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Nitric Oxide Levels in Erythrocyte of The Rabbits Which Implanted Ormocer Filling Materials into Connective Tissue

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The present aim of the study is to assess the alterations in nitric oxide ($NO^{\bullet}$) in erythrocytes of rabbits. $NO^{\bullet}$ levels were measured in all groups. Erythrocyte $NO^{\bullet}$ levels were of no significance in all comparisons. Ormocer filling materials do not cause an increase in reactive nitrogen species levels in erythrocytes of rabbits.

Key Words: Nitric oxide levels, Erythrocyte, Rabbits.

INTRODUCTION

Recently there have been considerable developments regarding the esthetic restorative material. They were used for the front teeth in the past. They have been the posterior teeth. Especially amalgam is replaced by the composites. Amalgam is not esthetic and has bad effects due to the presence of quick silver. This increases the use of composites and lead to the development of the new kinds of composites. For example, compomers which have the characteristics of composites and glass ionomers in order to improve the esthetic characteristics of glass ionomers[1].

$NO^{\bullet}$ is an inorganic free radical gas produced from L-arginine by a family of isoenzymes called NO synthases. Two of them are constitutively expressed and third one is inducible by immunological stimuli. It is the $NO^{\bullet}$ released by the constitutive enzymes that acts as an important signaling molecule in the cardiovascular and nervous systems and $NO^{\bullet}$ released by the inducible NO synthase (iNOS) is generated for long periods, by cells of the immune system among others and has been shown to be cytostatic/cytotoxic for tumor cells and a variety of microorganisms. $NO^{\bullet}$ is known, together with other reactive oxygen species (ROS), to induce cytotoxicity and cytostasis. Several studies on $NO^{\bullet}$ and hydrogen peroxide-induced oxidative damage have cited similarities between the two chemicals in their enzymatic generation, chemical interaction with macromolecules and resulting cytotoxicity[2-5].

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It is well known that $NO^{\bullet}$ possesses either antioxidant or pro-oxidant properties. It has been found that the concentrations of $NO^{\bullet}$, under non-pathological conditions, are in nanomolar levels and under conditions of oxidant injury in micromolar levels. Endogenous $NO^{\bullet}$ is of a double-edged role in specialized tissues and cells, which is an essential physiological signaling molecule mediating various cell functions, but also induces cytotoxic and mutagenic effects when present in excess. $NO^{\bullet}$ reacts rapidly with superoxide anion to form peroxynitrite, which may be cytotoxic by itself or easily decompose to the highly reactive and toxic hydroxyl radical and nitrogen dioxide ($NO^{\bullet}_2$)[2,3].

Reactive nitrogen species (RNS) have been implicated in the pathogenesis of a large number of diseases such as diabetes mellitus, cancer, rheumatoid arthritis, systemic lupus erythematosus, Behçet's disease, infectious diseases, sinusitis and atherosclerosis and in aging[6-9].

To our best of knowledge, there are no available data on the studies of $NO^{\bullet}$ levels in rabbits with ormocer filling materials. Therefore, in the present study, aim is to investigate possible effects of ormocer filling materials on erythrocytes $NO^{\bullet}$ levels in rabbits, which implanted in connective tissue.

EXPERIMENTAL

Seven female rabbits were used for this study. The animals were kept in cages under standardized conditions, fed with standard hard diet pellets and housed in controlled air conditioned and humidity, environment. The animals were acclimatized for at least 7 d before experiment.

Animals were anaesthetized with 20 mg/kg ketamin hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) under sterile conditions and 2 cm longitudinal skin incision was made on the dorsal skin. The surgical site was shaved along the dorsal skin surface and then scrubbed with chlorhexidine before and after. Then test materials holding freshly prepared materials were inserted subcutaneously into rabbits in the implant test.

Preparation of direct restorative materials: Ormocers (Admira-Voco) were kept refrigerated until required for sample preparation. Cylindrical samples (2 mm in diameter × 4 mm length) were prepared in open-ended Teflon moulds which were cleaned with ethanol to minimize bacterial contamination. Each restorative material formed into blocks 1 mm thick and 8 mm long, polymerized according to the manufacturer's directions. The resin was then cured with a light-curing unit (Hilux 320, Benlioglu, Turkey) by exposure of both ends of the mould to light for 40 s. Then test materials holding freshly prepared materials were inserted subcutaneously into rabbits in the implant test.

Biochemical measurements: One mL of blood was taken from both orsal auricular veins of the rabbits with a 24 G angiocath before implantation and at 1 and 7 d after implantation. Erythrocyte sediments were prepared for the analyses. Erythrocytes were then hemolyzed by diluting with deionized water (50-fold) and

the analyses were carried out in this hemolysed supernatant fraction. Hemoglobin values of the samples were measured by a GEN-S counter hematology analyzer. Hemolysed samples were kept at -80 ºC until biochemical determinations.

Since the half-life of NO is very short, NO levels in the erythrocytes were determined by measuring levels of nitrite plus nitrate. NO values obtained by this procedure represent the sum of nitrite and nitrate. First, nitrate transforms nitrite by nitrate reductase. Then, total nitrate + nitrite concentrations were measured as NO according to the Griess method using Griess reagent as previously described[10]. Biochemical measurements were carried out at room temperature using a spectrophotometer (CECIL CE 3041, Cambridge, UK).

Statistical analysis: Results are given as mean ± SD. All parameters were analyzed by one-way variance analysis test. LSD (Least significant difference) multiple range test was used to compare the mean values. (Acceptable significance was recorded when p values were < 0.05). Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 11.5).

RESULTS AND DISCUSSION

All parameters are shown in Table-1. As seen from the Table, erythrocyte $NO^{\bullet}$ levels were of no significance in all comparisons ($p > 0.05$).

TABLE-1
MEAN ± SD OF ERYTHROCYTE NITRIC OXIDE LEVELS IN ALL GROUPS

	The baseline values	The first day values	The seven day values
NO (µmol/g Hb)	63.3 ± 6.1	58.5 ± 2.9	59.5 ± 6.7

The baseline values = before treatment (control group), The first day values = after 1st treatment day, The seven day values = after 7th treatment day.

Occupational exposure to dental monomers has been associated with contact dermatitis, asthma, drowsiness, headache, anorexia and decrease in gastric motor activity and with induction of localized and generalized motor and sensory neuropathy[11-13].

Oxidants such as superoxide radical ($O^{\bullet-}_2$), hydroxyl radical ($OH^{\bullet-}$) are produced in metabolic and physiological processes and harmful oxidative reactions may occur in organisms. Oxidative stress is an imbalance between the production of free radicals that contain unpaired electrons and antioxidant defences buffering the oxidative damages. Oxidative effects of free radicals are controlled by exogenous antioxidants such as vitamins C and E and also by endogenous antioxidants. Under some conditions, increases in oxidants and decreases in antioxidants cannot be prevented and oxidative/antioxidative balance shifts towards the oxidative stress[14]. When the oxygen free radicals are not removed by natural scavengers, damage occurs through peroxidation of structurally important PUFA within the phospholipids structure of the membranes. Lipid peroxidation decreases both the fluidity and the barrier function of

membranes, resulting in disturbances in structural organization, enzymic inhibition and possible death of the cell. In addition, lipid peroxides are able to inhibit protein synthesis, block macrophage function and alter chemotactic activity[15-17]. In previous studies, it was found that erythrocyte malondialdehyde levels were of no significance in all groups. This may indicate a decrease in oxygen free radical levels[16-18].

In general, $NO^{\bullet}$, a free radical produced by iNOS, appears to regulate several steps of the inflammatory process. As a potent vasodilator, $NO^{\bullet}$ modulates the early vascular responses of the acute inflammatory reaction. In addition, $NO^{\bullet}$ is one of the cytostatic-cytotoxic defence mechanisms against a pathogen, in the non-specific immune response. Free radical production by the interaction of $NO^{\bullet}$ with $O^{\bullet-}_2$ has both protective (microbial killing, neutralizing $O^{\bullet-}_2$) and toxic effects by the formation of the peroxynitrite ($ONOO^-$), which is now generally considered a more toxic species than either $NO^{\bullet}$ or $O^{\bullet-}_2$ alone and $OH^{\bullet-}$. Furthermore, $NO^{\bullet}$ synthesized by activated inflammatory cells regulates the functions of other cells involved in the inflammatory process and appears to act as a secondary mediator of some actions of proinflammatory cytokines, such as interleukin-1[15].

It is found that erythrocyte $NO^{\bullet}$ levels were of no significance in both the 1st day values and the 7th day values compared to the baseline values (control group). The ormocer filling materials decrease NOS activities that results in decreased $NO^{\bullet}$ synthesis. So, oxidative stress decreases in these conditions.

In conclusion, this is the first study that investigates possible effects of ormocer filling materials on erythrocyte $NO^{\bullet}$ levels in rabbits, which implanted into connective tissue. Present results showed that ormocer filling material did not induce oxidative stress, on the contrary, it prevented formation of NO. However, further experimental and clinical studies on the possible effect of ormocer filling material on oxidant/antioxidant system are needed.

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