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ORIGINAL PAPER

Investigation of extracellular medium osmolality depending on zinc application and incubation time on A549 cancer cells

Duygu Tarhan[1] · Nural Pastaci Özsobaci[1] · Dilek Düzgün Ergün[2] · Alev Meltem Ercan[1]

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Abstract
Changes in the osmolality of the extracellular medium (ECM) affect cell volume and cellular processes such as cell migration and proliferation. Not only may high concentrations of zinc (Zn) lead to cell death by apoptosis, but Zn is also a physiological suppressor of apoptosis. The aim of our study was to examine whether Zn and regulation of extracellular osmolality had an effect on the lung cancer cell line (A549) and how to be changed in ECM according to elements and osmolality depending on incubation time and Zn application. Our study consisted of four groups: cell-free medium, ECM of cancer cell after 24 h incubation (24hECM), ECM of cancer cell after 48 h incubation (48hECM), and ECM of cancer cell after 48 h incubation with $ZnCl_2$ (48hECM + Zn). ECM osmolality was measured by using osmometer, and the levels of chromium (Cr), iron (Fe), and magnesium (Mg) elements were analyzed using ICP-OES device for all groups. According to the result of the analysis, a statistically significant difference was found when osmolality and element values of ECM of 24hECM and 48hECM groups were compared with the values of the 48hECM + Zn group. It was observed that there was a decrease in the levels of Cr, Fe, and Mg with Zn application and incubation period in ECM. The regulation of ECM osmolality is a promising method due to biophysical effects on cancer cells. In our study, we speculated that the understanding of the effects of Zn and osmolality with the relationship between ECM and cancer cell might lead to the discovery of biophysical approaches as a novel therapeutic strategy.

Keywords A549 · Osmolality · Zinc · Extracellular medium · IC50

✉ Duygu Tarhan
duygu.tarhan@iuc.edu.tr

[1] Cerrahpasa Faculty of Medicine, Department of Biophysics, Istanbul University-Cerrahpasa, Istanbul, Turkey

[2] Faculty of Medicine, Department of Biophysics, Istanbul Aydin University, Istanbul, Turkey

1 Introduction

Extracellular and intracellular fluid compartments constitute a large part of the organism. The extracellular medium contains various proteins, polysaccharides, and macro- and micro-molecules [1–3]. The osmotic pressure of the extracellular medium changes due to the exchange of substances between the inside and outside of the cell [4]. Most fundamentally, osmotic pressure is determined by the number of particles of solutes. The movement of water from a medium with a low dissolved substance concentration to a medium with high substance concentration is called osmosis [5]. The osmosis ability and osmotic pressure of solutions are measured by osmol (Osm). Osmolality, the number of osmoles in one kilogram of water, can be assessed for understanding osmotic pressure. If a substance is soluble in water, the boiling point and osmotic pressure of the water increase as the freezing point and vapor pressure of the water decrease [3–7]. These properties of the solution are known as colligative properties and are related to the osmotic pressure determined by the total number of particles in the solution. Osmotic pressure measurement with the osmometer is carried out using any of the colligative properties [5]. The most widely used method in physiological fluids is the measurement of the osmotic pressure at the freezing point of the fluid. The concept of osmolality, in which the laws of physics are used, is very important for people whose body consists of 60% liquid part [5–7]. In a healthy person, there is an osmotic balance between intracellular and extracellular fluids [8]. Besides, changes in the osmolality of the extracellular medium affect cell volume and cellular processes such as cell migration and proliferation [9–11]. At the same time, the concentration of micro- and macro-elements in the extracellular medium changes throughout the vital functions of the cell.

Zinc (Zn), chromium (Cr), iron (Fe), and magnesium (Mg) are very important micro- and macro-elements for the cell [12, 13]. Cr is required for insulin activity and also may affect the amount of energy for cell [14]. Fe is a physiologically essential metal, because it plays a role in the structures of many enzymes (NADH dehydrogenase, peroxidase, tryptophan pyrrolase, catalase, guanylate cyclase, and nitric oxide synthase) and serves as the carrier for oxygen [12, 14, 15]. Moreover, Mg is an important macro-element served as the cofactor for many enzymes (i.e., glucokinase, phosphomevalonate, hexokinase, mevalonate kinase, squalene synthase) [16, 17]. In addition to all this, Zn has an important role in cell division, cell growth, differentiation, and development. It also helps to maintain intracellular ion homeostasis and essential in members of signaling molecules, enzymes, and transcription factors [12, 18]. On the other hand, Zn can be toxic and plays a role in the regulation of apoptosis in cancer cells depending on the dose amount and incubation time [19]. The aim of our study was to examine how to be changed in the extracellular medium according to micro–macro elements and osmolality depending on incubation time and Zn application. By investigating these changes in the extracellular medium, it is a study that we would like to show that extracellular medium is very important in obtaining information about cell behavior as much as an intracellular medium. In addition, our study is a novel therapeutic strategy by examination of the effects of zinc and osmolality on the relationship between extracellular medium and cancer cells.

2 Material and methods

2.1 Cell line and culture conditions

Human non-small-cell lung cancer cell line A549 were grown in completed RPMI 1640 medium (PAN Biotech, Germany; Lot: 4,200,319). RPMI 1640 medium contains 2 mM L-glutamine, 1.5 g/L $NaHCO_3$, 10 mM HEPES, 4.5 g/L glucose, and 1 mM Sodium pyruvate. For obtaining completed RPMI 1640 medium (cRPMI), RPMI 1640 medium is supplemented with 10% fetal bovine serum (FBS; Cegrogen Biotech, Germany; Lot: C1515) and 1% penicillin–streptomycin (Gibco, Grand Island, USA; Lot: 2,058,871). Cell cultures were kept in a humidified incubator maintained at 37 °C and 5% CO_2. The medium was replaced every 48 h. The cells were trypsinized (Gibco, Grand Island, USA; Lot: 2,063,511) and reduced once 80% confluence was obtained. Equal numbers of cells for each group were seeded into 6 sterile wells (3×10^5 cells per well). All processes were repeated 6 times. $ZnCl_2$ (Merck, Germany; Lot: B0974516344) in powder form was dissolved in cRPMI medium. 2 ml of fresh cRPMI medium or Zn + cRPMI medium was added to the cells seeded in the wells according to groups.

2.2 Determination of cell number and viability

After the A549 cell culture in the carbon dioxide incubator was taken into the laminar flow cabinet under sterile conditions, the cells were removed from the surface with Trypsin–EDTA and collected in a 15-mL centrifuge tube. After centrifugation at 1200 rpm for 5 min at 37 °C, the supernatant was aspirated, and a fresh medium was placed on the cell pellet at the bottom and pipetted at least 20 times with a sterile automatic pipette. The cell suspension transferred into a sterile 1.5-mL Eppendorf tube in a laminar flow cabinet was mixed with 0.4% trypan blue (Amresco, Solon, Ohio; Lot: 2275C214) at a ratio of 1:1 and left for 5 min. Thoma slides (Western Germany; 0.100 mm, 0.0025 mm^2) and coverslips (Isolab, Germany; 24 × 24 mm) were sterilized with 70% alcohol before counting. With the help of a sterile automatic pipette, the sample taken from the homogeneous mixture of cells and trypan blue was carefully spread between the slide and coverslip. When viewed under a light microscope (Leitz-Diavert, Germany), the Thoma slide consists of 16 large squares, each of which is 5 × 5 square. The total number of cells was calculated by counting the cells (N) in 16 large squares on the Thoma slide with a microscope. Since the membrane integrity and permeability of living cells are not impaired, trypan blue cannot enter into the cell. Based on this feature, when viewed under a microscope, dead cells were stained as blue, while living cells were unstained. Accordingly, the calculation of % vitality was calculated according to the formula below.

$$Number\ of\ cell/ml = N \times 10^4 \times Dilution\ factor$$

$$Total\ number\ of\ cell = (Number\ of\ cell/ml) \times Total\ volume$$

$$Cell\ viability(\%) = (Number\ of\ living\ cell/Total\ number\ of\ cell) \times 100$$

2.3 Determination of half maximal inhibitory concentration (IC50)

It was aimed to find the most effective dose and duration of application for the Zn element to be applied to the A549 lung cancer cell line. In this direction, cells were planted in sterile 6 wells with equal and 3×10^5 cells in each well, and after a 24-h incubation period with a fresh medium, the doses were applied as 0, 5, 10, 20, 50, 100, 200, and 300 μM $ZnCl_2$.

Different doses of Zn groups were incubated for 24, 48, and 72 h as three separate sets. Each incubation process was repeated three times while determining the effective application time. At the end of the incubation period, the viability of the cells was calculated using trypan blue. According to the data obtained, the half-maximum inhibitory concentration (IC50) values for the applied dose amounts of Zn were calculated separately for all three incubation periods (24, 48, and 72 h) using the GraphPad Prism 8.3.1 (GraphPad Software, Inc., San Diego, USA) (Table 1).

2.4 Application of Zn

According to the experimental protocol, Zn-cRPMI-1640 medium solution was obtained by dissolving the Zn element in the powder form of zinc chloride ($ZnCl_2$) and in cRPMI-1640 medium was applied to the A549 cells. After 24 h of incubation of cell, the medium was replaced with cRPMI-1640 containing fresh 61-μM $ZnCl_2$ and incubated for 48 h under standard cell culture conditions (37 °C and 5% CO_2).

2.5 Study design

Our study on A549 lung cancer cell line consists of four groups: Group 1, cell-free medium (Medium; cRPMI medium; M); Group 2, extracellular medium (ECM) of cancer cell after 24 h incubation (24hECM); Group 3, extracellular medium of cancer cell after 48 h incubation (48hECM); and Group 4, extracellular medium of cancer cell after 48 h incubation with $ZnCl_2$ (48hECM + Zn).

2.6 Osmolality measurement

At the end of the incubation period of each group, the extracellular mediums were transferred to sterile Eppendorfs with the help of sterile pipettes. Standard solutions (0–400 mOsm/kg) (KNAUER, Berlin, Germany; Lot: Y124118H1) were used before osmolality measurements. Osmolality measurements of extracellular media (150 μL) were measured immediately after the incubation period. The osmolarity of each medium was measured using freezing point depression with a Semi-Micro Osmometer (KNAUER, Berlin, Germany; 7400).

Table 1 IC50 values of $ZnCl_2$ in A549 cells

	24 h		48 h		72 h	
Zn	IC50 (μM)	R^2	IC50 (μM)	R^2	IC50 (μM)	R^2
	128.1	0.440	61	0.300	51.68	0.361

For 24 h, 48 h, and 72 h incubation times; IC50: the half-maximum inhibitory concentration

2.7 The element analysis of extracellular medium

The analysis of Cr, Fe and Mg elements has been carried out by utilizing an inductively coupled plasma-optical emission spectrophotometer (ICP-OES, England; Thermo iCAP 6000series) at Trace Element Analysis Laboratory at Biophysics Department, Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa. The parameters of ICP-OES device are presented in Table 2. Extracellular medium samples were kept at − 80 °C until measurements and were diluted (1:5) with distilled water in sterile tubes by using an automatic pipette.

Standard solutions of Cr, Fe, and Mg elements were prepared from proper stock solutions containing 1000 ppm (mg/L) (Inorganic Ventures, USA; Cr Solution Lot: N2-CR668253; Fe Solution Lot: N2-FE667623; Mg Solution Lot: M2-MG658972). Distilled water was used as blank solution. Test solutions containing 2000 mg L-1 for each element (Thermo Fisher Scientific; Cambridge; Lot: 10-21GS) were used for the quality assurance of ICP-OES analysis. In the study, the appropriate wavelengths of Cr (267.716 nm), Fe (259.940 nm), and Mg (285.213 nm) elements were used to analyze by the ICP-OES device. The three point calibration graph was obtained from the ICP-OES device using blank and standard solutions. Linear and reproducible calibration curves were obtained for analysis and the recovery of the analyzed quality control was between 98.4% and 102% (Table 3). The trace element analyses of the prepared extracellular medium samples were carried out. Element levels were expressed as mg/L for extracellular medium samples.

2.8 Statistical analysis

Statistical analysis was performed with the software package SPSS 21 program for Windows. All data were expressed as means ± standard deviation (SD). The values of $p < 0.05$ were considered statistically significant. One-way ANOVA test, which is a parametric test, was applied for datasets (osmolality, Cr, Fe, and Mg) with normal distribution as a result of the normality test. When post hoc analysis of variance was performed in the one-way ANOVA test, Tukey test was applied for homogeneous variance (for Cr and Fe datasets), and Tamhane's T2 test was applied for non-homogeneous variance (for osmolality and Mg datasets). On the other hand, the Pearson correlation test were used for the determination of correlation between osmolality values and elements levels. All IC50 values were done by using GraphPad Prism.

Table 2 ICP-OES device parameters for element analysis

Parameters	Assigned value
Plasma gas flow rate	15 L/min
Argon carrier flow rate	0.5 L/min
Sample flow rate	1.51 L/min
Flush pump rate	100 rpm
RF Power	1150 W
Nebulizer flow	0.70 L/min
Analysis pump rate	50 rpm
Pump relaxation time	5 s

ICP-OES inductively coupled plasma-optical emission spectrophotometer

Table 3 The results of method validation for Cr, Fe, and Mg elements

Elements	QC	LOD (mg/L)	LOQ (mg/L)	Correlation coefficient	Expected concentration (mg/L)	Measured concentration ($n=3$) (mg/L)	Precision (%RSD)	Recovery (%)
Cr	QC-1	0.003	0.008	0.9999	0.500	0.495	0.967	99
	QC-2				1.000	1.005	1.771	100.5
Fe	QC-1	0.014	0.041	0.9998	0.500	0.510	2.088	102
	QC-2				1.000	0.990	1.030	99
Mg	QC-1	0.000	0.001	0.9998	0.500	0.492	1.268	98.4
	QC-2				1.000	1.008	0.292	100.8

QC quality control, *LOD* limit of detection, *LOQ* limit of quantitation, *RSD* relative standard deviation

ICP-OES inductively coupled plasma-optical emission spectrophotometer, *Cr* chromium, *Fe* iron, *Mg* magnesium

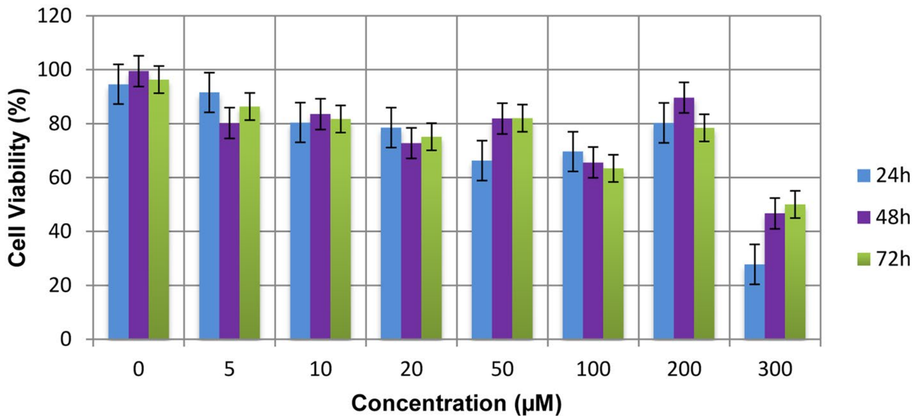

Fig. 1 The cell viability (%) of A549 cell line for $ZnCl_2$ (0, 5, 10, 20, 50, 100, 200, 300 μM) in the different incubation time (24 h, 48 h, 72 h)

3 Results

In our study, the results of cell viability (%) were obtained for $ZnCl_2$ on A549 cancer cells according to different doses (0, 5, 10, 20, 50, 100, 200, 300 μM) and different incubation times (24 h, 48, 72 h) (Fig. 1). IC50 values were calculated separately for the 24 h, 48 h, and 72 h incubations for Zn application (Table 1). For the Zn application in the study groups, 61 μM $ZnCl_2$ was applied according to the IC50 value in 48 h of incubation.

After 24 h of incubation of A549 cells (24hECM), the osmolality value of the extracellular medium was statistically higher ($p < 0.001$) than the osmolality value in the cell-free medium (M; cRPMI). On the other hand, after 48 h of incubation of the cells, the osmolality values of the Zn applied (48hECM + Zn) ($p < 0.001$) and non-applied (48hECM) ($p < 0.01$) groups were statistically significantly lower than that of M group (Table 4).

There was a numerical decrease in the Cr values of ECM when the values of the cell-free medium were compared with the other groups. However, the Cr value of only the 48hECM + Zn group was statistically significantly lower than the Cr value of cRPMI ($p < 0.05$). Likewise, there was a numerical decrease in the Mg values when the 24-h ECM

Table 4 The Osmolality, Cr, Fe, and Mg values of the extracellular medium of A549 cells

Groups	Osmolality (mOsm/kg)	Cr (mg/L)	Fe (mg/L)	Mg (mg/L)
M	318.67 ± 2.422	0.0140 ± 0.008	0.177 ± 0.033	5.428 ± 0.358
24hECM	398.50 ± 7.064***	0.0138 ± 0.015	0.139 ± 0.060	6.928 ± 2.965
48hECM	266.00 ± 23.03**	0.0068 ± 0.001	0.040 ± 0.014***	4.785 ± 1.316
48hECM + Zn	179.33 ± 5.279***	0.0045 ± 0.002*	0.020 ± 0.008***	4.631 ± 0.223*

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Cr chromium, *Fe* iron, *Mg* magnesium

All data is means ± standard deviation (M ± S.D.)

M:Cell-free medium, cRPMI; 24hECM: Extracellular medium of cancer cell after 24 h incubation

48hECM: Extracellular medium of cancer cell after 48 h incubation

48hECM + Zn: Extracellular medium of cancer cell after 48 h incubation with 61 μM $ZnCl_2$

and 48-h ECM groups were compared with cRPMI, with the increase in the incubation time of A549 cells. However, this decrease was not statistically significant. On the other hand, in the Mg values, after 48 h of incubation (48hECM + Zn) with Zn application, there was a statistically significant decrease compared to cRPMI ($p < 0.05$). When we evaluated the Fe values, another important trace element for ECM and cells, there was a statistically significant decrease when 48-h ECM and 48-h ECM + Zn groups were compared with cRPMI ($p < 0.001$). However, although the Fe values of the 24hECM group were lower than the Fe value of cRPMI, it was not statistically significant (Table 4). In addition to these, the values of osmolality were positively correlated with the Cr values (r: 0.549; $p < 0.01$), the Fe values (r: 0.670; $p < 0.001$), and the Mg values (r: 0.561; $p < 0.01$) (r: correlation coefficients).

4 Discussion and conclusions

Osmolality is the concentration of a solution as osmoles per kilogram of water. In dilute solutions such as extracellular and intracellular fluids in the body, it is a crucial parameter. The small changes in the concentrations of the extracellular medium can lead to large osmotic pressures across the cell membrane relatively [4–6]. An impermanent solute (that will not permeate the cell membrane) for each milliosmole concentration gradient is exerted across the cell membrane about 19.3 mm Hg of osmotic pressure. There is an osmotic balance between the extracellular and intracellular mediums in normal conditions [5]. A force can move water across the cell membrane when the extracellular and intracellular mediums are not in osmotic balance. As a result of these forces, the small changes in the concentration of impermanent solutes in the extracellular medium can cause large changes in cell volume and structure. It is known that the factors that can cause extracellular and intracellular volumes to change markedly are excess or loss of amounts of fluid [5, 8].

The cell mediums can be classified into three as isotonic, hypertonic, and hypotonic according to the differences between the solute amounts of the medium [5]. If the extracellular medium is an isotonic solution, there is an osmotic equilibrium between the intracellular and extracellular compartments of the cell, and no osmosis occurs between the intracellular and extracellular compartments. If the extracellular medium is a hypertonic solution, the osmolality of the extracellular medium increases. Therefore, osmosis occurs water out from the cell to the extracellular medium, and the cell shrinks. On the other hand, if the extracellular medium is a hypotonic solution, osmolality decreases and water flows into the cell from the extracellular medium (the cell swells) [4–7]. It is known that the extracellular medium contains various proteins, polysaccharides, and macro- and micro-molecules [3]. Therefore, the osmolality of the extracellular medium changes due to the transport between the inside and outside of the cell [4]. Cell membrane structure, the preservation of the integrity of the cell membrane, membrane permeability, cell volume, and transport mechanisms and the balanced relationship between the intracellular and extracellular mediums of cells are very important biophysically. Therefore, the osmolality of the extracellular medium gives us very important information about the structure of the cell, such as the cell membrane, transport mechanisms, and cell volume. In our study, we examined the changes in the extracellular medium osmolality of both according to the incubation times of cancer cells and the IC50 value of Zn applied. We consider that due to the changes in the extracellular osmolality, we could obtain information about the intracellular and cell membrane

transport systems and the cell membrane. According to our osmolality results (Table 4), the osmolality of the extracellular medium (24-h ECM: 398.50 $\pm$ 7.064 mOsm/kg) after 24 h of incubation of A549 cancer cells was higher than that of cRPMI (cell-free medium, M: 318.67 $\pm$ 2.422 mOsm/kg) ($p < 0.001$). However, after 48 h of incubation, the osmolality of the extracellular medium of A549 cancer cells (48-h ECM: 266.00 $\pm$ 23.03 mOsm/kg) was lower than that of 24-h ECM and cRPMI ($p < 0.01$). A549 cells may have caused the extracellular medium to become hypertonic by transferring substances to the extracellular medium during the first 24 h of incubation or by increasing the passage of water from the medium into the cell. While the anabolic and catabolic activities of the cells continue, the 48-h incubation period shows that the extracellular medium has become hypotonic with the passage of substances into the cell. In the case of application of Zn with IC50 value (48hECM + Zn: 179.33 $\pm$ 5.279 mOsm/kg), it increased the substance transfer into the cell, further reducing the osmolality of the extracellular medium and rendering it hypotonic. In this case, we may consider that the incubation period of A549 cancer cells may be sufficient for the amount of Zn we applied during the 48-h incubation period in order to disrupt the cell membrane integrity of the A549 cancer cells due to decreasing in the osmolality of the extracellular medium. We estimate that apoptotic cell markers and morphological examinations should be performed after 48 h of Zn-applied incubation to more clearly explain the effect of Zn in the cell. However, the osmolality of the extracellular medium can obtain very quickly the information that A549 cancer cells can shrink and not protect the membrane integrity at the incubation time and Zn dose we applied.

Cr is one of the physiologically essential trace elements that has an important role in the insulin activity and also its effects on protein, lipid, and carbohydrate metabolism. Not only insulin has a major role about regulator of cell metabolism but also is a growth factor. For many years, it is known that several cancer cells require insulin for optimal cell growth [20]. A wide range of in vivo and in vitro studies have clearly established that insulin could affect tumor progression [21, 22]. For all these reasons, the Cr element, which is important in insulin activity, also has an effect on cancer cells. In our study, there was no significant difference between the Cr level of the extracellular medium after 24 h of incubation of the cancer cell (24hECM: 0.0138 $\pm$ 0.015 mg/L) and cRPMI (M: 0.0140 $\pm$ 0.008 mg/L), but a significant decrease was observed after 48 h of incubation (48hECM: 0.0068 $\pm$ 0.001 mg/L). There was also a statistically significant decrease between 48 h Zn application (48hECM + Zn: 0.0045 $\pm$ 0.002; $p < 0.05$) and cRPMI. We consider that when Zn application is added during the 48-h incubation process, cancer cells take Cr into the cell and cause a decrease in the Cr level in the extracellular medium. With the therapeutic effect of Zn here, the need for Cr in cancer cells may have increased.

The homeostasis of Fe is regulated via several mechanisms, from recycling by macrophages to absorption by enterocytes and storage in hepatocytes [14, 15]. Because Fe deficiency or excess can induce a variety of biological processes, it has dual properties, which may facilitate cell death or tumor growth. Several studies have shown that Fe is closely related to the development and occurrence of cancer cells. Besides, cancer cells tend to have an increased dependence on Fe [23–25]. In our study, we analyzed that the level of Fe in the extracellular medium decreases as the incubation period increases. 24-h ECM (0.139 $\pm$ 0.060 mg/L) and 48-h ECM (0.040 $\pm$ 0.014 mg/L; $p < 0.001$) Fe levels were decreased compared to Fe level of cRPMI (M: 0.177 $\pm$ 0.033 mg/L). Besides, Fe levels in ECM decreased significantly with Zn application (48-h ECM + Zn: 0.020 $\pm$ 0.008; $p < 0.001$). Our study confirms the previous studies that indicated cancer cells need more Fe elements depending on the incubation period.

On the other hand, the relationship between Mg and cancer is complex. Mg has both pro- and anti-tumor effects. We need more studies to establish firm conclusions about cancer and Mg [26]. However, our study is demonstrated that the Mg levels of ECM decreased in 48-h ECM (4.785 ± 1.316 mg/L) and 48-h ECM + Zn 4.631 ± 0.223; $p < 0.05$) according to cRPMI (M:5.428 ± 0.358 mg/L) and 24-h ECM (6.928 ± 2.965 mg/L). This decrease may present us that A549 cancer cells take Mg elements for their metabolism activity into the cell inside from ECM. However, there is an important point about incubation time. The Mg level of ECM is higher than all other groups (M, 48hECM and 48hECM + Zn) after 24 h of incubation (24hECM). In 24-h incubation, Mg is not needed from the ECM, even Mg is transported from the cell to the outside. However, after 48 h of incubation, we consider that the cancer cell needs Mg and transfers it from the ECM into the cell.

We are of the opinion that our study, in which we performed 24-h and 48-h incubations in the extracellular medium of A549 cancer cells and Zn application at IC50 value, can provide an insight into studies that will be directly focused on ECM. In many studies on the relationship between osmolality, osmotic pressure, and ECM, the importance of osmolality for the cell has been demonstrated from different perspectives [27–31]. By changing the osmolality of the extracellular medium in which the cell is located, the osmotic pressure on the cell can be increased/decreased, causing the membrane structure to deteriorate, leading to the death of the cells. Likewise, studies on trace element amounts in the extracellular medium and especially the therapeutic effects of Zn application show that this subject can be used quite effectively in the extracellular medium as well as in the intracellular medium [19, 31].

In conclusion, we examined how the osmolality and Cr, Fe, and Mg change in 24-h ECM, 48-h ECM, and cell-free media according to the incubation time of the extracellular medium. In addition to different incubation times, we have determined the amount of Zn that can lead A549 cancer cells to apoptosis, due to the IC50 dose study we have done for Zn in A549 cancer cells. In this way, we analyzed that with the Zn we applied at the IC50 value in the 48hECM + Zn group, Cr, Fe, and Mg levels decreased in the extracellular medium in correlation with osmolality. When we compared the 48-h ECM and 48-hECM + Zn groups, we observed that Zn had significant effects in the extracellular medium, regardless of the incubation period. In this way, the information we gained about the extracellular environment also gave information about cell behavior. Therefore the regulation of extracellular medium osmolality is a promising method due to the biophysical effects on cancer cells. In our study, we speculated that the understanding of the effects of Zn and osmolality with the relationship between extracellular medium and cancer cell may lead to the discovery of biophysical approaches as a novel therapeutic strategy.

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Declarations

Conflict of interest The authors declare no competing of interest.

References

1. Su, S.B., Poon, T.C., Thongboonkerd, V.: Human body fluid. BioMed Res. Int. 918793 (2013). https://doi.org/10.1155/2013/918793

2. Hill, L.L.: Body composition, normal electrolyte concentrations, and the maintenance of normal volume, tonicity, and acid-base metabolism. Pediatr. Clin. North Am. **37**, 241–256 (1990). https://doi.org/10.1016/s0031-3955(16)36865-1
3. Ruth, J.L., Waterhouse, J.: Organization within the body: from molecules to body compartments. Anaesth. Intensive Care Med. **7**, 466–472 (2006). https://doi.org/10.1053/j.mpaic.2006.09.003
4. Bianchetti, M.G., Simonetti, G.D., Bettinelli, A.: Body fluids and salt metabolism Part I. Ital. J. Pediatr. **19**;35(1):36 (2009). https://doi.org/10.1186/1824-7288-35-36
5. Hall, J.E., Guyton, A.C.: Guyton and Hall Textbook of Medical Physiology. Philadelphia, USA (2011)
6. Wassner, S.J.: Body composition: salt and water. Pediatr. Rev. **27**, 181–188 (2006). https://doi.org/10.1542/pir.27-5-181
7. Waymouth, C.: Osmolality of mammalian blood and of media for culture of mammalian cells. In Vitro **6**(2), 109–127 (1970). https://doi.org/10.1007/BF02616113
8. Yue, B.: Biology of the extracellular matrix: an overview. J. Glaucoma. Suppl. **1**, S20–S23 (2014). https://doi.org/10.1097/IJG.0000000000000108
9. Watt, F.M., Fujiwara. H.: Cell-extracellular matrix interactions in normal and diseased skin. Cold Spring Harb. Perspect. Biol. **3**(4):a005124 (2011). https://doi.org/10.1101/cshperspect.a005124
10. Gonzalez-Molina, J., Mendonça da Silva, J., Fuller, B., Selden, C.: The extracellular fluid macromolecular composition differentially affects cell-substrate adhesion and cell morphology. Sci. Rep. **9**(1):8505 (2019). https://doi.org/10.1038/s41598-019-44960-3.
11. Nallanthighal, S., Heiserman, J.P., Cheon, D.J.: The role of the extracellular matrix in cancer stemness. Front. Cell Dev. Biol. **7**, 86 (2019). https://doi.org/10.3389/fcell.2019.00086
12. Bogden, J.D., Klevay, L.M.: Clinical Nutrition of the Essential Trace Elements and Minerals. New York, USA (2000)
13. Cefalu, W.T., Rood, J., Pinsonat, P., Qin, J., Sereda, O., Levitan, L., Anderson, R.A., Zhang, X.H., Martin, J.M., Martin, C.K., Wang, Z.Q., Newcomer, B.: Characterization of the metabolic and physiologic response to chromium supplementation in subjects with type 2 diabetes mellitus. Metabolism **59**(5), 755–762 (2010). https://doi.org/10.1016/j.metabol.2009.09.023
14. Bárány, E., Bergdahl, I.A., Bratteby, L.E., Lundh, T., Samuelson, G., Skerfving, S., Oskarsson, A.: Iron status influences trace element levels in human blood and serum. Environ. Res. **98**(2), 215–223 (2005). https://doi.org/10.1016/j.envres.2004.09.010
15. Handelman, G.J., Levin, N.W.: Iron and anemia in human biology: a review of mechanisms. Heart Fail. Rev. **13**(4), 393–404 (2008). https://doi.org/10.1007/s10741-008-9086-x
16. Szewczyk, B., Poleszak, E., Sowa-Kućma, M., Siwek, M., Dudek, D., Ryszewska-Pokraśniewicz, B., Radziwoń-Zaleska, M., Opoka, W., Czekaj, J., Pilc, A., Nowak, G.: Antidepressant activity of zinc and magnesium in view of the current hypotheses of antidepressant action. Pharmacol. Rep. **60**(5), 588–589 (2008)
17. Barbagallo, M., Belvedere, M., Dominguez, L.J.: Magnesium homeostasis and aging. Magnes Res. **22**(4), 235–246 (2009). https://doi.org/10.1684/mrh.2009.0187
18. Zadrozna, M., Gawlik, M., Nowak, B., Marcinek, A., Mrowiec, H., Walas, S., Wieteche-Posluszny, R., Zagrodzki, P.: Antioxidants activities and concentration of selenium, zinc and copper in preterm and IUGR human placentas. J. Trace Elem. Med. Biol. **23**(2), 144–148 (2009). https://doi.org/10.1016/j.jtemb.2009.02.005
19. Provinciali, M., Pierpaoli, E., Bartozzi, B., Bernardini, G.: Zinc induces apoptosis of human melanoma cells, increasing reactive oxygen species, p53 and fas ligand. Anticancer Res. **35**(10), 5309–5316 (2015)
20. Vigneri, R., Goldfine, I.D., Frittitta, L.: Insulin, insulin receptors, and cancer. J. Endocrinol. Invest. **39**(12), 1365–1376 (2016). https://doi.org/10.1007/s40618-016-0508-7
21. Milazzo, G., Giorgino, F., Damante, G., Sung, C., Stampfer, M.R., Vigneri, R., Goldfine, I.D., Belfiore, A.: Insulin receptor expression and function in human breast cancer cell lines. Cancer Res. **52**(14), 3924–3930 (1992)
22. Frittitta, L., Cerrato, A., Sacco, M.G., Weidner, N., Goldfine, I.D., Vigneri, R.: The insulin receptor content is increased in breast cancers initiated by three different oncogenes in transgenic mice. Breast Cancer Res. Treat. **45**(2), 141–147 (1997). https://doi.org/10.1023/a:1005801713713
23. Chen, Y., Fan, Z., Yang, Y., Gu, C.: Iron metabolism and its contribution to cancer (Review). Int. J. Oncol. **54**(4), 1143–1154 (2019). https://doi.org/10.3892/ijo.2019.4720
24. Wang, Y., Yu, L., Ding, J., Chen, Y.: Iron metabolism in cancer. Int. J. Mol. Sci. **20**(1), 95 (2018). https://doi.org/10.3390/ijms20010095
25. Forciniti, S., Greco, L., Grizzi, F., Malesci, A.: Laghi, L: Iron metabolism in cancer progression. Int. J. Mol. Sci. **21**(6), 2257 (2020). https://doi.org/10.3390/ijms21062257

26. Castiglioni, S., Maier, J.A.: Magnesium and cancer: a dangerous liason. Magnes. Res. **24**(3), 92–100 (2011). https://doi.org/10.1684/mrh.2011.0285
27. Kiehl, T.R., Shen, D., Khattak, S.F., Jian Li, Z., Sharfstein. S.T.: Observations of cell size dynamics under osmotic stress. Cytometry. A. **79**(7):560–569 (2011). https://doi.org/10.1002/cyto.a.21076
28. Miermont, A., Lee, S.W.L., Adriani, G., Kamm, R.D.: Quantitative screening of the effects of hyper-osmotic stress on cancer cells cultured in 2- or 3-dimensional settings. Sci. Rep. **9**, 13782 (2019). https://doi.org/10.1038/s41598-019-50198-w
29. Park, K.G., Chetty, U., Scott, W., Miller, W.: The activity of locally applied cytotoxics to breast cancer cells in vitro. Ann. R. Coll. Surg. Engl. **73**, 96–99 (1991)
30. Mercill, D.B., Jones, N.R., Harbell, J.W.: Human tumor cell destruction by distilled water. An in vitro evaluation. Cancer **55**, 2779–2782 (1985)
31. Shiozaki, A., Ichikawa, D., Kosuga, T., Marunaka, Y., Otsuji, E.: Regulation of osmolality for cancer treatment. J. Physiol. Sci. **67**(3), 353–360 (2017). https://doi.org/10.1007/s12576-017-0528-x

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