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Effects of some heavy metals on the activities of carbonic anhydrase enzymes from tumorous and non-tumorous human stomach

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Abstract

In this study, *in vitro* effects of certain heavy metals on the human carbonic anhydrase enzyme were examined. Inhibitory effects of metal ions (Pb^{2+}, Cu^{2+}, Fe^{2+}, Cr^{2+}, Al^{3+}, Ni^{2+}, Mn^{2+}, Cd^{2+}, Zn^{2+}, and Mg^{2+}) were observed in tumorous and non-tumorous tissue. IC_{50} values were calculated for metals. The Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} and Mg^{2+} IC_{50} values of tumorous tissue were calculated as 0.034 mM, 0.426 mM, 0.597 mM, 0.878 mM and 2.52 mM, respectively. The Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} and Mg^{2+} IC_{50} values of non-tumorous tissue were calculated as 0.067 mM, 0.991 mM, 1.065 mM, 1.724 mM and 6.13 mM, respectively. Carbonic anhydrase activity was measured as described by Wilbur and Anderson. Hydratase activity was used to determine IC_{50} values. In this study, tumorous and non-tumorous human stomach tissues were selected due to the fact that among the diseases, stomach cancer has one of the highest mortality rates. Stomach cancer, a type of cancer affecting the digestive system, is a fatal disease in living systems. The effects of metals on the CA enzyme were investigated due to the extremely important role that CA enzymes play in living beings.

Keywords: Carbonic anhydrase, heavy metals, tumorous, inhibition

1. Introduction

Gastric cancer is the fourth most common cancer in the world and the second leading reason for cancer-related death worldwide (WANG *et al*. 2011). Almost 3 million people are diagnosed with cancer in the digestive system and 2.2 million of those diagnosed die each year. Gastric cancer is the type of cancer with the highest mortality among cancers of the digestive system. Every year, more than 600.000 people die from gastric cancer (WINAVER 2005).

Carbonic anhydrase (CA: EC 4.2.1.1) is a common metaloenzyme that is found in the highest vertebrates, including humans. In the animal kingdom there are 16 isozymes of carbonic anhydrase. These include cytoplasmic isoenzymes (CA I, CA II, CA III, CA VII and CA XIII), mitochondrial (CA VA and CA VB); CA VI is found in saliva and milk, CA IV, IX, XII and XIV are membrane-bound, CA VIII, CAX and CA XI are non-catalytic. It has been clarified that there is no expression of CA XV in human beings and other primates, although it is plentiful in rodents and other higher vertebrates (WINAVER 2005; EKINCI *et al*. 2010; HILVO *et al*. 2005; EKINCI *et al*. 2007). One of the genes highly upregulated by hypoxia is that encoding CA IX. CA IX is a high activity tumor-associated membrane enzyme predominantly found in hypoxic tumor tissues being absent from most normal tissues except for a low level of expression in the gastrointestinal tract. CA IX was demonstrated to be a druggable target for the development of novel anticancer therapies and as a tumorous progression marker (OZENSOY and SUPURAN 2007). Metal ions occur naturally in the Earth's crust and are incorruptible and indestructible.

A small amount of these metals enter the body through food, drinking water and air. Trace elements such as heavy metals (eg copper, selenium, zinc) are necessary for the human body to maintain its natural metabolism. However, at higher concentrations they may be toxic (EKINCI *et al*. 2007; CEYHUN *et al*. 2011).

An important concern of environmental toxicology is the exposure to heavy metals. A large number of heavy metals are poisonous to humans, animals and plants. Humans face a significant risk of suffering from health hazards related to toxic metals as a result of bioaccumulation (HURA and HURA 2006). Human body accumulates heavy metals through the consumption of plants and this is an important pathway for the entry of toxic heavy metals into the human body. Some essential nutrients in the body can be seriously depleted by contaminated heavy metals and this is further

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responsible for decreasing immunological defences, intrauterine growth retardation, impaired psychosocial faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (ARORA *et al*. 2008). The objective of this study was to assess the effect of some heavy metals on carbonic anhydrase enzymes from non-tumorous and tumorous inflicted human stomach tissue.

2. Materials and methods

The homogenate was prepared according to the procedure outlined by DEMIRDAG *et al* (2012). Human gastric specimens of tumorous and remote non-tumorous locations were obtained from the Pathology Departments of the Research Hospital of Atatürk University after operation and stored at -80ºC until usage. 20 grams of thawed samples of the human gastric system were cut separately into small pieces with a knife and the fragments were homogenised with liquid nitrogen. The homogenate was taken to two volumes buffer solution (50mM Tris-HCl, pH=7.4) and 30 mL hexane was added to solution to solve the lipids. The homogenate was filtered through four layers of cheesecloth and then lipid fraction was removed from the homogenate using a Separatory Funnel. The homogenate was then centrifuged at 13.000 rpm for 1 hour and the pellet was discarded (DEMİRDAG *et al*. 2011). The homogenate was used for the determination of the effects of metals. All the purification steps were performed at 4°C.

2.1. Hydratase activity assay

Carbonic anhydrase hydratase activity was assayed by following the hydration of CO_2 according to the method described by Wilbur and Anderson (1948). CO_2-hydratase activity was calculated as an Enzyme Unit (EU) using the equation $(t_0\text{-}t_c/t_c)$ where t_0 and t_c are the times for pH change of the non-enzymatic and the enzymatic reactions, respectively.

2.2. In vitro inhibition effect of heavy metals on enzyme activity

In this study, the activities of the effluents were defined by the hydratase method, in which CO_2 was used as the substrate. For Pb^{2+}, Cu^{2+}, Fe^{2+},Cr^{2+}, Al^{3+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, and Mg^{2+} inhibition studies were performed using hydratase activity. All compounds were tested in triplicate at each concentration used. Different inhibitor concentrations were used. Control tube activity in the absence of inhibitor was taken as 100%. For each inhibitor an Activity%-[Inhibitor] graph was drawn. The curve-fitting algorithm allowed us to obtain the IC_{50} values (CEYHUN *et al*. 2011, DEMİRDAG *et al*. 2012). Regression analysis graphs were drawn for IC_{50} using inhibition % values by a statistical package (SPSS-for windows; version 10.0) on a computer (Student t-test; n: 3).

3. Results and Discussion

Up until this time a large number of authors have studied the effects of several substances including metals on CA enzyme activity (DEMIRDAG *et al*. 2012; SENTURK *et al*. 2012; EKINCI *et al*. 2012). The aim of this study was to determine the effects of heavy metals on the CA enzyme in tumorous and non-tumorous inflicted human stomach tissue. Enzyme activities are altered through a high level of chemical substances and drugs, which in turn affect the metabolism (EKINCI *et al*. 2012; KOZ *et al*. 2012). Chemicals are known to usually inhibit or activate certain body enzymes *in vivo* or *in vitro* and affect the metabolic pathways (CIFTCI *et al*. 2002; LUTZ *et al*. 1996).

Organic impurities and toxic heavy metals in the present era of technology found in water, air and soil contamination lead to a wide range of environmental and health problems. These impurities enter the human metabolism through the food chain and lead to bioaccumulation. Some metals such as copper, iron, manganese and zinc may be useful in small amounts for the metabolic pathways. For example, the zinc ion is an important metal for the CA of the active region. At the same time, the ion exists in some of the active sites of enzymes' such as sorbitol dehydrogenase. In addition to the well-known element iron is a vital element for the structure of haemoglobin. Conversely, if these metals in the metabolism of living creatures are found in high concentrations, the toxicological effects may be life-threatening and perhaps even fatal. In addition, some metals are not necessary for biological functions. They have toxic effects such as metals cadmium, lead and mercury (COBAN *et al*. 2007; LUTZ *et al*. 1996).

The inhibition effects of numerous substances such as medical drugs, various metals, anions and pesticides have been reported in literature. Normal enzyme activity is changed with high levels of chemicals and this affects the metabolism, particularly inhibition of specific enzymes and the effects can be both dramatic and systemic (RASPANTI *et al*. 2009; DEMIRDAG *et al*. 2011).

We report here the first study on the inhibitory effects of metal ions on tumorous and non-tumorous stomach tissue CA activity by hydratase method. The previous report by Ekinci *et al*.(2007), investigated metal ions (including Hg^{2+}, Co^{2+} and Pb^{2+}) by using esterase activity method, NPA hydrolysis assay for monitoring CA inhibition. DEMIRDAG *et al*.(2011) investigated the effects of certain metals on the activity in purified sheep liver tissue on CA II isozymes and they used both the esterase activity and hydratase activity method. K_i values for metals Zn^{2+}, Cu^{2+}, Cd^{2+} and Al^{3+} were calculated as 3.91 mM, 151 mM, 6.7 mM and 1.34 mM, respectively using the esterase activity

method. IC_{50} values for metals Zn^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+} and Ni^{2+} were found to be 0.058 mM, 0.00041 mM, 0.66 mM, 0.75 mM and 1.44 mM, respectively with the method of hydratase activity.

In this study, tumorous and non-tumorous homogenate human stomach tissues were prepared and the effects of certain heavy metals on the CA enzyme were examined. According to this study (Table 1), heavy metals were found to inhibit the enzyme cancerous tissue at much lower concentrations.

Heavy metals inhibition of the enzyme found that tumorous tissues have approximately two fold lower concentrations than non-tumorous tissues. When it comes to a live subject matter of the enzyme metabolism of CA and importance will be understood to have more adverse effects on heavy metals and cancer patients. This study shows that heavy metals in cancerous patients inhibit the CA enzyme at a much lower concentrations. Although it is impossible to avoid the effects from the metals in this technological age, cancer patients are recommended to stay away from heavy metals.

Table 1. Inhibition values for some metal ions of tumorous CA, non-tumorous CA, hCA I, and hCA II.

Type of metals	For tumorous tissue average values of IC_{50} (mM)	For non-tumorous tissue average values of IC_{50} (mM)	Human CA I values of K_i (mM)[a]	Human CA II values of K_i (mM)[a]
Cu^{2+}	0.034 ±0.00036*	0.067±0.0014*	0.90	0.16
Zn^{2+}	0.426±0.00042	0.991±0.023	4.41	2.78
Ni^{2+}	0.597±0.00049	1.065±0.014	-	-
Cd^{2+}	0.878±0.0015	1.724±0.029	2.18	0.24
Mg^{2+}	2.52±0.063	6.13±0.116	-	-

Mean from at least three determinations. * $P > 0.1$ vs. control, Student's *t* test. (n=3)

[a] From Ref. 23.

In this study cancerous human stomach and non-cancerous tissues were selected due to the fact that cancer has one of the highest mortality rates among diseases. Inhibitor effects of metals examined under *in vitro* conditions. For each inhibitor IC_{50} values were defined. Inhibitory effects of Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} and Mg^{2+} metals were observed in both tumorous and non-tumorous tissue. IC_{50} values were calculated for every metal. Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} and Mg^{2+} IC_{50} values of non-tumorous tissue were calculated as 0.067, 0.991, 1.065, 1.724, and 6.13 mM, respectively. Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} and Mg^{2+} IC_{50} values of tumorous tissue were calculated as 0.034, 0.426, 0.597, 0.878 and 2.52 mM, respectively. However, any inhibition effect for Pb^{2+}, Fe^{2+}, Cr^{2+} and Al^{3+} metals were not detected in both tumorous and non-tumorous tissues.

According to the data, we found that metal ions had an inhibition at a lower concentration on the enzyme CA from tumorous tissue than that of non-tumorous tissue. In this study we showed that metals had toxic effects in lower concentrations in cancerous tissues. People are constantly exposed to metals in different ways. In the present study, we can conclude that metals, especially in patients that are suffering from cancer, have negative effects on health.

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