EFFECT OF CIGARETTE SMOKE ON SALIVARY PROTEIN TYROSINE NITRATION

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Nitration of tyrosine and tyrosine-containing proteins and their roles in pathophysiology have just recently been reviewed. Despite low yields of tyrosine modifications, nitration of tyrosine residues may inactivate important proteins. Nitrotyrosine can be formed by various nitrating agents, including peroxynitrite. Thus, the occurrence of nitrotyrosine-containing proteins in vivo should be regarded as a general indication of tissue damage induced by reactive nitrogen species such as peroxynitrite. This strongly suggests that peroxynitrite could be formed in vivo under certain pathophysiological conditions. Our aim in this study was to elucidate the effect of cigarette smoke (CS) on nitrotyrosine formation in saliva proteins. We exposed saliva to CS, in vitro, and used Western Blotting (WB) and monoclonal anti-nitrotyrosine antibody to assess the level of saliva protein nitration. As saliva contains extensive amounts of nitrites, it was no surprise that at basal levels, saliva proteins, albumin and alpha-amylase, were already nitrated. The WB also revealed that with continuous exposure to CS the tyrosine nitration of both albumin and alpha-amylase is declining significantly after 3 h. A quite similar effect was seen after exposure to aldehydes but to a less extent as compared to CS. Exposure of Nitrotyrosine-modified BSA (BSA-N) to aldehydes, produced a similar effect, meaning a decrease in tyrosine nitration. These findings might be explained by the possible ability of CS aldehydes to reduce protein-bound nitro group to an amine. Another proposed mechanism is that CS unsaturated aldehydes react with proteins mainly through Michael addition reaction, leading to the generation of stable aldehyde-protein adducts (APA). Thus, it may react with nitro groups of saliva proteins, like albumin or alpha-amylase, to generate APA, which ultimately, may not be recognized by our antibody. Another possible mechanism, is interaction between the aldehydic group with the hydroxyl group of the 3-nitrotyrosine, forming a hemiacetal, which is not recognized by the antibody. This mechanism might explain the difference in the "denitration" effects caused by the saturated aldehyde acetaldehyde, which exists in large amounts in CS, and unsaturated aldehydes. Therefore, it is possible that the main player in the CS smoke 'denitration' effect on salivary proteins, is the aldehydic group and not the double bond of unsaturated aldehydes.