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Nitric Oxide Synthase Activity on Erythrocyte of The Rabbits Which Implanted Composite Resin Filling Materials into Connective Tissue

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There have been considerable developments regarding the esthetic restorative material. Especially amalgam is replaced by the composites. Amalgam is not esthetic and has bad effects due to the presence of silver. This increases the use of composites and lead to the development of the new kinds. The aims of present study were to assess the alterations in nitric oxide synthase activity in erythrocytes of rabbits. Nitric oxide synthase activities were measured in all groups. Erythrocyte nitric oxide synthase activities were significantly increased in both the first day values and the seven compared to the baseline values. Reactive nitrogen species may play an important role in the cells and tissue damage due to composite resin-generated oxidative stress.

Key Words: Lipid peroxidation, Composite resin, Oxidative stress, Nitric oxide, Nitric oxide synthase.

INTRODUCTION

Recently there have been considerable developments regarding the esthetic restorative material. They were used for the front teeth in the past as well as they have been posterior teeth. Especially amalgam is replaced by the composites. Amalgam is not esthetic and has bad effects due to the presence of silver. This increases the use of composites and lead to the development of the new kinds. 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 a third 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

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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 H_2O_2-induced oxidative damage have cited similarities between the two chemicals in their enzymatic generation, chemical interaction with macromolecules and resulting cytotoxicity[2-4].

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 nonpathological 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 ($NO^{\bullet}_2$) (see the equations below)[2,3].

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^-$$
$$ONOO^- + H^+ \rightarrow ONOOH$$
$$ONOOH \rightarrow OH^{\bullet -} + NO_2^-$$

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[5-8].

To our knowledge, there are no available data studies on nitric oxide synthase (NOS) activities in rabbits with composite resin filling materials. Therefore, in the present study, we aimed to investigate possible effects of composite resin filling materials on erythrocytes nitric oxide synthase activities in rabbits, which implanted that into connective tissue.

EXPERIMENTAL

Seven female rabbits (5 kg and 3.5 months) 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 anaesthetised with 20 mg/kg ketamin hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) under sterile conditions. A 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: Composites were kept refrigerated until required for sample preparation. Cylindirical samples (mm in diameter × 4 mm length) were prepared in open-ended Teflon molds which were cleaned with ethanol to minimize bacterial contemination. Each restorative material formed into blocks 1mm thick and 8 mm length, polymerized according to the manufacturer's directions. The resin was then cured with a light-curing unit (Hilux 320, Benlioglu,Tr.) by exposure of both ends of the mold to light for 40 s. Then test materials holding freshly prepared materials were inserted subcutaneously into rabbits in the implant test.

Biochemical measurements: 1 mL of blood was taken from both dorsal 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 hemolyzed supernatant fraction. Hemoglobin (Hb) values of the samples were measured by a GEN-S counter hematology analyzer. Hemolyzed samples were kept at -80 ºC until biochemical determinations.

Nitric oxide synthase activity was determined by the diazotization of sulfanilic acid by $NO^{\bullet}$ in acidic pH medium and subsequent coupling to N-(1-napthyl) ethylene diamine. To 0.1 mL of sample, 0.2 mL of 0.2 M arginine was added and the mixture was incubated at 37 ºC for 1h, after which 0.2 mL of 10 mM HCl, 100 mM sulfanilic acid and 60 mM N-(1-napthyl) ethylene diamine was added. Absorbance at 540 nm was measured[9] after 0.5 h.

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-1, Nitric oxide synthase (NOS) activities were measured in all groups. Erythrocyte NOS activities were significantly increased in both the first day values ($p < 0.05$) and the seven ($p < 0.005$) and compared to the baseline values.

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[10,11].

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

	The baseline values	The first day values	The seven day values
NOS (IU/g Hb)	207.9 ± 11.8	245.3 ± 21.0[a]	254.3 ± 28.1[b]

[a]$p < 0.05$, [b]$p < 0.005$ *vs.* the baseline values; The baseline values: before treatment (control group); The first day values: after 1st treatment day; The seven day values: after 7th treatment day.

Since composite resin was introduced by Bowen[12] into the field of dentistry, it has become one of the most widely accepted materials in restorative dentistry. Although its physical properties are excellent, it is clinically well known that composite resin occasionally elicits unfavourable effects on the human dental pulp[11,13].

Oxygen free radicals (OFR) are capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, protein and free amino acids, lipids and lipoproteins, carbohydrates and connective tissue macromolecules. These species may have impaired on such cell activities as membrane function, metabolism and gene expression. Propagation of this chain reaction results in a repeated chain reaction[14]. When the balance between OFRs production and the antioxidative defense mechanisms is impaired, OFRs levels may increased. When the OFRs 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[14,15]. In our previous studies, we found that erythrocyte malondialdehyde level, an important indicator of oxidant stres, was significantly higher in both the first day values and the seven values than those of the baseline values (control group). These may indicate increased OFRs. OFRs are shown to damage the microcirculatory endothelia of all organs[14-16].

In general, $NO^{\bullet}$, a free radical produced by inducible nitric oxide synthase, 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 $O_2^{\bullet-}$ has both protective (microbial killing, neutralizing $O_2^{\bullet-}$) and toxic effects by the formation of the peroxynitrite ($ONOO^-$), which is now generally considered a more toxic species than either $NO^{\bullet}$ or $O_2^{\bullet-}$ alone and hydroxyl radical ($^{\bullet}OH$). 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[13]. We found that erythrocyte NOS activities were significantly higher in both the first day values and the seven values than those of the baseline values (control group). The erythrocytes cannot synthesize NOS. The blood flow to inflamed regions increases. Possibly, NOS released by injured cells may be absorbed by erythrocytes. The composite resin filling materials increase NOS activities that results in increased $NO^{\bullet}$ synthesis, which combines with $O_2^{\bullet-}$ to form $ONO^{\bullet-}$. So, these conditions increase oxidative stress.

In conclusion, this is the first study that investigates possible effects of composite resin filling materials on erythrocyte NOS activities in rabbits, which implanted that into connective tissue. The present results confirm the presence of oxidative stress, possibly because of the excess production of the reactive oxygen species and reactive nitrogen species in both the first day values and the seven values in rabbits. However, further experimental and clinical studies on the subject are needed.

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