Plasminogen activation system in oral cancer: Relevance in prognosis and therapy (Review)

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Received March 12, 2015; Accepted May 4, 2015

DOI: 10.3892/ijo.2015.3021

Abstract. Research on carcinogenesis and progress in cancer treatment have reduced mortality of cancer patients. Mortality rates decreased by 1.5% per year from 2001 through 2010 for most types of cancer in men and women. However, oral cancer is still a significant global health problem since incidence and mortality rates are increasing. Oral cavity cancer is ranked the 8th in men and the 14th in women based on data collected between 2006 and 2010 by the National Institute of Health. Furthermore, an increasing incidence of head and neck neoplasms, particularly the tongue cancer among young adults has been reported recently. It is most likely due to increasing human papillomavirus (HPV) infection or the early start of tobacco and alcohol consumption. Treatment of oral cancer patients is mainly surgical and often leads to esthetic and functional deformities, with severe impact on the quality of life. Thus, novel form of treatments and selection of patients with high and low risk of mortality is of high priority for clinical studies. The expression of proteolytic enzymes in tumor and stromal tissues has been shown to have prognostic significance in many human cancers. Mechanisms of action of the individual components have been extensively studied in tumor cells in vitro and in animal models. In several experimental tumor models in animals some degrees of tumor control have been achieved. Specifically, inhibitors of urokinase (uPA) and matrix metalloproteinases (MMP)

Contents

1. Introduction
2. Plasminogen activation system
3. Plasminogen activation system in oral cancers
4. Plasminogen activation system as potential therapeutic target in oral cancer
5. Summary

Introduction

Dynamic research on carcinogenesis and cancer treatment has finally started to reduce mortality of cancer patients. Death rates decreased by 1.5% per year from 2001 through 2010 for most cancers in men and women for all major racial and ethnic groups (1). Oral cavity cancer is the 8th most common cancer in men and the 14th in women based on data collected between 2006 and 2010 by the National Institute of Health (NCI) (1). However, oral cancer is still a significant global health problem since incidence and mortality rates are increasing requiring constant search for novel approaches for treatment. Furthermore, an increasing incidence of head and neck neoplasms, particularly tongue cancer among young adults has been reported recently. It is most likely due to increasing human papillomavirus (HPV) infection or the early start of tobacco and alcohol consumption (2-4).

Meaningful drug designs for therapeutic intervention of oral cancers necessitate an exhaustive understanding of the role of all the factors involved in complicated mechanism of tumor formation and progression. The expression of proteolytic enzymes in tumor and stromal tissues has been shown to have prognostic significance in many human cancers. Mechanisms of action of the individual components have been extensively studied in tumor cells in vitro and in animal models. In several experimental tumor models in animals some degrees of tumor control have been achieved. Specifically, inhibitors of urokinase (uPA) and matrix metalloproteinases (MMP)
reduce growth or size of cancers (5-7). We review literature on prognostic value of the expression of proteolytic enzymes and mechanism of action of their inhibitors.

2. Plasminogen activation system

The plasminogen activation system (PAS) contains the elements defined below. Plasminogen (PLA) is a proenzyme that is cleaved by urokinase plasminogen activator (uPA) or tissue plasminogen activator (tPA). Cleaved PLA becomes an active form called plasmin. Plasmin is a strong proteolytic enzyme able to digest proteins of connective tissue and basement membranes. It is also able to activate other latent proteolytic enzyme, such as procollagenase broadening the spectrum of proteins hydrolyzed. Plasmin plays a fundamental role in tissue remodeling, tumor invasion and development of distant metastasis and angiogenesis (8-10).

uPA and tPA activators. Both uPA and tPA are weak proteolytic enzymes with one known function i.e. activating plasminogen to plasmin. Urokinase is implicated in pericellular proteolysis during tissue remodeling, wound healing, angiogenesis, but tPA mediates intravascular thrombolysis (11-13).

Inhibitors of plasminogen activators. There are few known protein inhibitors of uPA/tPA: PAI-1, PAI-2 and protein nexin. Most relevant appears to be PAI-1 existing as active, non-active-latent and/or cleaved non-active forms. Unique to this serpin protein is the close association between its conformational and functional properties. PAI-1 is a highly specific inhibitor of tPA and uPA, however, it is not a stable protein but converts quickly from active into the latent form, with a half-life of $t_{1/2} = 2$ h. This transmission is associated with insertion of the reactive loop (P4-P10') into the central $\beta$-sheet of the PAI-1 molecule. In such a conformation, P1-P1' and other sites are not accessible for reaction with tPA or uPA (Fig. 1).

PAI-1 has two different functions: i) it is a direct inhibitor of tPA and uPA blocking activation of plasminogen; ii) it binds with the adhesive glycoprotein vitronectin. PAI-1/vitronectin complex acts in tissue remodeling and metastasis. This function is independent of its proteinase inhibitory properties (6,12-14).

uPA receptor. The uPA receptor (uPAR) is a glycoprotein that binds uPA to the cell surface while uPA maintains its potential to activate plasminogen to plasmin. High numbers of uPA receptors on the surface of cancer cells, if occupied by uPA, enhance the proteolytic activity in their proximity (6,15,16).

3. Plasminogen activation system in oral cancers

Urokinase in oral cancers. The ability of solid tumors to invade adjacent tissue and to metastasize depends on the uPA activated plasmin capable of digesting adjacent connective tissue and basement membranes (14). Overexpression of uPA in tumor tissues, as well as in serum, are well documented as prognostic markers of poor outcome in a wide range of malignancies (17). Yet, possible impact of uPA on the prognosis and outcome of an oral cancer was investigated only by a few laboratories.

Weng et al (18) investigated if polymorphisms of uPA, that can change expression of urokinase, could have impact on outcome of oral cancer. They investigated 253 patients with oral cancer and 344 healthy controls. Their findings revealed that it was no significant effect of uPA gene on the susceptibility to oral cancer. Duong et al (19) used 3-dimensional (3-D) cell invasion model for oral mucosa fibroblasts and oral cancer cell invasion into the basement membrane and connective tissue stroma. The oral mucosal fibroblasts and cell carcinoma were treated by uPA. They showed that the presence of uPA enhanced cell invasion. Yoshizawa et al (20) studied the relationship between the clinicopathological findings and expression of uPA in oral squamous cell carcinoma (OSCC). They examined 54 cases of OSCC using reverse immunochemical techniques. OSCC cases with high uPA level showed the worst survival rate (5-year overall survival rate, 29.4 vs. 77.8% for low level).

A high expression of extracellular matrix metalloprotease inducer (EMMPRIN) in cancer tissues has been linked with tumor invasion in cancers of oral cavity. In general the
EMMPRIN effect has been associated with induction of MMP activity, but it has also been demonstrated that EMMPRIN enhances uPA activity. Lescaille et al (21) investigated OSCC by treating cultured cells with EMMPRIN-enriched membrane vesicles. uPA expression was analyzed in cells and tumor tissue by qPCR, immunostaining, and the invasion ability was studied using Boyden chamber assay. They found that OSCC tumors overexpress EMMPRIN and uPA compared to dysplastic lesions. Squamous cell carcinoma (SCC-9) and cell line established from human dysplastic oral mucosa tissue (DOK), showed similar expression pattern. In SCC-9 and DOK cell lines EMMPRIN upregulated uPA and, as expected, MMP-2 and MMP-9. EMMPRIN also significantly increased cell invasion MMP and uPA dependently. It was concluded that the upregulation of uPA by EMMPRIN promotes oral tumor invasion.

Zhang et al (22) investigated the expression of seven biomarkers (among them proteolytic enzymes) of lymphatic spread in OSCC patient tissues. Tissue samples were obtained from 138 patients undergoing tumor resection. Using immunohistochemical staining techniques they found that all biomarker expressions were closely related with lymph node status and clinical stage. Urokinase in cancer tissues was significantly higher than in normal tissue. Also, expression of uPA was higher in metastatic tumor tissues than in non-metastatic tumours. Authors concluded that upregulation of MMP-1, MMP-2 and uPA might predict lymphatic dissemination for OSCC patients at relatively early stage.

uPA in oral cancer. Tissue plasminogen activator has been shown activated in various cancers including oral malignancy. However, it seems that it is not associated with the ability of cancer to metastasize or the prediction of outcome (23-26).

PAI-1 in oral cancer. Similarly to uPA, elevated levels of PAI-1 in tumor tissues are accepted as prognostic markers of poor outcome in a many human cancers (27-32). It was suggested that PAI-1 may be required for effective angiogenesis as well as tumor growth (26,33-35). PAI-1 may also increase tumor growth by way of the inhibition of apoptosis (36). It is paradoxical at first glance, since proteolysis via plasmin, activated by uPA, enhances tumor growth and invasion, while PAI-1 is known to inhibit urokinase. However, effects of PAI-1 on cancer depend on it concentration and thus are not related only to inhibition of proteolysis. PAI-1 binds with high affinity to the N-terminal of vitronectin (VN) to which uPAR also binds, but with lower affinity (37). It was postulated that PAI-1 can free the cells from the extracellular matrix (ECM) (36). At high concentrations, PAI-1 might displace VN from the uPAR-VN complex. Thus, the tumor cell can detach from VN and the ECM, enhancing cell migration and metastasis (38). When cancer cell culture was treated with PAI-1 it inhibited spontaneously inducing apoptosis, that was reversed by antibodies which neutralized active PAI-1 (36). Other mechanisms have been proposed, such as inactivation of caspase-3, upregulation of Bcl-2, c-jun/ERK, Bcl-XL and downregulating Bax and Bcl-X8 after internalization of uPA/uPAR/PAI-1 complex (39). PAI-1 can be proangiogenic or anti-angiogenic depending on its concentration (35,40,41). In experimental animal tumor models, physiological levels of PAI-1 are needed for vascularization of tumors, but in PAI-1 knockout (PAI-1-/-) mice angiogenesis was decreased. Increased levels of PAI-1 by adenoviral PAI-1 gene transfer to cancer cells increases tumor angiogenesis (42). Further increase of PAI-1 levels reduces angiogenesis as it was shown in chicken chorioallantoic membrane angiogenesis model and some animal models requiring supraphysiological levels of PAI-1. The high concentration of PAI-1 inhibits uPA on the angiogenic vessel preventing its progression into the tumor body (35,43-45).

Weng et al (18) investigated whether polymorphisms of PAI-1 associated with the risk of developing clinical stage III or IV cancer and lymph node metastasis. Large group of patients (253 patients with oral cancer and 344 healthy controls) were analyzed by polymerase chain reaction-restriction polymorphism. It was found that patients with oral cancer with one or more 5G allele of PAI-1 gene have a lower risk of developing clinical stage III or IV and lymph node metastasis in comparison to those with 4G/4G homozygotes. The 4G allele of 4G/5G insertion/deletion polymorphism in the promoter region 675 bp, upstream from the transcription start sequence of the PAI-1 gene, produces higher plasma PAI-1 levels in humans (46). Similar result was found by Vairaktaris et al (47). They suggest that the 4G allele results in higher PAI-1 expression and is a contributing factor in early stages of oral carcinogenesis. They reported also that increased PAI-1 stimulates development of early stages of OSCC via increase of cell detachment that favors metastasis. However, in advanced OSCC PAI-1 might delay tumor progression by inhibiting vascularization.

uPAR in oral cancer. Strong correlations between poor prognosis in numerous malignances and levels of uPA and uPAR have been reported (48-50). Nozaki et al (51) concluded that uPAR is needed for invasion and metastasis of highly malignant oral cancer cells (OSC-19). Moreover, treatments of these cells with antisense oligonucleotides that target uPAR dramatically reduced uPAR mRNA expression. Consequently, pretreatment with oligonucleotides inhibited progression of OSC-19 cells in animal experimental models. They suggested that uPAR is a promising therapeutic target in oral cancer.

Yoshizawa et al (20) investigated the binding of uPA to uPAR in relation to cancer invasion and metastasis in OSCC patients. Using immunohistochemical techniques to assess the expression of uPA, uPAR in 54 cases and in six cell lines derived from OSCC using reverse transcriptase-polymerase chain reaction (RT-PCR), they showed a positive correlation with potential of cancer invasion. Specifically, tumors showing elevated levels of uPA+/uPAR+/maspin metastasized more frequently and had the worst survival rate (5-year survival rate, 29%). Tumors with an expression of uPA+/uPAR+/maspin+, exhibited the most favorable 5-year survival rate of 78%. Similar results were observed in expression of uPA+/uPAR+ in cells with stronger invasive potential than in the cells lines derived from the lower grades. Also, lower expression of maspin was observed in the cell lines derived from high grades of OSCC. It seems that levels of uPA, uPAR and maspin may be useful prognostic markers in OSCC patients (20). Role of maspin in oral carcinoma is not understood. Maspin (mammary serine protease inhibitor) protein belongs to the serpin (serine protease inhibitor) superfamily (52,53).
The exact molecular function of maspin in carcinogenesis is currently unknown (54,55).

In a different study (56) expression of uPAR in the malignant OSCC in 34 the primary oral cancers were examined immunohistochemically. The urokinase receptor expression was detected in 29.4% cases. uPAR expression correlated with the mode of invasion. In highly invasive tumors both uPA and uPAR were positive. Moreover, they found that uPA/uPAR/PAI-2 cases almost always showed secondary invasion. In highly invasive tumors both uPAR expression correlated with the mode of invasion. They concluded that the PAS plays a major role in the invasion and metastasis of OSCC, and might be a powerful prognostic marker (56). This was the only literature found describing PAI-2 involvement in oral cancers.

Shi et al (57) examined the contribution of uPAR to protein expression in a human OSCC using tissue microarray. Overexpression of uPAR relative to vector control cells revealed a significant correlation between uPAR and pl30cas expression. They suggested that protein tyrosine kinase c-Src was responsible for the phosphorylation of pl30cas in response to uPAR high expression. Also, the Rho family GTPase Cdc42, but not Rac1, were activated, suggesting a pathway leading to actin reorganization, filopodial protrusion and enhanced motility in uPAR overexpressing OSCC. These results suggest additional mechanism, different than participation of uPAR in proteolysis, could modulate OSCC invasive activity.

4. Plasminogen activation system as potential therapeutic target in oral cancer

Inhibition of uPA in oral cancer. Despite existing highly specific uPA inhibitors such as inhibitor CJ-463 (benzylsulfonyl-D-Ser-Ser-4-aminobenzylamide) (58,59), benzamidine, p-benzamidine, amiloride (60-62), 6-substituted 2-naphthamidine inhibitors (63), EGCG (64,65), PAI-1 (35,66), there has been no attempt to therapeutically inhibit urokinase in oral cancers by binding such inhibitors to its active site, rather almost exclusively research was done on biologicals (plant derived products).

Quercetin is a flavonol found in many fruits, vegetables, leaves and grains (67,68). Quercetin has been shown to have a wide range of pharmacological properties, including antioxidant activities. Lai et al (69) found that quercetin significantly reduced expression of several enzymes (COX-2, MMP-2, -7, -9, -10, VEGF and others) including uPA in human oral cancer cell (SAS) culture after 12- and 24-h treatment.

Curcumin is the principal curcuminoid of turmeric, a widely used Indian spice. Turmeric contains two other curcuminoids: desmethoxycurcumin and bis-desmethoxycurcumin. Curcumin exists in several tautomeric forms, with enol form being more energetically stable (70). Published literature provides many examples describing use of curcumin in therapy including for malignancy (71,72). Zhen et al (73) investigated effects of curcumin on the activation of EGFR and its downstream signaling molecules in SCC-25 oral cell invasion. They showed that curcumin inhibited SCC-25 cell proliferation and inhibited SCC-25 cell invasion by downregulation of uPA and uPAR, MMP-2 and MMP-9 expression.

Green tea polyphenols are cancer chemopreventive and cancer treatment agents studied for many years. Epigallocatechin-3-gallate (EGCG), which is the most abundant polyphenol in green tea, has been proven to suppress many cancers in animal and epidemiological studies (74-76). Human oral cancer cell line OC2 cells were treated with EGCG resulted in a dose-dependent inhibition of the invasion and migration of OC2 cells. Ho et al (77) performed gelatin zymography and casein zymography to evaluate the impact of EGCG on MMP-2, -9 and uPA secretion by OC2 cells. EGCG decreased the expression of MMP-2, -9 and uPA concentration-dependently. Authors suggested that EGCG might inhibit the invasion and migration of human oral cancer cells because of the decreased productions of proteinases.

Balcalein is a flavonoid, originally isolated from the roots of Scutellaria baicalensis and Scutellaria lateriflora with anticancer activity (78-80). Apigenin, present in many plants, is a natural product belonging to the flavone class, structurally similar to balcalein and 4,5,7-trihydroxyflavavnone. These flavones were reported to possess anticancer activity as well (81). Yang et al (82) treated cultured OSCC cells with 5,6,7-trihydroxyflavanone (baicalein), 7-hydroxyflavanone (apigenin) and 4,5,7-trihydroxyflavavnone. They reported that flavone treatment of cultured cells resulted in decrease of uPA, and MMP-2 as measured by zymography and western blot analysis in a concentration-dependent manner. Furthermore, in choriocallantoic membrane assay flavones reduced blood vessel formation.

Inhibition of PAI-1 in oral cancer. PAI-1 is playing an important role in many pathological processes such as fibrosis, thromboembolic diseases, atherosclerosis and cancer. The inactivation of PAI-1 by small organic molecules or peptides has been observed in vitro and in vivo models. PAI-1 has been postulated as a potential therapeutic target for pathological conditions including cancer (6,83,84).

PAI-1 can be inhibited either by antibody or small molecule inhibitors. The monoclonal antibodies can inactivate PAI-1 most effectively by preventing formation of the Michaelis complex between substrate (uPA) and PAI-1. Some other mechanisms were also postulated such as: acceleration the transition from active to the latent form or by inducing turnover of the PAI-1 protease complex as a substrate (85-88).

Several small molecule inhibitors have been found to inhibit PAI-1, but the mechanisms seem to be different. For example AR-H029953XX (89) and PAI-039 (90) inhibitors bind to the hydrophobic cleft region around α-helices D and E and β-strand 1A (Fig. 2). This part of PAI-1 molecule acts as a flexible joint when β-sheet A opens and the reactive center loop of PAI-1 is inserted as β-strand 4A. In that way inhibition of PAI-1 occurs not by prevention of the interaction between PAI-1 and the substrate, but by inhibiting the formation of a stable covalent complex, that must be created to act on uPA or tPA. A different binding site was proposed for PAI-749 small molecule inhibitor that is blocking formation of the initial Michaelis complex between PAI-1 and tPA or uPA (91). Yang et al (82) treating cultured OSCC cells with 4,5,7-trihydroxyflavanone and 7-hydroxyflavanone found that these inhibitors downregulate expression of PAI-1. Surprisingly, treatment with 5,6,7-trihydroxyflavanone increased PAI-1 expression. These examples do not include
all known inhibitors but rather are intended to illustrate complexity of PAI-1 inactivation.

Our previous study revealed that a black tea extract (containing mostly theaflavins) as well as the isolated theaflavins (theaflavin-3'-gallate, theaflavin-3,3'-digallate), were potent inhibitors of PAI-1 while the other two i.e., theaflavin and theaflavin-3-gallate did not show inhibitory activity (92). From computer molecular modeling of complex formation, we found that the most likely place of binding would be in the proximity of Arg346-Met347 (P1, P1′) i.e. at the active site of PAI-1 (86,92).

We have found no literature on therapeutic use of PAI-1 inhibitors in oral cancer. However, zhang et al (93) summarized evidence on associations between black tea consumption and oral cancer by analyzing 57 articles based on 87 datasets containing ~50,000 cases. In general, high tea consumption reduced risk of oral cancer and dose response meta-analysis indicated that even an increase in tea consumption reduce risk of oral cancer. They also reported that there was no effect on gastric, rectal, colon, lung, pancreatic, liver, breast, prostate, ovarian or bladder cancers.

Lee et al (94) studied bioavailability of theaflavins after thoroughly rinsing brewed black tea (2 g of black tea leaves in 100 ml) in the mouth for 2-5 min. They found that high concentrations of theaflavins (C_{max} = 0.6-1.8 μM) were observed in saliva 1 h after washing. They concluded that these results indicated that using black tea could be a convenient, slow-release source of theaflavins in the prevention of oral cancer and dental caries.

Therefore, it is plausible that anticancer activity of theaflavins could be related to inhibition of PAI-1. However, many mechanisms of black tea action have been proposed for prevention of the cancer formation and growth. These include the modulation of signal transduction pathways limiting the inhibition of cell proliferation and transformation, initiation of apoptosis of cancer cells, and inhibition of tumor invasion and angiogenesis (95). Because of this multiplicity of action of black tea polyphenols, demonstration that anticancer activity of theaflavins is related to PAI-1 inactivation need to be better evaluated and verified in the future.

**Inhibition of uPAR in oral cancer.** High expression of urokinase plasminogen activator receptor (uPAR) is involved in progression and metastasis of oral cancer (51,96,97). One of the possibilities of inactivation of uPAR is silencing this gene by RNA interference (siRNA). Liang et al (98) introduced

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**Figure 2. Inhibition of uPA and PAI-1 by small molecular chemicals.** (A) Ribbon model of uPA in complex with amiloride [1f5k] (106). (B) Surface of uPA and stick model of amiloride in specificity pocket of uPA. All known inhibitors of uPA bind in this part of the urokinase. Inactivation of PAI-1 is more complex. It can bind to the hydrophobic cleft region around α-helices D and E as shown on C and D or in the proximity of active site of PAI-1 as shown in E and F (CDE-096, a carbamoyl derivative of a synthetic digalloyl polyphenol compound) [4g8r] (107). Inhibitor of PAI-1 can bind in the proximity of its active site [theaflavin] (86,92). Proteins are shown in rainbow colors, protein surface in gray, inhibitors are presented as stick model, carbons in green, oxygen in red, nitrogen in blue, fluorine in light blue, hydrogens are omitted for clarity.
siRNA to downregulate the expression of uPAR in the highly malignant OSCC cells and demonstrated that siRNA leads to the efficient inhibition of endogenous uPAR mRNA as well as protein expression. Consequently, silencing of uPAR resulted in an impressive reduction of cell proliferation, adhesion, migration and invasion in vitro. They conclude that RNAi-directed targeting of uPAR can be used in therapy of oral cancer, especially in preventing cancer cell invasion and metastasis. Similar approach was used by Nozaki et al (51). They found that uPAR is required for invasion and metastasis of highly malignant oral cancer cells (OSC-19). When cells were treated with antisense oligonucleotide targeting uPAR a dramatic reduction of uPAR mRNA was observed. Also, cells pretreated with antisense oligonucleotide or siRNA targeting uPAR, reduce progression of OSC-19 cells in vitro.

Inhibition of epidermal growth factor receptor (EGFR) is a molecular target in anticancer therapies. Curcumin is a non-specific inhibitor of EGFR and reduces growth, invasion and metastasis in some cancer cells. Zhen et al (73) investigated whether curcumin would influence proliferation and invasion in SCC-25 cell line. They also explored the effect of curcumin on the activation of EGFR and its downstream signaling molecules Akt, ERK1/2 and STAT3. Furthermore, they examined the inhibition effect of curcumin on EGFR-induced EGFR phosphorylation and SCC-25 cell invasion. Curcumin inhibited SCC-25 cells proliferation and induced G2/M phase arrest, and cell invasion, in a dose-dependent manner. They also observed downregulation of uPAR and other proteins such as: MMP-2, MMP-9 and uPA. The authors suggested that curcumin reduced SCC-25 uPAR expression most likely by inhibiting the phosphorylation of EGFR and EGFR downstream signaling molecules Akt, ERK1/2 and STAT3.

5. Summary

Further progress regarding the application of plasminogen activation system as a therapeutic target and prognostic marker in oral cancer will depend not only on understanding of the role of each member in carcinogenesis but also on interaction between them (99). For example, assessment of net proteolytic balance is needed instead of simple statement that some elements are overexpressed. Not only uPA/PAI-1 balance should be measured but also other proteolytic enzymes (MMP-2, -9) involved in invasion and metastasis should be quantified. Another very important factor is expression of PAI-1 in oral cancer, it can exist in three distinct forms. It is essential to measure each PAI-1 form since active, latent and cleaved variety have completely different effects on inhibition of urokinase driven activation of plasminogen, but they might play a role in cell signaling. Our understanding of cell invasion and metastases of oral cancer cells by mechanisms other than proteolysis is far from complete and should be investigated further.

In addition to age, gender and ethnicity could be the differentiating factors at least in some cases of cancer formation and progression (100). For example in the Supplementation in Vitamins and Mineral Antioxidants Study (SU.VI.MAX) 12,741 French adults (7,713 women and 5,028 men) were given ascorbic acid (120 mg), vitamin E (30 mg), β-carotene (6 mg), selenium (100 µg) and zinc (20 mg), or placebo daily for a follow-up time of 7.5 years. Supplementation reduced total cancer rate and total cancer death in men but in women an increase of skin cancer including melanoma was observed (101). It was reported also that functional polymorphisms of EGFR is a prognostic markers in colon cancer but has opposite
prognostic consequences in males and females (102). It has been shown also that sex hormone receptors are expressed in some cancers and play role in gene expression involved in carcinogenesis (103). Thus, it is important to attract the attention toward the impending role of sex hormones in oral carcinogenesis in future research. Furthermore, cancer risk for women taking female hormone therapy should be investigated.

Use of biologicals to alter gene expression or to inhibit enzymes in humans is relatively easy to convince ethics committees, especially if these are constituents of food. However, one should keep in mind that their bioavailability could be biased since they could be degraded/transformed and thus not detected or difficult to follow, as can be seen in case of curcumin. Curcumin application could be beneficial albeit it is not stable under different conditions. For example: it is degraded in aqueous solution where, ~90% decomposed within 30 min (0.1 mol/l phosphate buffer, pH 7.2 at 37˚C) and we cannot define to the bioactive compounds such as ferulic acid, feruloyl-methane and vanillin (Fig. 3) (104) and we cannot define.

It is degraded in aqueous solution where, ~90% decomposed within 30 min (0.1 mol/l phosphate buffer, pH 7.2 at 37˚C) and we cannot define which of all of them, or combination of some, are responsible for the benefits. Use of specific and stable compound could be more beneficial in treatment of oral cancer and prediction of its outcome.

Acknowledgements

The present review was supported in part by grants from the Poznan Medical University, the Frank Stranahan Endowed Chair and the Children Miracle Network.

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