper levels decreased. *Conclusions:* ESWL treatment of the parotid gland may increase lipid peroxidation and decrease antioxidant enzyme activity in adjacent tissues such as the facial nerve. It also causes a decrease or increase in many mineral levels of the facial nerve, which is an undesirable condition for normal physiological function.

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Sialolithiasis is the most common disease of the salivary glands. Calculi in the parotid glands have been noted to occur in 1.2% of the population.[1,2] The incidence of medical hospital admissions for symptomatic sialolithiasis in the white English population was recently reported to be 31.5 per million per year.[3] Among whites, 80% of salivary gland calculi are located in the submandibular glands, 10% in the sublingual glands, and 10% in the parotid glands.[1] A diagnosis of sialolithiasis is the result of a careful consideration of both the patient's history of typical symptoms and findings on clinical examination. Sonography is the first choice for imaging in this disorder.[4] No pharmacological treatment is available for dissolving salivary gland stones, and dilation of the efferent and salivary ducts is usually successful only in cases of small, distally located calculi. As a result, surgical extirpation of the salivary gland, or sialoadenectomy, was the treatment of choice for sialolithiasis until a few years ago.[5] The major disadvantage of surgery is the risk of damaging the facial nerve.

The mechanism of ESWL-induced cellular damage is still controversial. One of the mechanisms discussed for tissue damage is free radical formation during ESWL treatment.[6,7] In addition to mechanical fragmentation of the calculus, each shock wave that is generated by 18,000–24,000 V of electrical energy may result in some biochemical events, and homolytic cleavage of molecules may take place that leads to formation of free radicals.[6,8] Because of their reactivity, ROS can damage other molecules and cell structures. Injuries to adjacent organs have been reported in <1% of patients.[9] These injuries include pulmonary contusion,[10] pancreatitis,[11] bile duct injury,[12] bowel perforation,[13] aortic aneurysm rupture,[14] retroperitoneal hemorrhage,[15] cardiac arrhythmias, and gastric erosions.[16] However, the cellular and biochemical mechanisms underlying this phenomenon remain unclear.[17] Massive hemoptysis and death from pulmonary damage due to vascular and alveolar rupture has been reported in laboratory animals after a single exposure of the thoracic region to ESWLs.[18] This potential of ESWLs to cause pulmonary damage poses a real problem during lithotripsy in children because of the close proximity of the kidneys to the lungs.[6]

Normal cell functions and integrity of cell structures may be altered via the considerable reactivity of reactive oxygen species (ROS). The organism has enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and non-enzymatic factors, such as glutathione (GSH), vitamin C, and vitamin E

Abbreviations: CA, carbonic anhydrase; CAT, catalase; ESWL, extracorporeal shock-wave lithotripsy; GSH-Px, glutathione peroxidase; GSSG, glutathione disulfide; G6PD, glucose-6-phosphate dehydrogenase; LPO, lipid peroxidation; MDA, malondialdehyde; NADPH, nicotinamide adenine dinucleotide phosphate; NBT, nitroblue tetrazolium; SOD, superoxide dismutase; ROS, reactive oxygen species; TBA, thiobarbituric acid

Key words: ESWL, facial nerve, oxidative stress, rat, trace element levels

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antioxidant mechanisms, that work as scavengers for the harmful ROS. Radical-scavenging antioxidants are consumed by the increased free radical activity. Oxidative stress can be defined as an increase in oxidants and/or a decrease in antioxidant capacity. Excess amounts of ROS generated in inflamed tissues can cause injury to host cells and also induce DNA damage and mutations.[16] Oxidative DNA damage has been suggested to play a major role in the development of a broad range of neuropathological conditions, such as ischemia, edema, infarction, seizure disorders, aging, Alzheimer disease, and Parkinson disease.[17] In several studies, an increase in oxidative components, decreases in antioxidants, or both, have been reported in subjects with various acute or chronic diseases.[18] Although there have been some new developments that link free radicals to a variety of central nervous system diseases, it is extremely difficult to establish whether they actually cause the disorders or represent byproducts of tissue destruction caused by other primary factors.[19–21]

At least nine trace elements, iron, zinc, copper, manganese, iodine, chromium, selenium, molybdenum, and cobalt, are necessary for optimal metabolic function. These elements serve a variety of functions, including catalytic, structural, and regulatory activities in which they interact with macromolecules such as enzymes, pro-hormones, pre-secretory granules, and biological membranes. These micronutrients are involved, therefore, in all major metabolic pathways at levels that are so fundamental that the features of deficiency of many of them are protean and non-specific. The net achievement of the metabolic pathways for each element is delivery to its functional site(s) by exploiting its physicochemical characteristics to avoid interactions with other inorganic nutrients.[22]

In addition to its use in the treatment for stones in the upper urinary tract, in some clinics, there are electrohydraulic ESWL machines which are still in use and may be utilized for treatment of salivary stones. With the assumption that ESWL directed to the parotid gland also affects adjacent organs in rats, we designed our study to investigate the effect of short-term ESWL exposure of the parotid gland on oxidative stress and some trace element levels in the facial nerve.

METHODS

Animals. Twelve male Wistar albino rats, approximately 6 months of age, with an average body weight of 250–300 g, were obtained from the animal laboratory of Yüzüncü Yıl University. The animals were housed in individual cages. A 12/12-h light/dark cycle was maintained, and the rats were fed *ad libitum*. The study was approved by the local ethics committee of Yüzüncü Yıl University. All animals received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health.

Experimental Design. Rats were divided randomly into two groups, each consisting of 6 rats. Group 1 did not receive treatment and served as the control group. The left parotid gland of animals in group 2 was treated with 1000 18-kV shock waves (Multimed 2001 lithotripter; Elmed Co., located at the Department of Urology, Faculty of Medicine, Yüzüncü Yıl University, Van, Turkey) while they were anesthetized with 50 mg/kg ketamine. The animals in both groups were euthanized 72 h after the ESWL treatment, and the right facial nerve was harvested for determination of oxidant/antioxidant status and trace element levels.

Biochemical Analysis. ***Measurement of Malondialdehyde Level.*** A tissue specimen of 50 mg was homogenized in solution (0.15 mol/L KCl). After the homogenate was centrifuged at 1600 $\times$ *g*, the malondialehyde (MDA) levels in tissue homogenate supernatant were determined by the thiobarbituric acid (TBA) reaction, according to Yagi et al.[23] The principle of the method is based on measuring absorbance of the pink color produced by the interaction of TBA with MDA at 530 nm. Values were expressed as milligrams per deciliter.

SOD and GSH-Px Assays. The tissues were homogenized in physiological saline (1 g in 5 ml) using a homogenizer (Type 853202; B. Braun Melsungen AG, Germany) and then centrifuged at 4000 $\times$ *g* for 20 min (Labofuge 200; Heraeus, Germany). GSH-Px activity was measured by following changes in nicotinamide adenine dinucleotide phosphate (NADPH) absorbance at 340 nm and by measuring the decrease of H_2O_2 absorbance at 240 nm.[24] SOD activity was measured by the method based on the nitroblue tetrazolium (NBT) reduction rate. One unit of SOD activity was expressed as the enzyme protein amount that caused a 50% inhibition in the NBT reduction rate.[24]

Measurement of CAT Level. Biochemical analysis of erythrocyte CAT activity was performed with the method described by Aebi.[24] Briefly, the supernatant (0.1 ml) was added to a quartz cuvette containing 2.95 ml of 19 mmol/L H_2O_2 solution prepared in potassium phosphate buffer (0.05 mol/L, pH 7.00). The change in absorbance was monitored at 240 nm for 5 min using a spectrophotometer (UV-1201; Shimadzu, Japan).

Measurement of GSH Level. GSH levels in tissues were measured at 412 nm using the method described by Sedlak and Lindsay.[25] The samples were precipitated with 50% trichloroacetic acid (TCA) and then centrifuged at 1000 × *g* force for 5 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of Tris–ethylene-diamine tetraacetic acid buffer (0.2 M, pH 8.9), and 0.1 ml of 0.01 M 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). The solution was kept at room temperature for 5 min and then read at 412 nm on the spectrophotometer.

Measurements of Mineral Levels. Exactly 2.0 ml of the mixture of HNO_3/H_2O_2 (2/1) was added to 0.7 g of the tissue samples. The mixture was placed into a water bath at 70°C for 30 min and stirred occasionally. Then 1.0 ml of the same acid mixture was added, and the mixture was transferred into a Teflon vessel bomb for the microwave oven. The bomb was closed, and the solution was placed inside the microwave oven. Radiation was applied for 3 min at 450 W. After addition of 0.5 ml of the same acid mixture, radiation was repeated for 3 min. After cooling for 5 min, 2.0 ml of 0.1 mol/L HNO_3 was added, and the solution was transferred into a Pyrex tube. After centrifugation, the clear solution was used for the determination of copper, cobalt, magnesium, manganese, lead, cadmium, and iron.[6] The levels were determined by atomic absorption spectrophotometer measurements with a spectrophotometry device (UNICAM-929; Unicam, Ltd., Cambridge, UK).

Statistical Analysis. Results were expressed as mean ± standard deviation (SD). The Kruskal–Wallis test was used for comparison of groups. When significant differences were observed ($P < 0.05$), the Tukey multiple comparison test was used to determine differences between groups. Statistical analyses were carried out using SPSS software, version 13.0 for Windows (SPSS, Inc., Chicago, Illinois).

RESULTS

Lipid peroxidation product MDA and antioxidant GSH levels increased, and the activities of antioxidant enzymes, such as SOD, CAT, and GSH-Px, decreased in the facial nerves of ESWL-treated rats (Table 1). The levels of iron, lead, manganese, and cobalt increased, and magnesium, cadmium, and copper levels decreased (Table 2).

Table 1. MDA and GSH levels, and the activities of SOD, GSH-Px, and CAT enzymes in facial nerves of control and ESWL-treated rats ($n = 6$).

	Groups	
	Control	ESWL treatment
SOD (U/mg)	756.47 ± 32.44	585.53 ± 28.02*
MDA (mg/dl)	90.08 ± 9.53	195.13 ± 1.93*
GSH-Px (mIU/mg)	0.89 ± 0.03	0.58 ± 0.05*
GSH (mIU/mg)	58.79 ± 2.75	91.53 ± 1.77*
CAT (EU/g Hb)	125.17 ± 6.07	73.37 ± 3.37*

Results are expressed as mean ± standard deviation.
**Significant difference (P < 0.05) between treatment and control groups.*

Table 2. Some mineral and heavy metal concentrations in facial nerves of control and ESWL-treated rats ($n = 6$).

	Control	ESWL of facial nerves
Co (μg/dl)	0.17 ± 0.01	0.45 ± 0.03*
Pb (μg/dl)	0.60 ± 0.03	0.76 ± 0.04*
Cd (μg/dl)	0.075 ± 0.01	0.31 ± 0.06*
Mg (μg/dl)	45.55 ± 13.0	19.04 ± 0.84*
Mn (μg/dl)	0.01 ± 0.00	0.05 ± 0.00*
Fe (μg/dl)	3.01 ± 0.46	4.7 ± 0.3*
Cu (μg/dl)	0.94 ± 0.03	0.30 ± 0.01*

Results are expressed as mean ± standard deviation.
**Significant difference (P < 0.001) between treatment and control groups*

DISCUSSION

This study was designed to assess the effect of short-term ESWL directed at the parotid gland on oxidative stress and some trace element levels in the facial nerve of rats. Calculi are unusual sequelae of inflammation of the salivary gland. They develop as a result of debris mineralization, but their exact etiology remains unknown. Burnstein et al. performed X-ray diffraction and chemical analyses of six parotid gland stones and found that the three major crystalline components were apatite, magnesium-substituted whitlockite, and octacalcium phosphate.[26] Until a few years ago, the standard treatment for sialolithiasis was conservative, consisting of antibiotics, sialogogues, and gland massage to wash out the calculi. Dilation of the duct was performed occasionally in cases of stenosis.[1] If symptoms persisted, subtotal parotidectomy, even with its associated risks, especially facial nerve injury, was the treatment of choice. Since the introduction of piezoelectric ESWL for the treatment of sialolithiasis, this method of therapy has become the primary treatment modality in some clinics.[27] ESWL has shown such reliability that it has even been used to treat pediatric patients for sialolithiasis.[1,31]

Generation of ROS is a part of normal life, and their interaction with host antioxidant defense systems appears to exert a significant influence on cellular chemistry in health and disease. We found that the lipid peroxidation product MDA increases in the facial nerve after the ESWL treatment. Because excess production of ROS is very harmful to tissues, ROS are expected to induce tissue damage in the facial nerve. Our results show that neighboring tissues, such as the facial nerve, might also be affected by ESWL treatment of the parotid gland. It has also been shown that normal facial nerve cell functions and the integrity of cell structures may be disrupted via the considerable reactivity of ROS.[27] Proteins, membrane lipids, and nucleic acids are targets of free radical–mediated injury. Free radicals may act by peroxidation of membrane lipids, inactivation of enzymes by oxidation of sulfhydryl groups, depolymerization of polysaccharides, and disruption of nucleic acids.[19,21]

A variety of antioxidant defense systems exist to prevent the formation of these highly reactive and toxic oxygen species. These include SOD, GSH-Px, CAT, and other free radical scavengers, such as GSH. In our study, GSH levels increased, and the activities of antioxidant enzymes, such as SOD, CAT, and GSH-Px, decreased in facial nerves of ESWL-treated rats. The increased formation of ROS and decreased antioxidant defense is defined as oxidative stress, which is widely recognized as an important feature of many diseases. The decrease in antioxidant enzyme activities might be due to their use against free radical destruction and/or their inhibition by free radical species.[29] SOD and CAT are cellular antioxidants, which protect cells from oxidative stress. SOD catalyzes the dismutation of the superoxide anion radical to hydrogen peroxide, and CAT catalyzes the decomposition of hydrogen peroxide to water. GSH-Px, on the other hand, catalyzes the reduction of hydrogen peroxide by GSH. GSH is readily oxidized to glutathione disulfide (GSSG) by the GSH-Px reaction.[30] GSSG can be reduced by the NADPH- dependent reaction catalyzed by glutathione reductase. A decrease in SOD, CAT, and GSH-Px enzyme activity probably results in accumulation of superoxide anion radical and hydrogen peroxide, which react with metal ions to promote additional radical generation, with the release of the particularly reactive hydroxyl radical. Hydroxyl radicals react with lipids, DNA, and proteins, causing a loss of cell integrity, enzyme function, and genomic stability.[31] Because glucose-6-phosphate dehydrogenase (G6PD) catalyzes the first step of the pentose phosphate pathway, which provides the NADPH necessary for conversion of oxidized glutathione (GSSG) to GSH, the increased tissue GSH level was probably due to the increased G6PD activity that caused the increased production of the GSH.

Human life depends on the existence of appropriate amounts of various trace elements. Some of the diseases that may be affected by trace element deficiency include multiple sclerosis, various cancers, arthritis, goiter, Down syndrome, and mental retardation.[32] Deficiency in any of these trace elements leads to undesirable pathological conditions that can be prevented or reversed by adequate supplementation.[33] We found that levels of iron, lead, manganese, and cobalt increased, and magnesium, cadmium, and copper levels decreased in the facial nerves of ESWL-treated rats. Although iron is an essential nutritional mineral for all life forms, it is known that excess iron and iron deficiency also lead to oxidative DNA damage.[34] The accumulation of iron in the central nervous system has been linked to a variety of neurodegenerative disorders, including Alzheimer disease, Huntington disease, and Hallervorden–Spatz syndrome.[35] Copper deficiency affects various physiological functions that may be important in immunological defense to pathogenic challenge.[36] Copper is also a highly toxic metal that has been associated with several neurodegenerative disorders.[35] Iron increases oxidative stress, affects endothelial function, promotes inflammation, downregulates nitric oxide production, and induces renal dysfunction.[37] Manganese is essential for normal physiological functioning in humans and animals.[38] It also plays a role in the free radical scavenging activity of SOD. Cadmium is a ubiquitous toxic heavy metal and, unlike organic compounds, it is not biodegradable and has a very long biological half-life.[39] Magnesium is the fourth most abundant cation in the body and plays a pivotal role as an enzyme cofactor in biosynthesis of proteins and mineral administration. Its metabolism is connected with bone, and it is indispensable for osteogenesis and mineralization.[40] Subacute magnesium deficiency has been shown to cause lymphopoietic neoplasms in young rats.[41]

We conclude that short-term ESWL treatment of the parotid gland may increase lipid peroxidation and decrease antioxidant enzyme activity in adjacent tissues such as the facial nerve. It also causes a decrease or increase in many mineral levels in the facial nerve, which is an undesirable condition for normal physiological function. However, more studies are needed to verify and clarify the roles of oxidative stress, antioxidant enzyme activities, and trace element levels in the pathogenesis of ESWL.

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