Journal of Bioequivalence & Bioavailability
ISSN: 0975-0851

Ayrapetyan et al., J Bioequiv Availab 2012, 4:7
DOI: 10.4172/jbb.1000123

Open Access

# Na/K Pump $\alpha_3$-Isoform-Dependent Cell Hydration Controlling Signaling System Dysfunction as A Primary Mechanism for Carcinogenesis

**Sinerik Ayrapetyan[1]*, Liana Yeganyan[1], Gagik Bazikyan[2], Rafayel Muradyan[3] and Flora Arsenyan[3]**

[1]UNESCO Chair-Life Sciences International Postgraduate Educational Center, Yerevan, Armenia
[2]National Center of Oncology, Yerevan, Armenia
[3]NAS RA the Scientific Technological Center of Organic and Pharmaceutical Chemistry SNPO, Yerevan, Armenia

## Abstract

More than 40 years cell over-hydration serves as a diagnostic marker for carcinogenesis. However, the nature of cell volume controlling mechanism dysfunction of which leads to over hydration and abnormal cell proliferation is not clear yet. The individual roles of $Na^+/K^+$ pump isoforms having different affinity to ouabain ($\alpha_1$-low, $\alpha_2$-midle and $\alpha_3$-high affinity) in cell hydration of different organs of healthy (H) and sarcoma-180 tumor (ST) carrying (SC) mice were studied. The tissue hydration in all organs in SC animals was higher. The pathology-induced cell hydration was accompanied by increase in $\alpha_3$ receptors affinity to $^3$H-ouabain in excitable and decrease in non-excitable cells. $10^{-11}$ M ouabain leads to dehydration while $10^{-8}$ and $10^{-6}$ M to hydration in SC mice, including ST. Tissue hydration in H and SC mice has different sensitivity to anti cancer drug-cisplatin (cisPt): in H mice it has organo-specific effects while in SC mice it leads to dehydration in all tissues, including ST. This dehydration was accompanied by increase of receptors' affinity to ouabain which was more pronounced in case of $\alpha_3$-receptors. At $10^{-6}$ M ouabain concentration cisPt has hydration effect on muscles and dehydration effect on non-excitable tissues in both H and SC mice, including ST. Cell hydration is suggested as a universal diagnostic marker for cell pathology. $Na^+/K^+$ pump $\alpha_3$ isoform-dependent cell hydration controlling signaling system dysfunction is supposed to be a primary mechanism for generation of carcinogenesis. Endogen ouabain circulating in mammalian blood, by its dehydration effect would have antitumor property, and its deficit would promote carcinogenesis.

**Keywords:** Cancer; Cisplatin; Hydration, $Na^+/Ca^{2+}$ exchange; $Na^+/K^+$ pump; Ouabain

## Introduction

Hydration is a fundamental parameter which determines cell's functional state [1-3]. It is capable of shifting the pattern of cellular metabolism: cell swelling triggers proliferation, while cell shrinkage promotes the apoptosis [4]. Our early studies have shown that membrane proteins having enzymatic, receptor and channel-forming properties, are present in functionally active and inactive (reserve) states, the ratio of which changes depending on cell volume (membrane packing) [2]. On the other side, cell hydration leads to activation of intracellular enzymes' activity by "protein folding" mechanism [5].

It is now well established that cancerous tissue is markedly overhydrated and can contain up to 90% water. Over-hydration of cancer cells serves as one of the essential diagnostic parameters [6]. Notwithstanding the introduction in 1971 by Raymond Damadian of nuclear resonance technique for detecting over-hydrated - cancer cells [7], the nature of the metabolic mechanism the dysfunction of which brings to cell's over-hydration leading to carcinogenesis is not clear yet.

Among the number of mechanisms involved in cell volume regulation $Na^+/K^+$ pump has fundamental role in this process. The importance of its role is dictated by the fact that $Na^+$ gradient serves as an energy source for a number of secondary ionic transporters, such as $Na^+/Ca^{2+}$, $Na^+/H^+$, $Na^+$/sugars, amino acids & osmolytes [8].

There are two enzyme systems actively involved in metabolic regulation of cell hydration, associated with cation transport across surface membranes and ionic adsorption in cytoplasm: transport ATPases, which are the translocating structure and are fueled by the free energy derived from ATP hydrolysis, and kinases, which may regulate translocation via phosphorylation of the transporter molecules through the membrane as well as phosphorylation of associated regulatory adsorption properties of intracellular structures. The close talking between these two enzyme systems is realized through the intracellular signaling systems, the dysfunction of which leads to generation of cell pathology, accompanied by corresponding changes of cell hydration. As $Na^+/K^+$ pump is the most ATP-utilizing machine in the cell it serves as a main regulator of all other ionic pumps' and kinases' activity. Therefore, factors able to change the balance between ATP hydrolysis and ATP production system (mitochondria), by changing $Na^+/K^+$ pump activity, could switch on the intracellular signaling systems-induced modulation of cell katabolic and anabolic processes. Hence, the dysfunction of $Na^+/K^+$ pump can be considered as a common gate for cell pathology, including cancer. However, which of $Na^+/K^+$ pump isoforms in particular serves as the primary mechanism dysfunction of which could lead to apoptosis in excitable cells and enhance proliferation in non-excitable cells stays unclear.

The second ionic transporting mechanism in cell membrane, having a crucial role in cell volume regulation is $Na^+/Ca^{2+}$ exchange [9-12]. It is known that there is a close correlation between the electrogenic $Na^+/K^+$ pump and electrogenic $Na^+/Ca^{2+}$ exchange, which has been described in the pioneering work by Baker et al. [13]. At present close

***Corresponding author:** Sinerik Ayrapetyan, UNESCO Chair-Life Sciences International Postgraduate Educational Center, Armenia, Tel: 374-106-241-70; Fax: 374-106-241-70; E-mail: info@biophys.am

**Received** October 03, 2012; **Accepted** October 26, 2012; **Published** October 31, 2012

**Citation:** Ayrapetyan S, Yeganyan L, Bazikyan G, Muradyan R, Arsenyan F (2012) Na/K Pump $\alpha_3$-Isoform-Dependent Cell Hydration Controlling Signaling System Dysfunction as A Primary Mechanism for Carcinogenesis. J Bioequiv Availab 4: 112-120. doi:10.4172/jbb.1000123

correlation between these two ion transporting mechanisms on the level of different $Na^+/K^+$ pump isoforms is described by prof. Blaustein's group who discovered and characterized different isoforms of $Na^+/K^+$ pump and indicates a key role of high affinity ouabain receptors ($\alpha_3/\alpha_2$) in regulation of $Ca^{2+}$ exchange [14,15]. However, the detailed mechanism of correlation between these isoforms, especially $\alpha_3$ one and $Ca^{2+}$ exchange is not defined. Askari and coworkers suggested that $\alpha_3$ isoforms mainly perform signaling function and through protein interactions activate the intracellular signaling cascade in cells [16]. From this point of view was explained the nanomolar ouabain induced inhibition of cell proliferation and apoptosis in breast cancer cells [17]. It increases the endocytosis and degradation of $Na^+/K^+$-ATPase in LLC-PK1, human breast (BT20), and prostate (DU145) cancer cells and the expression of $Na^+/K^+$-ATPase dictates the growth of regulatory effects of ouabain on cells [18].

The fact that ouabain in nanomolar concentrations stimulates the increase of intracellular concentration of cAMP was shown in different tissues of mammals [19]. Our earlier work also indicated that $\alpha_3$ isoforms could serve as a gate for activation of intracellular signaling cascades. Nanomolar concentration of ouabain elevates intracellular cAMP content, the latter, in turn, stimulates $Na^+/Ca^{2+}$ exchange in reversal mode without affecting $Na^+/K^+$ pump activity [20]. It is known that in mammals blood constantly circulates endogen ouabain-like hormone in nanomolar concentration. It can be suggested that $\alpha_3$ isoforms by mentioned pathway could have a strong dehydration effect on cell by cAMP-dependent phosphorylation-induced contraction of cytoplasmatic filaments and cytoskeleton as well as by activation of electrogenic $Na^+/Ca^{2+}$ exchange in reverse mode, functioning in stoichiometry 3:1. Therefore, $\alpha_3$ isoform-dependent signaling system activation-induced cell dehydration could be responsible for nanomolar ouabain induced inhibition of proliferation. It is suggested that healthy cells by means of cGMP-dependent electroneutral $Ca^{2+}$ pump [21] in membrane are able to compensate $Ca^{2+}$ influx through $Na^+/Ca^{2+}$ exchange, but not its dehydration effect. As it is known there is a reciprocal relation between expression of $Na^+/Ca^{2+}$ exchange and $Na^+/K^+$ pump proteins in development [22]. Therefore, it is predictable that in early postnatal period cAMP-dependent $Na^+/Ca^{2+}$ exchange – cGMP-dependent $Ca^{2+}$ pump cascade could have pivotal role in regulation of cell hydration. While in maturation in norm this regulation is realized mainly through $Na^+/K^+$ pump.

It is known that in aging $Na^+/K^+$ pump dysfunction leads to weakening of $Na^+/Ca^{2+}$ exchange in forward mode. As a result an increase of intracellular $Ca^{2+}$ content takes place, having stronger inhibitory effect on $\alpha_3$ isoform due to its higher affinity to $Ca^{2+}$ [13,23]. Therefore, the working hypothesis in present work is that the dysfunction of $\alpha_3$ leads to the failure of intracellular signaling system responsible for cell adaptive function, which could be the primary reason for over hydration in cancer cells and abnormal proliferation.

With the purpose of testing this suggestion the comparative study of dose-dependent $^3$H-ouabain binding with membrane and cell hydration in healthy(H) and sarcoma-180 tumor carrying (SC) mice' different tissues, including the tumor tissue were studied, as well as these parameters' sensitivity to antitumor drug cisplatin (cisPt).

## Materials and Methods

### Chemicals

As a physiological solution (PS) Tyrode's solution of following composition (in mM): 137 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 5 $C_6H_{12}O_6$, 11.9 $NaHCO_3$, 0.42 $NaH_2PO_4$ was used adjusted to pH=7.4. All chemicals were obtained from "Medisar" Industrial Chemical Importation Company (Yerevan, Armenia).

Different doses of ouabain ($10^{-11}$–$10^{-6}$ M) were prepared on the basis of the same stock physiological solution. For experiments radioactive [$^3$H]-ouabain having 12 Ci/mM specific activity, and non radioactive one (both from PerkinElmer, Boston, MA, USA) was used.

During isotopic measurements scintillation mixture (Bray's scintillation solution) of following composition was used: 4 g PPO (2,5-diphenyloxazole), 0.2 g POPOP (1,4-bis[-2-(5-phenyloxazolyl)]-benzene), 200 ml 98% ethanol, brought to final volume of 1000 ml with p-dioxane ("Medisar" Industrial Chemical Importation Company (Yerevan, Armenia)).

### Animals

Exogamic (fusion from parents that are not closely related) white male mice with the average weight of 18-20g were used for the experiments. The animals were purchased from the Animal Resources Centre at the Life Sciences International Postgraduate Educational Center (LSIPEC, Yerevan, Armenia). All procedures performed on animals were carried out following the protocols approved by Animal Care and Use Committee of Life Sciences International Postgraduate Educational Center (LSIPEC, Yerevan, Armenia).

### Experimental setup

Exogamic male mice with the average weight of 18-20 g were used for the experiments. Animals were regularly examined, kept under control of the veterinary in LSIPEC and reserved in a specific pathogen free animal room under optimum conditions at temperature of 22 ± 2°C and were fed ad libitum on a standard lab chow and water.

Anticancer activity of cisPt was studied on the model of transplantable mice tumor – sarcoma-180 (Sarcoma-180, Crooker's sarcoma).

Tumor tissues removed from sarcoma-180 carrying animal were transplanted to experimental animals. The experiments consisted of two phases: at first sarcoma transplanted animals were studied, and then experiments on normal healthy mice were performed for each ouabain concentration. Animals were divided into groups (each group consisted of 10 mice): 1. control, healthy (H); 2. cisPt, healthy (H), 3. control, sarcoma carrying (SC) 4. cisPt, SC.

During 6 days each mouse in the groups 1 and 3 (after 48 hours of the tumor transplantation) was intraperitoneally injected with 0.5 ml PS, and in the groups 2 and 4 (after 48 hours of the tumor transplantation) they were intraperitoneally injected with 0.5ml (concentration 0.1 mg/ml) of Cisplatin (cisPt; Cisplatin Ebeve, Austria).

### Tissue preparation

Twenty-four hours after the last injections (PS or cisPt) each experimental animal was intraperitoneally injected with 0.5ml PS containing different concentrations of ouabain ($10^{-11}$ – $10^{-6}$M). The mice in control groups were injected with PS, not containing ouabain molecules. 30 minutes after this injection the animals were dissected, then organs were isolated. To avoid the anesthetic effect on initial cell hydration in present experiments [14] we preferred to use the sharp freezing method [23]. The experimental animals were immobilized by dipping their heads into liquid nitrogen (for 3-4 s) which led to the freezing of the heads and death of mice (Takahashi and Aprison, 1964).

At the full absence of animals' somatic reflexes upon the extra stimuli tissue samples were removed.

### Estimation of cell hydration

Determination of the water content of different tissues was performed by the traditional "tissue drying" method [23]. For the estimation of cell hydration, the tissue slices with similar shape and weight, after determination of wet mass (w.m.), were dried in thermostatically controlled oven (Factory of Medical Equipment, Odessa, Ukraine) for 24 hours at 105°C. The quantity of water in 1 g of dry mass (d. m.) of tissue was derived by the following equation: (w.m.–d.m.) / d.m. and expressed as water content g/g dry mass.

### Isotope measurement

Radioactive [$^3$H]-ouabain is usually used to estimate the number of $Na^+/K^+$ pump units in the membrane. It is supposed that each binding site in membrane binds one molecule of ouabain [13].

After 30 min of [$^3$H]-ouabain intraperitoneal injection (0.5 ml per mouse) the mice (n=10 in each experimental groups) were decapitated and different organs were removed. The mice in control groups were injected with PS, not containing ouabain molecules. Control and experimental tissue slices with similar shape and weight were removed. After that, the slices were washed threefold for 10, 5 and 5 min respectively to remove any radioactive ouabain absorbed by the intracellular spaces and not bound with receptors. Then, the tissue pieces were placed in special vials and each sample was homogenized in 50 µl 68% $HNO_3$ solution. Then Bray's scintillation fluid was added (2ml) and the radioactivity of mixture was counted by "Wallac 1450" liquid scintillation and luminescent counter (Finland production). The radioactivity of samples was calculated as counted per minute (CPM)/mg. The results were averaged on the basis of the weight of each individual tissue sample.

### Statistical analysis

The mean and standard error of tissue samples hydration index and [$^3$H]-ouabain binding in different samples was calculated and the statistical probability was determined by Student's paired t-test by means of computer program Sigma Plot (Version 8.02A, San Jose, CA, USA).

## Results

### Cell hydration and dose-dependent $^3$H-ouabain binding with membrane in different tissues of H and SC mice

The data on initial cell hydration of different tissues of H and SC mice are presented in Table 1. As these data indicate, the hydration of all investigated tissues in SC mice is higher than in H mice. These data allow us to consider the increase of cell hydration as a universal marker for cells pathology. Liver being the main organ having detoxification function in organism is predicted to have a comparatively higher (59%) cell hydration in SC mice.

The comparative study of dose-dependent (10-11-10-6 M) 3H-ouabain binding with cell membrane in H and SC mice as well as in sarcoma tumor tissue (ST) indicates that in healthy animals only in heart muscle 3 components of ouabain binding having different dose-dependent kinetics can be distinguished. In case of non excitable cells it is difficult to distinguish these components. While in case of SC animals in heart muscle the differences of dose-dependent kinetics of binding with receptors are slightly distinguished but in non-excitable tissues they become visible. These data can probably be explained by the fact that high affinity receptors in non excitable cells are localized deeper in membrane than low affinity ones and only cell hydration-induced swelling makes these receptors accessible for $^3$H-ouabain binding (Figure 1, only data on heart muscle and lung are presented as examples of excitable and non-excitable tissues, respectively). The curve of dose-dependent ouabain effect on cell hydration in both types of tissue is multi-component, which indicates on heterogeneity within the three distinguished α subunit isoforms.

| Organ | Healthy mice | Sarcoma carrying mice | Δ% |
|---|---|---|---|
| **Heart muscle** | 2.39 ± 0.1 | 3.3 ± 0.1 | ↑38.14 |
| **Skeletal muscle** | 2.36 ± 0.23 | 3.87 ± 0.21 | ↑64,23 |
| **Lung** | 2.2 ± 0.15 | 3.31 ± 0.03 | ↑50.47 |
| **Liver** | 2.05 ± 0.12 | 3.27 ± 0.15 | ↑58,93 |
| **Spleen** | 2.23 ± 0.13 | 2.8 ± 0.14 | ↑25.84 |
| **Kidney** | 2.05 ± 0.13 | 3.36 ± 0.19 | ↑63,76 |

**Table 1:** Tissue hydration of different organs of healthy and sarcoma-180 carrying mice.

To find out the individual role of different pump isoforms in determination of cell hydration in norm and pathology effects of $10^{-6}$ ($\alpha_1$), $10^{-8}$ ($\alpha_2$), and $10^{-11}$ M ($\alpha_3$) ouabain concentration on tissue hydration in H and SC animals were studied. Data presented in Table 2A indicate that in H mice $10^{-11}$ M ouabain injection has organo-specific effect on tissue hydration while $10^{-8}$ M injection has hydration effect on all investigated tissues. The same effect was more pronounced in all tissues (except heart and lung tissues) at $10^{-6}$ M concentration.

From these data it is clear that hydration effect observed at $10^{-8}$ and $10^{-6}$ M concentrations is due to pump inactivation. The reverse effect in heart muscle and lung probably can be explained by increase of muscle contractility. But this explanation is not reliable in case of $10^{-11}$ M.

In SC mice (Table 2B), the tissues of which had higher initial hydration compared to healthy ones, $10^{-11}$ M ouabain has dehydration effect on all investigated tissues (except lung and kidney) including ST while $10^{-8}$ and $10^{-6}$ M concentrations as in case of H mice (Table 2A), in all tissues (except heart muscle) led to increase of hydration. It is worth noting that in sarcoma tumor hydration was less sensitive to $10^{-6}$ M ouabain concentration than to $10^{-11}$ and $10^{-8}$ M concentrations. The pronounced dehydration effect of $10^{-11}$ M ouabain in SC mice and $10^{-8}$ and $10^{-6}$ M concentration hydration in both H and SC, indicate on existence of pathology-sensitive $\alpha_3$-receptors-dependent cell hydration controlling mechanism.

Study of $^3$H-ouabain binding with $\alpha_3$($10^{-11}$ M), $\alpha_2$($10^{-8}$ M) and $\alpha_1$($10^{-6}$ M) receptors in H and SC mice's tissues showed that though in SC mice all tissues were hydrated compared to H tissues (Table 2) $^3$H-ouabain binding with $\alpha_3$ receptors in muscle membranes was increased while in non-excitable tissues it was decreased (Table 3).

Such differences between excitable and non-excitable tissues were not observed at level of $\alpha_2$ and $\alpha_1$ receptors. It is worth to note that in liver tissue, where cell pathology-induced increase of hydration was more pronounced, ouabain binding with $\alpha_3$ receptors was significantly depressed. Observed opposite effects of cell pathology on ouabain binding with $\alpha_3$ receptors in excitable and non-excitable tissues are new

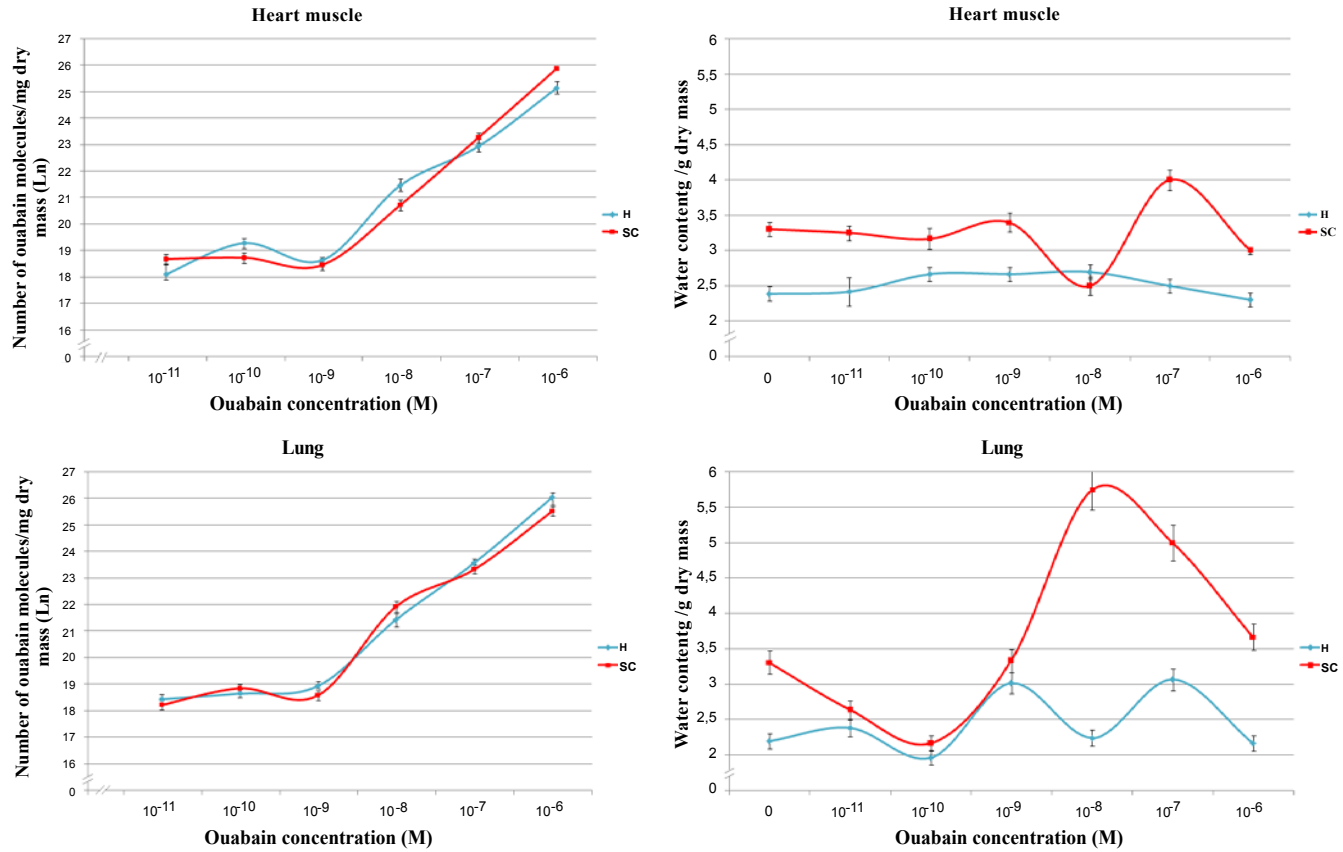


**Figure 1:** Dose-dependent ouabain effect on cell hydration (water content) (right side) and ouabain binding with membrane (left side) in healthy (H) and sarcoma-180 tumor carrying (SC) mice.

and extremely interesting from the point of view of understanding the pump dysfunction-induced activation of proliferation in non-excitable and activation of apoptosis in excitable cells. Presented data indicated that $\alpha_3$ receptors' affinity, as well as cell hydration dependent on these receptors are more sensitive to pathology.

To check this statement in the next series of experiments the effect of antitumor drug cisplatin (cisPt) on cell hydration and dose-dependent ouabain binding in H and SC mice's tissues was studied.

## CisPt effect on cell hydration and ouabain binding with membrane in H and SC mice's tissues

The data on the effect of cisPt (0.002 mg/g w.w animals) on tissue hydration in H mice which are presented in table 4 show that it has dehydration or hydration effect on cells depending on the type of tissue. But only in case of $10^{-6}$ M ouabain-injected animals ($Na^+/K^+$-pump inactivated) cisPt has specific hydration effect on excitable and dehydration effect on non-excitable tissues. These data indicate that cisPt has pump-independent specific hydration effect on excitable and dehydration effect on non-excitable cells.

The study of cisPt effect on different tissues' hydration in SC animals showed that (except heart muscle) it had dehydration effect on all tissues, including ST (Table 4B). The strongest dehydration effect was observed in kidney (33%), skeletal muscle (31%) and liver (24%). On ST cisPt had 15% dehydration effect.

As can be seen from presented data, $10^{-11}$ M ouabain in SC animals depressed the dehydration effect of cisPt, including ST. For example, in kidney dehydration effect even changed to hydration (6%). From these data it can be concluded that in SC animals $10^{-11}$ M ouabain and cisPt have dehydration effect on cell by different pathways.

At $10^{-8}$ M ouabain concentration cisPt in SC mice also had dehydration effect, except heart muscle. It is extremely interesting that at $10^{-6}$ M ouabain, when pump was inactive, cisPt had dehydration effect on both healthy and SC excitable tissues and dehydration effect on non-excitable ones. It is worth noting that cisPt dehydration effect on ST at $10^{-6}$ M was just 3%. The differences between cisPt effects in two types of cells can be explained by different nature of mechanism involved in cell volume regulation.

To find out which of $Na^+/K^+$ pump isoforms is responsible for cisPt-induced modulation of cell hydration in different tissues of H and SC animals the comparative study of cisPt effect on dose-dependent ouabain binding with cell membrane in tissues of H and SC animals was performed.

From the curves of dose-dependent ouabain binding with membrane of H tissues in absence and presence of cisPt (Figure 2 left side) and data obtained in SC animals (Figure 2 right side) can be seen that the dose-dependent ouabain binding with membrane in tissues of SC have significantly higher sensitivity to cisPt than H tissues. The differences of this sensitivity were pronounced in ouabain binding with

a)

| Healthy | Tissue hydration (water content g/g dry mass) | | | | | | |
|---|---|---|---|---|---|---|---|
| Organ | Ouabain concentration (M) | | | | | | |
| | 0 | $10^{-11}$ | Δ% | Ouabain $10^{-8}$M | Δ% | Ouabain $10^{-6}$M | Δ% |
| Heart muscle | 2.389 ± 0.1 | 2.422 ± 0.02 | ↑1.38 | 2.7 ± 0.26 | ↑13.02 | 2.306 ± 0.05 | ↓3.47 |
| Skeletal muscle | 2.357 ± 0.23 | 2.338 ± 0.12 | ↓0.8 | 3.361 ± 0.25 | ↑42.6 | 3.421 ± 0.3 | ↑45.14 |
| Lung | 2.199 ± 0.15 | 2.385 ± 0.15 | ↑8.4 | 2.243 ± 0.06 | ↑2.01 | 2.167 ± 0.3 | ↓1.46 |
| Liver | 2.054 ± 0.12 | 2.337 ± 0.22 | ↑13.78 | 2.547 ± 0.18 | ↑24.01 | 2.92 ± 0.17 | ↑42.16 |
| Spleen | 2.225 ± 0.13 | 1.567 ± 0.08 | ↓29.57 | 2.444 ± 0.25 | ↑9.84 | 3.147 ± 0.3 | ↑41.44 |
| Kidney | 2.05 ± 0.13 | 1.722 ± 0.08 | ↓16.04 | 2.878 ± 0.19 | ↑40.32 | 3.09 ± 0.15 | ↑50.66 |

b)

| Sarcoma carrying | Tissue hydration (water content g/g dry mass) | | | | | | |
|---|---|---|---|---|---|---|---|
| Organ | Ouabain concentration (M) | | | | | | |
| | 0 | $10^{-11}$ | Δ% | Ouabain $10^{-8}$M | Δ% | Ouabain $10^{-6}$M | Δ% |
| Heart muscle | 3.3 ± 0.1 | 3.25 ± 0.01 | ↓1.5 | 2.5 ± 0.01 | ↓24.24 | 3 ± 0.01 | ↓9.09 |
| Skeletal muscle | 3.87 ± 0.21 | 2.64 ± 0.14 | ↓31.78 | 4.63 ± 0.33 | ↑19.64 | 4.41 ± 0.23 | ↑13.95 |
| Lung | 3.31 ± 0.03 | 3.62 ± 0.21 | ↑9.37 | 5.75 ± 0.18 | ↑73.72 | 3.67 ± 0.23 | ↑10.88 |
| Liver | 3.265 ± 0.15 | 2.95 ± 2.27 | ↓9.64 | 3.88 ± 0.35 | ↑18.84 | 3.94 ± 0.22 | ↑20.67 |
| Spleen | 2.8 ± 0.14 | 2.7 ± 0.14 | ↓3.57 | 2.93 ± 0.33 | ↑4.64 | 3.14 ± 0.23 | ↑12.14 |
| Kidney | 3.357 ± 0.19 | 3.52 ± 0.16 | ↑4.86 | 3.56 ± 0.16 | ↑6.05 | 3.44 ± 0.16 | ↑20.47 |
| Sarcoma 180 | 4.8 ± 0.33 | 4.4 ± 0.4 | ↓8.33 | 5.66 ± 0.28 | ↑17.92 | 4.97 ± 0.32 | ↑3.54 |

**Table 2:** The effect of ouabain (at $10^{-11}$, $10^{-8}$ and $10^{-6}$M concentrations) on tissue hydration of different organs: **a)** healthy mice; **b)** sarcoma-180 carrying mice.

| Organ | Ouabain concentration (M) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^{-11}$ | | | $10^{-8}$ | | | $10^{-6}$ | | |
| | H | SC | Δ% | H | SC | Δ% | H | SC | Δ% |
| Heart muscle | 18.11 ± 0.4 | 18.67 ± 0.1 | ↑3.09 | 21.46 ± 0.64 | 20.72 ± 0.1 | ↓3.46 | 25.14 ± 0.49 | 25.87 ± 0.1 | ↑2.9 |
| Skeletal muscle | 16.65 ± 0.38 | 17.28 ± 0.39 | ↑3.79 | 18.81 ± 0.5 | 20.03 ± 0.87 | ↑6.52 | 23.75 ± 0.5 | 24.67 ± 0.26 | ↑3.87 |
| Lung | 18.45 ± 0.75 | 18.23 ± 0.59 | ↓1.21 | 21.44 ± 1.06 | 21.92 ± 0.29 | ↑2.24 | 26.04 ± 0.77 | 25.53 ± 0.29 | ↓1.99 |
| Liver | 17.39 ± 0.38 | 15.79 ± 0.48 | ↓9.16 | 18.77 ± 0.55 | 18.97 ± 0.35 | ↑1.08 | 24.25 ± 0.45 | 23.52 ± 0.08 | ↓3.02 |
| Spleen | 18.49 ± 0.63 | 17.32 ± 1.14 | ↓6.31 | 21.56 ± 0.46 | 20.64 ± 1.22 | ↓4.3 | 24.99 ± 0.48 | 25.19 ± 1.81 | ↑0.77 |
| Kidney | 17.06 ± 0.61 | 16.95 ± 0.43 | ↓0.66 | 19.94 ± 0.6 | 19.64 ± 0.09 | ↓1.5 | 24.51 ± 0.52 | 24.61 ± 0.66 | ↑0.42 |
| Sarcom-180 | | 16.64 ± 0.68 | | | 19.88 ± 0.75 | | | 24.4 ± 0.22 | |

**Table 3:** $10^{-11}$M, $10^{-8}$M and $10^{-6}$M ouabain binding with $\alpha_3$, $\alpha_2$ and $\alpha_1$ receptors in tissues of different organs of healthy (H) and sarcoma-180 carrying (SC) mice.

a)

| Organ | Ouabain concentration (M) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | $10^{-11}$ | | | $10^{-8}$ | | | $10^{-6}$ | | |
| | control | cisPt | Δ% | control | cisPt | Δ% | control | cisPt | Δ% | control | cisPt | Δ% |
| Heart muscle | 2.39 ± 0.1 | 1.93 ± 0.15 | ↓19.07 | 2.42 ± 0.02 | 1.38 ± 0.12 | ↓42.89 | 2.7 ± 0.26 | 1.99 ± 0.13 | ↓26.1 | 2.3 ± 0.05 | 2.47 ± 0.19 | ↑7.3 |
| Skeletal muscle | 2.36 ± 0.23 | 3.08 ± 0.21 | ↑30.47 | 2.34 ± 0.06 | 2.76 ± 0.12 | ↑18.23 | 3.36 ± 0.25 | 3.25 ± 0.29 | ↓3.2 | 3.42 ± 0.3 | 3.76 ± 0.27 | ↑9.95 |
| Lung | 2.2 ± 0.15 | 1.21 ± 0.11 | ↓45.06 | 2.39 ± 0.15 | 0.88 ± 0.11 | ↓63.17 | 2.24 ± 0.06 | 1.11 ± 0.12 | ↓50.5 | 2.17 ± 0.17 | 1.69 ± 0.16 | ↓22.15 |
| Liver | 2.05 ± 0.12 | 2.27 ± 0.12 | ↑10.24 | 2.34 ± 0.22 | 2.32 ± 0.19 | ↓0.74 | 2.55 ± 0.18 | 2.98 ± 0.2 | ↑17.2 | 2.92 ± 0.17 | 2.43 ± 0.17 | ↓16.94 |
| Spleen | 2.23 ± 0.13 | 2.13 ± 0.12 | ↓4.12 | 1.57 ± 0.17 | 1.6 ± 0.15 | ↑2.13 | 2.44 ± 0.13 | 1.55 ± 0.07 | ↓36.6 | 3.15 ± 0.26 | 1.75 ± 0.14 | ↓44.4 |
| Kidney | 2.05 ± 0.13 | 2.05 ± 0.12 | ↓0.12 | 1.72 ± 0.08 | 2.41 ± 0.13 | ↑39.85 | 2.88 ± 0.19 | 2.46 ± 0.18 | ↓14.6 | 3.09 ± 0.15 | 2.67 ± 0.19 | ↓13.73 |

b)

| Organ | Ouabain concentration (M) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | $10^{-11}$ | | | $10^{-8}$ | | | $10^{-6}$ | | |
| | control | cisPt | Δ% | control | cisPt | Δ% | control | cisPt | Δ% | control | cisPt | Δ% |
| **Heart muscle** | 3.3 ± 0.1 | 3.34 ± 0.08 | ↑1.3 | 3.25 ± 0.1 | 2.55 ± 0.07 | ↓21.54 | 2.5 ± 0.4 | 2.88 ± 0.15 | ↑15 | 3 ± 0.12 | 3.05 ± 0.18 | ↑1.67 |
| **Skeletal muscle** | 3.87 ± 0.21 | 4.07 ± 0.18 | ↓30.91 | 3.53 ± 0.14 | 4.78 ± 0.16 | ↑15.37 | 4.62 ± 0.33 | 4.74 ± 0.16 | ↓6.72 | 4.41 ± 0.23 | 4.81 ± 0.31 | ↑1.46 |
| **Lung** | 3.31 ± 0.03 | 2,83 ± 0.13 | ↓14.39 | 2.64 ± 0.21 | 3.1 ± 0.14 | ↑17.54 | 5.75 ± 0.18 | 3.25 ± 0.15 | ↓43.48 | 3.67 ± 0.15 | 2.5 ± 0.19 | ↓31.81 |
| **Liver** | 3.27 ± 0.15 | 2.4 ± 0.17 | ↓26.43 | 3.62 ± 0.27 | 2.98 ± 0.1 | ↓17.58 | 3.88 ± 0.35 | 3.58 ± 0.2 | ↓7.76 | 3.94 ± 0.15 | 3.92 ± 0.12 | ↓0.55 |
| **Spleen** | 2.8 ± 0.14 | 2.42 ± 0.12 | ↓13.69 | 2.95 ± 0.06 | 1.88 ± 0.19 | ↓36.54 | 2.93 ± 0.13 | 2.33 ± 0.01 | ↓20.33 | 3.14 ± 0.19 | 2.86 ± 0.2 | ↓8.98 |
| **Kidney** | 3.36 ± 0.19 | 2.25 ± 0.2 | ↓32.98 | 2.69 ± 0.16 | 2.85 ± 0.25 | ↑5.81 | 3.56 ± 0.16 | 3.17 ± 0.24 | ↓10.94 | 3.44 ± 0.15 | 2.88 ± 0.18 | ↓16.36 |
| **Sarcoma-180** | 4.8 ± 0.33 | 4.08 ± 0.35 | ↓15.16 | 4.41 ± 0.4 | 4.78 ± 0.15 | ↓8.37 | 5.66 ± 0.23 | 4.74 ± 0.23 | ↓16.25 | 4.98 ± 0.32 | 4.81 ± 0.36 | ↓3.25 |

**Table 4:** The effect of cisPt on tissue hydration of different organs: a) healthy mice; b) sarcoma-180 carrying mice.

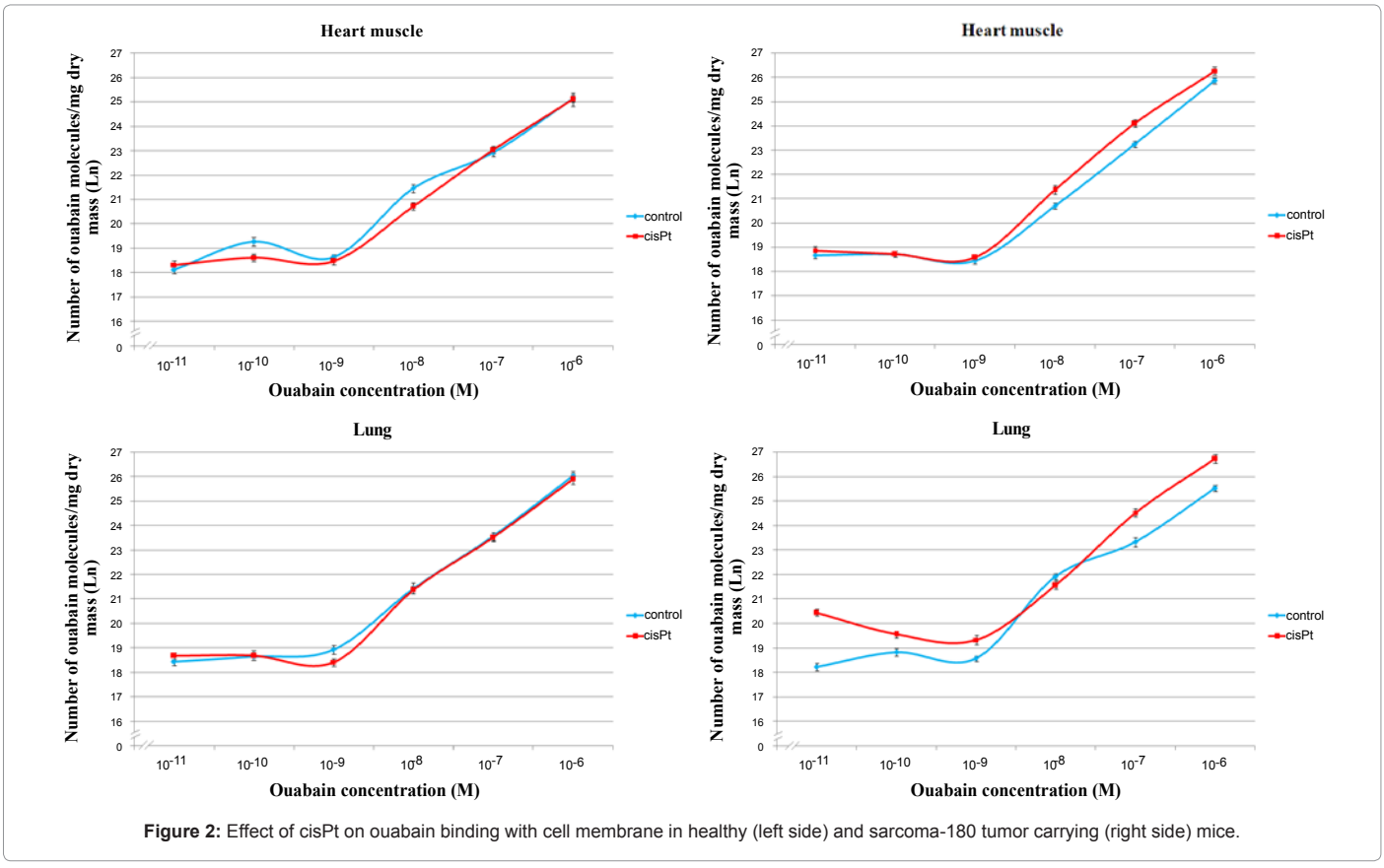


**Figure 2:** Effect of cisPt on ouabain binding with cell membrane in healthy (left side) and sarcoma-180 tumor carrying (right side) mice.

$\alpha_3$ receptors. In all studied excitable and non excitable tissues of SC animals, including ST (Figure 3), cisPt increased ouabain binding with membrane, but the most pronounced activation effect was expressed on ouabain binding with $\alpha_3$ receptors (except heart muscle tissue where the effect is pronounced on $\alpha_2$ and $\alpha_1$ receptors).

As can be seen from data presented in Table 5 cisPt basically had elevation effect on ouabain binding at $10^{-11}$ M and $10^{-6}$ M ouabain concentrations in H (Table 5A) and SC (Table 5B) mice. But this effect was more pronounced in SC, including ST at $10^{-11}$ M ouabain injection. At $10^{-8}$ cisPt had organ-specific effect on ouabain binding and had no effect on ST.

## Discussion

As it is known cell's over-hydration is responsible for abnormal proliferation. Data presented in this work have shown that in pathology (SC mice) hydration increases in all tissues. This indicates that cell hydration increase could serve as an extra-sensitive diagnostic parameter for cell pathology. As was noted above there are two metabolic cascades controlling osmotic gradient on membrane: ionic transport mechanism and intracellular signaling system, regulating adsorption properties of intracellular macromolecules and organs. The question is which of these two mechanisms is the one more sensitive to cell pathology the dysfunction of which leads to decontrolling of cell hydration and generating carcinogenesis.

It is known that there are 3 mammalian α subunit isoforms ($\alpha_1$ to $\alpha_3$) of $Na^+/K^+$ pump which are products of different genes but have ≈90% sequence identity, different expression patterns, and different kinetics, and they are differently regulated [9-12,13]. It is documented

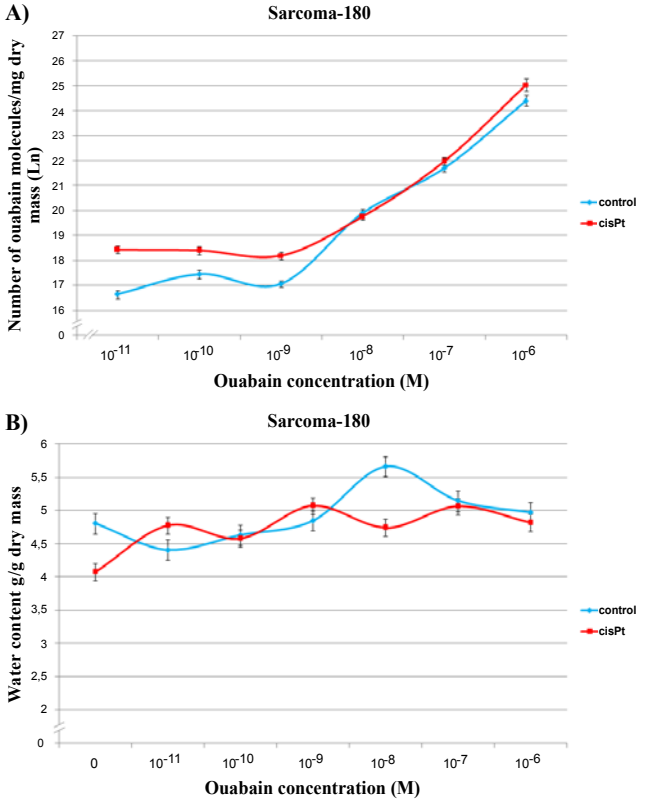


**Figure 3:** The effect of cisPt on ouabain binding with cell membrane of cancer tissue (number of ouabain molecules/mg dry mass (Ln)) (A) and the effect of cisPt on cell hydration of sarcoma-180 tissue (B).

that the low affinity isoforms ($\alpha_1$) have a "housekeeping" function: they control, primarily, $Na^+$ in bulk cytosol while the real function of $\alpha_2$ and $\alpha_3$ isoforms and their functional significance, are uncertain [15,23]. It is suggested that $\alpha_2$ and $\alpha_3$ may regulate $Na^+$ and, indirectly, $Ca^{2+}$ in a restricted cytosolic space between the cell membrane and reticulum. These isoforms may thereby modulate reticulum $Ca^{2+}$ content and $Ca^{2+}$ signaling [14]. By $\alpha_2$ pump isoform-induced activation of $Na^+/Ca^{2+}$ exchange in reversal mode, the generation of hypertension in mammals was explained [13].

A relationship between cancer and $Na^+/K^+$ pump $\alpha_3$ isoforms has been repeatedly documented: it is acquiring special interest, as accumulating evidence now indicates that the altered cellular ionic homeostasis may be involved in the abnormal cell proliferation that is a hallmark of the malignant transformation. The matter has aspects that are seemingly paradoxical. On one hand, the $Na^+/K^+$ pump $\alpha_3$ isoforms' dysfunction promotes cell proliferation in non- excitable cells. On the other hand, $Na^+/K^+$ pump inhibition in excitable cells triggers apoptotic death pathways. Somehow, therefore, tumor cells regulate the cell signaling machinery to promote proliferation while at the same time protecting themselves from apoptosis. A remodeling of $Na^+/K^+$ pump $\alpha_3$ and $\alpha_2$ isoforms is thus increasingly considered important in the process of malignant transformation and it is thus only to be expected that alterations of higher affinity to ouabain $Na^+/K^+$ pump isoform should have a role in the process of carcinogenesis [17]. It is known that $\alpha_3$ isoforms' function closely correlates with intracellular signaling systems [16,17,21]. Our previous data showed that nanomolar concentration of ouabain could stimulate cAMP dependent $Na+/Ca^{2+}$ exchange in reverse mode without changing $Na^+/K^+$ pump activity [20]. It is known that the energy for this transporting mechanism is the difference between electrochemical gradients of $Na^+$ and $Ca^{2+}$ ($\Delta E_{Na/Ca}=E_{Na}-E_{Ca}$). Therefore, low ouabain-induced activation of $Na^+/Ca^{2+}$ exchange in reverse mode can be explained by decrease of intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$) in result of its adsorption by intracellular structure. Obtained data indicate that $10^{-11}$ M ouabain has dehydration effect on tissues of SC mice, including ST, while the concentrations, which inhibit $Na^+/K^+$ pump ($10^{-8}$ and $10^{-6}$ M), have tissue hydration effect. This can be explained also by activation of electrogenic $Na^+/Ca^{2+}$ exchange in reverse mode, having dehydration effect on cells. This suggestion is in accordance with our recent data on nM concentration-induced heart muscle and brain tissue dehydration which is induced by activation of 45 $Ca^{2+}$ uptake through $Na+/Ca^{2+}$ exchange [23]. It is worth mentioning that as SC mice were in young age they still had high capacity of intracellular $Ca^{2+}$ buffering system because of which the $\alpha_3$ receptors activation could lead to cell hydration while in higher concentration ouabain-injected animals we had only hydration effect on tissues (Table 2B).

Data obtained in present work indicate that it is ouabain binding with $\alpha_3$ receptors that suffers most in cell pathology. Appearing of high affinity receptors in pathology in non-excitable tissues when the cells are over-hydrated correspond to our earlier data that the number of these receptors is more sensitive to hypotonic solution [2]. Such direct correlation between hydration and number of these receptors can be explained by Blaustein's data that $\alpha_3$ isoforms are localized deeper in membrane [12]. From this point of view probably in non-excitable tissues the localizing of these receptors is deeper than in excitable cells. It is obvious that for final explanation of these differences a more detailed investigation is needed.

Even though pathology leads to increase of cell hydration both in excitable and non-excitable tissues the correlation between cell hydration and number of binding $^3$H-ouabain with $\alpha_3$ receptors is opposite: i.e. in excitable tissues it increases, while in non-excitable ones it decreases. As the number of ouabain receptors increases by cell swelling in hypotonic medium [2], it is obvious that its decrease can be explained by decrease of receptors affinity to agonist, which can be increase of $[Ca^{2+}]_i$. It is suggestible that the elucidation of differences in signaling system of excitable and non-excitable cells would make clear the reason of $\alpha_3$ dysfunction-induced activation of apoptosis in excitable and proliferation in non-excitable cells (carcinogenesis).

It is widely known that the risk of different medical disorders including cancer increases with aging. Our previous data have shown that aging leads to inhibition of dose-dependent ouabain binding with $\alpha_3$ isoforms which is accompanied by inhibition of $Ca^{2+}$ efflux from the cell leading to accumulation of $[Ca^{2+}]_i$ [23-25]. Probably age-dependent dysfunction of $\alpha_3$ pump isoforms could be responsible for age-dependent increase of cancer risk. Moreover, as $\alpha_3$ isoform has much higher affinity to intracellular $Ca^{2+}$ than $\alpha_2$ and $\alpha_1$ isoforms [13], it is predictable that any pathology-induced intracellular accumulation of $Ca^{2+}$ ions could inhibit $\alpha_3$ function first of all.

The experimental results on antitumor drug cisPt effect on cell hydration and ouabain binding in H and SC animals demonstrated the higher cisPt-sensitivity of $\alpha_3$ receptors in cell pathology. Although cisPt is widely used in clinics, the detailed mechanism of its antitumor effect is not fully understood. It is widely recognized that antitumor effect of cisPt is realized by modification of DNA structure. However, the question of its effects on membrane transporting function as well as

a)

| Organ | Ouabain concentration (M) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^{-11}$ | | | $10^{-8}$ | | | $10^{-6}$ | | |
| | control | cisPt | Δ% | control | cisPt | Δ% | control | cisPt | Δ% |
| Heart muscle | 18.11 ± 0.41 | 18.33 ± 0.55 | ↑1.2 | 21.46 ± 0.64 | 20.72 ± 0.52 | ↓3.45 | 25.14 ± 0.49 | 25.11 ± 0.28 | ↓0.13 |
| Skeletal muscle | 16.65 ± 0.38 | 16.58 ± 0.83 | ↓0.4 | 18.8 ± 0.51 | 19.8 ± 0.28 | ↑5.3 | 23.75 ± 0.51 | 24.05 ± 0.68 | ↑1.25 |
| Lung | 18.45 ± 0.75 | 18.67 ± 0.33 | ↑1.21 | 21.44 ± 1.06 | 21.37 ± 0.53 | ↓0.33 | 26.04 ± 0.77 | 25.91 ± 0.22 | ↓0.5 |
| Liver | 17.39 ± 0.38 | 17.66 ± 0.68 | ↑1.54 | 18.77 ± 0.55 | 19.97 ± 0.38 | ↑6.38 | 24.25 ± 0.45 | 24.65 ± 0.55 | ↑1.64 |
| Spleen | 18.49 ± 0.63 | 18.69 ± 0.67 | ↑1.15 | 21.56 ± 0.46 | 21.04 ± 0.55 | ↓2.43 | 24.99 ± 0.48 | 25.43 ± 0.29 | ↑1.75 |
| Kidney | 17.06 ± 0.61 | 17.52 ± 0.18 | ↑2.69 | 19.94 ± 0.61 | 19.92 ± 0.15 | ↓0.1 | 23.75 ± 0.52 | 24.55 ± 0.42 | ↑0.26 |

b)

| Organ | Ouabain concentration (M) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^{-11}$ | | | $10^{-8}$ | | | $10^{-6}$ | | |
| | control | cisPt | Δ% | control | cisPt | Δ% | control | cisPt | Δ% |
| Heart muscle | 18.67 ± 0.11 | 18.88 ± 0.76 | ↑1.1 | 20.72 ± 0.11 | 21.37 ± 0.81 | ↑3.17 | 25.87 ± 0.11 | 26.27 ± 0.65 | ↑1.56 |
| Skeletal muscle | 17.28 ± 0.39 | 18.41 ± 0.74 | ↑6.49 | 20.03 ± 0.87 | 20.62 ± 0.25 | ↑2.9 | 24.67 ± 0.26 | 24.75 ± 0.42 | ↑0.33 |
| Lung | 18.23 ± 0.59 | 20.45 ± 0.35 | ↑12.18 | 21.92 ± 0.29 | 21.57 ± 0.61 | ↓1.62 | 25.52 ± 0.29 | 26.73 ± 0.81 | ↑4.74 |
| Liver | 15.79 ± 0.48 | 18.52 ± 0.73 | ↑17.26 | 18.97 ± 0.35 | 20.39 ± 0.23 | ↑7.46 | 23.52 ± 0.08 | 25.06 ± 0.44 | ↑6.54 |
| Spleen | 17.32 ± 1.14 | 19.81 ± 0.46 | ↑14.4 | 20.64 ± 1.22 | 21.22 ± 0.37 | ↑2.84 | 25.19 ± 1.81 | 26.41 ± 0.41 | ↑4.87 |
| Kidney | 16.95 ± 0.43 | 19.09 ± 0.81 | ↑12.67 | 19.64 ± 0.09 | 20.62 ± 0.32 | ↑5.08 | 24.61 ± 0.66 | 25.21 ± 0.69 | ↑2.45 |
| Sarcoma-180 | 16.64 ± 0.68 | 18.43 ± 0.54 | ↑10.76 | 19.88 ± 0.75 | 19.75 ± 0.34 | ↓0.66 | 24.41 ± 0.33 | 25.04 ± 0.26 | ↑2.63 |

**Table 5:** The effect of cisPt on ouabain binding with cell membrane (number of ouabain molecules/mg dry mass (Ln)) of different organs of a) healthy mice; b) sarcoma-180 carrying mice.

on intracellular signaling systems controlling cell hydration and cell proliferation is not sufficiently elucidated. Obtained data demonstrate that cisPt has dehydration effect on all tissues (except that of heart muscle) in SC animals, including sarcoma tumor (Table 4B). According to literature data on the close correlation between cell hydration and cell proliferation [3] cisPt-induced cell dehydration can be considered as one of the essential pathways through which its inhibition effect on cell proliferation in tumor tissues is realized. However, the nature of membrane mechanism as well as intracellular signaling system responsible for cisPt-induced cell dehydration remains unclear. Comparing cisPt effect on tissue hydration in H and SC animals it can be concluded that the target for cisPt should be a pump-independent cell volume regulating mechanism leading to hydration of excitable tissues (muscles) and dehydration of non-excitable tissues (lung, liver, spleen, kidney, sarcoma). A potential candidate for such a mechanism could be also Na+/$Ca^{2+}$ exchange in forward and reverse modes, respectively. However, to prove this suggestion the study of $Na^+/Ca^{2+}$ exchange in these tissues is necessary and is the subject for our current study. The disappearance of differences of cisPt effect on tissue hydration at $10^{-6}$ M ouabain concentration in H and SC animals could serve as strong evidence that these differences are due to different initial activity of $Na^+/K^+$ pump i.e. because in H mice's tissues pump is active while in SC and tumor tissue pump is inactivated.

The fact that cisPt, having dehydration effect on cells has an elevation effect on ouabain binding with cells membrane in all tissues of SC animals indicates that it has increasing effect on receptors affinity to ouabain (Figure 2 and Table 5). From the data on cisPt effect on dose-dependent binding of 3H-ouabain with cell membrane of H and SC animals (Figure 2) it can be concluded that the effect of cisPt on receptors affinity depends also on initial functional activity of cell, probably on cell hydration. For example, in muscles of H animals it has depressing effect on receptors affinity while in SC animals it has elevation effect on these receptors' affinity. It is worth to note that cisPt effect on $\alpha_3$ receptors in SC animals, especially in sarcoma tumor, was more pronounced. The fact that the elevation effect of cisPt on the affinity of ouabain receptors appeared at the level of $\alpha_1$ also, (Figure 2) when the $Na^+/K^+$ pump was in inactive state (at $10^{-6}$ M ouabain), indicates on pump independency of this effect.

Thus, all the above presented data bring us to the following conclusions:

- Cell hydration, extra sensitive to pathological changes of cells, could serve as a universal diagnostic parameter for cell pathology;
- $\alpha_3$-dependent intracellular signaling system controlling cell hydration is a primary mechanism dysfunction of which causes decontrolling of cell hydration and activation of abnormal proliferation in non-excitable cells leading to carcinogenesis;
- $\alpha_3$-dependent intracellular signaling system controlling cell hydration in excitable and non-excitable tissues has different nature.
- $\alpha_3$ isoform serves as a target of antitumor drag - cisPt, which leads to increase of $\alpha_3$ isoform's affinity to ouabain;
- Taking together the fact that in mammalian blood endogen ouabain-like species in nM concentration constantly circulate, and the data presented on low ouabain dehydration effect on tissues, including ST (Table 2B), it can be concluded that the deficit of the endogen ouabain could be a promotional factor for cell over-hydration and carcinogenesis.

Thus, presented results give new insight into the role of $\alpha_3$-dependent cell hydration controlling signaling system in the generation of cell pathologies, including carcinogenesis. Further detailed elucidation of

the mechanism of this intracellular signaling system could provide us with new avenues for cancer diagnosis and treatment.

### References

1. Ayrapetyan SN (1980) On the Physiological Significance of Pump Induced Cell Volume Changes. Adv Physiol Sci 23: 67-82.
2. Ayrapetyan SN, Suleymanyan MA, Saghyan AA, Dadalyan SS (1984) Autoregulation of the Electrogenic Sodium Pump. Cell Mol Neurobiol 4: 367-383.
3. Haussinger D (1996) The role of cellular hydration in the regulation of cell function. Biochem J 313: 697-710.
4. Lang F (2007) Mechanisms and significance of cell volume regulation. J Am Coll Nutr 26: 613S-623S.
5. Parsegian VA (2005) Van der Waals Forces: A Handbook for Biologists, Chemists, Engineers, and Physicists. Cambridge University Press, Cambridge, UK.
6. Damadian R, Minkoff L, Goldsmith M, Stanford M, Koutcher J (1976) Field Focusing Nuclear Magnetic Resonance (FONAR): Visualization of a Tumor in a Live Animal. Science 194: 1430-1432.
7. Damadian R, US Patent 3,789,832, field 17 March 1972.
8. Kostyuk PG, Mironov SL, Doroshenko PA (1982) Energy profile of Ca channel in the membrane of mollusc neurons. J Membrane Biol 70: 181-189.
9. Baumgarten CM (2006) Cell volume regulation in cardiac myocytes: a leaky boat gets a new bilge pump. J Gen Physiol 128: 487-489.
10. Takeuchi A, Tatsumi S, Sarai N, Terashima K, Matsuoka S, et al. (2006) Ionic Mechanisms of cardiac cell swelling induced by blocking Na+/K+ pump as revealed by experiments and simulation. J Gen Physiol 128: 495-507.
11. Blaustein NP, Lederer WJ (1999) Sodium/calcium exchange: Its physiological implications. Physiol Rev 79: 763–854.
12. Juhaszova M, Blaustein MP (1997) $Na^+$ pump low and high ouabain affinity alpha subunit isoforms are differently distributed in cells. Proc Natl Acad Sci U S A 94: 1800-1805.
13. Baker PF, Blaustein MP, Hodgkin AL, Steinhardt SA (1969) The influence of calcium on sodium efflux in squid axons. J Physiol 200: 431-458.
14. Adams RJ, Schwartz A, Grupp G, Grupp I, Lee SW, et al. (1982) High-affinity ouabain binding site and low-dose positive inotropic effect in rat myocardium. Nature 296: 167–169.
15. Lucchesi PA, Sweadner KJ (1991) Postnatal changes in Na,K-ATPase isoform expression in rat cardiac ventricle. Conservation of biphasic ouabain affinity. J Biol Chem 15: 9327–9331.
16. Xie Z, Askari A (2002) Na(+)/K(+)-ATPase as a signal transducer. Eur J Biochem 269: 2434-2439.
17. Kometiani P, Liu L, Askari A (2005) Digitalis-induced signaling by $Na^+/K^+$-ATPase in human breast cancer cells. Mol Pharmacol 67: 929-936.
18. Harwood S, Yaqoob M (2005) Ouabain-induced cell signaling. Front Biosci 10: 2011-2017.
19. Tian L, Hires SA, Mao T, Huber D, Chiappe ME, et al. (2009) Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nat Methods 6: 875-881.
20. Saghyan AA, Avetisian TH, Ayrapetyan S (1996) The pH-dependence of magnetic field 2 (MF) induced changes of Ca-uptake by Helix Neurons. Radiobiology and Radioecology. (5), Moscow, 714-717.
21. Blaustein MP, Zhang J, Chen L, Song H, Raina H, et al. (2009) The pump, the exchanger, and endogenous ouabain: signalling mechanisms that link salt retension to hypertension. Hypertension 53: 291-298.
22. Brini M, Carafoli E (2011) Calcium signaling and disease: Preface. Biofactors 37:131.
23. Heqimyan A, Narinyan L, Nikoghosyan A, Deghoyan A, Yeganyan L, et al. (2012) Age-dependency of high affinity ouabain receptors and their magneto sensitivity. Environmentalist J 32: 228-235.
24. Ayrapetyan SN (2012) Cell hydration as a universal marker for detection of environmental pollution. Environmentalist J 32: 210-221.
25. Narinyan L, Ayrapetyan G, Ayrapetyan S (2012) Age-dependent magneto-sensitivity of heart muscle hydration. Bioelectromagnetics 33: 452-458.