

## THE SERUM OF CARCINOMA PATIENTS POWERFULLY ACTIVATES CARBONIC ANHYDRASE II

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## СЫВОРОТКА КРОВИ ОНКОЛОГИЧЕСКИХ БОЛЬНЫХ АКТИВИРУЕТ КАРБОАНГИДРАЗУ II

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The effects of the serum of cancer patients on carbonic anhydrase (CA) I and II activity have been studied. In total 2134 samples of the serum from patients with different types of cancer as well as non-cancer patients and healthy persons have been analyzed. CA activity was assayed by a stopped-flow method with a rapid kinetic device HI-TECH SF51MX. All the sera of cancer patients under study, no matter of the type of cancer, turned out to be able to activate CA II. The activating effect was registered at a 1 : 100 dilution and increased progressively up to 1 : 10 dilution reaching the peak of the activation. The results suggest the presence of some endogenous CA II activators in the serum of cancer patients. *Key Words:* carbonic anhydrase, cancer patients, serum.

Изучали влияние сыворотки крови больных раком на активность очищенной карбоангидразы (КА) типа I и II. Всего было проанализировано 2134 образца сыворотки крови больных с опухолями различной локализации, пациентов с неопухоловой патологией и здоровых лиц. Показано, что сыворотка крови больных раком (независимо от локализации опухоли) во всех случаях активизирует КА II. Активирующее действие отмечается уже при разведении 1 : 100 и постепенно усиливается, достигая пика при разведении 1 : 10. Результаты исследования указывают на присутствие в сыворотке крови этих больных эндогенных активаторов КА II.

*Ключевые слова:* карбоангидраза, онкологические больные, сыворотка крови.

Carbonic anhydrase (CA) is a zinc-enzyme that is detected in red blood and other cells, playing the main role in maintaining the acid-base equilibrium. This enzyme has 12 isozymes, most of them having well established physiological functions. Our previous studies have proved that isozyme I located in the red blood cells and in vascular walls is involved in the modulation of vascular processes [1]. CA II isozyme, a cytosol enzyme of the red blood and secretory cells modulates the secretory processes [2, 3]. Our previous research has shown that NSAIDs known as cyclooxygenase inhibitors are, at the same time, CA II activators both *in vitro* and *in vivo* [4–6]. CA is also activated by vasoconstrictive prostaglandins while vasodilating prostaglandins inhibit this enzyme [7, 8]. While anticancer chemotherapy increased CA II activity [9], carcinogenic substances reduce it [10]. Other studies showed low CA II activity in cancer patients [11].

Recent studies suggest that aspirin and other NSAIDs known as CA II activators antagonize CA II inhibition induced by carcinogenic substances [12, 13]. Our previous data have shown strong activation of purified and erythrocyte CA II by sialic acid,  $\alpha$ 1-anti-

chymotrypsine [14] and p185<sup>Her2</sup> oncoprotein [15]. The aim of the present study was to analyse the effects of the serum of cancer patients on purified CA II activity.

The experiments were approved by the local human ethics committee and informed consent was obtained from each patient. We selected 9 groups of cancer patients with histologically confirmed diagnosis (see Table) and another 2 groups, of healthy volunteers ( $n = 221$ ) and non-cancer patients ( $n = 208$ ).

In all the patients blood was collected by venous puncture. The serum was separated and its effects were measured at 1 : 100, 1 : 50, and 1 : 10 dilutions.

CA II activity was assessed by the stopped-flow method [16] by measuring the enzymatic activity of CO<sub>2</sub> hydration. pH change was assayed colorimetrically using a rapid kinetic spectrophotometer HI-TECH SF-51MX (England), equipped with a mixing unit and a system of two syringes supplying the reagents. The time required for pH drop in the reagent mixture from initial value of 7.5 to the final value of 6.5 was referred to as the reaction time.

CA activity was calculated as follows:

$$A = \frac{T - T_0}{T} [\text{EU/ml}]$$

where  $T_0$  — the uncatalyzed reaction time;

and  $T$  — the catalyzed reaction time (in the presence of CA II).

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Abbreviation used: CA — carbonic anhydrase.

**Table.** Groups of cancer patients under study

Group	Tumor site	Type of tumor	Number of patients
1	Esophagus	squamous cell carcinoma	92
		adenoid cystic carcinoma	67
		primary esophageal lymphoma	30
2	Colon and rectum	stage A (limited to mucosa)	71
		stage B2 (through serosa)	117
3	Liver	hepatocellular carcinoma	79
		cholangiocarcinoma	63
		hepatoblastoma	45
		angiosarcoma	14
4	Pancreas	adenosquamous carcinoma	62
		lymphoma	71
		sarcomatoid carcinomas:	
		spindle cell carcinoma	12
		malignant giant cell carcinoma	9
		pleomorphic giant cell carcinoma	7
5	Lung	round cell anaplastic carcinoma	5
		adenocarcinoma	109
		epidermoid carcinoma	62
6	Breast	small cell carcinoma	48
		invasive ductal	168
		infiltrating lobular	59
7	Ovary	epithelial	116
		stromal	41
		germ cell tumors	32
8	Testis	germinal cell tumors	102
		gonadoblastoma	46
9	Prostate	adenocarcinoma	88
		squamous cell carcinoma	54
		transitional cell carcinoma	36

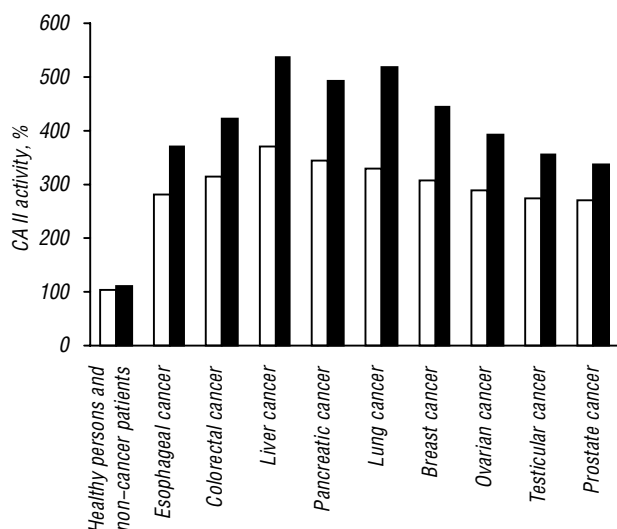
CA activity in the presence of serum was assessed by adding the serum dilutions to the purified isozyme. The percentage of CA activation was calculated as:

$$\frac{A_1 - A}{A} \times 100 (\%)$$

where A — purified CA II activity and A<sub>1</sub> — CA II activity in the presence of serum.

To determine whether the CA II activity was affected by serum, a repeated measure ANOVA was performed. Probabilities of *P* < 0.05 were considered significant.

As shown in the figure, the serum of healthy persons and non-cancer patients did not significantly modify purified CA II activity regardless of the serum dilution. On the contrary, the serum of cancer patients, no matter the type of cancer, activated CA II in all the cases. The



**Figure.** Effects of sera at 1 : 50 (□) or 1 : 10 (■) dilution on purified CA II activity. Basal CA II activity in each group of patients is assumed as 100%

activating effect is present at 1 : 100 dilution and increases progressively up to 1 : 10 dilution. There were no recorded false-positive results in any case. The activation attained 100% at a 1 : 50 dilution and exceeded 200% at a 1 : 10 dilution.

Therefore, the sera of cancer patients, whatever the particular type of cancer, activate purified CA II. Our data testify to the existence of certain powerful activators of CA II in the serum of these patients, while the sera of non-cancer patients and healthy persons are devoid of such an activity.

Our previous studies showed powerful dose-response CA II activation by sialic acid and α1-antichymotrypsine [14], carcinoembryonic antigen [17], and p185<sup>Her2</sup> oncoprotein [15]. Certain cancers are accompanied with hypergastrinemia [18] and/or hyperhistaminemia [19]. Our previous studies showed activating effects of both gastrin and histamine upon CA II *in vitro* and *in vivo* [20, 21]. Meanwhile, the activating effects of the sera of patients under study exceeded the effects attributed to the particular agents mentioned above.

The data obtained suggest the presence of some endogenous CA II activators in the serum of cancer patients. The activation of CA II suggests its involvement in carcinogenesis. Our previous studies have proved that carcinogenic substances reduced CA II activity in parallel with increasing pH<sub>i</sub>, while anticarcinogenic agents increased CA II activity concomitantly reducing pH. CA II and CA IV activation by NSAIDs followed by intracellular pH drop could also explain the effectiveness of NSAIDs as chemopreventive and adjuvant therapy of cancer.

The diagnostic significance of CA II assay is far from being elucidated. Nevertheless, our data suggest that assaying CA II could contribute to further elaboration of the screening tests aimed at the diagnosis of cancer [22, 23]. However, the activatory effects of cancer serum upon CA II do not seem to correlate with localization, staging or histological type of the tumor.

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