Presence of arsenic in different types of MTA and white and gray Portland cement

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Objective. The presence of arsenic in various types of mineral trioxide aggregate (MTA) and Portland cements were evaluated to verify if they comply with the ISO-recommended limit for water-based cements of 2 mg arsenic/kg material.
Study design. An amount of 5 mL of hydrochloric acid was added to 2 g each of MTA and Portland cement to be analyzed. After 15 minutes, the material was filtered and the volume of supernatant was diluted with reagent-grade water up to 40 mL. Atomic absorption spectrophotometry readings were performed in triplicate.
Results. The following mean values were obtained: CPM (Egeo, Buenos Aires, Argentina) 11.06 mg/kg; CPM sealer (Egeo) 10.30 mg/kg; MTA-Obtura (Angelus, Londrina, PR, Brazil) 0.39 mg/kg; Experimental MTA: 10.30 mg/kg; White MTA-Angelus (Angelus) 1.03 mg/kg; Gray MTA-Angelus (Angelus) 5.91 mg/kg; ProRoot-MTA (Dentsply/Tulsa Dental Specialties, Tulsa, OK) 5.25 mg/kg; Gray Portland cement (Votorantim Cimentos, Cubatão, SP, Brazil): 34.27 mg/kg; and White Portland cement (Cimento Rio Branco, Rio de Janeiro, RJ, Brazil) 0.52 mg/kg.
Conclusion. All tested materials presented arsenic in their composition. The form of arsenic was not analyzed nor the toxicity of the arsenic found. Only MTA-Obtura, White MTA-Angelus, and White Portland cement presented arsenic levels below the limit set in the ISO 9917-1 standard. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106: 909-913)**

Mineral trioxide aggregate (MTA) was first presented as a root perforation repair material in 1993.[1] The material is composed primarily of calcium, silicon, bismuth, and oxygen. Gray MTA also contains small amounts of iron and aluminum.[2] Unhydrated MTA is composed of tricalcium and dicalcium silicate and bismuth oxide. On hydration, the main products formed are calcium silicate hydrate, calcium hydroxide, monosulphate, and ettringite.[3] Several studies have demonstrated the excellent biological properties of MTA[4-7] and its good marginal adaptation[8] and sealing ability.[1,9] MTA was introduced to the US market under the brand name ProRoot MTA (Dentsply/Tulsa Dental Specialties, Tulsa, OK).

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It has been reported that Portland cement type 1 is the main component of MTA with addition of bismuth oxide at 4:1 ratio to provide radiopacity.[10] Great similarity between MTA and Portland cement has been demonstrated in respect to the composition of the basic elements,[11,12] the antimicrobial action,[11] and the biological properties.[13-15] This knowledge and the abundant availability of the raw material led several manufacturers, such as Angelus in Brazil and Egeo in Argentina, to develop new commercial brands of MTA. MTA-based root canal sealers with enhanced consistency, such as MTA-Obtura from Angelus, are also currently available. A new experimental MTA with improved handling characteristics has been developed and tested at the Department of Physics and Chemistry of the São Paulo State University, Campus of Ilha Solteira, Brazil, with recently published data on calcium release and pH.[16]

Portland cement is the worldwide covenant designation for one of the most widely employed materials in construction. Although its use was described by the Romans, Portland cement was better characterized by Joseph Aspdin, who patented the material with the denomination of Portland cement in 1824 because it resembled the color of the stones of the Isle of Portland

in the south of England, which was the material used in construction at the time.[17]

This material is manufactured by a clinkering process or partial fusion of raw materials. This process includes limestone decarbonization at 400 to 600°C; formation of dicalcium silicate, tricalcium aluminate, and tricalcium aluminoferrite between 800 and 1200°C; and production of tricalcium silicate at 1400°C by the reaction of dicalcium silicate with free lime[18] forming clinker. Additives may be included and, depending on the type of additive, Portland cement is thus classified into different types.

However, Portland cement may contain some heavy metals and contaminants. There has been a special concern regarding the presence of arsenic in this material. Arsenic is a metalloid encountered in water, air, and soil in both inorganic and organic forms and in different stages of oxidation (–3, 0, +3, +5). The trivalent and pentavalent stages of oxidation are the most toxic. The toxicity of arsenic lies on the hepatic, renal, and peripheral nervous system level and it may also cause skin problems and digestive, glandular, blood, and respiratory disorders.[19]

According to ISO 9917-1[20] standard, entitled *Water-based cements - Part 1: Powder/liquid acid-base cements* (2003), a material to be used in dental procedures should contain no more arsenic than 2 mg/kg of cement. Duarte et al.[21] recently evaluated the release of arsenic from Portland cements and MTAs, among which were gray ProRoot-MTA and MTA-Angelus, and found levels well below the ISO-specified limit. However, these authors evaluated the *release* and not the *presence* of arsenic, leaving unanswered the question of whether the arsenic levels in these materials are within standard limits. In a *Letter to the Editor*, Primus[22] questioned the results and conclusions of Duarte et al.,[21] stating that, while Portland cements contain arsenic in their composition, ProRoot-MTA does not contain this metalloid because this material is specially manufactured by Dentsply under controlled, clean, and segregated conditions to ensure freedom from contamination.

However, as far as it could be ascertained, no study has yet investigated whether commercial and experimental MTA compositions including Portland cement contain arsenic levels within the ISO-recommended limit. Therefore, the purpose of this study was to quantify, by atomic absorption spectrophotometry, the amount of arsenic in different commercial and experimental MTA formulations and gray and white Portland cement.

MATERIAL AND METHODS

The tested materials were the following:

1. CPM and CPM sealer (Egeo, Buenos Aires, Argentina). CPM is a white MTA product. The CPM sealer is a white MTA mixed with a proprietary viscous vehicle.
2. White MTA-Angelus, Gray MTA, and MTA-Obtura (Angelus, Londrina, PR, Brazil). MTA-Obtura is a mixture of white MTA with a proprietary viscous liquid.
3. ProRoot-MTA (Dentsply/Tulsa Dental Specialties, Tulsa, OK).
4. Experimental MTA[15] (Department of Physics and Chemistry of the São Paulo State University, Campus of Ilha Solteira, Brazil).
5. Gray Portland cement (Votorantim Cimentos, Cubatão, SP, Brazil).
6. White Portland cement (Cimento Rio Branco, Rio de Janeiro, RJ, Brazil).

The methodology employed for analysis of arsenic was based on ISO 9917-1 standard.[20]

Three grams of each MTA material and Portland cement were homogenized and placed in a plastic package and sealed. The material was compressed in the package in order to obtain a thin disk. The cement disks were then stored dry at 37°C for 24 hours and thereafter ground into a fine powder. Precisely, 2 g of each material were weighed and mixed with 5 mL of concentrated 20% acid hydrochloride solution, pH less than 1 (Merck SA, Rio de Janeiro, RJ, Brazil). After 15 minutes, each solution was filtered and the supernatant was transferred to a volumetric flask, which was filled with reagent grade water to the 40-mL level. The blank and test samples for construction of the standard curve were prepared according to ISO 2590 standard.[23]

Arsenic quantification was performed using an atomic absorption spectrophotometer (model 1475; Varian, Victoria, Australia) equipped with a hydride generator (VGA model 76; Varian, Victoria, Australia), arsenic hollow cathode lamp and deuterium lamp for background correction, and operating at 193.7-nm wavelength with 5-mA lamp operation current. All measurements were made in triplicate. Arsenic levels (in mg/kg) were obtained by means of a standard curve constructed with 0, 2.5 ppb (parts per billion), 5 ppb, 10 ppb, 15 ppb, and 20 ppb.

RESULTS

The amount of arsenic (in mg/kg weight) obtained from each material is summarized in Table I.

DISCUSSION

Arsenic has been considered as one of the heavy metals that might be an MTA contaminant. Previous studies using x-ray spectroscopy to compare the components of Portland cement and MTA did not detect the presence of arsenic.[11,12] This fact might be due to

Table I. Amount of arsenic in mg/kg and μg/g of weight of material in each sample

Material	Amount of arsenic, mg/kg	Amount of arsenic, μg/g
CPM	11.06	11.06
CPM sealer	10.30	10.30
MTA-Obtura	0.39	0.39
Experimental MTA	10.13	10.13
White MTA-Angelus	1.03	1.03
Gray MTA-Angelus	5.91	5.91
ProRoot-MTA	5.25	5.25
Gray Portland cement	34.27	34.27
White Portland cement	0.52	0.52

MTA, mineral trioxide aggregate.

technique limitation because if this ion is not marked to be analyzed, the equipment will not detect it or else the amount of arsenic in relation to the other ions is too small and not sufficient to be detected by this methodology. In the present study, where atomic absorption spectrophotometry was employed, CPM, CPM sealer, Experimental MTA, gray MTA-Angelus, gray ProRoot-MTA, and gray Portland cement presented arsenic levels above those recommended by the ISO 9917-1 standard,[20] which refers to water-based dental cements and states that arsenic content in these materials should be limited to 2 mg/kg of material. It should be highlighted that in this methodology the ion is removed from the material and only its presence is analyzed. The ISO 9917-1 standard[20] was adopted in this study because it is the only international quality standard that specifies the amount of arsenic that a material should present to be considered as safe for use in the human oral cavity.

The toxicity of arsenic depends on the compound. The median lethal dose (LD_{50}) administered orally, *per* kg body weight, is 2 to 3 mg for arsenic trioxide, 20 mg for calcium arsenate, 600 mg for sodium arsenate, 10 to 50 mg for lead arsenate, and 100 mg for Paris green.[24] In rats, a dose of 15 mg/kg body weight of arsenic trioxide administered orally has been shown to be toxic.[25] Other studies,[26,27] however, found that a dose of 8 mg/kg body weight of arsenate would be sufficient to cause the same toxic effect if administered via intramuscular or intraperitoneal injection. Most of the materials tested in the present study had arsenic levels above the limit recommended by the ISO 9917-1[20] specification (maximum 2 mg/kg material); only MTA-Obtura, white MTA-Angelus, and white Portland cement met the standard for usage with respect to arsenic content. However, it is unclear whether the arsenic is released from the materials and would have a toxic action.

Duarte et al.[21] analyzed the release of arsenic from gray MTA-Angelus, gray ProRoot-MTA, gray Portland cement, and white Portland cement and observed minimal release, in parts per billion (ppb). In the present study, all 4 materials tested by those authors were evaluated and presented different amounts of arsenic: gray MTA-Angelus (5.91 mg/kg material), gray ProRoot-MTA (5.25 mg/kg material), gray Portland cement (34.27 mg/kg material), and white Portland cement (0.5 mg/kg material). Although Primus[22] claimed that ProRoot-MTA is manufactured under well-controlled conditions and is free of contamination, in the present study, arsenic was detected in this material in levels above those recommended by the ISO 9917-1 standard.[20]

The greater amount of arsenic in gray Portland cement and regular MTA may be attributed to differences in product manufacturing. White Portland cement is distinguished from other cements by its color. The white color is achieved by using raw materials with low ferric oxide and manganese contents and special manufacturing conditions, especially regarding product cooling and grinding.[17] Comparing white and gray MTA, a greater amount of ferric salts has been observed in the gray type of the cement.[28] The higher arsenic content does not necessarily indicate a greater release of arsenic from gray MTAs because these materials have a higher content of ferric salts,[28] which stabilize the arsenic.[29] This may explain why the release of arsenic from the various MTA preparations and Portland cements was minimal in a previous study.[21] One component found in MTA that is not present in Portland cement is bismuth oxide,[2] which is added to provide radiopacity. However, the presence of bismuth oxide in MTA composition seems not to affect arsenic release, given that Duarte et al.[21] found no difference in the release of this metal while comparing different types of MTA containing bismuth oxide and Portland cement without bismuth oxide. In addition, if the arsenic present in MTA and Portland cements was released in high amounts, these materials would present toxicity and genotoxic action because arsenic combines with the thiol group of proteins, inactivating them. Trivalent arsenic replaces the 2 hydrogen atoms in the thiol group and binds to a sulfur molecule, forming a chelate complex of dihydrolipoyl arsenite and preventing the reoxidation of dihydrolipoyl, which is essential for continuous enzyme activity, thus inhibiting this major step of enzyme activity. As a result, the blood levels of pyruvate increase, energy production is reduced, and cell damage occurs.[30] The excellent biocompatibility of MTA and Portland cement demonstrated in several studies[6,7,13-15] can be attributed to calcium hydroxide formation after hydration.[3] It also

shows that the arsenic is not released in toxic amounts from MTA and Portland. Even when present in larger amounts in these materials, its release is minimal, as demonstrated by Duarte et al.,[21] who observed that arsenic is stabilized by iron, as also reported elsewhere.[29]

It must be emphasized that MTA is used in very small amounts, less than 1 g, in clinical endodontic procedures. Thus, the 34.27 mg of arsenic per kilogram of material recorded for Gray Portland cement correspond to 34.27 μg of arsenic per gram of cement. Considering that the median lethal dose (LD_{50}) for arsenic trioxide administered orally is 2 to 3 mg per kg body weight, the toxic dose for an individual weighing 70 kg would be 140 to 210 mg. This is considerably above the arsenic content in the Portland cement used for a root end filling (approximately 35 μg). This provides a significant safety margin.

Arsenic also shows a carcinogenic effect and the theory proposing alterations in DNA repair seems to be attractive because trivalent arsenic compounds, such as arsenite, can bind strongly to both dithiol and sulphydryl groups. These protein bonds may cause inhibition of DNA repair, mutations at key genetic sites, or an increase in cell proliferation, which might induce subsequent mutations by inhibition of DNA repair.[30] In terms of genotoxicity, neither gray/white MTA nor white/gray Portland cements have shown genotoxic effect,[31,32] which demonstrates the stability of the these arsenic compounds.

Further research is required to investigate whether the ISO 9917-1 standard[20] is applicable to Portland cement–type root end filling materials.

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