Tryptophan and its metabolites in patients with oral squamous cell carcinoma: preliminary study

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Abstract

Purpose: It has been showed that tryptophan (TRP) degradation has been linked to modulation of cancer cell proliferation. The aim of our study was to estimate the concentration of TRP and its derivatives, such as anthranilic (AA) and kynurenic acid (KYNA) in plasma, saliva, squamous cell carcinoma (SCC) tissues and healthy oral mucosa in patients with oral SCC.

Material and methods: The study was performed on plasma, non-stimulated, mixed saliva and squamous cell carcinoma tissues and healthy oral mucosa in patients with oral SCC. The concentration of TRP and its metabolites were determined by high-performance liquid chromatography (HPLC).

Results: In plasma the concentration of TRP was 33.73±2.52 μM, of KYNA was 26.97±5.35 nM and of AA was 32.40±2.30 nM. In saliva the concentration of TRP was 3.81±0.62 μM, of KYNA was 8.06±1.86 nM and of AA was 20.41±10.77 nM. In cancer tissues the levels of TRP (30.21±5.88 μM), KYNA (15.85±1.82 nM) and AA (265.32±151.45 nM) were higher in respect to the concentration of TRP (13.28±0.62 μM), KYNA (12.75±2.28 nM) and AA (31.68±8.89 nM) in normal tissues. The increase in the content of TRP, KYNA and AA in cancer tissues reached 127.48±5.95%, 24.31±4.35% and 737.50±206.96%, respectively.

Conclusions: Our study has demonstrated the change of TPR metabolism, which is reflected by the increase TRP, AA and KYNA concentrations in patients with oral squamous cell carcinoma. We can suppose that these substances may be one of many factors responsible for cancer development.

Key words: oral cancer, tryptophan metabolites.

Introduction

Cancers of the oral cavity represent approximately 2.5% of all malignant neoplasms in Poland. Squamous cell carcinoma (SCC), which arises from the oral mucosal lining, accounts for over 90 percent of these tumors [1,2]. The most common site for oral carcinoma is the tongue, which accounts for around 40 percent of all cases of caries of the oral cavity proper. These tumors most frequently occur on the posterior lateral border and ventral surface of the tongue. The floor of the mouth is the second most common oral location. Less-common sites include the gingiva, buccal mucosa, labial mucosa and hard palate [3].

Despite of advances in surgery, radiotherapy, and chemotherapy, the five-year survival rate among patients with oral cancer has not improved significantly over the past several decades and it remains at about 50 to 55 percent [4]. So there is a need for more data which not only will help to improve new therapeutic oncologic modifications but also will be useful for finding potential substances to easier diagnose, treat and monitor of oral cancer.

L-tryptophan (TRP), essential amino acid, is metabolized in 95% via kynurenine pathway [5]. The first of TRP metabolite is N-formylkynurenine which is further catabolized to kynurenine (KYN) by constitutive intracellular formylase. KYN is transformed to a number of metabolites such as anthranilic (AA) and kynurenic acid (KYNA), which are biological active substances. AA plays an important role in the regulation of immunological processes [6,7] as well shows antibacterial activity [8]. However, KYNA has been identified as an essential neurotransmitters' agonist [9,10].

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Table 1. **Baseline characteristics of patients**

	Patient 1	Patient 2	Patient 3	Patient 4
Age (yrs)	49	52	81	45
Gender	F	M	M	M
Diagnosis	Carcinoma planoepitheliale keratodesa G_2	Carcinoma planoepitheliale keratodesa G_2	Carcinoma planoepitheliale keratodesa G_2	Carcinoma planoepitheliale keratodesa G_2
Localization	Carcinoma fundi cavi oris sinistri	Carcinoma buccae dextri	Carcinoma buccae dextri	Carcinoma linguae dextri
Staging	$T_3N_{2A}M_0$	$T_4N_{2B}M_0$	$T_4N_1M_0$	$T_3N_1M_0$
WBC [$10^3/\mu l$]	6.0	6.42	6.6	6.6
RBC [$10^6/\mu l$]	4.63	3.90	4.44	4.54
HGB [g/dl]	14.6	12.8	12.6	14.6
HCT [%]	43.3	36.5	37.8	42.6
PLT [$10^3/\mu l$]	196	242	339	324
ISE Na^+ [mmol/l]	130	140	142	140
ISE K^+ [mmol/l]	3.76	4.50	3.83	4.50

The literature has showed that AA can induce liver cancer in mouse offspring, which mother had administered AA transplacentally [11]. Moreover, the increased concentration of AA was found in urea of patients with bladder cancer. This observation and later studies have proved that AA is potential carcinogen [12].

Because of data have showed that TRP degradation has been linked to a modulation of proliferation of cancer cells we decided to study it level in plasma, saliva and tissues (normal and cancer) patients with oral carcinoma, which are of the most frequent. Nevertheless, there are many studies in this subject, the pathogenesis of this disorder is still unknown.

In our previous study we have found TRP metabolites in human saliva. At the same time we have not observed any changes in indoleamino 2.3-dioxygenase activity, enzyme responsible for TRP metabolism, in saliva of these patients [13].

Thus, the aim of our study was to estimate certain TRP derivatives in plasma, saliva and salivary glands (normal and cancer) in patients with oral carcinoma.

Material and methods

Specimen collection and patient details

Baseline characteristics of the patients who were included in the study shows *Tab. 1*. These patients met the following criteria: absence of other diseases in which production of saliva is impaired (including: diabetes, Sjögren syndrome) and there was no administration of any pharmaceuticals which could affect saliva production. None of the patients had received blood transfusion for at least 3 months or any drugs which could affect the function of the immune system. The TNM classification according to UICC convention was used to evaluate clinical tumor stage. All patients were informed about the aim of the study. Written consent was obtained from each subject and Local Ethical Committee approved the experimental protocol.

A cancer tissue specimens were obtained from patients who were classified for surgical treatment and underwent surgery at the Department of Maxillofacial Surgery Medical University of Białystok, Poland. Normal epithelium (control tissue) was received from the margin of these resections of these carcinomas.

Samples were homogenized in ice-cold with 2 M TCA and centrifuged at 12000 g for 15 minutes at 4°C. The supernatant fluid was passed through a WATERS 0.45 μM filters. The concentrations of TRP, KYNA and AA were determined by high-performance liquid chromatography (HPLC) [14].

Blood sampling

Venous blood was drawn in the morning between 7 and 8 am and put into a tube containing 3.8% sodium citrate (citrate/ /blood =1:9). Hematological (red and white cells count, hematocrit, hemoglobin, plates) and biochemical (the concentrations of sodium and potassium) parameters were assayed by standard laboratory methods.

In order to estimate tryptophan, kynurenic and anthranilic acid concentration, the plasma was deproteinized with 2 M $HClO_4$ and centrifuged at 12000 g for 15 minutes at 4°C. The supernatant fluid was passed through a WATERS 0.45 μM filters. Samples were stored at -80°C until assayed. TRP and its metabolites were determined by high-performance liquid chromatography (HPLC) [14].

Saliva sampling

Samples of non-stimulated mixed saliva were taken from patients each morning between 7 and 8 am, 10 min after mouth washing MilliQ water using the spitting method. The saliva samples were immediately treated 2 M $HClO_4$ and after 15 min of incubation with acid at 4°C, samples were centrifuged 30 min 12000 g. The supernatant was collected in -80°C for measurement of concentrations of TRP and its products degradation by high-performance liquid chromatography (HPLC) [14].

Statistical analysis

The values are expressed as the mean ±SEM or as a real values.

***Table 2*. The real values of TRP and its metabolites as well the ratio of KYNA/TRP and AA/TRP in patients with oral squamous cell carcinoma**

		Patient 1	Patient 2	Patient 3	Patient 4
TRP	plasma [nM]	27.91	31.10	38.10	37.79
	saliva [nM]	5.03	4.68	2.43	3.09
	control tissue [pmol/g tissues]	14.98	13.30	11.66	13.48
	carcinoma tissue [pmol/g tissues]	43.83	18.67	36.03	22.29
KYNA	plasma [nM]	36.38	22.32	35.14	14.05
	saliva [nM]	9.92	10.32	6.20	12.67
	control tissue [pmol/g tissues]	17.91	6.89	12.40	13.78
	carcinoma tissue [pmol/g tissues]	15.16	17.91	11.02	19.29
KYNA/TRP ratio	control tissue	1.20	0.52	1.06	1.02
	carcinoma tissue	0.35	0.96	0.31	0.87
AA	plasma [nM]	12.67	9.50	11.09	9.50
	saliva [nM]	12.45	40.92	15.84	4.48
	control tissue [pmol/g tissues]	52.80	10.56	36.96	26.40
	carcinoma tissue [pmol/g tissues]	253.44	36.96	696.96	73.92
AA/TRP ratio	control tissue	3.52	0.79	3.17	1.96
	carcinoma	5.78	1.98	19.34	3.32

***Figure 1*. The increase of TRP and its metabolites in cancer tissues**

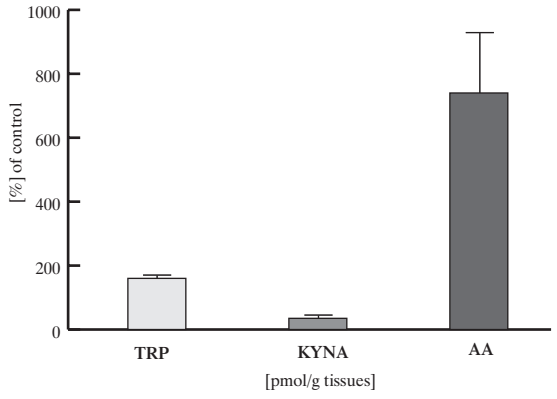
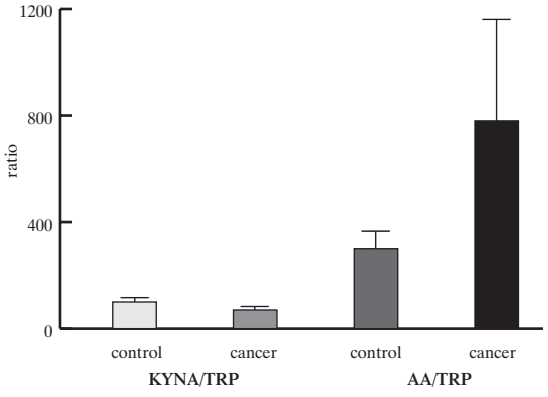


***Figure 2*. The ratio of KYNA/TRP and AA/TRP in cancers tissues**



Results

The estimation of TRP and its metabolites via degradation of kynurenine pathway is presented in *Tab. 2*. In plasma the concentration of TRP was 33.73±2.52 μM, of KYNA was 26.97±5.35 nM and of AA was 32.40±2.30 nM. In saliva the concentration of TRP was 3.81±0.62 μM, of KYNA was 8.06±1.86 nM and of AA was 20.41±10.77 nM. In cancer tissues the levels of TRP (30.21±5.88 μM), KYNA (15.85±1.82 nM) and AA (265.32±151.45 nM) were higher in respect to the concentration of TRP (13.28±0.62 μM), KYNA (12.75±2.28 nM) and AA (31.68±8.89 nM) in normal oral mucosa. The increase in the content of TRP, KYNA and AA in cancer tissues reached 127.48±5.95%, 24.31±4.35% and 737.50±206.96%, respectively (*Fig. 1*).

The ratio of KYNA/TRP was 95.00±14.84 in control tissue (normal epithelium) and 62.25±17.01 in cancer tissue. The ratio of AA/TRP was 236.00±63.09 in control tissue and 760.50±399.00 in cancer tissue (*Fig. 2*).

Discussion

For the first time we have observed the increase of TPR and its metabolites in the human oral cancer tissues. Among the all studied substances the enhanced of AA was the highest. Its concentration was almost 8.5 times higher in cancers' tissues in comparison to level observed in normal tissues. This high concentration, observed only in tumor, suggests that AA can be produced by cancer cells. In the literature is not any information about a role of this substance in oral carcinogenesis.

However, the data showed that AA has been implicated in carcinogenesis of the liver [15]. This observation is in line with the finding of Fujii and Watanabe who have demonstrated that transplacentally administration of AA for 1 year induced liver tumor in male mouse offspring [11]. In 1960s years several groups of workers suggested that AA has got carcinogenetic properties and it is responsible for development of human bladder cancer [12]. The authors confirmed, if bladder cancer is caused by agents presented in the urine, e.g. AA, it should be

also involved in the protective mechanism against carcinogenesis [12]. Thus, on the one hand the high level of AA can be a way of protection of cancer cells against their own toxins, on the other hand it can be implicated in oral carcinogenesis. The mechanism of activity of AA against cancer development is probably used in anticancer therapy according to the last data which has showed that effect in a new compounds designed as the anthranilic acid scaffold. The antiproliferative activity of AA was observed in vitro and in vivo in cancer experimental models [16,17].

We have also observed the elevation of TRP concentration almost 2.3 times in the oral carcinoma tissues in comparison with control healthy mucosa. Since TRP is known as an essential amino acid necessary for a variety of metabolic processes, e.g. protein biosynthesis and its availability and also it is necessary for rapidly-dividing tumors [18]. Thus, observed accumulation of TRP concentration in our study may be explained as self-protection of tumor cells.

KYNA concentration has showed the lowest increase in cancer tissues. Physiologically it is an antagonist of ionotropic glutamate receptors. Recent it was found that glutamate antagonists inhibit a proliferation of different human tumor cells [19]. We cannot exclude that small increase of KYNA concentration may be result of inhibition of its synthesis by cancer cells and thereby it can be a form of self-protection. So, it can suggest that KYNA is involved in regulation of carcinogenesis as well other disorders. Our recent study has showed the increase of saliva concentrations of KYN and KYNA in patients with both diabetes and hypertension in comparison to healthy volunteers and patients with hypertension, or diabetes alone [13].

In conclusion, our study has demonstrated the change of TRP metabolism which has been reflected by the increase of TRP, AA and KYNA concentrations in patients with oral squamous carcinoma. Moreover, we have showed the shift of amino acid transformation pathway on AA side. Our results have indicated accumulation of these substances in squamous cell cancer. Because their concentrations are lower in plasma and saliva we cannot exclude that cancer cells synthesize both of them locally. Thus, we can suppose that these substances may be one of many factors responsible for cancer development. Moreover, further studies are needed to prove it.

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