BIOCOMPATIBILITY EVALUATION OF 3 DIFFERENT INTRACANAL MEDICAMENTS (A HISTOLOGICAL STUDY)

Abeer A Elgendy*, Doaa A. Labah** *and* Fawzy A. Darweesh**

ABSTRACT

Background: To analyze tissue responses of Wistar rats to calcium hydroxide (calcipast), calcium hydroxide iodoform (calcipaste fort) and 2% chlorhexidine gel intracanal medicaments at different implantation time intervals.

Methods: 90 specimens were randomly implanted subcutaneously of rats dorsal surface with a polyethylene tubes containing the following substances; calcium hydroxide (calcipast), 2% chlorhexidine gel and calcium hydroxide iodoform (calcipaste fort) intracanal medicaments (test groups). However subcuateneous implantations of 30 specimens of empty polyethylene tubes (control group) were performed; Rats were distributed according to time intervals of evaluation at 1, 2 and 4 weeks. The histological sections were stained with Harris hematoxylin and eosin then evaluations were made in a digital Microscope at X40, X100, and X400 magnifications for quantitative evaluations of inflammatory cells.

Results: It was observed that between the tested materials, calcipast fort (group 4) showed the least inflammatory rats tissue reaction, followed by chlorohexidine (group 3) then calcipast group (group 2) at all tested time intervals in comparison to control group (group1).

Conclusion: Calcipast fort showed good biocompatibility when compared with chlorohexidine and calcipast intracanal medications.

KEYWORDS: Biocompatibility, Calcium hydroxide, iodoform and Chlorhexidine

INTRODUCTION

Endodontic treatment is used to control infection and treat the recurrent infection of the root canals [1]. But, chemical and mechanical root canal preparations may not be able to promote complete cleansing of the root canal system due to its complex anatomy[2]. To avoid this problem, intracanal medicaments have been used as an underlying therapy in endodontic treatments after preparing the root canal system [3, 4]. Calcium hydroxide is the most common intracanal medication in the root canal treatment of

* Associate Professor, Department of Endodontic, Faculty of Oral and Dental Medicine, Zagazig University
** Associate Professor, Department of Oral Biology, Faculty of Oral and Dental Medicine, Zagazig University

teeth because of its ability to neutralize bacterial endotoxins and stimulate periapical healing [5]. CALCIPAST is ready to use calcium hydroxide paste. It doesn't required additional mixing, and it is straight applied from the syringe into the wanted part by the means of attached applicator [3]. Calcipast forte is Calcium hydroxide paste with the addition of iodoform and chlorophenol, camphor and barium sulfate. Calcipast fort is used in dental treatment as a material for root canal filling temporally. It has drying effect on root canal liquids. It has remineralizing effect on dental tissues due to its high calcium hydroxide content. It supports restoration of destructed periapical tissues [6, 7].

Chlorhexidine (CHX) is a potent antimicrobial frequently used in endodontic [8] .It has a broad antibacterial effect against *E. faecalis* and *Candida albicans* with an immediate action on bacteria, chlorhexidine can be absorbed and released from dental tissues, resulting in an antibacterial activity [9]. Dentistry requires contact with variable restorative and auxiliary dental materials with different composition; these materials may cause leakage and release substances which have allergenic reaction when interact with the tissues causing hypersensitivity effect producing changes in both the surrounding materials and tissues [10, 11].

Histologically, nonspecific tissue reactions caused by endodontic materials are normally investigated following the implantation of the test material into various tissues of animals. The test material may be directly injected or implanted (either directly or within polyethylene tubes) into different tissues [12, 13]. When used as an intracanal medication, it remains in close contact with the periapical tissues, usually for a different time periods. so, knowledge of tissue reaction in its presence is of great importance. also, longitudinal observation of these reactions seems to be a relevant factor for explanation of the type of response that host tissues can present in the presence of this substance. so, the aim of our study was to evaluate the behaviour of rat dorsal submucosa to the empty polyethylene tubes, and tubes containing calcipast, calcipast fort and 2% chlorhexidine solution at 1, 2, and 4 weeks time intervals.

MATERIALS AND METHODS

Animals from the Medical Experimental Research Center (MERC) at Faculty of Medicine, Mansoura University, Egypt. Rats were about 2 months at the time of experiment and were housed in polycarbonate cage with wire lids in standard condition under light standard conditions (light: dark, 13h–11h) and libitum. Animal experiments were conducted with respect of criteria of the investigation and ethics committee of the community guidelines dealing with experimental animals. Sixty male rats (Albino rats) weighting about 180–200g were used according to the Animal Research: Reporting of In Vivo Experiments 2013 (ARRIVE guidelines) [14, 15]. Rats were divided according to the study period. The cages were cleaned on a daily basis, and free access to food and water was allowed.

Filling of polyethylene tubes with test materials and Implantation into the rats:

120 sterile polyethylene tubes measuring 10mm in length and 1.3mm in diameter) were used which were classified into equal 4 groups (each of 30) according to the test material used as the following:

Group 1 (Control): Empty polyethylene tubes.

Group 2: Polyethylene tubes filled with Calcipast (CERKAMED medical company Poland).

Group 3: Polyethylene tubes filled with chlorohexidine (ICPA health product Ltd India)

Group 4: Polyethylene tubes filled with Calcipast fort (CERKAMED medical company Poland)

Back skin of animals were removed and disinfected with 5% iodine solution. The backs of animals were incised four incisions using a no. 15

blade in a head-to-tail alignment orientation. The skin was reflected. Implantation materials were put into spaces created by blunt dissection in which the empty polyethylene tubes were implanted at upper right of rat dorsum, the polyethylene tubes filled with calcipast were implanted at upper left of rat dorsum, the polyethylene tubes filled with 2% chlorohexidene were implanted at lower right of rat dorsum, and the polyethylene tubes filled with calcipast fort were implanted at lower left of rat dorsum. Each animal received the three materials evaluated in the study and empty polyethylene tubes used as controls. To avoid materials interactions, the tubes were placed at least 3 cm from each other. The skin was closed with 4/0 silk suture (Johnson & Johnson Produtos Profissionais, Ltda.SP, Brazil). After surgical implantation rats in each group were sacrificed after different 3 time intervals 1, 2 and 4 weeks.

The specimens Preparation

After completing each time interval (1, 2 and 4 week), 20 rats were sacrificed by giving over doses of anesthetics. back skin was shaved, and the tubes were removed together with the connective tissues around them. The specimens were fixed in a 10% formalin solution. After fixation a section oriented parallel to the long axis of the tube, this aspect was oriented to be section in the paraffin block. Sections of 6 um thickness was taken and become ready for staining with hematoxylin & eosin.

Histological study

Evaluations were done by a digital Microscope (LEICA DM LB2 Soft Ware: Leica Qwin V3) at X400 magnifications. Counting of inflammatory cells was made in five separate areas of sections at X400 magnifications. An average value for each material was obtained from the sum of cells counted in five separate areas.

Statistical methods

Data was analyzed using Statistical Package for Social Science software computer program version 26 (SPSS, Inc., Chicago, IL, USA). Data were presented as mean and standard deviation. One way Analysis of variance (ANOVA) and tukey were used for comparing quantitative parametric data of more than two different groups. P value less than 0.05 was considered statistically significant.

Histological results fig. (1)

After completion of 1 week implantation period:

Control group (embty tube) showed thin inflammatory wall associated with few macrophages and neutrophils cells infiltration. The subcutaneous tissue reaction to **Calcipast group** demonstrated cellular inflammatory response in this group with mild to moderate inflammatory reaction. **Chlorohexidine group** showed mild to moderate inflammatory reaction around the tube with mild inflammatory cells infiltration, **Calcipast forte group** showed mild to moderate inflammatory reaction around the tube with mild inflammatory cells infiltration. It was reported that the least mean value of tissue reaction was observed with calcipast fort followed by control group then chlorohexidene group however the highest tissue reaction mean value was found with cacipast group with significant difference between all groups.

After 2 weeks

Control group showed mild inflammatory cells infiltration. **Chlorohexidine group** showed obvious decrease in inflammation with regression of inflammatory cells number, **Calcipast forte group** showed obvious decrease in inflammation with regression of inflammatory cells number. **Calcipast group** showed similar reaction to the previous group but with a higher degree regarding inflammatory cells. As presented in **table (1)** the least mean value of tissue reaction was observed
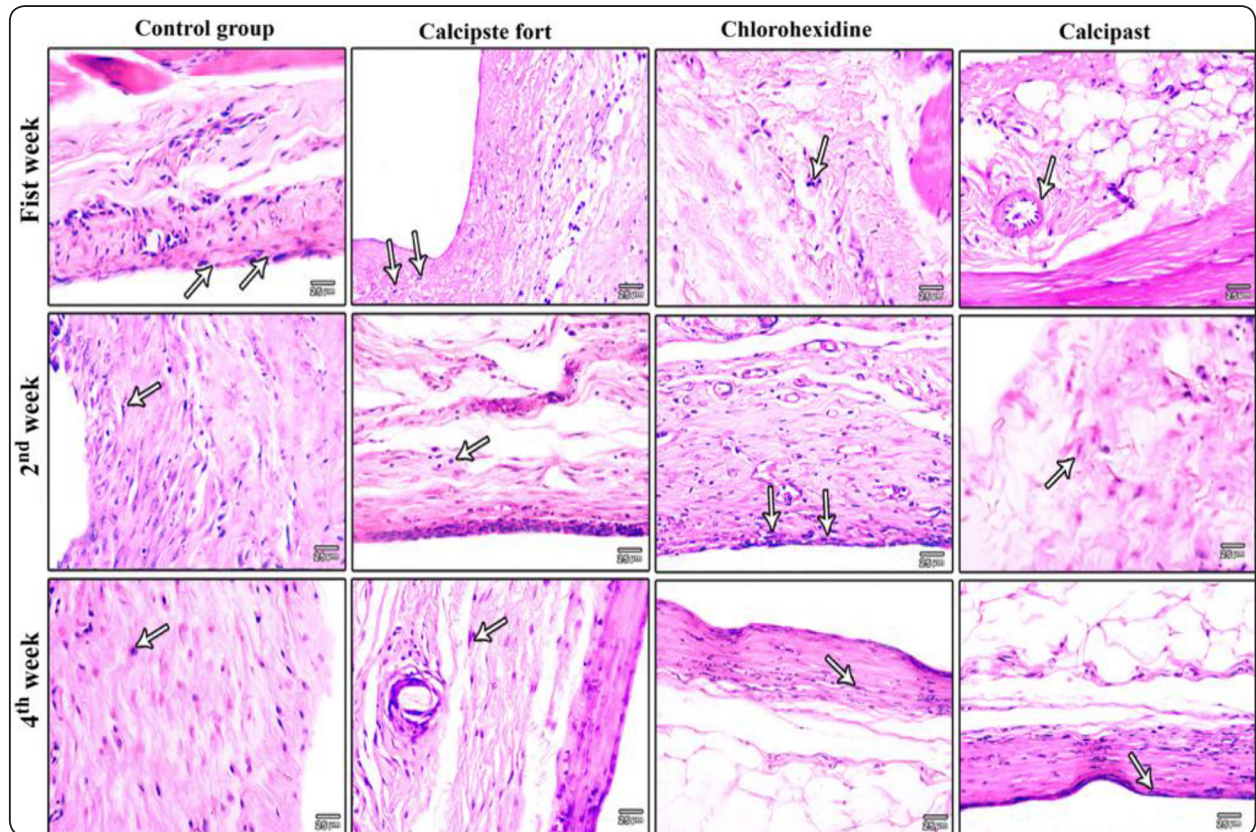
Fig. (1) Photomicrograph of H&E staining showing tissues surrounding the tube of control, Calcipaste forte, Chlorohexidine and Calcipast groups after one, two and three weeks . After one week, control tube showing slight inflammatory response within and around the thin capsule with minimal inflamatory cells infiltration (arrow), Calcipaste forte tube show moderate degree of inflammatory response (arrow head), Chlorohexidine tube show intense inflammatory reaction (arrow), Calcipaste tube show moderate inflammatory reaction (arrow). After two week, Control tube show decrease of the inflammatory reaction (arrow), Calcipaste forte tube show marked decrease of inflammatory reaction (arrow), Chlorohexidine tube show intense inflammatory reaction (arrow), Calcipaste tube show relatively thick inflammatory capsule (arrow). After 3 weeks, control tube show normal connective tissue capsule, Calcipaste forte tube show mild inflammatory response and moderate fibrous connective tissues capsule. Chlorohexidine tube show moderate inflammatory response (arrow), Calcipaste tube showing moderate inflammatory cells infiltration (arrow). (H& E X400).

with control group followed by calcipast fort then chlorohexidene however the highest tissue reaction mean value was found with cacipast group with significant difference between all groups.

At the period of 4 weeks:

Control group demonstrated little inflammatory reaction. **Chlorohexidine group** showed progressive healing with obvious decrease of the inflammatory reaction by decrease of lymphocytes and macrophages infiltration. Also **Calcipast forte** group showed progressive healing. **Calcipast group** showed restoration of the normal tissue. The least mean value of tissue reaction was observed with control group followed by calcipast fort then chlorohexidene however the highest tissue reaction mean value was found with cacipaste group with significant difference with control group, group 3 and group 4 however there was no significant difference between calcipast fort group when compared to both a chlorohexidene and control group.

Statistical results

The mean values and standard deviations of inflammatory response at different intervals among the different intracanal medicaments were shown in Table (1) and Figure (2).

After the first week of tubes implantation it was observed that calcipast fort showed the least mean value of inflammatory reaction followed by control group however after 2and 4 weeks least mean value of tissue reaction was observed with control group followed by calcipast fort there was no significant difference between them at all time intervals. The higher inflammatory reaction mean value was chlorohexidine and the highest mean value was observed with calcipast group. There was a significant difference (P<0.001) between them and also between calcipast fort and control groups. The same results were obtained after 4 weeks except there was no significant difference between calcipast fort and chlorohexidine.

Regarding the effect of different time intervals on tissues inflammatory reaction, the results were represented in table (2) and figure (2)

The mean values of inflammatory response were decreasing over time in all test groups. The highest mean value of tissue reaction was observed after one week then decreased after 2 weeks and the least value was after 4 weeks

TABLE (1): Aspects related to tissue reaction between the tested materials and time intervals (data presented as mean ± standard deviation (X̄±SD); significance level 5% (p<0.05)

Time (week)	Mean values (x) ± standered deviation (SD) of different groups				P
	Control	Calcipast	Chlorohexidine	Calcipast fort	
1week	0.883±0.147	5.495±0.916 [A]	2.185±0.364 [AB]	0.719±0.120 [BC]	<0.001*
2 weeks	0.491±0.082	4.677±0.780 [A]	1.474±0.246 [AB]	0.646±0.108 [BC]	<0.001*
4 weeks	0.437±0.073	3.572±0.595 [A]	0.825±0.138 [AB]	0.510±0.085 [B]	<0.001*

Data expressed as mean±SD, *: significanCe≤0.05

A: Significance vs. Control, B: Significance vs. Calcipast, C: Significance vs. Chlorohexidine
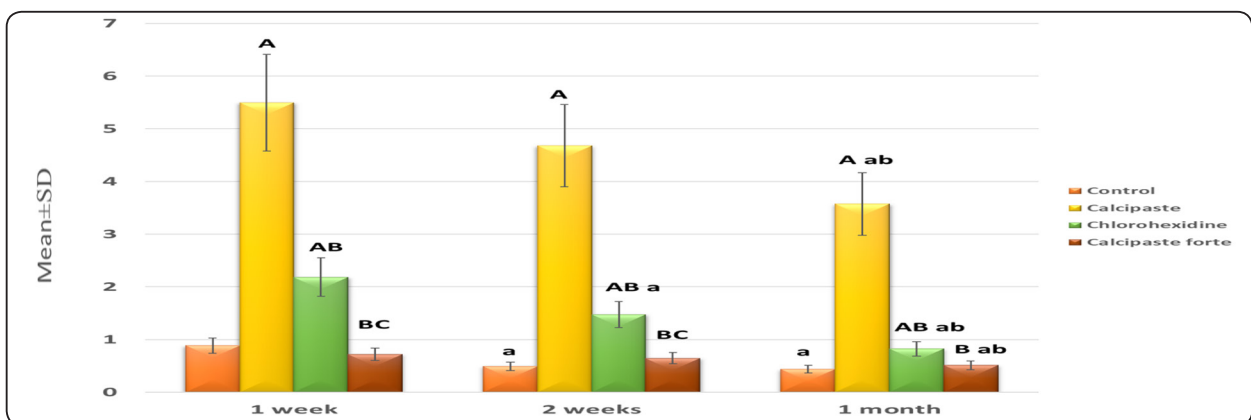

Fig. (2): Histogram showing mean tissue reaction of control group and tested materials at 1, 2, and 4 weeks

Table (2): Effect of implantation time intervals on tissue response of tested materials

Tested groups	Mean values (x) ± standered deviation (SD) of different groups at different time intervals			P
	1week	2 weeks	4 weeks	
Control	0.883±0.147	0.491±0.082 [a]	0.437±0.073 [a]	<0.001*
Calcipast	5.495±0.916	4.677±0.780	3.572±0.595 [ab]	<0.001*
Chlorohexidine	2.185±0.364	1.474±0.246 [a]	0.825±0.138 [ab]	<0.001*
Calcipast fort	0.719±0.120	0.646±0.108	0.510±0.085 [ab]	<0.001*

*Data expressed as mean±SD, *: significance ≤0.05 a: Significance vs. 1 week, b: Significance vs. 2 weeks*

Group I (Control group): There was a significant difference (P<0.001) between tissue reaction mean value after one week in comparison to tissue reaction mean values of 2 and 4 weeks however there is no significant difference between 2 and 4 weeks time intervals.

Group II (Calcipast group): There was a significant difference (P<0.001) between tissue reaction mean value after 4 weeks in comparison to tissue reaction mean values of 1 and 2 weeks. However there is no significant difference between 1 and 2 weeks

Group III (Chlourohexidene group): There was a significant difference (P<0.001) between tissue reaction mean value between all time intervals

Group IV(Calcipast fort group): There was a significant difference (P<0.001) between tissue reaction mean value between 4 weeks time interval compared to 1 and 2 weeks however there is no significant difference between 1 and 2 weeks.

DISCUSSION

The surviving bacteria after root canal instrumentation proliferate between visits. so, intracanal medicaments with antimicrobial effect must be used between visits to kill possible remaining microorganisms, especially in case of necrotic pulp[16,17]. Calcium hydroxide has antimicrobial property so it is used in endodontic as intracanal medication between visits. Using of calcium hydroxide as a temporally dressing in the presence of large chronic periapical lesions can allow an environment more favorable to healing [18]. Chlorhexidine has been highly used as intracanal dressing due to its rapid antimicrobial effect with wide antibacterial effect [19-22].

In the present study, each medicament biocompatibility was evaluated through subcutaneous implantation of polyethylene tubes containing the tested materials in rats. This method is the most commonly used to evaluate biocompatibility in preliminary in vivo studies. In addition, the use of rats (*albino rats*) provides more safe Treatment and related results over a short period of time because there is an accelerated metabolism of these animals [23, 24]. Using of tubes is preferred to prevent both irritation and material diffusion to the surrounding tissues [25-30]. This allows simulation of the clinical conditions of medications applied in the root canal treatment [28].

Silveira et al. concluded that a material is considered biocompatible if the inflammation is decreased to be insignificant with time. In our study, empty tube was used as negative control because it is an inert material and the formation of connective tissue was allowed in contact with its surface [28, 30-32]. The stipulation of time intervals of 1, 2 and4 weeks, contemplated the commonly used periods in endodontic, allowing noticing histological responses at short, medium and long term periods, and was in agreement with the protocol of other studies that analyzed tissue response [33-35].

Regarding the effect of time interval on the tested intracanal medicaments, the obtained results indicated that all materials presented a statistically significant decrease in the inflammatory reaction at more long time intervals, inflammatory reaction of all groups tested, including the control group at 1week, was more intense than at 2 and 4 days, as reported by a previous study [36] .The primary inflammatory reaction may be explained by the aggressiveness suffered during the surgical process and not only by the substances under analysis.

The reaction of rats subcutaneous tissues to calcium hydroxide paste used in association with different substances may be attributed to antimicrobial activity. In our study the tissue reaction of chlorohexidine is better when compared to calcipast in all tested time intervals if the antimicrobial property of tested materials was regarded; this result is in accordance to **Ballal V.et al** they reported. The antibacterial effect of calcium hydroxide against Candida albicans and Enterococcus faecalis is less than chlorohexidine after 3 days. The result was explained by the possibility of the dilution of calcium hydroxide as time passed. But when the effect of chlorhexidine on these two pathogens, was evaluated alone. The efficacy of the chemical increased from 24 to 72 hours. But our results in disagreement with **de Souza-Filho F.J.et al,** they concluded that Calcium hydroxide has antimicrobial effect better than chlorhexidine after 1 week and this contradiction may be dueto difference in the form of calcium hydroxide they used.

In our study the addition of iodoform to calcium hydroxide (calcipast fort) give less inflammatory reaction when compared to calcium hydroxide and chlorohexidine at all tested time intervals this may be due to increased antibacterial effect of calcipast fort and this in agreement with de **Souza-Filho F.J. et al.** who reported addition of iodoform increase the antibacterial effect of calcium hydroxide and give better biocompatibility. This may be due to the addition of calcium hydroxide to iodoform

in oily preparations are non-water soluble that provide little solubility and diffusion of paste within tissue[39]. Also the least tissue reaction obtained by calcipast fort in our study is in accordance to **Jara M, et al**, they reported that addition of camphorated monochlorophenole and iodoform to calcium hydroxide result in potent antibacterial effect of calcium hydroxide against Enterococcus faecalis [40].

CONCLUSIONS

Addition of iodoform and camphorated mono-chlorophenol to calcium hydroxide (calcipast fort) intracanal medicament improve its biocompatibility.

RECOMMENDATIONS

More research work must be performed to evaluate antibacterial effect of calcipast fort and clinical evaluation with cases associated with bone resorption ca be performed. Also bond strength of different sealer types after using of calcipaste fort intracanal medicament can be examined.

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