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Research Article

The Inhibition Effect of Four Different Iron Supplements on the Initiation of Dental Caries

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Abstract

Previous studies either done on experimental animals or laboratory ones revealed the possible inhibition effect of iron supplements on the initiation of dental caries.

The purpose of this *in vitro* study was to examine the inhibition effect of four iron supplements on the initiation of dental caries in human teeth.

Materials and Methods: In this study, four pharmaceutical products of iron supplements were used in the same concentration (100%); their names were (fre-in-sol, ferotonic, feromin and ferose). One hundred twenty extracted human teeth were distributed randomly into 6 groups (n = 20 teeth for each group). Four groups were from the four pharmaceutical iron products, in addition to a positive and a negative control groups. Mutants streptococci bacteria (6715) grown in Todd Hewitt Broth were used. Assessment of decalcification and cavitation was done daily for 60 days.

Results: Results of this *in vitro* study showed that three different iron - supplement products had inhibition effect on Mutants streptococci bacteria and delayed the initiation of the dental caries. All pharmaceutical iron supplements have cario-static effect with the exception of ferose product. After proper inspection and examination for experimental teeth in all groups, it was found that the mean dates for decalcification varied with lowest for the positive control (10 days) and the highest was for feromin. Cavitation was initiated in two groups; the positive control and ferose groups. The mean of the first day of cavitation was after 55 days.

Conclusion: It was concluded that some pharmaceutical iron - supplement products may have inhibition effect on the *in vitro* mutants streptococci bacteria and delayed the initiation of the dental caries in human teeth.

Keywords: *Dental Caries; Iron Supplements; Streptococcus Mutants*

Introduction

One of the major public health issues globally is dental caries. There is a gradual shift from ideal preparation and restorations to interceptive and preventive management of caries [1]. Caries propagation is associated with the ability of dental plaque to produce lactic acid to dissolve minerals in teeth. Streptococcus mutants, lactobacilli and other bacteria utilize the lactic acid to create a low-pH environment [2]. Streptococcus mutants produces an enzyme glucosyltransferase (gtf), is most virulent factor in the caries activation [3]. A recent study by Laila A stated that the prevalence of dental caries among the children in the Riyadh, Saudi Arabia was 74% [4].

Caries can continue throughout life so preventive measures must be a part of any treatment philosophy. Preventive methods may include fluoride, fissure sealants, dietary advice and patient education, as well as recall visits [5]. Another possible caries preventive method is by using certain types of minerals [6].

Some previous animal experimental studies concluded that when iron is mixed with cariogenic diet it reduces the incidence of dental caries [7-8]. In addition, Rosalen., *et al*. [9] and Meguel., *et al*. [10] found that iron decreases the caries initiation and propagation in the de-salivated rats. Moreover, Larson., *et al*. [11] found that iron supplemented diet, either in food or in drinking water have cario-static effect attributable primarily to local action on the teeth.

Devulapalle and Mooser [12] have shown that iron ions are strong inhibitors of the glucosyltransferase enzyme (GTF) that considered the most significant virulent factor of dental caries. Gierat-Kucharzewska and Karasinski [13] and Clarke., *et al*. [14] have shown that iron plays an important role in the inhibition of dental caries initiation. In addition, Berlutti., *et al*. [15] concluded that iron could have a key role in guarding the oral tissues from pathogenicity of Streptococcus mutants. It well known fact that pediatricians prescribe different iron supplements for children diagnosed with anemia, it might be interesting to study the effect of other iron supplements products on the initiation of dental caries. Therefore, the aim of this *in-vitro* study was to evaluate the effect of four different iron supplements on the initiation of dental caries.

Materials and Methods

Teeth Preparation

Human permanent premolars, which are caries and restoration free and which are extracted for orthodontic purpose, of sample size of one hundred and twenty teeth are selected for this study. These teeth were debrided from any soft tissue attachments and stored using thymol at room temperature until used. Cold cure acrylic resin is used to mount the teeth, covering till the cemento-enamel junction. A colored nail polish is used to paint on the selected area on the buccal surface of the premolar teeth and the remaining whole coronal part was covered with transparent nail polish. After the varnish dries, the colored areas were removed to leave one exposed enamel surface of approximately 0.5 cm^2 in area. Teeth were randomly distributed to six groups as seen in (Table 1).

Group number	Group name (n = 20\group)	Media (µl)		
		Bacteria	10% Sucrose	Iron
First	Positive control	100	100	000[a]
Second	Negative control	000[a]	100	000[a]
Third	100% fre-in-sol	100	100	100
Fourth	100% ferotonic	100	100	100
Fifth	100% feromin	100	100	100
Sixth	100% ferose	100	100	100

Table 1: *Distribution of teeth to experimental and control groups (total = 120 teeth).*

[a]100 ml dH_2O

Iron Sources

Four different products of iron supplements were used. These products were: (1) Fre-in-sol (Bristol Myers Squibb Company, New Jersey, USA), (2) Ferotonic (Ram Pharmaceutical, Amman, Jordan), (3) Feromin (Riyadh Pharma, Riyadh, KSA) and (4) Ferose (Spimaco Al Qassim Pharmaceutical Plant, Saudi Arabia). In only a high concentration 100%. But generally, these iron supplements supplied in two concentrations (100% and 50%). The 100% concentration means that the content of the bottle was used directly with no dilution.

Whereas, 50% concentration means that the solution in the bottle was prepared by adding 50% of the product to 50% distilled water (dH_2O) in sterile containers. A total of four experimental groups were made.

Artificial Caries Experiment

Six 24-well ELISA plates (2 ml volume/well) were used. Each group was assigned single plate. The mounted teeth were placed in ELISA well containing immersed solution media of 1.00 ml of THB containing MS bacteria strain (6715), 100 µl of 10% sucrose and 100 µl iron products. No iron is used for the positive control group. Negative control group has no bacteria. For both control groups, 100 µl dH_20 was used. Incubation at 37°C of ELISA plates are done in an anaerobic chamber.

Caries Progress Evaluation

The progress of dental caries initiation and progression was evaluated by visual examination (decalcification) and tactile examination by dental explorer (for cavitation). The decalcification of hard tissues and caries propagation were recorded for 60 days daily and the evaluations were done by two examiners and the lower values was taken independently.

Statistical Analysis

Data was statistically analyzed using descriptive statistics to show the general behavior of the data. Chi-square test was used to determine the relationship between the groups. If the chi-square was not valid due to the limited number of sample parameters, then Fisher exact test was used. The significant level is maintained with probability ($p = 0.05$).

Results

Visual Examination

Results show that all teeth in all groups (except the negative control) showed decalcification in some teeth. Negative control group was the only group that never developed any decalcification during the storage time of this *in vitro* study. Table 2 summarizes the descriptive data of the visual examination. The means of the first day of decalcification was 12 days for group one (positive control) and all teeth of this group (20 teeth) developed decalcification. Additionally, group three (100% fre-in-sol) showed only two teeth developed decalcification. In this group the means of the initial day of decalcification was 29 days. For both groups four (100% ferotonic) and group five (100% feromin) showed three teeth decalcified. In these two groups, the means of the initial day of decalcification was 45 days. Furthermore, in group six (100% ferose), sixteen teeth showed decalcification and the means of initial day of decalcification was 35 days.

Group number	Group name	Number of decalcified teeth	Means of the decalcified dates
First	Positive control	20	12
Second	Negative control	0	0
Third	100% fre-in-sol	2	29
Forth	100% ferotonic	3	45
Fifth	100% feromin	3	45
Sixth	100% ferose	16	35

Table 2: *The descriptive statistics (number and mean) of decalcification date for all groups using visual examination after 60 days.*

The percentage of sound teeth per group at the end of the study using visual examination is shown in table 3. All teeth in positive control group developed decalcification. In group six (100% ferose), 4 teeth (20%) did not show decalcification. Group four (100% ferotonic), five (100% feromin) showed 85% of teeth were sound (n = 17). Additionally, Table 3 shows that group three (100% fre-in-sol) showed 90% of the teeth were sound (n = 18). At the end of the experiment, 44 teeth were decalcified and 76 teeth were sound.

Group	Name	Number of decalcified teeth	Number of sound teeth	Percent of sound teeth
First	Positive control	20	0	00%
Second	Negative control	0	20	100%
Third	100% fre-in-sol	2	18	90%
Fourth	100% ferotonic	3	17	85%
Fifth	100% feromin	3	17	85%
Sixth	100% ferose	16	4	20%
overall		44	76	63.5%

Table 3: *Number and percentage of sound teeth per group at the end of the study using visual examination.*

Multiple comparisons between groups (number of decalcified teeth) diagnosed by visual examination using chi-square test is shown in table 4. Statistical analysis between different groups showed Significant difference (P = 0.0001) between group one (positive control) and all other groups except group six (100% ferose) which showed marginal significant difference (P = 0.053). Other significant difference was found between group six (100% ferose) and all other groups (P = 0.000).

Groups	Second	Third	fourth	fifth	Sixth
First	0.000	0.000	0.000	0.000	0.053
Second	-	0.244	0.115	0.115	0.000
Third	-	-	0.500	0.500	0.000
Fourth	-	-	-	0.669	0.000
Fifth	-	-	-	-	0.000
sixth	-	-	-	-	-

Table 4: *Multiple comparisons between groups (number of teeth decalcified) diagnosed by visual examination using chi-square test.*

If number of cells had expected frequency were more than 20%, the exact fisher test was used.

Tactile Examination

Table 5 summarizes the descriptive data of the tactile examination used for the diagnosis of cavitations. It shows that only five teeth developed cavitation. Four teeth were from group one (positive control) and one tooth from group six (100% ferose). The means on the first day of cavitation's was 57 days. Furthermore, data shows that 80% of group one (positive control), 95% from group six (100% ferose) and all other groups were sound. Statistical analysis between different groups is shown in Table 6. Significant difference between group one (positive control) and all other groups (P = 0.024) except group six (100% ferose) which showed no significant difference (P = 0.091).

Group	Name	Number of carious teeth	Means of the first day of cavitations	Percentage of remaining sound teeth %
First	Positive control	4	57	80%
Second	Negative control	0	0	100%
Third	100% fre-in-sol	0	0	100%
Fourth	100% ferotonic	0	0	100%
Fifth	100% feromin	0	0	100%
Sixth	100% ferose	1	57	95%

Table 5: *The descriptive statistic (number and means) on the first day of cavitation and number and percentage of sound teeth using tactile examination. (*Experimental period = 60 days).*

Groups	second	Third	Fourth	Fifth	Sixth
First	0.024	0.024	0.024	0.024	0.091
Second	-	1.000	1.000	1.000	0.500
Third	-	-	1.000	1.000	0.500
Fourth	-	-	-	1.000	0.500
Fifth	-	-	-	-	0.500
sixth	-	-	-	-	-

Table 6: *Multiple comparisons between group's means of number of cavitations by using chi-square test by tactile examination (If number of cells had expected frequency more than 20%, the exact fisher test was used).*

Discussion

The objective of this study was to investigate the dental caries initiation on an *in vitro* model when supplemented with four different iron products. An *in vitro* caries model helps in controlling different factors (e.g. saliva and host factors) that may play a role in the initiation and progression of dental caries. An *in vitro* model was used previously [16-20]. In addition, orthodontically extracted premolar teeth were collected from young patients. The *in-vivo* studies which will be conducted in the future will depend on the results of *in vitro* models and helps in performing the human clinical trials.

The iron products used in this study are used widely for the treatment of anemia [21]. In future, there might be additional iron products available which will investigate their effect on the initiation of dental caries. In this study, only 100% of the products were supplied and the 50% dilution was not used. It is possible that different iron concentration may lead to different results. Therefore, a better control will be to standardize the iron concentration per product as done in this study.

The experimental groups, positive and negative control groups were used in this study. The positive control model we used is effective in producing decalcification and/or cavitation's. The negative control group helps to rule out error that might occur due to contamination. Thus, results showed that both positive and negative control groups served their objectives.

The diagnosis of dental caries is one of the fundamental to the practice of dentistry. Early stage of dental caries is difficult to diagnose [22-23]. Caries is diagnosed by examination of teeth including tactile inspection, radiograph and visual examination by dental explorer [22].

Visual Examination

In this study, visual examination was used for the diagnosis of decalcified teeth. Results showed that the positive control group developed decalcification of all teeth and negative control group teeth did not show any decalcification. This shows that positive and negative control groups used are good and without any error. If contamination occurs, negative control group might have developed decalcification in some teeth. This may ensure to a certain limit, the elimination of contamination possibility in this study.

Results showed that teeth in groups containing pharmacological formula of fre-in-sol, ferotonic and feromin developed less decalcification as compared to ferose groups which is attributed to different iron pharmacological formula used. Fre-in-sol, ferotonic and feromin contain ferrous sulphate and ferose contain iron (III) – hydroxide polymaltose complex. Torell (1988) [24] reported that application of different iron product in term of content have different cariostatic effect. For ferrous sulphate groups, it was found to have cariostatic effect on experimental hamster studies when applied topically to the teeth as when added to the drinking water or the diet [9,10,22,25] had demonstrated that the iron sources containing ferrous sulphate reduced the development of dental caries in intact and desalivated rat.

For the ferose group, ferose contain iron III – hydroxide polymaltose complex. There is no cariostatic effect compared to other ferrous sulphate groups because of the hydrolysis of maltose that produced two molecules of glucose. Another possible effect is the formation of complex between hydroxide polymaltose and iron that may lead to have very few iron to react with the dental enamel [24].

Tactile Examination

In most studies, Dental explorer was used for diagnosis of cavitation (Tactile examination) [16,26,27,28]. This examination is taught to transfer the microorganisms from one site to other and in addition, the dental explorer causes irreversible damage in the early demineralized area [29,26]. Although all teeth in group one and 16 teeth from group six developed decalcification, the result showed that only five teeth developed cavitation. Four teeth were from group one (positive control) and one tooth from group six (100% ferose). There is a significant difference noticed between group one (positive control) and all other groups except group six (100% ferose) and these findings were confirmed by visual examination. At the end of 60 days of study, the teeth is diagnosed for decalcification/cavitations in the last three days by tactile examination, it is the only reason to extend the experimental study for few more days.

Effect of Iron on dental caries

Results confirmed those of the previous studies that showed a cariostatic effect of ferrous sulphate [10,12]. Iron is able to cover the enamel surface with acid protective layer. This was agreed with the results of [16,30]. It can be assumed that these protective layers will be firmly bound to the organic parts of the enamel as hydrous iron oxides and clay materials have great affinity to organic materials [31]. This affinity has been utilized in the clinical work to increase the adhesion between composite resins and dentine by mordanting with ferric chloride [32].

Gels and crystals of hydrous iron oxides are capable of adsorbing various ions and of nucleating various crystalline substances. The adsorption of calcium and phosphate ions is of special importance because iron has a repairing function [24]. This can be achieved by nucleating the precipitation of salivary calcium and phosphate ions as apatite crystals or other phosphates on the enamel surface. Therefore, the iron may have the effect of replacing minerals which can have been dissolved during the acid phases of the caries process. In addition, owing to the above-mentioned affinity to organic materials iron ought to be able to mediate the fixation of remineralized particles to the organic parts of the enamel [24].

Many studies have proven the possibility of iron in the remineralization of human enamel. Bachra and van Harskamp (1970) [33] in his study has concluded that very low concentrations of iron could initiate the precipitation of calcium apatite from meta stable calcifying buffer systems. Further it is noticed that re-mineralized caries lesion contains more iron than intact enamel surface layers [24].

Conclusion

Based on the results obtained in this *in vitro* study, it is concluded that different iron supplement products used in this study play cariostatic effect in the development of the dental caries with the exception of 100% ferose.

Conflict of Interest

There are no conflicts of interest.

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