Analysis of heavy metal contents in gray and white MTA and 2 kinds of Portland cement: a preliminary study

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Objective. The levels of 10 heavy metals (arsenic, bismuth, cadmium, chromium, copper, iron, lead, manganese, nickel, and zinc) in gray Portland cement (GPC), white Portland cement (WPC), gray MTA (GMTA), and white MTA (WMTA) were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES).
Study design. One gram of each material was digested with 80°C "aqua-regia" (7 mL of 60% HNO3 and 21 mL of 35% HCl), filtered, and analyzed by ICP-AES. The analysis was performed 6 times and the data were analyzed statistically.
Results. Arsenic and lead concentrations were the highest in GPC ($P < .05$). GPC had much more of 7 heavy metals than the other 3 cements ($P < .05$). GMTA and WMTA had higher purity than GPC and WPC ($P < .05$), particularly when arsenic content was considered.
Conclusion. If a clinician is considering using Portland cement versus MTA, the differences in purity may be considered. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:642-646)**

Mineral trioxide aggregate (MTA) has been used as root-end filling material,[1-3] a root or furcal perforation repair material,[4,5] and a pulp-capping material.[6] Despite the superior characteristics of MTA, such as biocompatibility and physical properties, the high cost has been regarded as one of the disadvantages of MTA. Therefore, many studies have been performed to analyze the composition,[7-9] biocompatibility,[10-12] and physical properties[13-15] of Portland cement as a substitute for MTA and the results were comparable to MTA.[10-15] However, in the waste industry, Portland cement is used as a waste conditioner for the safe disposal of sludge containing arsenic and other heavy metals.[16] Regarding the concerns of the arsenic content in Portland cement, 1 previous study[17] demonstrated that MTA and Portland cement are both safe for use in clinical practice, whereas another investigator[18] commented that Portland cement should not be used without verifying its safety for medical use. In addition to the arsenic content, Portland cement has been reported to contain more heavy metals, such as manganese and strontium, than MTA, which may induce rejection, inflammation, or allergic reactions.[19] Another study[20] also showed that variable amounts of chromium, nickel, and cobalt were contained in some commercial Portland cements. However, until now, there has been only 1 study that measured the contents of various heavy metals in MTA and Portland cement.[19] This study, however, did not clearly describe the sample treatment method for digestion of MTA and Portland cements. Furthermore, heavy metals such as cadmium, chromium, cobalt, lead, and silver were not identified. Because there might be a difference in the measured value of heavy metals according to the sample treatment methods and it is necessary to have accurate information regarding the heavy metal content of MTA and Portland cement for clinicians, an accurate and better designed method to assess the heavy metals of MTA and Portland cement is very important.

Therefore, this study used the sample treatment method of "aqua regia" (1:3 mixture of nitric acid and hydrochloric acid) and inductively coupled plasma-atomic emission spectrometry (ICP-AES) to measure the concentrations of 10 heavy metals (arsenic, bis-

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Received for publication Jul 10, 2009; returned for revision Dec 2, 2009; accepted for publication Dec 9, 2009.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2009.12.017

muth, cadmium, chromium, copper, iron, lead, manganese, nickel, and zinc) in gray Portland cement (GPC), white Portland cement (WPC), gray MTA (GMTA), and white MTA (WMTA). The null hypothesis of this study was that there is no remarkable difference in heavy metal contents among GPC, WPC, GMTA, and WMTA.

MATERIALS AND METHODS

Sample treatment

The materials tested in this study were GPC (Lafarge Halla Cement Corp., Seoul, Korea), WPC (Union Corp., Seoul, Korea), GMTA (ProRoot MTA, Dentsply Tulsa Dental, Tulsa, OK), and WMTA (ProRoot MTA tooth colored formula, Dentsply Tulsa Dental). One gram of each sample, to the nearest of 0.001 g, was weighed and transferred to the reaction vessel (50 mL HDPE bottle, Pyrex, Lowell, MA). A mixture of 7.0 mL of nitric acid (HNO_3; 60% by weight) and 21.0 mL of hydrochloric acid (HCl; 35% by weight) was added to the reaction vessel and left to stand for 2 hours. After the vessel was slightly capped, it was transferred to the hood containing an exhaust vent and heated to 80°C by placing on the heating block (SCP science, Baie D'Urfé, Québec, Canada) and allowed to equilibrate at 80°C for 150 minutes. The temperature of the mixture was raised slowly to eliminate losses of traces by abrupt boiling. After reaction of the sample and the mixture acids, the reaction vessel was cooled to room temperature. Then, this mixture was filtered through Whatman No. 42 filtration paper (Whatman plc, Whatman House, Maidstone, UK). A blank test was carried out in parallel by the same procedure, using the same quantities of all reagents but omitting the test sample.

Contamination control during analytical operation

To eliminate leaching trace metals from the walls of containers (sample bottle) and pipette tips, they were soaked in an 8:1:1 vol/vol mixture of water, nitric acid, and hydrochloric acid for more than 48 hours, rinsed several times with ultrapure water having a resistance of 18 MΩ, dried at room temperature in a clean bench, and stored in a clean air environment before use. The analyst wore polyvinyl chloride gloves during cleaning as well as analytical operation.

Reagents

A total of 1000 mg/L of stock standard solutions of 10 heavy metal species (arsenic, bismuth, cadmium, chromium, copper, iron, lead, manganese, nickel, and zinc) (SCP science, Baie D'Urfé, Québec, Canada) were purchased and diluted into 5 different concentrations. Deionized water, having a resistance of 18 MΩ, was obtained with a 3-stage ion-exchange filter system. All reagents used in the experiment were of analytical grade or higher.

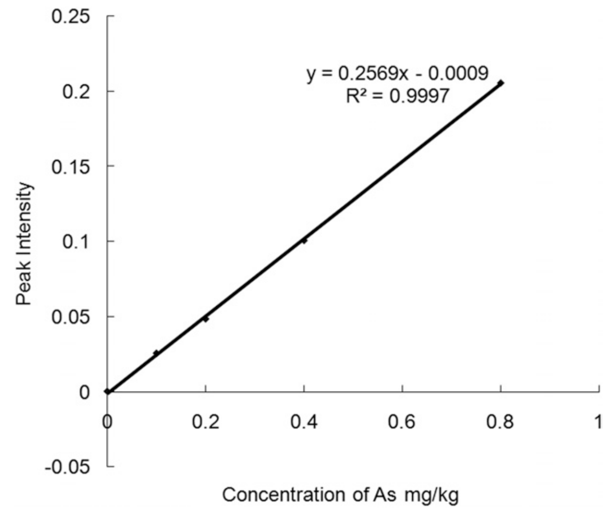

Fig. 1. Calibration curve for arsenic determination.

Calibration

The equipment was calibrated using a "5-point" calibration method. Once the spectrometer was calibrated, the calibration was verified with the use of standard solutions as described previously. One representative calibration curve (for arsenic) is shown in Fig. 1.

Determination of elements

The resulting filtrates were analyzed by ICP-AES (ICPS-7510, Shimadzu, Kyoto, Japan). The conditions for the analysis were 1.2 kW of power from a radiofrequency generator, a plasma argon flow rate of 1.2 L/min, a cooling gas flow of 14 L/min, a carrier gas flow of 0.7 L/min, an entrance slit of 20 μm, an exit slit of 30 μm, an observation height of 15 mm, and an integration time lapse of 5 seconds. The concentrations of the 10 heavy metals (arsenic, bismuth, cadmium, chromium, copper, iron, lead, manganese, nickel, and zinc) were analyzed by ICP-AES with 6 repeats.

Statistical analysis

The results were analyzed statistically using a Kruskal-Wallis test followed by an LSD (least significant difference) test. Differences with a P value less than .05 were considered significant.

RESULTS

Arsenic and lead

Arsenic was detected in all 4 cements (Fig. 2 and Table I). The arsenic concentration was the highest in GPC (25.01 mg/kg) and the lowest in GMTA (2.16

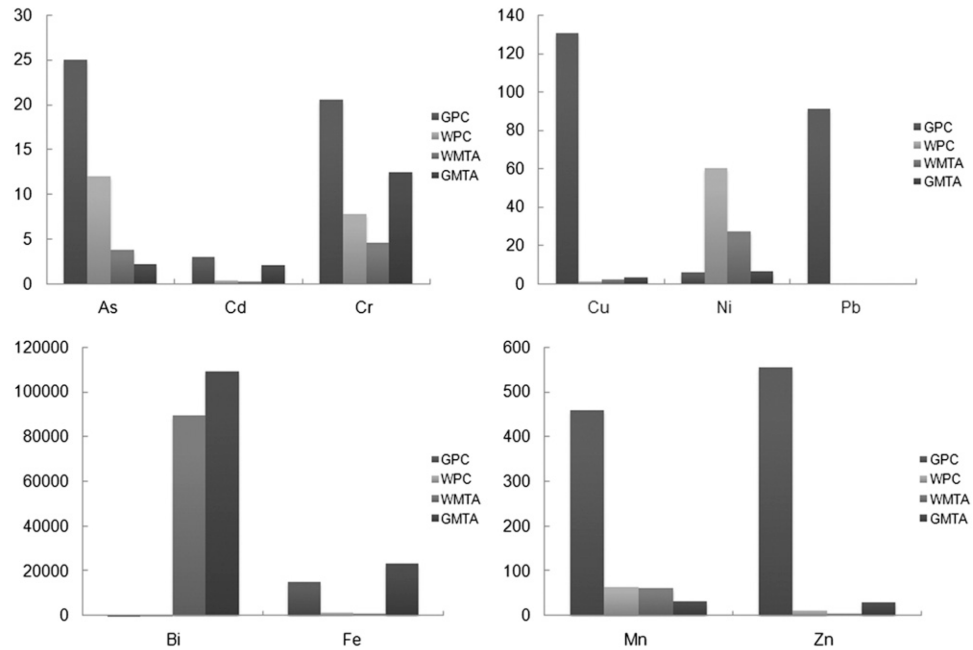

Fig. 2. Concentrations (in mg/kg) of the 10 heavy metals (arsenic, bismuth, cadmium, chromium, copper, iron, lead, manganese, nickel, and zinc) in gray Portland cement (GPC), white Portland cement (WPC), gray MTA (GMTA), and white MTA (WMTA).

Table I. Concentration of 10 heavy metals in GPC, WPC, GMTA, and WMTA (mg/kg)

	GPC	*WPC*	*WMTA*	*GMTA*
Arsenic	25.01 ± 2.99[a]	12.05 ± 0.92[b]	3.75 ± 1.39[c]	2.16 ± 0.46[d]
Bismuth	4.54 ± 3.91[a]	7.08 ± 5.73[a]	89520.25 ± 38265.7[b]	108917.47 ± 21801.45[b]
Cadmium	2.97 ± 0.23[a]	0.35 ± 0.28[b]	0.23 ± 0.19[b]	2.09 ± 0.04[c]
Chromium	20.58 ± 1.53[a]	7.78 ± 0.54[b]	4.53 ± 0.75[c]	12.44 ± 0.19[d]
Copper	130.47 ± 11.08[a]	1.38 ± 0.69[b]	2.09 ± 0.66[b]	3.20 ± 0.21[c]
Iron	15196.83 ± 910.91[a]	1317.83 ± 102.81[b]	1108.33 ± 100.23[c]	23229.16 ± 349.61[d]
Lead	91.00 ± 2.64	Not detected	Not detected	Not detected
Manganese	459.83 ± 114.84[a]	64.40 ± 15.54[b]	61.66 ± 13.46[b]	31.75 ± 0.61[c]
Nickel	5.79 ± 0.2[a]	60.34 ± 1.93[b]	27.40 ± 1.12[c]	6.43 ± 0.07[d]
Zinc	553.95 ± 50.09[a]	10.51 ± 7.72[b]	4.40 ± 0.38[c]	28.92 ± 0.64[d]

GPC, gray Portland cement; *WPC,* white Portland cement; *WMTA,* white mineral trioxide aggregate; *GMTA,* gray mineral trioxide aggregate. The same superscript represents no statistically significant difference ($P < .05$).

mg/kg). Both types of MTA had statistically less arsenic than the 2 Portland cements ($P < .05$). Lead was detected only in GPC.

Other heavy metal elements

GMTA had less or statistically equal contents of 7 heavy metals (except bismuth, iron, and nickel) compared with GPC. WMTA had less or statistically equal contents of 9 heavy metals compared with WPC (except bismuth). The manganese concentration was the lowest in GMTA. WPC contained the highest nickel concentration and GMTA had the highest iron concentration ($P < .05$). WMTA had the least amount of 4 trace metals of the materials tested (cadmium, chromium, iron, and zinc). Thus, the null hypothesis was rejected.

DISCUSSION

Regarding the concerns of the sample treatment methods for the extraction of arsenic in MTA and Portland cements, Duarte et al.[17] used an extraction technique with a mild acid (diluted HCl) and reported a negligible amount of arsenic (0.0002 mg/kg) in Pro-Root MTA. Monteiro Bramante et al.[21] used a strong acid solution (20% HCl) for 15 minutes for dissolution of MTA and De-Deus et al.[22] also used strong acids (a mixture 1:1 of hydrochloric and nitric acids plus tetrafluoroboric acid) to ensure dissolution of the MTA and Portland cement. In the present study, we used a 1:3 mixture of HCl and HNO_3 for the detection of trace elements, which is recommended by the ISO 11466, and found that the measured arsenic content was 25.01 mg/kg in GPC, 12.05 mg/kg in WPC, 3.75 mg/kg in WMTA, and 2.16 mg/kg in GMTA, respectively. However, our results cannot be compared directly with previous studies[17,19,21,22] because the sample treatment methods were not identical. In other words, Duarte et al.[17] measured the arsenic content that had leached out of MTA, whereas the other 3 studies,[21,22] including ours, measured the total content of arsenic contained in MTA itself.

Interestingly, WMTA contained higher concentrations of arsenic than GMTA in this study, which is in agreement with the study of De-Deus et al.[22] Also, lead was detected only in GPC. Cadmium and chromium were detected in both MTAs, which were not detected by Dammaschke et al.[19] The differences were also shown in contents of copper, iron, manganese, and nickel in MTA between the present study and the study of Dammaschke et al.[19] This could be attributed to the differences of sample treatment methods. Another possible explanation of the differences of heavy metal contents shown in this present study and previous studies[17,19,21,22] is the regional difference of sample manufacturers, which allows for differences in the compositions of the raw materials for Portland cement production. The measurements of the heavy metal content in WPC showed interesting results. Namely, WPC contained lower concentrations of iron and manganese than GPC. This explains the color differences between WPC and GPC because iron and manganese are well-known chromophores.[19] The concentrations of the 4 heavy metals (cadmium, copper, lead, and manganese) in WPC showed no significant difference with those in WMTA, and even the concentrations of 6 heavy metals (bismuth, cadmium, chromium, copper, iron, and zinc) were lower in WPC than in GMTA. Another interesting finding of this study was that WMTA and WPC contained higher amounts of nickel than GPC and GMTA. It is possible that the raw material for WPC and WMTA is similar but different from the raw materials for GPC and GMTA. Future studies on the chemical constitution and biological behavior of WPC are needed. Aqua regia (mixed use of HCI and HNO_3) has been used widely for the determination of heavy metals in many fields including dental[23] and soil science.[24] The advantage of the aqua regia method was reported to be ease of application, cost-effectiveness, and consistency of the results.[25] Aqua regia was also known to be able to release more heavy metals such as iron and manganese than single acid alone (HNO_3).[26] To the best of our knowledge, our study is the first to use aqua regia for determination of various heavy metals in MTAs and Portland cements. The 10 heavy metals investigated in this study were selected considering the previous studies[17,21,22] (arsenic) and the study of Dammaschke et al.[19] (cadmium, chromium, copper, iron, lead, manganese, nickel, and zinc), which has been the only article that investigated the various kinds of heavy metal contents of MTA and Portland cement so far. Omitting other toxic heavy metals such as barium, uranium, cerium, mercury, cobalt, silver, strontium, titanium, and indium, which was studied in a previous study,[19] could be one of the limitations of the present study. The analysis of these heavy metals will soon be carried out in our affiliation as a successive study.

As was mentioned in the introductory paragraphs, one of the motivations of this study was to give information regarding the heavy metal contents of MTA and Portland cement to the clinicians who consider the use of Portland cement and MTA. In a comparison of GMTA versus GPC and WMTA versus WPC, GMTA had less or statistically equal contents of 7 heavy metals (arsenic, cadmium, chromium, copper, lead, manganese, and zinc) compared with GPC. Likewise, WMTA had less or statistically equal contents of 9 heavy metals (arsenic, cadmium, chromium, copper, iron, lead, manganese, nickel, and zinc) compared with WPC. These differences in heavy metal contents could be considered before use of Portland cement and MTA by the clinicians. It was found that GMTA or WMTA contained considerably fewer heavy metals (arsenic, cadmium, chromium, copper, lead, manganese, and zinc) than GPC. However, there have been previous studies that reported good cell response[10,11] and tissue response[12] of Portland cement that were comparable to those of MTA. Considering these results, it is unlikely that the release of these heavy metals from Portland cement would present a severe health risk because these heavy metals are also present in an ordinary diet, and the ingestion of large quantities is needed to cause health problems.[27,28] De-Deus et al.[22] reported that for the human body, poisoning by inorganic arsenic can be lethal at oral doses higher than 60 mg/kg. This is considerably higher than the amount contained in Portland cement used for root-end filling or perforation repair. In this respect, the use of Portland cement can be regarded as having a significant safety margin in relation to the arsenic content. However, the possible effects of various heavy metals in Portland cement and MTA should be investigated in future studies. In addition, future studies on the effect of heavy metal content on the biocompatibilities and mechanical properties of MTA and Portland cement are also needed.

CONCLUSION

In the present study, 4 materials (GPC, WPC, GMTA, and WMTA) were tested to determine the total contents of heavy metals, such as arsenic, bismuth, cadmium, chromium, copper, iron, lead, manganese, nickel, and zinc. These tests were not a measure of what could be extracted in vivo, but the total trace metal contents. Lead (91 mg/kg) was detected only in GPC. Arsenic was present in all materials, but particularly in GPC and WPC (25 mg/kg and 12 mg/kg, respectively), and less than 4 mg/kg in GMTA and WMTA. GPC had significantly more arsenic, cadmium, chromium, copper, lead, manganese, and zinc than WPC, GMTA, and WMTA. Both GMTA and GPC had 2 to 3 mg/kg of cadmium. The results of the present study also showed that WPC and WMTA have much less iron but more nickel (60 and 27 mg/kg, respectively) compared with GPC and GMTA. If a clinician is considering the use of Portland cement in clinical practice, the presence of trace heavy metals and their lower radiopacity may be considered.

REFERENCES

1. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod 1993;19:591-5.
2. Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. J Endod 1995;21:109-12.
3. Torabinejad M, Smith PW, Kettering JD, Pitt Ford TR. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. J Endod 1995;21:295-9.
4. Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod 1993;19:541-4.
5. Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasam SP. Use of mineral trioxide aggregate for repair of furcal perforations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;79:756-63.
6. Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP. Using mineral trioxide aggregate as a pulp-capping material. J Am Dent Assoc 1996;127:1491-4.
7. Song JS, Mante FK, Romanow WJ, Kim S. Chemical analysis of powder and set forms of Portland cement, gray ProRoot MTA, white ProRoot MTA, and gray MTA-Angelus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:809-15.
8. Islam I, Chng HK, Yap AU. X-ray diffraction analysis of mineral trioxide aggregate and Portland cement. Int Endod J 2006;39:220-5.
9. Camilleri J, Montesin FE, Di Silvio L, Pitt Ford TR. The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use. Int Endod J 2005;38:834-42.
10. De Deus G, Ximenes R, Gurgel-Filho ED, Plotkowski MC, Coutinho-Filho T. Cytotoxicity of MTA and Portland cement on human ECV 304 endothelial cells. Int Endod J 2005;38:604-9.
11. Ribeiro DA, Duarte MA, Matsumoto MA, Marques ME, Salvadori DM. Biocompatibility in vitro tests of mineral trioxide aggregate and regular and white Portland cements. J Endod 2005;31:605-7.
12. de Morais CA, Bernardineli N, Garcia RB, Duarte MA, Guerisoli DM. Evaluation of tissue response to MTA and Portland cement with iodoform. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:417-21.
13. Santos AD, Moraes JC, Araujo EB, Yukimitu K, Valerio Filho WV. Physico-chemical properties of MTA and a novel experimental cement. Int Endod J 2005;38:443-7.
14. Islam I, Chng HK, Yap AU. Comparison of the physical and mechanical properties of MTA and Portland cement. J Endod 2006;32:193-7.
15. De-Deus G, Petruccelli V, Gurgel-Filho E, Coutinho-Filho T. MTA versus Portland cement as repair material for furcal perforations: a laboratory study using a polymicrobial leakage model. Int Endod J 2006;39:293-8.
16. Singh TS, Pant KK. Solidification/stabilization of arsenic containing solid wastes using Portland cement, fly ash and polymeric materials. J Hazard Mater 2006;131:29-36.
17. Duarte MA, De Oliveira Demarchi AC, Yamashita JC, Kuga MC, De Campos Fraga S. Arsenic release provided by MTA and Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:648-50.
18. Primus CM. Comments on "arsenic release provided by MTA and Portland cement" by Duarte MA, et al. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:416-7; author reply 7-8.
19. Dammaschke T, Gerth HU, Zuchner H, Schafer E. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. Dent Mater 2005;21:731-8.
20. Tandon R, Aarts B. Chromium, nickel and cobalt contents of some Australian cements. Contact Dermatitis 1993;28:201-5.
21. Monteiro Bramante C, Demarchi AC, de Moraes IG, Bernadineli N, Garcia RB, Spangberg LS, et al. Presence of arsenic in different types of MTA and white and gray Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:909-13.
22. De-Deus G, de Souza MC, Sergio Fidel RA, Fidel SR, de Campos RC, Luna AS. Negligible expression of arsenic in some commercially available brands of Portland cement and mineral trioxide aggregate. J Endod 2009;35:887-90.
23. Petoumeno E, Kislyuk M, Hoederath H, Keilig L, Bourauel C, Jager A. Corrosion susceptibility and nickel release of nickel titanium wires during clinical application. J Orofac Orthop 2008;69:411-23.
24. Ewen C, Anagnostopoulou MA, Ward NI. Monitoring of heavy metal levels in roadside dusts of Thessaloniki, Greece in relation to motor vehicle traffic density and flow. Environ Monit Assess 2009;157:483-98.
25. Tarvainen T, Kallio E. Baselines of certain bioavailable and total heavy metal concentrations in Finland. Applied Geochemistry 2002;17:975-80.
26. Pavlíčková J, Zbíral J, Čižmárová E, Kubáň V. Comparison of aqua regia and HNO3-H2O2 procedures for extraction of Tl and some other elements from soils. Anal Bioanal Chem 2003;376:118-25.
27. Anderson RA. Chromium as an essential nutrient for humans. Regul Toxicol Pharmacol 1997;26:S35-41.
28. Schumann K. Safety aspects of iron in food. Ann Nutr Metab 2001;45:91-101.

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