Cigarette Smoke Effects on Salivary Antioxidants and Oral Cancer – Novel Concepts

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Oral squamous cell carcinoma is the most common malignancy of the head and neck, with a worldwide incidence of over 300,000 new cases annually [1]. The disease is characterized by a high rate of morbidity and mortality (about 50%) [1–4]. The major inducer of oral SCC is exposure to tobacco, considered to be responsible for 50–90% of cases worldwide [5–7]. The incidence of oral SCC in cigarette smokers is four to seven times higher than in non-smokers; when alcohol is also consumed this incidence is even higher. Moreover, compared with non-smokers, the higher cigarette smoke-related risk for oral SCC is manifested by a reduction in the mean age of development of the disease by 15 years [8,9].

The "field cancerization" concept is the currently accepted explanation for the carcinogenic effect of cigarette smoke on oral mucosa [10]. According to this theory, there is a constant and direct attack of various cigarette smoke reagents on the oral epithelial cells, which gradually accumulate and cause a step-wise malignant transformation. It has been suggested that free radicals, reactive oxygen species and reactive nitrogen species in the inhaled cigarette smoke induce this gradually evolving process, initially expressed by dysplastic lesions of the mucosa, are then transformed into *in situ* carcinoma lesions and eventually result in full-blown infiltrating and metastasizing oral SCC. Further credence for the suggested role of free radicals in the pathogenesis of evolving oral SCC is found in a recent study [11] demonstrating that ROS, such as hydroxyl radical, are formed in the human oral cavity during areca quid chewing, and that the activity might cause oxidative DNA damage to the surrounding tissues. In this respect the salivary anticarcinogenic capacity, which has only recently been recognized, may be based on its antioxidant system.

The protective role of saliva on oral cancer

The anticarcinogenic capability of saliva was demonstrated in a study published in 1997, in which saliva was shown to significantly inhibit the initiation and progression of oral cancer in an animal model [12]. Further support for this salivary capacity was given by Nishioka et al. [13], who, using the AMES test (mutagenicity test where the genotoxicity of a number of structurally diverse DNA-interactive telomerase inhibitors is examined), found that saliva inhibited the mutagenicity of well-known oral cancer inducers such as cigarette smoke, 4-nitroquinoline 1-oxide and benzopyrene. Moreover, it was recently reported that patients with oral lichen planus (a premalignant lesion) have lower salivary antioxidant capability [14]. Previous studies [15] demonstrated that saliva inhibits the production of radical oxygen species, the superoxide free radical (O_2^-) and hydrogen peroxide (H_2O_2) from betel quid tobacco, a most potent inducer of oral cancer. The significant inhibition of all enzymatic activities following a single cigarette (probably due to the interactions between smoke aldehydes and -SH groups of the enzyme molecules) was recently demonstrated [16]. Moreover, the percentage of enzymatic inhibition showed a negative correlation with the basal level of salivary-reduced glutathinone. The results emphasize that not only is one cigarette sufficient to impair the salivary enzymatic activities, but it also strengthens the proposed protective role of GSH against the noxious biochemical effects of cigarette smoke.

Salivary antioxidant system

Saliva is the first biological medium met by external materials taken into the body as part of food, drink, or inhaled volatile ingredients. During evolution, various defense mechanisms developed in the saliva aimed at combating penetrating bacteria, viruses or fungi, and protecting against chemical or mechanical attack. Moreover, even after swallowing, saliva has a mucosal protective capacity within the gastrointestinal tract [17,18]. An extensive amount of research has been devoted to the immunologic defense mechanism of saliva, primarily based on secretory immunoglobulin A and the protein-enzymatic defense system. That, in turn, is based on the enzyme lysozyme and other components, such as histatin, lactoferrin, proline-rich protein, mucin, etc. The soft tissue integrity

Oral cancer is induced mainly by cigarette smoke and occurs in the oral epithelium

SCC = squamous cell carcinoma
ROS = reactive oxygen species

GSH = glutathinone

defense system, in which the epidermal growth factor plays a pivotal role, has also been evaluated thoroughly [17].

Recently, the importance of an additional salivary defense system has become clear: the antioxidant defense system, which appears to lose efficiency with advanced age [19]. Similar to other biological systems, the salivary antioxidant system includes various molecules and enzymes, the most important of which are the uric acid molecule and the peroxidase enzyme, both of them water-soluble. The lipid-soluble antioxidants carried by lipoproteins, whose concentration in saliva is very low, contribute no more than 10% of the total salivary antioxidant capacity [20–22]. Uric acid, the most important antioxidant molecule in saliva [21,23,24], contributes approximately 70% of the total salivary antioxidant capacity [21], with the antioxidant role of the ascorbic acid molecule being secondary [21,24].

The correlation between concentrations of uric acid in both saliva and plasma points to the latter as the origin of salivary uric acid [23]. In the enzymatic salivary antioxidant system, peroxidase is by far the most important enzyme. However, superoxide dismutase, a zinc-attached enzyme that catalyzes the reaction $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$, exists in a few isoenzymes in saliva and has a secondary antioxidant role [25]. Other detectable salivary enzymes, such as catalase, glutathione peroxidase and glutathione reductase, have only marginal antioxidant significance [25]. Two peroxidase enzymes are found in saliva: salivary peroxidase, which in structure and antigenic characteristics resembles the lactoperoxidase found in bovine milk [18,26,27], and myeloperoxidase, produced by leukocytes in inflammatory regions of the oral cavity [28,29]. Despite the importance of peroxidase in saliva, it accounts for only 0.01% of the total salivary protein and exists in equilibrium between a monomeric state and multimolecular aggregates [18,26,30]. Salivary peroxidase has a dual role: a) it controls the level of hydrogen peroxide secreted by bacteria and leukocytes present in the oral cavity, and b) has a specific antibacterial activity, inhibiting the metabolism and proliferation of various bacteria in the oral cavity.

Hydrogen peroxide, toxic to both oral and gastrointestinal mucosa, oxidizes the thiocyanate ion (SCN^-), a detoxification product of cyanide secreted by saliva (mainly parotid saliva). Similar to glutathione in other biological systems [28,31], thiocyanate is the electron-donating component and, in the reaction $SCN^- + H_2O_2 + H^+ \rightarrow HOSCN + H_2O$, is catalyzed by peroxidase. Two potent antibacterial oxidizing products evolve out of this observation: hypothiocyanous acid (HOSCN) and its conjugated hypothiocyanite anion ($OSCN^-$). The antibacterial activity of these ions stems from their inhibiting capability of sulfhydryl groups containing enzymes of the bacteria that are vital for glycolysis [8,9,28]. In short, the accumulated antibacterial activity of the combination of peroxidase, hydrogen peroxide, and thiocyanate is much more potent than that of hydrogen peroxide alone [32]. Moreover, oxidizing the hydrogen peroxide catalyzed by the peroxidase combined with the $OSCN^-$ ion results in the production of a very potent antibacterial singlet-oxygen ROS [33].

The existence of much higher concentrations of various salivary molecular and enzymatic antioxidant parameters (superoxide dismutase, peroxidase, uric acid, and total antioxidant status) was recently demonstrated [34] in parotid saliva compared with submandibular/sublingual saliva. Another important finding was the distinction between the salivary antioxidant system and the immunologic and enzymatic protective systems, as represented by the salivary concentrations of secretory IgA and lysozyme, respectively. These findings suggest that the profound antioxidant capacity of saliva secreted from parotid glands is associated with the different physiologic demands related to eating (parotid predominance), to oral integrity maintenance (submandibular/sublingual predominance), or to the high content of deleterious redox-active transitional metal ions present in parotid saliva [35]. This may also signify that the oral cavity environment is only partially protected against oxidative stress during most of the day and night.

Moreover, a previous study published in 1997 demonstrated that rat saliva has a profound capacity for reducing redox activity rendered by transition metal ions, correlating well with its protein content [35].

Salivary peroxidase – the pivotal antioxidant enzyme inhibited by cigarette smoke

The peroxidase found in the oral cavity is the most important salivary antioxidant enzyme. As previously mentioned, this oral peroxidase is composed of two peroxidase enzymes, salivary peroxidase and myeloperoxidase. The term OPO is used here to denote the total activity of both isoforms, since the 2-nitrobenzoic acid-thiocyanate (NSB-SCN) assay used in these studies measured the activity of both enzymes. The SPO secreted from the major salivary glands, mainly the parotid gland [34], contributes 80% of OPO activity, while myeloperoxidase, produced by leukocytes in inflammatory regions of the oral cavity [28], contributes the remaining 20% of OPO activity. In structural and antigenic characteristics, SPO resembles the lactoperoxidase found in bovine milk [18,27]. OPO plays a dual role: a) it reduces the level of hydrogen peroxide (H_2O_2) excreted into the oral cavity from the salivary glands by bacteria and leukocytes, and b) it increases specific antibacterial activity by inhibiting the metabolism and proliferation of various bacteria in the oral cavity.

The oral and pharyngeal epithelium is constantly exposed to saliva, which contains advanced antioxidant/anticancer systems

In the reaction of the thiocyanate ion (SCN^-) + $H_2O_2 \rightarrow OSCN^- + H_2O$, which is catalyzed by the peroxidase, H_2O_2 oxidizes SCN^-, a detoxification product of cyanide secreted mainly from the parotid

IgA = immunoglobulin A
OPO = oral peroxidase
SPO = salivary peroxidase

gland. In this reaction, SCN^- acts as the electron-donating component, similar to GSH in other biological systems [28]. Two potent antibacterial oxidizing products evolve from this reaction: hypothiocyanous acid (HOSCN) and its conjugated anion, hypothiocyanite ($OSCN^-$). The cytotoxic properties of these salivary oxidants depend on the extracellular pH, and HOSCN can oxidize oxyhemoglobin into methemoglobin in erythrocytes, while both HOSCN and OSCN can oxidize intracellular reduced GSH [9]. The cytotoxic antibacterial activity of HOSCN and OSCN stems from their ability to react with sulfhydryl groups of bacterial enzymes that are vital for glycolysis, such as hexokinase, aldolase, and pyruvate kinase [9,28]. Under various oral stimuli, the peroxidase may act as a scavenger of H_2O_2 to produce molecular oxygen, but without producing OSCN plus HOSCN [36]. In any case, the accumulated antibacterial activity of the combination of peroxidase, H_2O_2, and SCN is much more potent than that of H_2O_2 alone [32].

This previously described circumstantial evidence, which emphasizes the possible significance of the effect of cigarette smoke on salivary antioxidants in the pathogenesis of oral cancer, initiated the performance of a relevant study in light of two facts demonstrated recently. The first is removal of H_2O_2 from the oral cavity, which is carried out by the OPO, may have special significance, as redox-active metal ions are uniquely and constantly secreted into the oral cavity via the parotid saliva [2,35]; such metals, in the presence of H_2O_2, may play a deleterious role in the oral cavity by enhancing the production of the ultimately aggressive hydroxyl free radicals, which may be injurious to the neighboring oral epithelial cells. The second finding was that cigarette smoke may attack antioxidant enzymes rather than molecules. This was recently shown to severely reduce the activity of various salivary antioxidant enzymes, but not antioxidant molecules, such as uric acid [21,37,38]. In fact, the study [39] demonstrated that after smoking a single cigarette, there was a sharp drop of OPO activity in both groups: 42.5% in smokers and 58.5% in non-smokers ($P < .01$). After 30 minutes, the level of activity returned to 90–100% of the pre-smoking level, presumably due to the secretion of new saliva into the oral cavity. The difference between the two groups was also observed after exposure of saliva to one cigarette in smoking flasks (*in vitro* studies); however, as expected, no recovery of activity was observed in either group. Similarly, the OPO activity loss was accompanied by increased carbonylation of the salivary proteins, an indicator of the oxidative damage to proteins. These results may be of great clinical importance, as heavy smokers smoke 20 cigarettes or more on a daily basis. Accordingly, most of the time the oral epithelium of heavy smokers is essentially unprotected by OPO against the deleterious effects of thiocyanate ions and hydroxyl free radicals produced by unremoved hydrogen peroxide in the presence of the salivary redox-active metal ions.

Destruction of these salivary systems by the smoke may play a pivotal role in the pathogenesis of the disease

In a very recent paper, the mechanism responsible for the inactivation of OPO by cigarette smoke was described [40]. In order to understand and elucidate the factor(s) in cigarette smoke that are responsible for cigarette smoke-associated inactivation of OPO, several oxidants and antioxidants were applied to saliva in the presence or absence of cigarette smoke. No protection for cigarette smoke-induced loss of OPO activity occurred in the presence of GSH, N-acetyl cystein, ascorbic acid or desferal. Exposure of saliva to purified aldehydes present in cigarette smoke had no effect on OPO activity. In addition, nicotine as well as ascorbic acid in the presence of FeCl3 also had no effect on OPO activity. Finally, the exposure of OPO in saliva to cyanate in levels present in cigarette smoke caused a marked 65–70% loss of OPO activity, which could have been prevented by pre-incubation of the saliva with hydroxocobalamine, a known chelator of cyanate. These results indicate that hydrogen cyanide, known to be present in significant amounts in cigarette smoke, is most probably the agent responsible for the cigarette smoke-associated loss of salivary OPO activity [40].

The findings described here may pave the way to unraveling the cigarette smoke-induced and saliva-mediated initiation and progression of oral cancer and, accordingly, to developing new means for prevention and/or treatment for this vicious and lethal cancer.

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Capsule

Multi-drug-resistant TB

A worrying characteristic of tuberculosis (TB) nowadays is its ability to generate "hot zones" of multi-drug-resistant strains of *Mycobacterium tuberculosis*. Blower and Chou have developed an amplifier model by which they can track the emergence and evolution of strains into hot zones that have serially accumulated resistance to several drugs, and they have verified their model with World Health Organization data. Paradoxically, it seems that high intensity control measures, which have most successfully reduced the incidence of sensitive strains, may have promoted the emergence of hot zones, even when the resistant strains are less fit and less transmissible. This is because if strains of bacteria escape cure, the victims of such resistant TB stay infectious for longer and the bacteria may thus go on to accumulate more modes of resistance. Nevertheless, there is time to act: The model also indicates that even after 30 years of poor drug control, only low levels of multi-drug resistance emerge. Regionally customized control programs could thus be developed to deal with local varieties and combinations of drug-resistant strains.

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