BIOCOMPATIBILITY OF CITRIC ACID IN DIFFERENT CONCENTRATIONS – EDEMOGENIC TEST

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ABSTRACT

Citric acid is a biologic organic acid used in the endodontic, periodontics and dentistry therapies. Concentration is an important factor associated with efficiency and biocompatibility of the acids. This study evaluated, by the Evans blue test, the irritating potential of citric acid (pH 1.0) in different concentrations (1%, 5%, 10% and 25%). Eight rats, per group, were anaesthetised and four experimental sites were designed in their backs. Injections of 2% Evans blue was given intravenously into the lateral caudal vein. 0.1ml of the solutions tests was injected intradermally into the experimental sites. The animals were killed 1/2, 1, 3 and 6 h after injection of the solutions, and each piece of skin containing the lesion was submerged in formamide and incubated at 45°C for 72 h. After filtration, the optical density was measured in a spectrophotometer (A^{620}). The data were statistically analysed by 2-way non-parametric test. There was no statistical difference between all solutions at the 1/2h, 3h and 6h times studied ($p<0.05$). At 1 h time, there was statistical difference between 10% and 25% ($p<0.05$), but not between 1% and 5% groups. The highest median values were observed at 3 h time specimens. When comparing the substances in each time period, the 5% and 25% groups had similar results. At 10% group was noted statistical difference between 3h and the other period of time ($p<0.01$). No differences were found between the specimens in the 1% group. The results showed the similarity of citric acid irritating potential in different time periods despite the increasing of concentration.

KEY WORDS*: Citric acid; biocompatibility; inflammation; endodontic therapy*

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INTRODUCTION

The success of the therapy depends on the physicomechanical and chemical preparation of the root canal system. This association constitute the complex protocol of the endodontic therapy that aims clean and shape the root canal systems, allowing the action of the intracanal medications as well as create conditions to properly seal the root canal.

In the irrigation and aspiration, it is important the capacity of cleaning and disinfection of the irrigating substance or solution act not only in organic composts but also in inorganic composts. Since Mc Comb and Smith (1975) related the presence of a layer adhered to the root canal walls, this step was included as a procedure in the endodontic therapy. Among the irrigation substances for the removal of "smear layer", are the chelating agents and organic acids (GAVINI et al., 1995; LOEL, 1975; MC COMB; SMITH, 1975; SOARES; SOUSA, 2003; STEWART, 1998). Despite the EDTA is the most chelating agent used in Endodontic, this substance has showed potential of irritation. Citric acid is the most organic acid used as chemical adjuncts in the cleaning of the root canal.

Sterret et al. (1993) demonstrated by of atomic absorption spectrophotometer analysis that the effect of citric acid on the dentin varies in accordance to its concentration (0%, 10%, 20%, 25%, 30%, 35%, 40% e 65%) and time application (1, 2 and 3 minutes). The results of the calcium quantification showed that the peak of demineralization occurred in the 25% and 30% concentrations. Gavini et al. (1995) also have investigated the demineralization effect of the citric acid of 25% and 50%, EDTA 17% and saline solution. The authors compared the loss of dentin mass, previously weight that were kept immersed in those solutions for 15 minutes, 24, 48 and 72 h, and then weighed again. It could be concluded that citric acid in the concentration of 25% and 50% were the most effective solutions in decalcifying dentin fragments. Recently, Soares et al (2000) and Souza et al. (2000) using the same method however, using different substracts, showed the effectiveness of the citric acid as demineralisation solution in comparison to EDTA and EGTA. As mentioned previously, considering the potential of citric acid for demineralization, it could be a valid alternative for use as irrigation solution in the "smear layer" removal.

Other important characteristic in the choice of an irrigation solution is the biological aspect. The effect of the citric acid application, in cavities, on the dental pulp was investigated by Lee et al.

(1971) and Cotton and Siegel (1977). The results of histopathology analysis, in both studies, demonstrated no severe or irreversible damage in the dental pulp. McInnes-Ledoux et al. (1985) studied the process of pulp response to three citric acid diluted solutions used for "smear layer" removal in class V cavities. The pulp response induced for 1%citric acid watery solution, 0.1% citric acid and 1% citric acid diluted in 30% of ethanol, applied in sockets cavities lined or not with Dycal was histologically analysed in three periods of time (3, 31 and 59 days). The severity and incidence of the superficial inflammation were decreasing by the time and also with the Dycal application. The authors suggest the use of citric acid in combination with a calcium hydroxide linning material.

Appling the Evans blue dye technique, Sousa (1999) analysed the tecidual reactions after application of EDTA, EGTA and citric acid in different periods of time (1/2, 1, 3 and 6 h). The citric acid was the less irritating substance despite the time period. Following, Soares and Souza (2003) evaluated the same solutions for 12, 24 and 48 h. In this study there was no statiscally significant difference between EGTA and citric acid group, however between EDTA and the others groups. In both studies, the EDTA was the most irritating substance independent of the time analysed.

Chan et. al. (1999) investigated the morphological alterations associated with the citric acid cytotoxic and cytostatic effects on cultured dental pulp cells. The concentration of citric acid had varied from 0.01% to 1% and the time maintenance from 1/2 hour to 3 h. The toxic effects of citric acid were associated with the pH reduction in the culture. The exposition of the cells to pure 1% citric acid (pH = 2.26) per 60s caused immediate cellular death. The cytotoxic and cytostatic effects of citric acid were classified as dependents of the concentration. Concentrations of 0.1%, 0.25%, and 0.5% of citric acid inhibited respectively 20%, 74% and 98% the cellular viability in number, when compared with the control group. The authors recommended that certain factors must be considered in the use of citric acid, such as the concentration and a controled application time.

In addition, it's essential to remember that the apical extrusion of these acids may alter some local physiological process. Considering the importance of biological aspect in the choice of irrigation solutions used during endodontic therapy, the aim of the present study was to evaluate the irritating potential of citric acid in different concentration and similar value of pH (1.0), in different periods of time 1/2, 1, 3 and 6 h through the physiochemical assay method.

MATERIAL AND METHODS

TEST SOLUTIONS:

Citric acid solutions (MERCK, Indústria Brasileira) in concentrations of 1%, 5%, 10% and 25%, and pH 1.0, were used. All the tested solutions were prepared in the Biochemical Laboratory of the Scholl of Dentistry of the University of São Paulo. The salts were weighed and diluted in deionised water, and their pH adjusted in pH meter (B371, Micronal, São Paulo, Brazilian Manufaturring)

PROCEDURES:

Thirty-two adult male Wistar rats (*Rattus novergicus*) weighting approximately 360g were used in this study. They were anesthetized with an association of cloral hydrate 10% (Manipulation Pharmacia, São Paulo, Brazil) and xylazine (Virbac do Brasil, São Paulo). Their backs were shaved and four experimental sites were designated. Their tails were washed and dried in order to facility the injections of 2% Evans blue (20 mg/kg; Merck, Germany), administered intravenously into the lateral caudal vein.

Immediately after, 0.1mL of the test solutions of citric acid in different concentrations were injected intradermally into the experimental sites with a rotation system.

Evaluation of the inflammatory exudade was accomplished in the periods of 1/2, 1, 3 and 6 hours. For each time period, 8 animals were used. After the respective period of time, the animals were killed by means of the injection of an excessive dose of anesthetic (100 mg/kg). The dorsal skin was dissected away and the skin lesions were punched out with a standard steel punch (3cm diameter). Each piece of shin containing the lesions was cut into small pieces and the dye was extracted with 10mL of formamide (Lab-Center-Brazil) for 72 h at 45ºC.

After 72 h, the solutions were filtrated with glass wool and the optical density was measured at 620 mm (A^{620}) in a spectrophotometer (UV Visible Spectrophotomer, Cary 50 Bio, USA). Transformation of the mean absorption of each sample or group in mg was obtained using the formula: mg = absorption x calculation factor (68) x total volume of formamide. The data was statistically analysed by 2-way non-parametric test (Friedman Repeated Measures Analysis of Variance on Ranks).

RESULTS

The data presented in tables showed the irritating potential of citric acid in different concentrations in association with the period of time 1/2, 1, 3 and 6 h. The value refers of the amount of dye extracted (mg) for all experimental groups and are presented in TABLE 1. The median and interquartilic semi-width of the amount of the dye extracted (mg) and the respective results of the non-parametric statistical test as to the comparison of time and substance are expressed in TABLE 2.

TABLE 1 – Amount of dye extracted (mg) in experimental groups.

Time	Animal	Critric acid 1%	Critric acid 5%	Critric acid 10%	Critric acid 25%
1/2h	1	136.68	80.92	112.88	116.96
1/2h	2	114.24	38.76	82.96	68.00
1/2h	3	113.56	119.68	212.16	139.40
1/2h	4	138.72	80.24	103.36	136.68
1/2h	5	161.84	112.88	125.80	157.76
1/2h	6	138.72	71.40	174.76	144.16
1/2h	7	96.56	199.24	175.44	85.68
1/2h	8	125.12	116.28	169.32	103.36
1h	1	154.36	216.92	109.48	173.40
1h	2	157.08	214.20	182.92	233.24
1h	3	119.68	127.84	147.56	180.20
1h	4	100.64	121.72	105.40	130.56
1h	5	102.00	147.56	129.88	138.72
3h	6	206.04	208.76	238.00	268.60
3h	7	189.72	244.80	267.24	123.76
3h	8	125.12	227.12	280.16	278.12
6h	1	137.36	184.28	201.96	167.28
6h	2	72.08	97.92	121.72	133.96
6h	3	316.88	170.00	172.04	294.44
6h	4	123.08	94.52	167.28	208.08
6h	5	89.08	124.44	110.84	127.16
6h	6	178.84	300.56	214.88	312.12
6h	7	79.56	174.76	161.84	165.92
6h	8	176.80	223.04	216.24	165.24

TABLE 2 - Medians and interquartilic semi-width of the amount of dye extracted (mg) and respective results of no parametric statistic test of the comparison the time and substance.

	Concentration				
Time	Citric acid 1%	Citric acid 5%	Citric acid 10%	Citric acid 25%	Result test of concetration
1/2h.	130.90± 32.64 a A	96.90 ± 80.24 a A	147.56± 64.60 a A	126.82 ± 44.88 a A	6.15 (p<0,05)
1h.	146.88± 36.72 a AB	137.70± 62.56 b AB	127.84± 52.70 a A	176.80± 1.34 b B	8.25 (p<0,05)
3h.	194.82± 84.66 a A	219.94±155.04 b A	238.00± 35.02 b A	247.52±120.36 b A	5.85 (p<0,05)
6h.	130.22±122.40 a A	172.38±103.20 b A	169.66± 52.70 a A	166.60±92.48 b A	5.70 (p<0,05)
Result test of time	7.42 (p<0.05)	10.91 (p<0.05)	17.98 (p<0.01)	11.63 (p<0.01)	

There was no statistical difference between the citric acid in different concentrations at the 1/2, 3 and 6 h times studied. At 1h time, there was statistical difference between 10% and 25% ($p<0.05$), but not between 1% and 5% groups.

Analysing the data and considering each substance in time period, it was observed that no difference was found between the specimens in the 1% group in all time period. However, in the 5% and 25% groups, there was also a significant difference between the 1/2h group with the others groups of the time period ($p<0.05$). In the 10% group, the citric acid showed statistically significant difference in the 3 h in relation with the other groups.

DISCUSSION

In science, the search of new composites and substances or the application of these for other uses is constant. The evaluation of the biocompatibility of the organic acids must be evaluated, considering the possibility of the unwanted extravasations of these acids

in the periapical tissues during biomechanical preparation, which may induce some adverse tissue response.

Studies have showed that citric acid is effective to remove the "smear layer" (GAVINI et al., 1995; SOARES et al., 2000; SOUSA et al., 2000). On the other hand, biocompatibility data is controversial, mainly when associated with its application in Periodontology, Dentistic and Endodontic. The reason for this diversity of results is due to the different methodologies, concentration, form and time of application and also the pH used in these experiments. All these modulating factors are closer related with the clinical behaviour. It is know that the concentration of this acid has a significant factor in its efficiency (GAVINI et al., 1995; STERRETT et al., 1993; STEWART, 1998) and biocompatibility (CHAN et al., 1999; COTTON; SIEGEL, 1977; KITCHINGS et al., 1984; LOEL, 1975; MCINNES-LEDOUX et al., 1985; REGISTER; BURDICK, 1975). So, this study considered both factors in the irritating potential of citric acid analysing the behaviour of different concentrations. The choice of the concentration and pH was associated with the literature results and the clinical use in dentistry.

The method used in this study is based in the evaluation of the exudate produced as a consequence of the increase of vascular permeability, due to the irritative potential of tested drugs and can be inferred throught spectrophotometer measurement of the dye extravased (UDAKA et al., 1970). Previous works had used this method to verify the irritating potential of several irrigant solutions of the root canal system (SOARES; SOUSA, 2003; SOUSA, 1999). This method also has been extensively applied in the evaluation of the anti-inflammatory effect of certain drugs (CATANZARO-GUIMARÃES, 1996).

The pH value of citric acid is speculated to be responsible for its deleterious effect in conjunctive tissue (CHAN et al., 1999; COTTON; SIEGEL, 1977). In pulp tissue this effect is characterized by the presence of disorganization of the odontoblasts, inflammation, necrosis and formation of micro abscesses. According to Chan et al. (1999) the toxic effects of the citric acid on cultured dental pulp cells, are associated with a decreasing pH value of the culture medium, that varied from 7.2 to 2.26. Toxic effects would be represented by cell membrane damage and impairment of mitochondrial functions.

In endodontics the acidity could be minimized by the use of calcium hydroxide sealer, which could neutralize this residual effect. This proposal is based in the data supplied in a study by McInnes-Ledoux et al. (1985) that recommend the use of citric acid in the removal of "smear layer", however, taking care to apply an appro-

priate lining. The calcium hydroxide contained in the Dycal could buffer the acid or seal the dentinal tubules, blocking the leakage of the acid through the pulp tissue.

In previously researches conducted in our laboratory, which compared the irritating potential of citric acid with others chelating agent used in endodontics, it was demonstrated that this acid is the less irritant (SOARES; SOUSA, 2003; SOUSA, 1999). The average value of irritating potential in Sousa (1999) study, were: EDTA (1447.33 mg), EGTA (770.59 mg), citric acid (329.8 mg) and saline solution (139.55 mg). It was observed significant difference ($p<0.01$) between all the groups. On the other hand, the average of the irritative potential described by Soares and Souza (2003) were: EDTA (477.69 mg), EGTA (199.25 mg), citric acid (218.07 mg) and saline solution (103.17 mg).

The results of the present study show that all solutions presented the highest means in 3 h period of time, denoting a greater irritation at this moment. This period also showed the differences of the responses between all concentrations. Initially (1/2 h) all substances were irritating to the conjunctive tissue and as time elapsed, it started to differentiate among itself. In relation to the concentration, the 1% and 10% citric acid solutions were the less irritating solutions. The 5% and 25% citric acid solutions were similar in 1, 3 and 6 h periods. Both were the most irritating solutions. Finally, the most biocompatible solution was the one of 1% citric acid.

CONCLUSION

The authors recommended caution in the clinical use of citric acid. We suggest whenever possible lower concentrations of this acid and the use of a calcium hydroxide sealer to minimize the possible residual acid effect. The use of the sodium hypochlorite, besides increasing the cleaning effect, also contributes to buffering the medium.

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