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Research Article

Determination of the Free Radical Scavenging and Antimutagenic Activity of Mineral Trioxide Aggregate, Root Canal Treatment Material Used In Dentistry

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Abstract:

Objectives: The purpose of this study is to evaluate the mutagenicity, antimutagenic and radical scavenging activities of Mineral Trioxide Aggregate (MTA), a material widely used in various dental procedures. Although there have been several studies that discuss MTA's antifungal and antibacterial properties, we believe this will be the first report to evaluate the antimutagenic and radical scavenging properties.

Methods: Free radical scavenging activity was determined according to the elimination of 1,1-diphenyl-2-picryl hydrazyl *(DPPH) radicals.* Antimutagenesis studies were performed with the *Salmonella*/microsome mutagenesis test. These assays were done on *Salmonella typhimurium* TA98 and TA100.

Results: MTA has low radical scavenging activity with a total of 8.95% and revealed strong antimutagenic effects at all of the tested concentrations.

Significance: Considering the results obtained in this study, MTA showed great antimutagenic attributes which may be considered a positive feature for a material widely used in dentistry.

Keywords: Mineral trioxide aggregate, Ames test, DPPH

1. Introduction

In carcinogenesis, DNA mutation plays a significant role. There has been observation of several oxidative DNA lesions in tumors that manifest damage in concordance with cancer etiology [1,2].

As mentioned earlier, mutation plays a significant role in carcinogenesis [3] and there is a strong relationship between mutagenicity and carcinogenicity [4]. Hence, it can be said that the possibility of cancer formation may be controlled if the mutation rate is decreased [3]. Furthermore, aside from playing a role in the uncontrolled cell division and carcinogenesis, the mutation of somatic cells may also have an effect on heart diseases and the development of degenerative genetic disorders such as atherosclerosis [5].

Due to the fact that cancer rates are rising gradually around the world, it is important that chemoprophylaxis and chemo-preventive determinants are evaluated to counter this threat [6]. Since DNA damage is highly important in different degenerative processes and diseases, it is necessary to identify and detect the antimutagenic agents that inhibit mutagenesis [7].

Since it is useful to obtain fast and efficient results, currently, the use of bacteria has become popular in studies that evaluate antimutagenic activities of various compounds. The Ames test is one of the ways to assess the mutation prevention of a compound employing bacteria [8] and used globally as a short-term reverse mutation test for genotoxic evaluation risks. This test is mainly used to screen different drugs and chemicals that have the capability of producing genetic damage (point mutations) [9]. There are strains able to detect frameshift and base-pair substitution mutations in the *Salmonella* strains, considering the histidine operon. Every mutation is formed in response to the mutagens acting through different mechanisms of action [9,10].

A common choice for endodontists, MTA is a biomaterial used in dentistry for various clinical applications [11,12]. Mahmud Torabinejad introduced MTA (Loma Linda University, California, US) to dental literature in 1993 [11]. Different components in MTA may be listed as; tricalcium oxide, tricalcium silicate, bismuth oxide, silicate oxide, tricalcium, and aluminate [11,13]. The US Food and Drug Administration approved this material in 1998 for endodontic use [12,13]. There are numerous uses of MTA, such as; perforation repairs [14], pulp capping [15], periapical surgeries [16], apexification treatments [17], regenerative procedures [18], pulpotomy and apexogenesis [19]. MTA is a

very useful material as it induces formation of new cementum in the periradicular tissues and dentin bridges in the pulp [12,18]. Moreover, recently, it has been found that MTA enhances periodontal ligament and alveolar bone regeneration [11].

Previously, MTA has been reported to have quite a number of significant properties such as; bioactivity, low solubility, hydrophilicity, sealing ability [12,18], radio-opacity [20], biocompatibility [18] and apatite-forming ability [21] which contribute to its usage in dentistry. Moreover, MTA shows antibacterial – antifungal [22], non-neurotoxic – non-mutagenic properties [23]. It also has no reactive properties with any restorative material [24]. There are numerous MTA studies that evaluate antibacterial [25]; antimicrobial [26]; and antifungal [27] properties. Considering all those useful properties mentioned above; this study was carried out to evaluate the antimutagenic and antioxidant properties of MTA. To our knowledge, this is the first study on this subject.

2. Materials and Methods

2.1. Mineral Trioxide Aggregate (MTA)

The MTA mixture (PRO $ROOT_{MTA}$) was obtained from Dentsply, Tulsa Dental, USA. PRO $ROOT_{MTA}$ is composed of Portland cement (75%), bismuth(III) oxide (20%) and gypsum (5-10%). In the mutagenicity and antimutagenicity tests 2.5, 5 and 10 mg/plate doses of MTA were used. MTA was used in 50 mg/ml concentration for the radical scavenging activity tests.

2.2. Bacterial strains

The Ames *Salmonella/*microsome mutagenicity assay was used to perform the antimutagenicity and mutagenicity tests. Different histidine dependent *Salmonella* strains were used in the Ames test, where each strain carried a peculiar mutation in different genes present in the histidine operon [9]. In the study, mutant strains of *S. typhimurium* TA 98 and TA 100 were used. These aforementioned strains were studied in light of Mortelmans and Zeiger [9], considering biotin and histidine need. Biotin and histidine requirements were in concordance with the excision repair capability, rfa mutation, mutation rates, and the presence of plasmid pkm101. We used a temperature of 37°C for 12-16 h for the incubation of bacterial stock, with a little agitation, and inoculated them in nutrient broth [28].

2.3. Determination of DPPH radical scavenging activity

Antioxidant activity of MTA was determined based on its ability to react with the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical [29]. Fifty µl of MTA (50 mg/ml) was added to 5 ml DPPH solution (0.004%) in ethanol. After incubation at room temperature for 30 min, the absorbance was determined at 517 nm. Ascorbic acid (5 mg/ml) and α-tocopherol (5 mg/ml) were used as positive controls.

2.4. Mutagenic and antimutagenic activity

The MTA cytotoxic doses were determined according to Mortelmans and Zeiger [9]. The MTA toxicity assay was carried out with *S. typhimurium* TA98 and TA100 [30]. Subcytotoxic doses were used in the mutagenicity and antimutagenicity tests.

The mutagenicity and antimutagenicity of MTA were studied according to the method of plate incorporation [10]. Further description on this subject was given by Sarac and Sen [31]. The instruments of positive controls were 4-nitro-o-phenylenediamine (4-NPD) (3 µg/plate) and sodium azide (NaN_3) (8 µg/plate) for *S. typhimurium* TA98 and *S. typhimurium* TA100, respectively. Distilled water was used as negative control. The subcytotoxic doses of MTA were as follows: 2.5, 5, and 10 mg/plate.

The mutagenicity inhibition was measured according to the following formula:

Inhibition = $[(M-S_1)-(M-S_0)] \times 100$

M: the number of plate/revertants that are induced by the mutagen

S_0: the spontaneous revertants' number

S_1: the number of revertants/plate induced by MTA plus the mutagen

The observations recorded for the antimutagenicity; low or none: 25% and less; moderate: 25-40%; and strong: 40% or more [32].

Every experiment was carried out in triplicate, and the resulting observations were given as mean ± SD.

3. Results

The DPPH radical scavenging method was used to evaluate the antioxidant activity of MTA (Table 1). The resulting activity was observed to be lower than the activity of α-tocopherol and ascorbic acid, and evaluated to have slight free radical scavenging activity.

Table 1. Free radical scavenging activity (%) of MTA.

Sample	Activity (%)*
MTA	8.95±0.53
α-tocopherol	91.67±0.07
Ascorbic acid	96.41±0.05

*****Values are given as mean ± S.D. of three parallel measurements

The cytotoxicity of MTA on *S. typhimurium* TA 98 and TA 100 was studied and the minimum cytotoxic dose was determined as 25 mg/plate. Thus the subcytotoxic doses of MTA (2.5, 5, and 10 mg/plate) were used in the mutagenicity and antimutagenicity tests. MTA was observed to have no mutagenic influence in the mutagenicity tests carried out through *S. typhimurium* TA98 and TA100, at the evaluated doses from 2.5 to 10.0 mg/plate. It was found that the MTA concentrations were effective to inhibit the mutagenicity of 4-NDP and NaN_3, having a linear dose-response relationship in antimutagenic activity observed with both mutagens (Table 2). It was also observed that there were antimutagenic effects ranging from 0.00-98.46% using TA98; and 68.43-87.17% using TA100.

Table 2. The antimutagenicity assay results of MTA for *S. typhimurium* TA98 and TA100 bacterial strains.

Test items	Concentration (mg/plate)	Number of revertants** TA98 Mean± S.D.	TA98 Inhibition (%)	TA100 Mean± S.D.	TA100 Inhibition (%)
Negative control		3.50±0.70[a]		45.5±6.36	
4-NPD*	3	266.66±28.86		-	
NaN_3*	8	-		681.33±27.22	
MTA	10	8.00±2.00	98.46	55.66±14.64	87.17
	5	20.33±2.08	96.08	84.33±10.06	80.56
	2.5	276.00±46.77	0.00	137.00±31.57	68.43

*4-NPD and NaN_3 were used as positive controls for S. *typhimurium* TA98 and TA100 strains, respectively.
** Values are given as mean ± S.D. of three parallel measurements

4. Discussion

Since the Ames test is highly efficient in determining potential gene mutations that may occur by extracts and drugs, this test was utilized in this study. The purpose of this study was to evaluate the antimutagenic potential of MTA by studying the effects on two histidine requiring strains of *S. typhimurium.* This process is carried out when the assay reacts through the detection of mutations present in the His−operon (→His+) particularly when *S. typhimurium* is growing in a His-poor medium [33].

There were reductions noticed in the base-substitution mutagenicity produced by NaN_3 as well as frame shift mutagenicity caused by 4-NDP. This shows that MTA undergoes various mechanisms. It was observed that antimutagenic activity was strongest at 10 mg/plate concentration on *S. typhimurium* TA98.

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 [34]. 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 [35].

Oral cancer stands fifth among the most commonly suffered cancer forms around the world; it is a life shattering disease [36]. 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 [37].

Cancer potential may be minimized if the mutation rate is decreased. An effective way to control this mutation rate is by avoiding exposure to carcinogens and mutagens [3]. Drugs that have antimutagenic characteristics are potentially able to reduce carcinogenesis and mutations in different diseases. MTA is becoming a material of more importance in dentistry day by day, used frequently both in endodontics and pediatric dentistry. Hence, the antimutagenic activity of MTA may be considered significant with respect to inhibition of mutagenesis in the oral tissues.

5. Conclusion

In this study, for the first time, MTA was found to have slight antioxidant and strong antimutagenic effects *in vitro*. With respect to these properties, as a widely used material in dentistry, MTA can be utilized safely in patients of all ages. Also, the antimutagenic and antioxidant properties may be considered promising for further studies in order to prevent cancerous changes in the oral cavity.

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