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Original research

Evaluation of antioxidant and antimutagenic activities of aluminum chloride

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Purpose

Hemostatic agents are used to control hemorrhage and the gingival crevicular fluid for dental applications. In this study; the antimutagenic and antioxidant activities of aluminum chloride ($AlCl_3$), a topical hemostatic agent used especially in the fields of dermatology and dentistry, were determined. To our knowledge, this is the first study that investigates these properties.

Materials and Methods

The antioxidant activity was determined by DPPH free radical scavenging and β-carotene-linoleic acid bleaching assays. The antimutagenic activity was evaluated with the Ames *Salmonella/* microsome mutagenicity test using *Salmonella typhimurium* TA98 and TA100 strains.

Results

The total antioxidant activity of $AlCl_3$, determined by β-carotene bleaching assay was found to be 25.59 ± 2.55% and the DPPH scavenging activity of $AlCl_3$ was determined as 17.49 ± 3.07%. $AlCl_3$ showed not any mutagenicity at the tested concentrations by the AMES test used *S. typhimurium* TA98 and TA100. This drug demonstrated antimutagenic effects at the test concentrations and the strongest antimutagenic activity was observed on 1.25 mg·mL^{-1}/plate concentration of $AlCl_3$.

Conclusion

$AlCl_3$ showed potent antimutagenic and antioxidant activities and these properties are significant for dentistry and dermatology.

Keywords: *$AlCl_3$; Ames; oxidative stress; DPPH; hemostatic*

Introduction

Mutations and rearrangements in DNA may give rise to development of many degenerative diseases such as atherosclerosis, autoimmune diseases, Alzheimer's disease, some types of diabetes, and aging (1,2). In cancer initiation and other stages of the carcinogenic process, mutation in somatic cells plays a key role (3).

Reactive oxygen species (ROS) made oxidative stress in the organism and these radicals caused oxidation of biomolecules and eventually cellular damage. The damage that ROS brings about on DNA, protein and lipids result in tissue injury (4,5). During oxidative stress lots of ROS are revealed and they are one of the most common reasons of intracellular DNA modifications (6,7). Oxygen radicals react with DNA and this interaction lead to oxidative damage of DNA (8). Following the reactions formed with free radicals, base changes in nucleic acid and chain breaks in DNA take place. If this change is not repaired, mutation and mutagenic DNA forms will be formed (9).

Cancer incidence may be reduced if mutation rates are decreased. The

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best way to decrease mutation rates in human beings is to prevent exposure to mutagenic and carcinogenic agents (10). The natural antimutagens, using control of mutagenity, can prevent cancer and other diseases caused by genotoxic agents (11,12).

Aluminum chloride is a topical agent used for hemostasis. It is formulated as $AlCl_3$ and has acidic character. It can be formulated in concentrations of 20-40% in water, alcohol, ether or glycerol as a protein coagulant (13). The 25% buffered aluminum chloride solution is marketed under the brand name Frenna AC Solution (Dharma Research, Inc., US).

Since there is a significant amount of protein in the blood, its protein coagulant property makes this agent a potent hemostatic agent (13). When blood is exposed to $AlCl_3$ a chemical reaction occurs between blood proteins and hydrochloric acid (HCl) that is believed to be formed by hydrolysis of $AlCl_3$ (13,14). This causes coagulation of the tissues, vasoconstriction, thrombus and occlusion of small blood vessels, tissue damage and thus activation of the extrinsic coagulation pathway, protein precipitation and coagulation (14,15).

The literature has reported use of this material especially in the fields of dermatology and dentistry (13). In dermatology, $AlCl_3$ is applied to bleeding areas with a cotton-tipped applicator after the wound is dried as much as possible. The tip is applied on the wound with a slight pressure and a twisting motion perpendicular to the skin. It is known to be used after curettages and shave and punch biopsies (15). It is also used as a successful contrast enhancer to differentiate between normal and cancer cells in Mohs micrographic surgical technique (16). In dentistry, it has been put to use in dental surgeries to obtain hemostasis and also as a medicament solution for gingival retraction cords to obtain proper impressions in the field of prosthetic dentistry (13,17). Furthermore, $AlCl_3$ is the most popular topical treatment applied to patients with Frey Syndrome who present with complaints about hyperhidrosis (18).

$AlCl_3$ is a preferred agent because it is an inexpensive, easy to use, easily stored material that does not require preparation (15). Despite these advantages, it has side effects such as painful paresthesia, burning sensation, tissue irritation and delayed wound healing; thus it must be applied with caution (15,19).

This study is aimed to determine the antimutagenic property of $AlCl_3$. The H_0 hypothesis of this study is that there is no difference between the different concentrations of $AlCl_3$ on the antimutagenic and antioxidant activities. To the best of our knowledge, this property has not yet been studied and this will be the first to be reported in the literature.

Materials and Methods

Frenna AC solution

The sample of $AlCl_3$ was provided as Frenna AC solution (25% $AlCl_3$) (Dharma Research Inc., US). The concentrations of 0.025, 0.25, 1.25 and mg·mL^{-1}/plate of $AlCl_3$ prepared with distilled water were used in the mutagenity and antimutagenicity tests. For antioxidant activity measurements, 125 mg·mL^{-1} concentration prepared with distilled water was used.

Microbial strains

The mutagenicity and antimutagenic activity of $AlCl_3$ was determined with Ames Salmonella/ microsome mutagenicity assay. In this test the mutant strains *S. typhimurium* TA98 and TA100, histidine dependent *Salmonella* strains were used (20).

Determination of DPPH radical scavenging activity

The radical scavenging activity of $AlCl_3$ was determined by DPPH free radical method (21). $AlCl_3$ was used at 125 mg·mL^{-1} concentration. Ascorbic acid (5 mg·mL^{-1}) and α-tocopherol (5 mg·mL^{-1}) were used as positive controls.

The β-carotene bleaching assay

The antioxidant activity of $AlCl_3$ was also determined by the *β*-carotene bleaching assay (22). $AlCl_3$ was used at 125 mg·mL^{-1} concentration; ascorbic acid (5 mg·mL^{-1}) and α-tocopherol (5 mg·mL^{-1}) were used as positive controls. Antioxidative activity of the $AlCl_3$ was compared with the positive controls ascorbic acid and α-tocopherol.

Mutagenic and antimutagenic activity

Mutagenic and antimutagenic activities of $AlCl_3$ were examined using the plate incorporation method (23) detailed by Sarac and Sen (24). Known mutagens 4-nitro-o-phenylenediamine (4-NPD) (3 µg/plate) and sodium azide (NaN_3) (8 µg/plate) were used as positive controls for *S. typhimurium* TA98 and *S. typhimurium* TA100, respectively. After determining the cytotoxic doses of $AlCl_3$, the subcytotoxic doses (0.025, 0.25, and 1.25 mg·mL^{-1}/plate concentrations) were studied for the activity assay. The antimutagenic activity (%) was calculated using the following equation:

Inhibition (%) = (A-B/A) * 100

A: The number of revertants per plate in the positive control,

B: The number of revertants per plate in the presence of mutagen and $AlCl_3$.

40% or more inhibition was determined as strong; 25-40% inhibition as moderate, and 25% or less inhibition was determined as low/none antimutagenic activity (11,25).

Statistical analysis

All tests were carried out in triplicates. Data were presented as mean ± SD. Dose dependent antimutagenic activity of AlCl3 is also evident from the correlation and regression analyses, the F- and t-tests were used. SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) and Microsoft Excel 2010 were used for the statistical evaluations. All hypotheses were tested at 0.05 significance level.

Results

This study evaluated the antioxidant and antimutagenic properties of $AlCl_3$, a buffered hemostatic agent, used in the fields of dermatology and dentistry.

H_0 hypothesis was accepted. DPPH assay was used to determine the free-radical-scavenging activity of $AlCl_3$ (Table 1). The radical scavenging activity of $AlCl_3$ was less than the

Table 1: *Free radical scavenging activity (%) of $AlCl_3$.*

Sample	Activity (%)
$AlCl_3$	17.49 ± 3.07
α-tocopherol	92.95 ± 0.54
Ascorbic acid	96.59 ± 0.06

Table 2: *Antioxidant activity (%) of $AlCl_3$ in β-carotene bleaching assay.*

Sample	Antioxidant activity (%)
$AlCl_3$	25.59 ± 2.55
Ascorbic acid	55.08 ± 2.95
α-tocopherol	91.99 ± 0.61

Table 3: *The antimutagenic activity of $AlCl_3$*

Test items	Concentration	Number of revertants			
		TA98		TA100	
		Mean ± SD	Inhibition (%)	Mean ± SD	Inhibition (%)
Negative control		5.66 ± 0.57		48.5 ± 2.12	
4-NPD† (μg/plate)	3	378 ± 29.66		-	
NaN_3† (μg/plate)	8	-		757 ± 33.2	
$AlCl_3$ (mg·mL^{-1}/plate)	1.25	231.33 ± 24.19	38.8	520 ± 20	31.00
	0.25	300.33 ± 20	20.54	542 ± 20.29	28.40
	0.025	370.66 ± 4.04	1.95	670.66 ± 18.07	11.40

†4-NPD and NaN_3 were used as positive controls.

activity of α-tocopherol and ascorbic acid. According to the results, $AlCl_3$ has moderate scavenging activity (17.49%).

Total antioxidant activity of $AlCl_3$ was evaluated using the *β*-carotene bleaching assay (Table 2). The total antioxidant activity of $AlCl_3$ was found to be lower than that of ascorbic acid and α-tocopherol. The antioxidant activity results showed that AlCl3 has moderate total antioxidant activity (25.59%).

In the AMES test, firstly, the cytotoxicity of $AlCl_3$ on *S. typhimurium* TA 98 and TA 100 was evaluated and the minimum cytotoxic dose was determined as 2.5 mg·mL^{-1}/plate concentration. In the mutagenicity and antimutagenicity tests the subcytotoxic doses of $AlCl_3$ (1.25, 0.25, and 0.025 mg·mL^{-1}/plate concentrations) were used. The AlCl3 at the tested concentrations showed no mutagenic effects in the Ames test (data not shown).

The antimutagenic activity of $AlCl_3$ against 4-NPD and NaN_3 was determined with the same strains (Table 3). 0.025, 0.25, and 1.25 mg·mL^{-1}/plate concentrations were used; and $AlCl_3$ was effective at 1.25 mg·mL^{-1}/plate concentration on TA98, and at 0.25 and 1.25 mg·mL^{-1}/plate concentrations on TA100. The strongest antimutagenic activity was determined at 1.25 mg·mL^{-1}/plate concentration on *S. typhimurium* TA98.

The antimutagenic activity of $AlCl_3$ increased dose dependently. $AlCl_3$ has moderate antimutagenic activity in higher test concentrations. The results showed no significant difference between the different doses of $AlCl_3$ in increasing the number of revertant colonies .

Discussion

The oxidation caused by toxic ROS on cellular structures such as DNA, lipids, proteins, carbohydrates and other biological molecules may result in DNA mutations and/or damage target cells or tissues which frequently leads to cell senescence and death (26,27). Natural antioxidants acquired from herbs and spices play a role in inhibition or prevention of the destructive consequences of oxidative stress (28).

Ames test is a short-term bacterial reverse mutation test specifically designed to investigate new substances and drugs that can cause damage genetically and lead to gene mutations (20). The *Salmonella* strains used in Ames test have different mutations in the histidine operon and each mutation is designed to respond to mutagens that have various mechanisms of action (20,23).

Genetically transferred metabolic disorders, various human diseases with age related and cancer, are resulted by mutations (29). The best way to decrease mutation rate in human beings is to lessen exposure to mutagenic and carcinogenic agents (10).

Cancer can be defined as an excessive multiplication of cells, which when followed by a cell invasion in the tissue surrounding it, spreads to other parts of the body. One of the chief characteristics of cancer is consistent cell proliferation, which disrupts the balance of the cell life cycle (30). Usually, cancer occurs when a mutation takes place in a cell and later it undergoes transformation turning into a malignancy of different stages by an acquisition (in a sequence) of further mutations (31).

Oral cancer stands fifth among the most commonly suffered cancer forms around the world; it is a life shattering disease (32). Oral cancer can be described as the cancer of pharynx and mouth, tongue, lips, palate, alveolar mucosa, floor of the mouth, tonsils, salivary glands, buccal mucosa, gingiva, and oropharynx (33).

Cancer potential may be minimised if the mutation rate is decreased. An effective way to control this mutation rate is by avoiding exposure to the ingestion of carcinogens and mutagens (10).

The use of antimutagenic and anticarcinogenic agents in daily life is the most effective method to prevent cancer and genetic diseases (10). The control of cellular mutagens by

natural antimutagenic agents can help prevent the mutations that eventually result in cancer and other diseases caused by genotoxic agents (11,12). Even though the antimutagenic activity of a drug does not definitely indicate that it is anticarcinogenic, it may certainly be considered a sign of anticarcinogenicity (34).

Conclusion

According to analysis performed within the present study, $AlCl_3$ was found to exhibit antimutagenic and antioxidant activity *in vitro*. The results showed that $AlCl_3$ was safe at the tested concentrations, and may represent an easily attainable antioxidant and antimutagenic source for dental applications. Moreover, the antioxidant and antimutagenic activity of $AlCl_3$, used as a hemostatic agent in dermatology and dentistry, has potential characteristics to provide prophylaxis against oxidations and mutations to an extent.

Türkçe Öz: *Alüminyum kloritin antioksidan ve antimutajenik aktivitelerinin değerlendirilmesi. Amaç: Hemostatik ajanlar, kanamayı ve dişeti oluğu sıvısını kontrol etmek amacıyla diş hekimliği uygulamalarında kullanılmaktadır. Bu çalışmada; özellikle dermatoloji ve diş hekimliğinde topikal hemostatik ajan olarak kullanılan alüminyum kloritin ($AlCl_3$) antimutajenik ve antioksidan aktiviteleri belirlenmiştir. Edinilen bilgilere göre bu çalışma bu aktivitelerin araştırıldığı ilk çalışma olma özelliğindedir. Gereç ve yöntem: Antioksidan aktivite DPPH serbest radikal giderim ve β- karoten- linoleik asit ağartma deneyi ile belirlenmiştir. Antimutajenik aktivite Ames Salmonella/mikrozomal mutajenite testi ile Salmonella typhimurium TA98 ve TA100 suşları kullanılarak değerlendirilmiştir. Bulgular: $AlCl_3$'ün β- karoten- linoleik asit ağartma deneyi ile belirlenen toplam antioksidan aktivitesi % 25.59 ± 2.55 olarak belirlenmiştir. DPPH serbest radikal giderim aktivitesi 17.49 ± 3.07% olarak tespit edilmiştir. $AlCl_3$'ün test konsantrasyonlarında, Salmonella typhimurium TA98 ve TA100 suşları ile yapılan Ames testine göre herhangi bir mutajenik etkiye sahip olmadığı belirlenmiştir. Bu ilaç test konsantrasyonlarında antimutajenik etki göstermiştir ve en güçlü antimutajenik aktivite 1.25 mg·mL^{-1}/petri konsantrasyonunda tespit edilmiştir. Sonuç: $AlCl_3$ antimutajenik ve antioksidan aktivite göstermiştir ve bu özellikler diş hekimliği ve dermatoloji için önem arz etmektedir. Anahtar Kelimeler: $AlCl_3$; Ames; oksidatif stres; DPPH; hemostatic*

Ethics Committee Approval: Not required.

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Author contributions: IRK participated in designing the study. GA and ZA participated in generating the data for the study. GA and ZA participated in gathering the data for the study. OK participated in the analysis of the data. ZK wrote the majority of the original draft of the paper. GA and ZA participated in writing the paper. All authors approved the final version of this paper.

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