FLUORIMETRIC DETECTION OF ALDEHYDE DEHYDROGENASE ACTIVITY IN HUMAN TISSUES IN DIAGNOSTIC OF CANCERS OF ORAL CAVITY

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Abstract: Aldehyde dehydrogenase (ALDH) is known to be susceptible to oxidation, and its assays require stabilization of the enzyme by thiols. Application of the fluorimetric method to assay the ALDH activity in human saliva demonstrated significant differences between procedures utilizing glutathione (GSH) and dithiothreitol (DTT) as stabilizing agents. It has been recently shown that average aldehyde dehydrogenase (ALDH3A1) activity in cancerous (oral cavity cancer) patients' tissues was higher than that found in the control group, what may indicate induction of tumor-specific ALDH.

Keywords: aldehyde dehydrogenase, enzyme oxidation, tissue, fluorimetric assay

Several classes of aldehyde dehydrogenases (ALDH, E.C.1.2.1.3) are present in various human tissues. ALDH3A1 isozyme oxidizes mainly long and medium chain aliphatic, as well as aromatic aldehydes (1). This isozyme is responsible for metabolism of many drugs as for example inactivation of cyclophosphamide, widely utilized in cancer chemotherapy (2). ALDH3A1 is located in stomach, lungs, cornea, sweat, hair, saliva (2) and oral cavity epithelium (a little amount) (3), so in the areas directly threatened with exogenous aldehyde. Moreover, very similar to ALDH3A1 isozyme (tumor-specific ALDH) can be also induced in some neoplastic states of different cancer (4).

It has been recently shown that ALDH3A1 activity in saliva of cancerous patient (oral cavity cancer) was higher than in a control group. Moreover, it has significantly decreased within 7 days after tumor removal surgery. During the next months ALDH3A1 activity slightly increased reaching the level of the control group (5).

EXPERIMENTAL

Tissue fragments preparation

Tissue samples were collected from surgical operation performed at the Holy Infant Clinical Hospital in Warsaw and stored at -80°C. After thawing, samples were homogenized in sucrose solution (0.25 M), buffered with 5 mM phosphate, pH 7.5, containing EDTA (1 mM) and DTT (2 mM). The homogenates were spun at $9000 \times g$ to remove the mitochondrial fraction. The resultant S-9 fraction (cytosol and microsomes) contained 0,2-1 mg protein/mL and was diluted 50-fold in the cuvette.

Apparatus and fluorimetric procedure

The fluorimetric assay is based on two artificial substrates, 6-methoxy-2-naphthaldehyde (MONAL-62), and 7-methoxy-1-naphthaldehyde (MONAL-71), allowing independent measurement of the activities of two principal cytosolic forms of human aldehyde dehydrogenase, respectively,

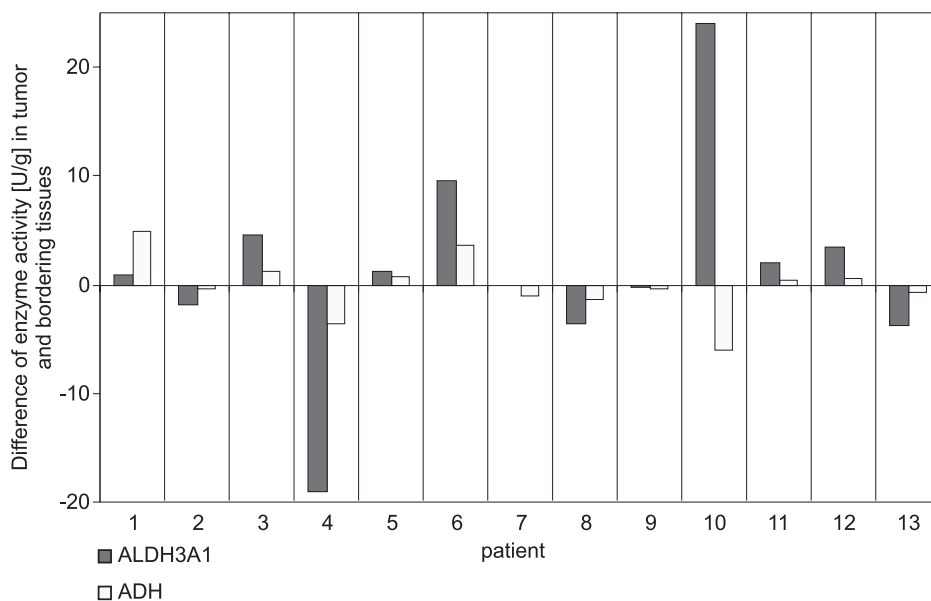* Corresponding author: E-mail: piowrona@tlen.pl

Figure 1. ADH less sensitive to 4-methylpyrazole inhibition and ALDH3A1 activity correlation.

ALDH3A1 (known as a tumor-associated ALDH) and ALDH1A1 in tissue fragments (6).

Fluorimetric assays were run on a thermostated Shimadzu 5001PC instrument. Instrumental settings were as follows: excitation wavelength was 310 nm, emission was monitored at 360 nm. The assays utilizes a highly fluorogenic naphthaldehyde substrate, 6-methoxy-2-naphthaldehyde (MONAL-62), acting with either NAD^+ or $NADP^+$ as co-substrates (Scheme 1). The estimated K_m values for the foregoing ALDH substrates are 0.24 μM for MONAL-62, 15 μM for NAD^+ and ~60 μM for $NADP^+$.

Fluorimetric assays were run in 50 mM pyrophosphate/HCl buffer, pH 8.1, at 25°C, in the presence of 0.5 mM EDTA and either 0.5 mM DTT, or 1 mM GSH. Typical substrate concentrations were 2-3 μM for MONAL-62, 100 μM for NAD^+ and 300 μM for $NADP^+$; thus nearly saturating ALDH3A1.

Table 1. The ALDH activity [U/g] examined by the fluorimetric method in tumor and bordering tissues of cancerous patients.

Patient	Tumor	Bordering tissues
1	1.153	0.263
2	0.435	2.248
3	5.387	0.761
4	2.788	21.808
5	1.920	0.730
6	10.397	0.752
7	0.879	0.927
8	2.731	6.299
9	0.022	0.209
10	24.096	0
11	2.163	0.176
12	3.444	0
13	1.117	4.847
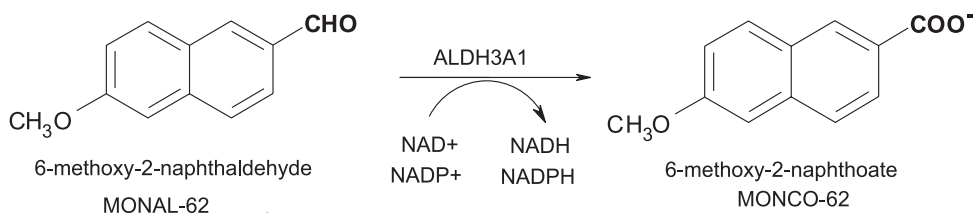
Scheme 1.

Table 2. Some clinical information about cancerous patients.

Patient	Sex	Age	Alcohol	Cigarettes	TNM[1]	Histopathological diversity level	Histopathological examination result	Tumor localization	Lymph gland metastasis	Radiation therapy	Radicality of surgery	Recursion
1	F	67	+/–	–	3/1/0	G3	*Carcinoma planoepitheliale spinocellulare non keratoides*	Lower gum, palate arch and throat	+	+	+	–
2	F	40	+/–	+	3/2/0	G2	*Carcinoma planoepitheliale spinocellulare partim keratoides*	Tongue (abscessive)	+	+	+	–
3	F	64			2/1/0	G2	*Carcinoma planoepitheliale spinocellulare partim keratoides*	Tongue (left side)	–	–	+	–
4	M	54	+	+	3/2/0	G2	*Carcinoma planoepitheliale spinocellulare keratoides*	Tongue and bottom of oral cavity	+	+	–	–
5	M	49	+	+	4/1/0	G2	*Carcinoma planoepitheliale spinocellulare non keratoides*	Bottom of oral cavity	+	+	–	–
6	F	65			2/1/0	G2	*Carcinoma planoepitheliale spinocellulare non keratoides*	Right side of tongue root	+	–	–	–
7	F	76	–	–	3/1/0	G1	*Carcinoma planoepitheliale spinocellulare partim keratoides*	Upper gum tumor dripping on mandible	+	–	+	+
8	M	57	+	–	3/1/0	G2	*Carcinoma planoepitheliale spinocellulare non keratoides invasivum*	Tongue (left side)	+	+	+	–
9	M	39	+	+	4/0/0	G1	*Fibroma myxomatosum*	Mandible	–	–	–	–
10	M	63	+	+	2/1/0	G2	*Carcinoma planoepitheliale spinocellulare non keratoides*	Tongue	+	–	+	–
11	M	48	+/–	+	3/2/0	G2	*Carcinoma planoepitheliale spinocellulare partim keratoides*	Bottom of oral cavity	–	+	+	–
12	F	70	–	–	3/2/0	G2	*Carcinoma planoepitheliale spinocellulare partim keratoides*	Upper gum tumor	–	+	–	+
13	M	59	+/–	+	3/3/0	G2	*Carcinoma planoepitheliale spinocellulare partim keratoides invasivum*	Bottom of oral cavity	–	+	+	+

[1]Tumor Nodules Metastasis

Purified reaction product(s) at concentrations of 3-5 μM were used as internal standards to obtain absolute reaction rates, which were calculated according to the formula:

$$\nu = \frac{dF}{dt} \frac{C_{st}}{F_{st}}$$

where:
dF/dt – fluorescence slope, C_{st} - standard concentration, F_{st} – fluorescence of the standard.

Specific activities were calculated as a ratio of the reaction rate to protein concentration, the latter determined by the Bradford method. Detection limit of the specific activity is estimated as ~0.05 U/g. The MONAL-62 unit is ca. 32% greater than the commonly used benzaldehyde unit.

RESULTS AND DISCUSSION

We have examined tumor and bordering tissue

of 13 patients suffering from oral cavity cancer (see Table 1). Average ALDH3A1 activity in cancerous patients tissues (tumor 4,35 U/g, C.V. 150%; bordering tissues 3,00 U/g, C.V. 199%) was higher than in a control group (0,14 U/g C.V. 150%). In 7 cases ALDH3A1 activity was higher in tumor than bordering tissues. In 90% of them ALDH3A1 activity in saliva significantly decreased within 7 days after tumor removal (average enzyme activity: 3,53 U/g, C.V. 170% before and 1,34 U/g, C.V. 130% after tumor removal, [5]). Moreover high activity of ALDH3A1 in tissues was correlated with high activity of ADH less sensitive to 4-methylpyrazole inhibition [ADH2, (7)]. Differences, observed between enzyme activities in tumor and bordering tissues for the examined group are presented in Figure 1. The positive values indicate higher enzyme activity in cancer in comparison to those in bordering tissue. Only in one case elevated ALDH3A1 activity was not accompanied with higher activity of ADH2 in related tissue.

Table 2 summarizes selected clinical information about 12 cancerous and 1 benign patient, whose data will not be described below. Among this cancerous group of people there were 6 women and 6 men aged between 40 and 76. Tumor was localized in 3 cases in the gum, in others in the tongue and bottom of oral cavity. Cancer stage was rated using TNM classification. The original tumor was qualified as T2 in 3 cases, T3 in 8 cases and T4 in the last one. Lymph gland metastases (N) were suspected in all cases (8 were confirmed in pathomorphological examinations). Distant metastases were not found. Pathomorphological examination recognized carcinoma planoepitheliale spinocellulare in all cases. Additional information keratoides (7 cases) or non-keratoides (5 cases) defined a state of tissue diversity. In 10 cases the grade of the cancer cells was rated G2, G3 in 1 case and G1 in 1 case. Surgery treatment was performed by tissue resection and removal of neck lymph nodes located on the same side as the tumor, and removal of supra-hyoid lymph nodes. Tissue deficits were refilled with material transplanted from the neighborhood or chest.

Small amount of cases results in difficulties in finding correlation between this information and variability of ALDH3A1 activity.

The aldehyde dehydrogenase ALDH1A1 isozyme activity, evaluated using a specific substrate 7-methoxy-1-naphthaldehyde (MONAL-71) as an indicator (6), was low in cancerous patients (in tumor 0,12 U/g, C.V. 170%; bordering tissues 0,08 U/g, C.V. 254%) as well as in the control group (0,06 U/g). In both groups, the ALDH1A1 activity in saliva was undetectable (5).

CONCLUSION

In most cases studied so far, ALDH3A1 activity in tumor and bordering tissues was higher than in a control group. This phenomenon may indicate the induction of this isozyme in malignant tissues of oral cavity cancerous patients. Differences between activity in cancerous and bordering tissues could be determined by clinical data, but requires further investigation.

Acknowledgment

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