ORIGINAL ARTICLE

RHODANESE AND ARGINASE ACTIVITY IN NORMAL AND CANCEROUS TISSUES OF HUMAN BREAST, ESOPHAGUS, STOMACH AND LUNG

Akram Jamshidzadeh PharmD*, Mahmoud Aminlari PhD**•, Hamid-Reza Rasekh PhD*

*Department of Pharmacology and Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, **Department of Biochemistry, School of Veterinary Medicine, Shiraz University, Shiraz, Iran*

Abstract

Background and Objectives-Differences in the activities or concentration of certain enzymes in cancerous cells and their normal counterparts have been well documented. The purpose of this investigation was to determine and compare the activities of arginase and rhodanese in selected normal and cancerous human tissues.

Methods-Cancerous tissue specimens were obtained from surgically resected organs and normal tissues were obtained from fresh frozen corpses in the Forensic Medicine Center, Shiraz, Iran. Rhodanese was assayed by the modified method of Sorbo and arginase activity was based on urea determination.

Results-In most cases, the activity of arginase in the cancerous tissues was higher than the normal tissues, but in the stomach cancer, the increase was significant ($p<0.05$). The comparison between the activity of rhodanese in the normal and cancerous tissues revealed variable results; the activity was higher in the breast cancer group while it showed significantly ($p<0.05$) reduced activity in the lung cancer.

Conclusion-The results presented in this study indicated that arginase activity was higher in the cancerous tissues than their normal counterparts and this might be consistent with the role of arginase in ornithine biosynthesis. Different patterns of rhodanese activity in the malignant tissues and its reduced activity especially in the lung cancer has no satisfactory explanation in the medical literature.

Keywords • Arginase • rhodanese • neoplasia

Introduction

The pattern of enzymatic alterations may be linked with the malignant state and the progression of cancerous cells in the tumor. Differences in the activities or concentration of certain enzymes between cancer cells and their normal counterparts might be useful as biological markers of malignancy and/or aggressiveness in particular tumors.[1] On the other hand, since the increase in the activities of certain enzymes is an indicator of prominence or abeyance of particular biochemical reactions or metabolic pathways, one might speculate that application of measures correlating to the activities of such enzymes will lead to elucidation of therapeutic approaches to cancer.[2]

The purpose of this study was to determine the activities of the enzymes rhodanese and arginase in normal and cancerous human tissues. Rhodanese (thiosulfate: cyanide sulfur transferase, EC 2.8.1.1) is found in both eukaryotes and prokaryotes. This mitochondrial enzyme is present in numerous tissues of mammals. Although the biochemical role of rhodanese *in vivo* is not fully understood, this enzyme has been implicated in the formation of iron-sulfur complexes[3,4] and cyanide detoxification.[5] Recent studies on the molecular properties of rhodanese indicate that this enzyme

•**Correspondence:** *M. Aminlari PhD, Department of Biochemistry, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. 1731, Fax: +98-71-680707. E-mail: aminlari@hafez.shirazu.ac.ir*

also plays an important metabolic function in the regulation of mitochondrial respiration rate.[6]

Furthermore, peculiar distribution of this enzyme in human[7] and animal tissues[8] suggests that cyanide metabolism is not the only, or even the major role of rhodanese. In particular, our study which indicates high level of rhodanese activity in the epithelial layer of certain organs such as the gastrointestinal[9] and respiratory tracts[10] as well as in the kidney cortex of animals[11], suggests that tissues having raised turnover rate or those involved in higher metabolic rate might contain higher rhodanese activities than normal tissues.

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1), which catalyzes the hydrolysis of L-arginine into L-ornithine and urea, was first detected in mammalian livers as the terminal enzyme of the urea cycle.[12] Arginase activity occurs also in other tissues, which are devoid of a complete urea cycle.[13-15] In the latter instance, the importance of arginase may be in the production of ornithine for the synthesis of the polyamines putrescine and spermine, which are required for normal cellular proliferation.[16,17] Several reports indicate that a higher activity of arginase is present in cancerous tissues.[18-21] The results obtained in this study indicate that in certain tumors, the levels of activity of rhodanese or arginase are different from normal tissues.

Materials and Methods

All chemicals were of analytical grade and were supplied by commercial sources. Cancerous tissue specimens were obtained from the surgically resected organs of patients referred to the Iranian Cancer Institute. In order to assure adequate diagnostic evaluation of the tissue, each sample was divided into two parts, one was sent for pathologie examination and the other for biochemical assessment. The samples were analyzed on a blind basis such that the personnel performing biochemical or pathological studies were unaware of the results of the other study group till completion of the research. The normal tissues were taken from fresh frozen corpses in the Forensic Medicine Center, Shiraz within 5-10 hours after death. These tissues were proven to be normal by the Center's pathologist. All normal tissues were taken from 5 males and 4 females.

Preparation of tissue extract

All samples, kept on ice, were transferred within 45 minutes to the laboratory; tissues were separated, stripped from adipose tissue, cleansed with physiological saline, and then bottled. Tissue extracts were prepared by freezing the samples in liquid nitrogen, homogenizing with a hand homogenizer and suspending the homogenates in 0.025 M sodium phosphate buffer, pH 7.2. The suspensions were centrifuged for 15 minutes at 400g in a MSE high-speed refrigerated centrifuge. Supernatants were used as the enzyme source. Samples with high enzyme activity or high protein content were appropriately diluted with phosphate buffer and the final results were multiplied by the dilution factor.

Determination of rhodanese activity

Rhodanese was assayed by the modified method of Sorbo.[22] The reaction mixture contained 16.8 mMol/L sodium thiosulfate, 40 mMol/L glycine buffer, pH 9.2, 6.7 mMol/L KCN, and 30 µL of enzyme solution in a final volume of 4 mL. The reaction was carried out for 20 minutes at 37°C and stopped by adding 0.5 mL 38% formaldehyde. In the control tubes formaldehyde was added prior to addition of enzyme solution. The concentration of thiocyanate was determined as follows (Sorbo, 1953): samples were mixed with 1 mL of ferric nitrate solution containing 0.025 g Fe $(NO_3)_3$, 9 H_2O in 0.74 mL water and 0.26 mL concentrated nitric acid. After centrifuging the mixture to remove the interfering turbidity, absorbances were measured at 460 nm against a blank which contained all reagents except that 30 µL water was substituted for the enzyme solution. The concentration of formed thiocyanate was obtained from a standard curve produced by treating solutions containing different concentrations of thiocyanate as described above. In this study, pH 9.2 was used instead of pH 7.4 (which is routinely used) in order to decrease the turbidity of solutions after addition of ferric nitrate. No significant differences were observed when purified rhodanese was assayed at pH 7.4 or 9.2. The unit of enzyme activity was defined as micromole thiocyanate formed per minute at 37°C and pH 9.2.

Determinants of arginase activity

A 0.02 mL of tissue extract was mixed with 0.5 mL saponin solution and incubated at 37°C for 10 minutes. About 0.02 mL of 10% $MnCl_2$ was added and the mixture was incubated for 10 minutes followed by addition of 0.2 mL 0.02 mol/L

Table 1. General characteristics of patients included in this study.

	Sex		Mean age	Type	Grading		
	Female	Male			Poor	Mod	Well
Breast (n=6)	6	0	56±5.5	4 Infiltrative lobular CA, 2 infiltrative ductal CA	4	1	1
Esophagus (n=7)	3	4	54±11	All SCC except one adenocarcinoma	4	1	2
Stomach (n=10)	6	4	54±9.4	All adenocarcinomas	5	3	2
Lung (n=5)	2	3	59±10	All SCC except one adenocarcinoma	2	2	1
Total (n=28)	17	11	55±9	---	15	7	6

SCC: squamous cell carcinoma, Poor: poorly differentiated, Mod: moderately differentiated, Well: well differentiated.

arginine solution. After mixing and incubating at 37° C for 20 minutes, one mL tungestic acid solution (equal parts of 2.2% sodium tungstate and 0.15 N sulfuric acid) and 0.26 mL 0.1 mol/L HCl was added and the mixture was further centrifuged. One mililiter of the supernatant was used for urea measurement. The amount of formed urea was determined according to the method described elsewhere.[23] About 2.5 mL of acid mix (phosphoric-sulfuric) and 0.5 mL 2% diacetylmonoxime was added to 1 mL aliquot of the reaction mixture, which had been stopped with tungestic acid and HCl. This mixture was shaken vigorously and heated for 30 minutes in a boiling water bath. The absorbance was read against a blank with a Bausch and Lomb Spec 20 (Arthur H. Thomas Co., Philadelphia, Pa) at a wavelength of 475 nm. One unit enzyme activity is the amount of enzyme required to produce 1 μmol urea per minutes at 37° C.

Protein determination
Protein content of tissue extracts was determined by the method of Lowry et al. (1951)[24] using crystalline bovine serum albumin as standard.

Statistical analysis
The data were expressed as mean±SD and normal tissues data were analyzed by one-way ANOVA by using SPSS/PC software, and Duncan's multiple range test was used to detect significant differences among enzyme activities of various tissues. The data illustrated in Table 2 were analyzed statistically using student's *t-test*.

Results

The activity of the enzymes arginase and rhodanese of the normal tissues of 5 male and 4 female subjects with mean age of 48±12 years was measured. Liver contained the highest activity of arginase (142±74 mU/mg) followed by kidney (18.4±10), skin (14±5) and duodenum (7.3±3.2). The liver arginase activity was significantly higher (p<0.05) than the other tissues. Rhodanese activity

Table 2. Arginase and rhodanese activities in normal and cancerous tissues of breast, stomach, esophagus and lung.

Different tissues	Protein in one gram tissue (mg)		Arginase activity (mU/mg protein)		Rhodanese activity (mU/mg protein)	
	Normal	Patients	Normal	Patients	Normal	Patients
Breast (N_{normal}=7, $N_{patient}$=6)	21±11.2	23.5±8.68	2.5±0.7	1.45±0.4	33±10	47.6±14
Esophagus (N_{normal}=7, $N_{patient}$=7)	46.4±26.7	16.3±7.47	3.5±0.6	6.3±1.8	38±14	29.8±12
Stomach (N_{normal}=9, $N_{patient}$=10)	41.1±19.1	37.8±16.3	2.3±0.9	6.2±2.1*	15±1.8	10.3±2.3
Lung (N_{normal}=7, $N_{patient}$=5)	84.5±38.8	44.6±16.9	3.5±0.8	4.5±1.65	19±3*	6±2.5

* p<0.05

of the liver (106±18 mU/mg) and kidney (92±11) were also significantly ($p<0.05$) higher than other normal tissues studied.

Basic characteristics of the patient groups are summarized in Table 1. The mean age of patients (17 females and 11 males) was about 55 years and most of the cancerous tissues were poorly differentiated according to the WHO International Histological Classification.[25]. Most common types of cancers were adenocarcinoma for stomach and squamous cell carcinoma for lung and esophagus cancers.

Table 2 shows the pathological features and activities of these two enzymes in normal and cancerous tissues.

The comparison between the activity of arginase in normal and cancerous tissues showed that although in most cases the activity of arginase in cancerous tissues was higher than the normal ones, only in the gastric carcinoma, this increase of activity was significant ($p<0.05$). The comparison between the activity of rhodanese in normal and cancerous tissues showed that only in lung cancer, the decrease in the activity of rhodanese is significantly ($p<0.05$) different from the normal tissues.

Discussion

Investigation on the pattern of distribution of enzymes in different tissues is of particular importance as this type of information can help localize certain biochemical processes that are unique to a tissue. In addition, such information might provide a basis for developing diagnostic and therapeutic approaches when these tissues are damaged or encounter a malignant state. Results obtained in this study are consistent with this notion. High activity of arginase in the liver is the result of participation of this enzyme in the urea cycle. However, prevalence of arginase found in this study indicates that it performs other functions in non-hepatic tissues. These include synthesis of precursors of polyamines[17] and proline.[26] These metabolites are required for normal cell differentiation[16] and collagen formation[27], respectively. The latter might explain a high arginase content in skin. Similarly, distribution of rhodanese in normal human tissue might indicate that cyanide detoxification, which is normally performed in the liver, might not be the only physiological role of rhodanese. In addition to liver, other organs are good sources of rhodanese. In these tissues, prevalence of rhodanese might be

related to other functions. Presence of high levels of rhodanese in certain human tissues found in this study as well as extensive studies performed in this laboratory which show high specific activity of rhodanese in epithelial layers of different parts of the gastrointestinal tract[9], respiratory tract[10] and kidney cortex of animals[11], indicate that certain physiological roles are performed by this enzyme in these tissues. Indeed, recent studies have clearly shown that rhodanese is involved in energy metabolism through its participation in regulation of mitochondrial electron transport[28] and in the biosynthesis of iron-sulfur centers.[3]

These considerations prompted us to determine and compare the level of arginase and rhodanese activities in normal and some cancerous human tissues. While there has been many studies on the level of arginase activity in different cancers, no report is available on the pattern of rhodanese activity in cancerous cell. For example, it has been reported that arginase specific activity is significantly greater in prostatic carcinoma than in prostatic hyperplasia.[29] Also, high level of arginase has been detected in gastric cancer.[21] The results of this study also indicate that the pattern of arginase activity in certain carcinomas is different from the normal tissues. Esophagus, stomach and lung carcinomas showed higher level of arginase than their normal counterparts (Table 2). In these tissues, higher arginase activity might be consistent with the role of this enzyme in ornithine biosynthesis as a precursor of polyamines required for cell replication.[30,31] Table 2 indicates a different pattern of rhodanese activity in the malignant tissues as compared with arginase. In these tissues, except for the breast, rhodanese activity is unexpectedly lower (significant only in lung) than the corresponding normal tissues. There is no satisfactory explanation in the medical literature for the controversial pattern of enzyme activities in breast tissue.

It is also to mention that the difference in the mean age of the patient and the control groups is not significant since the normal tissues were taken from nine cadavers, a perfect matching could not be expected. Moreover, the difference between protein levels of normal and cancerous tissues is not significant either, and the variance of this variable in normal tissues has been found to be high.

Since we have observed high rhodanese activity in certain tissues that are highly active in terms of cell division and energy requirements (epithelial

layers)[8], we presumed that an increase in rhodanese activity might also occur when cells become malignant. On the other hand, the labile sulphane-sulfur atom has been shown to have effects in biochemical systems, which suggests that it might have natural regulatory functions.[3] It has been suggested that defective sulfur metabolism might be related to carcinogenesis.[32] Tumors with high growth rates may have lower activities of the sulfur transferases than normal tissues or tumors with low growth rates.[33] Several reports have demonstrated remarkable anticancer effect of sulphane-sulfur compounds.[3] The results of the present study on rhodanese, although preliminary, might indicate a different sulfur transferase activity and sulphane-sulfur metabolism in malignant tissues and warrants more extensive studies on such tissues.

Acknowledgment

References

1 Mcintire KR. Tumor markers: how useful are they? *Hosp Pract.* 1984; **19:** 55-9.

2 Pamies RJ, Crawford DR. Tumor markers. An update. *Med Clin North Am.* 1996; **80:** 185-92.

3 Toohey JI. Sulphane-sulfur in biological systems: a possible regulatory role. *Biochem J.* 1989; **264:** 625-32.

4 Westley J. Cyanide and sulphane-sulfur. In: Venesland B, Conn EE, Knowles CJ, et al, eds. *Cyanide in Biology.* European Molecular Biology Organization. New York: Academic Press; 1981: 61-76.

5 Westley J, Alder H, Westley L, et al. The sulfur transferases. *Fund Appl Toxicol.* 1983; **3:** 377-82.

6 Ogate K, Volini M. Comparative properties of bovine heart and liver rhodanese and the regulatory role of the rhodanese in energy metabolism. *J Protein Chem.* 1986; **5:** 239-46.

7 Whitehouse DB, Pilz AJ, Porta G, et al. Rhodanese isozymes in human tissues. *Ann Hum Genet.* 1988; **52:** 1-10.

8 Aminlari M, Gilanpor H. Comparative studies of the distribution of rhodanese in different tissues of domestic animals. *Comp Biochem Physiol.* 1991; **66:** 673-7.

9 Aminlari M, Shahbazi M. Rhodanese (thiosulfate: cyanide sulfur transferase) distribution in the digestive tract of chicken. *Poultry Science.* 1994; **73:** 1465-9.

10 Aminlari M, Vaseghi T, Kargar MA. The cyanide-metabolizing enzyme rhodanese in different parts of the respiratory systems of sheep and dog. *Toxicol Appl Pharmacol.* 1994; **124:** 67-71.

11 Aminlari M, Gholami S, Vaseghi T, et al. Comparative studies on the distribution of the cyanide metabolizing enzyme rhodanese in different parts of the urogenital systems of sheep at pre-and post-natal stages of development. *Comp Biochem Physiol;* 2000.

12 Greenberg DM. Enzymes of urea cycle. In: Boyer PD, Lardy H, Myrback K, eds, *Enzymes.* Vol 4. 2 nd ed. New York: Academic Press; 1960: 257-67.

13 Pohjanpelto P, Holtta E. Arginase activity of different cells in tissue culture. *Biochem Biophys Acta.* 1983; **757:** 191-5.

14 Skoy S, Eriksson V, Eliasson E. Arginase, an s-phase enzyme in a human cell line. *Biochem Biophys Acta.* 1981; **672:** 33-44.

15 Schneider E, Dy M. The role of arginase in the immune response. *Immunol Today.* 1985; **6:** 136-40.

16 Pegy AE, McCann PP. Polyamine metabolism and function. *Am J Physiol.* 1982; **243:** 212-9.

17 Tobor CW, Tobor H. Polyamines. *Annu Rev Biochem.* 1981; **87:** 351-7.

18 Straus B, Cepelak L, Festa G. Arginase, a new marker of mammary carcinoma. *Clin Chem Acta.* 1992; **210:** 5-12.

19 Leu SY, Wang SR. Clinical significance of arginase in colorectal cancer. *Cancer.* 1992; **70:** 733-6.

20 Yang H, Li G, Huangs L, Qu S. Study of arginase activity in alveolar macrophages of patients with lung cancer. *Huran I Ko Ta Hsuch Pao.* 1997; **22:** 84-6.

21 Wu GW, Chung WN, Chi CW, et al. Immunohistochemical study of arginase in cancer of the stomach. *Virchows Arch.* 1996; **28:** 327-31.

22 Sorbo BH. Crystalline rhodanese. I. Purification and physicochemical examination. *Acta Chem Scand.* 1953; **7:** 1129-36.

23 Natelson S. *Techniques of Clinical Chemistry.* 3rd ed. Illinois: Springfield, ILL.,Thomas; 1971: 146-8.

24 Lowry OH, Rosenbrough NJ, Farr AL, et al. Protein measurement with Folin phenol reagent. *J Biol Chem.* 1951; **193:** 265-75.

25 Watanabe H, Jass JR, Sobin LH. *WHO International Histological Classification of Tumors.* New York: Springer-Verlag; 1990; 22-3.

26 Rodwell VW. Catabolism of protein and of amino acid nitrogen. In: Murray RK, Granner DK, Mayes PA, Rodwell VW, eds. *Harper's Biochemistry.* 24th ed. New York:Appleton & Lange; 1996: 334-7.

27 Rao RM, Mishra OP, Santhanam K, et al. Dermal wound healing in experimentally injuried rats: roles of arginase. *Nutr Rep Int.* 1980; **22:** 167-72.

28 Ogata K, Dai X, Volini M. Bovine mitochondrial rhodanese is a phosphoprotein. *J Biol Chem.* 1988; **267:** 2718-25.

29 Pretlow TG, Harris BE, Bradly EL, et al. Enzyme activities in prostatic carcinoma related to Gleason grades. *Cancer Res.* 1985; **45:** 442-6.

30 Heby O. Role of polyamines in the control of cell proliferation and differentiation. *Differentiation.* 1981; **14:** 1-9.

31 Luk GD, Casero RA. Polyamines in normal and cancer cells. *Adv Enz Regul.* 1987; **26:** 91-9.

32 Glode LM, Epstein A, Smith CG. Reduced γ-cystathionase protein content in human malignant leukemia cell line as measured by immunoassay with monoclonal antibody. *Cancer Research.* 1981; **41:** 2249.