The use of Portland cement in the repair of mandibular fractures in rats[1]

Uso de cimento Portland no reparo de fratura mandibular em ratos

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ABSTRACT

PURPOSE: To evaluate the bone healing of mandibular fractures following the use of Portland cement.

METHODS: Thirty-two male Wistar rats were divided into control and experimental groups. In the control group the rats were submitted to a mandibular fracture, which was reduced, and the soft tissues were sutured. In the experimental group the rats had the mandibular fracture reduced and maintained with the Portland cement. The animals were euthanized 7 and 21 days after surgery by injecting a lethal dose of anesthetic. The following variables were studied: weight of the animals, radiographic images, histopathological features and time of surgery. **RESULTS**: A weight loss was observed in the specimens of both groups at the different times of evaluation, a greater difference in weight before and after surgery being found in the experimental group, which was statistically significant ($p < 0.05$, $p = 0.041$). From the histological point of view, with a margin of error (5.0%) the only two significant differences ($p < 0.05$) recorded in the variables were “Material deployed” and “Bone resorption” during the evaluations at 7 and 21 days, respectively.

CONCLUSION: The Portland cement served to promote bone healing.

Keywords: Mandibular Fractures. Fracture Fixation, Internal. Bone Regeneration. Portland Cement. Rats.

RESUMO

OBJETIVO*:* Avaliar a reparação óssea de fratura mandibular após o uso do cimento Portland (CP).

MÉTODOS: Trinta e dois ratos machos Wistar foram divididos em grupo controle e grupo experimental. No grupo controle os ratos foram submetidos à fratura, redução e manutenção dos seguimentos com sutura dos tecidos moles. No grupo experimental foram submetidos a fratura, redução e manutenção dos segmentos fraturados com CP e sutura dos tecidos. Os animais foram eutanasiados com sete e 21 dias de pós-operatório através da injeção de dose letal dos anestésicos adotados. As variáveis estudadas foram: peso dos animais, avaliação tomográfica, avaliação histológica e tempo cirúrgico.

RESULTADOS: Perda de peso foi observada nos espécimes de ambos os grupos nos diferentes intervalos de tempo considerados, sendo maior a diferença de peso antes e após cirurgia para o grupo experimental, que foi estatisticamente significante ($p<0,05$; $p=0,041$). Do ponto de vista histológico para a margem de erro fixada (5,0%) as duas únicas diferenças significativas ($p<0,05$) foram registradas nas variáveis: “Material implantado” na avaliação com sete dias e “Reabsorção óssea” na avaliação com 21 dias.

CONCLUSÃO: O cimento Portland atuou promovendo a reparação óssea.

Descritores: Fraturas Mandibulares. Fixação Interna de Fraturas. Regeneração Óssea. Cimento Portland. Ratos.

Introduction

Trauma is a social problem. Mortality rates from external causes have increased in recent years. Facial trauma is considered one of the major injuries seen in trauma centers because of its emotional consequences and the possibility of deformity, as well as the economic impact of these traumas cause on a health system[1]. Mandible fractures have a prevalence ranging from 24.3% to 48% depending on the profile of the center, its geographicall location and country. In most cases, these patients need to undergo a procedure for treating these fractures[2].

Currently, the treatment of facial fractures ranges from follow-up, maxillomandibular immobilization by locking wire and/or rubber bands to direct fixation of the fracture with titanium plates or the use of resorbable materials to keep the fractured bone reduced and fixed to enable bone healing to occur. Of these options, the stable internal fixation with plates and screws or titanium screws is widely used for the treatment of various types of fractures and its success has not been called into question[3].

Despite the excellent prognosis, stable internal fixation has some disadvantages, in particular the need to remove the material after the phase of bone repair in some cases and the need for targeted training in its use in others, as well its high cost.

In view of these drawbacks, new materials, or even ones already tested in other areas of health, should be evaluated and developed to fill this gap in the treatment of mandibular fractures. In this regard, Portland cement is presented as an option, as it has been successfully tested in dentistry and other areas, but not in traumatology[4].

Portland cement is the technical name of the material commonly known in the building industry as cement. Portland cement was created and patented in 1824 by an English builder named Joseph Aspdin. Cement is a fine powder with binder properties, which hardens under the action of water, and is therefore a hydraulic binder. Once hardened, even under the action of water, it does not subsequently decompose. It is composed of clinker with the addition of other substances, which contributes to its properties and facilitates its use. Thus the present study sought for a new alternative for treating mandibular fratures that could induce the healing of such fractures using Portland cement as an adjuvant material for fracture fixation.

Methods

This study was approved by the Ethics Committee of the University of Pernambuco (CEUA/UPE No. 005/09). The sample was composed of 32 male Wistar rats. The animals were randomly divided into 2 groups - control and experimental. The animals that underwent fracture reduction and maintenance of the segments with the suture of soft tissues comprised the control group and the ones in which the tested material (Portland cement) was used comprised the experimental group. The animals were evaluated at 7 and 21 days after surgery, when they were euthanized.

For general anesthesia Ketamine Hydrochloride (Dapalen® - Vetbrands) and Xylazine Hydrochloride (Anasedanâ® - Vertbrands) diluted 1:1 was used at a dose of 0.1 ml per 100 g body weight in the thigh muscle of the animal. Local anesthesia was obtained with a local infiltration of 0.2 ml of lidocaine HCl (Xylocaine® 1:200,000 - AstraZeneca) at a concentration of 1:200,000. Antisepsis of the area was performed with a 10% solution of polyvinylpyrrolidone-iodine (povidone degermante - Johnson waxes). Linear submandibular incision was made approximately 10 mm on the right side of the mandible, through the skin and periosteum using a #15 blade (Feather Safety Razon CO., LTD. Medical Division) mounted on a #3 cable scalpel. The dissection was performed by detaching the masseter muscle and periosteum, following which the flap was retracted with delicate retractors. Once access to the body of the mandible had been obtained, the fracture was performed unilaterally with #700-701 steel drills and shifters. The fracture was then reduced and fixed with Portland cement in the experimental group. The suture was performed with 4.0 nylon wire in all procedures, which also helped in the reduction and fixation.

The Portland cement paste used in the experimental group was prepared immediately before use with a strict control of the humidity. The mixture was inserted into the fracture through a metal instrument.

After surgery the wound was cleaned. In order to minimize the risk of infection the antibiotic enrofloxacin was applied intramuscularly in 24/24h (0.5 ml/10kg) and analgesic dipyrone was administered intramuscularly for two days, 12/12h (0.2 ml/10kg) to alleviate postoperative pain. The animals were killed with a lethal dose of the general anesthetic ketamine hydrochloride (Dopalen® - Vetbrands) and Xylazine hydrochloride (Anasedanâ® - Vetbrands) at 7 and 21 days postoperatively, when bone repair was evaluated.

The mandible was surgically removed after the euthanasia in both groups. Following separation of the mandible by fibrous symphysis, the skin and soft tissues were removed in order to obtain a better fixation of the material during the histological process.

Radiogical study

Cone Beam Computed Tomography i-CAT® New Generation (Imaging Sciences International, Hatfield, Pennsylvania, USA) was used to obtain the radiographic images. Two rats were housed on an acrylic platform, enabling them to be fixed in order to carry out the imaging. The images of the fracture region were taken in the sagittal, coronal and axial planes. The thickness of the sections was 1 mm and the distance between the sections was also 1 mm for these reconstructions.

Next, the sections of each rat were selected and organized in a template. The subjective evaluation of each rat was saved in PDF (Portable Document Format) and a film of the cross-sections was selected for evaluation.

The images, coded and evaluated in a dark environment, were analyzed individually in random order, size 1:1 in a 17-inch monitor using Adobe Photoshop®. The images of each rat were analyzed separately. The evaluations of the digital imaging and CT scans were performed by two radiologists. A session for calibration of the examiners was held in order to explain to them the chosen method of assessment. The radiologists produced a report for each CT rat study.

Histological study

The specimens were fixed in 10% formalin for 24 hours and subsequently decalcified in formic acid solution and sodium citrate for approximately 5 days. After decalcification, they were washed in water for 24 hours, dehydrated in increasing alcohol solutions (70-100%), cleaned in xylene baths and embedded in paraffin. The specimens were sectioned into slices with a thickness of 5µm and stained with hematoxylin and eosin.

The phenomena of inflammation and repair were analyzed based on the criteria proposed by Kaplan, Hirsberg[5], described as follows: 0 = Absent, 1 = <15 cells/field, 2 = 15-50 cells/field and 3 => 50 cells/field. To classify the conditions of granulation tissue, foreign body reaction, clotting, osteoid, mature bone, biocompatibility, resorption and cartilage, the following criteria were adopted: 0 = absent, 1 = Present.

Statistical analysis

The statistical analysis of data was done using the chi-square test or Fisher's Exact test when it was not possible to use the former, and the Mann-Whitney test.

The margin of error used for interpreting the statistical tests was 5.0%. Data were entered in the Excel spreadsheet, and SPSS version 13 was used to obtain the statistical calculations.

Results

The mean weight was less when the pre- and postoperative times of evaluation of the two groups were compared. In the experimental group, at 7 days the weight had decreased from 345.50 ± 54.54 to 305.17 ± 43.69, and at 21 days from 408.17 ± 71.77 to 377.33 ± 67.03. In the control group, at 7 days the weight had fallen from 348.50 ± 20.06 to 375.50 ± 12.19; and at 21 days from 378.00 ± 47.41 to 372.17 ± 35.32.

Tables 1 and 2 present the results of the histological data for each group at the different times of evaluation.

TABLE 1 – Evaluation of the histological features of the initial phase of repair according to group and time of evaluation

Time of evaluation/ Histological features	Group				P value
	Experimental		Control		
	N	%	N	%	
TOTAL	**12**	**100.0**	**12**	**100.0**	
• **Inflammation**					
7 days - Absent	2	33.3	6	100.0	$p^{(1)} = 0.061$
15 - 50 cells/Field	1	16.7	-	-	
> 50 cells/Field	3	50.0	-	-	
21 days - Absent	5	83	6	100.0	$p^{(1)} = 1.000$
15 - 50 cells/Field	-	-	-	-	
> 50 cells/Field	1	16.7	-	-	
• **Granulation Tissue**					
7 days - Absent	6	100.0	6	100.0	**
Present	-	-	-	-	
21 days - Absent	6	100.0	6	100.0	**
Present	-	-	-	-	
• **Foreign Body Reaction**					
7 days - Absent	6	100.0	6	100.0	**
Present	-	-	-	-	
21 days - Absent	6	100.0	6	100.0	**
Present	-	-	-	-	
• **Blood Clot**					
7 days - Absent	3	50.0	6	100.0	$p^{(1)} = 0.182$
Present	3	50.0	-	-	
21 days - Absent	5	83.3	5	83.3	$p^{(1)} = 1.000$
Present	1	16.7	1	16.7	

(**): Not determined owing to absence of categories.
(1): Fisher´s Exact Test.

TABLE 2 – Evaluation of the histological features of the final phase of repair according to group and time of evaluation.

Time of evaluation/ Histological features	Experimental Group		Control Group		P value
	N	%	N	%	
TOTAL	**12**	**100.0**	**12**	**100.0**	
Osteoid					
7 days – Absent	2	33.3	1	16.7	$p^{(1)}$= 1.000
Present	4	66.7	5	83.3	
21 days – Absent	1	16.7	1	16.7	$p^{(1)}$= 1.000
Present	5	83.3	5	83.3	
Cartilage					
7 days – Absent	6	100.0	6	100.0	**
Present	-	-	-	-	
21 days – Absent	6	100.0	4	66.7	p=0.455
Present	-	-	2	33.3	
Mature bone					
7 days – Absent	4	66.7	4	66.7	$p^{(1)}$=1
Present	2	33.3	2	33.3	
21 days – Absent	1	16.7	2	33.3	$p^{(1)}$=1
Present	5	83.3	4	66.7	
Bone resorption					
7 days – Absent	2	33.3	2	33.3	$p^{(1)}$=1
Present	4	66.7	4	66.7	
21 days – Absent	-	-	5	83.3	p=0.015*
Present	6	100.0	1	16.7	

(*): Significant at 5.0%.

Figure 1 presents evaluations of the histological data comparing the times of evaluation in the experimental and control groups.

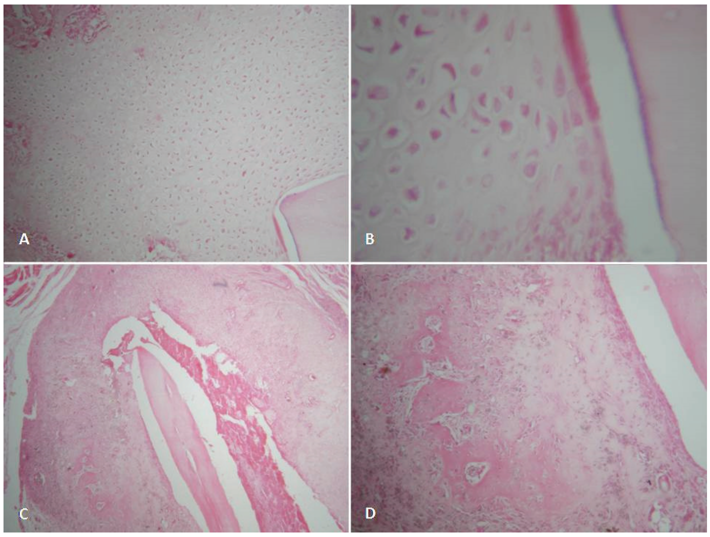

FIGURE 1 – **A**: Bone repair of the fracture showing cartilage formation in the control group at 21 days (HE 100x); **B**: Close-up of the cartilage formation (HE 400x); **C**: Fragment of the fractured bone envolved by a bone callous in development with granules of the material inserted in the experimental group after 21 days (HE 40x); **D**: Close-up of the bone formation parallel to the fractured bone (HE 100x).

The mean duration of surgery was 10.42 minutes in the experimental group and 7.08 in the control group, a difference that was statistically significant ($p < 0.05$).

According to the evaluation of the CT scans, in the control group there are suggestive images of a fracture line in all specimens, and in the experimental group, in addition to the fracture line, a hyperdense material is apparent, suggesting the presence of the material used for testing (Portland cement). In one animal in the experimental group hyperdense material was found in the soft tissue (Figures 2 and 3).

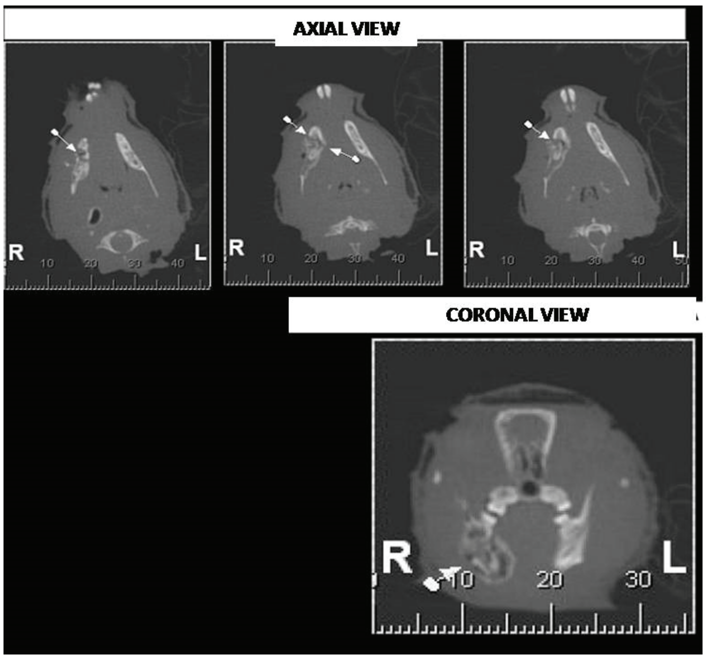

FIGURE 2 – CT scan of the experimental group at 21 days (the white arrows show the fracture line).

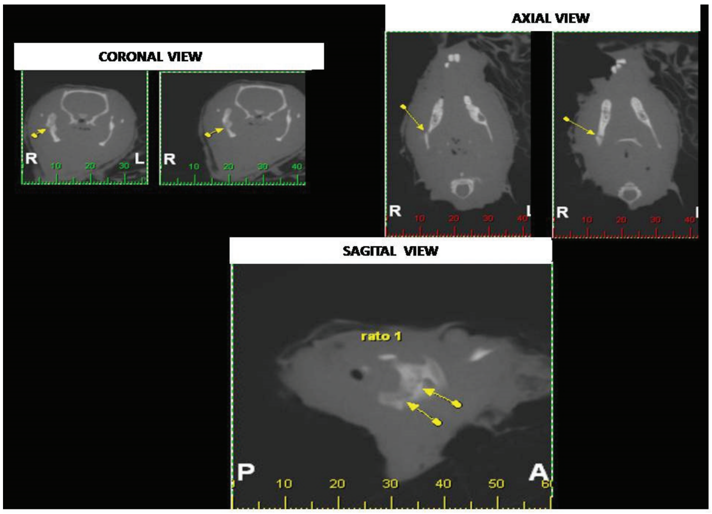

FIGURE 3 – CT scan of the control group at 21 days (the white arrows show the fracture line).

Discussion

Currently the treatment of facial fractures ranges from follow-up, maxillomandibular immobilization by locking steel wire and/or rubber bands to direct fixation with titanium plates or the use of resorbable materials. Stable internal fixation with plate and screws or titanium screws only for the various types of fracture is the most widely accepted technique in the wolrd today[3].

Despite the excellent prognosis stable internal fixation has a number of disadvantages, which emphasizes the need to remove the material after the phase of bone repair in some cases and the need for targeted training in its use, in addition to its high cost.

In view of these drawbacks, new materials, even those already tested in other areas of health, should be evaluated and developed to fill this gap in the treatment of mandibular fractures.

In this connection, Portland cement is presented as an option as it has been successfully tested in other specialties of dentistry, but not in traumatology[4]. Because studies of innovative treatments constitute a pressing need and also the fact such studies in humans involve ethical concepts, the use of animals for this purpose is of great value in discovering new forms of treatment.

Research involving laboratory animals nowadays is recommended, but each animal model has advantages and limitations depending on the experiment. When considering an appropriate experimental model, one intuitively seeks an animal similar to humans.

The ideal species for the study were the ones most closely related to humans, making monkeys one option. However, these animals are large and therefore difficult and expensive to acquire with regard to the structure of the bioterium and the experiment itself[6].

The choice of rats as an experimental model has advantages such as low cost, easy handling and quick response of tissues that warrant its use[7], despite the extrapolation of results being partially impaired.

There is no study using this technique for mantaining the fractured mandibular segment with Portland cement, hence the choice of methodology in the present study.

In cases of favorable mandibular fractures the animal's own muscles help in the reduction and fixation of the fractured segments[8]. Thus it was decided in this study to perform a favorable linear fracture, following which its reduction and fixation could be achieved by the muscles in the control group and by Portland cement in the experimental group. This study model has a limitation in that the placement of a maxillo-mandibular plate is not feasible.

When analyzing the time of evaluation, it may be suggested that the surgical technique chosen is feasible and easy to reproduce in the animal model, since the average surgical time was approximately 10.42 minutes. The explanation for the difference in duration of the surgery between the two groups being statistically significant could be the fact that the experimental group required an extra step for the placement of cement.

During insertion of the cement it was observed that there is a possibility of soft tissue impregnation, which could account for the inflammation of this tissue. It is hypothesized that the use of cement only in human fractures would not be sufficient for treating them, since the action of muscle may lead to the displacement of the fracture.

Thus a lower profile plate associated with the use of the cement could be used for purposes of reduction and fixation of fractured segments in which the cement would be an element for stimulating bone repair.

It is worth noting that when the cement is being placed the blood may change the time required for the material to take, so it is recommended that the fracture be isolated with gauze until this process begins. We could thus state that the use of an accelerator associated with Portland cement would be a breakthrough in traumatology in that it would reduce the number of implants and facilitate bone repair in a shorter time.

The weight of the animals was seen to decrease until the seventh day after surgery, the weight loss being greater in the experimental group. These results also corroborate the literature reviewed[9-11], suggesting that the weight gain could be extended to all rats for each group if the time between surgery and euthanasia was greater, as Luz, Araujo[9] has shown in different periods of evaluation, namely 7, 15, 30 and 90 days. The research model was thus shown to be safe for the preservation and quality of life of animals until the end of the evaluation period, since there was no excessive loss of weight that could indicate inadequate nutrition and animal suffering. The minimum weight loss could be attributed to the rats' post-trauma and pain in executing their movements.

When the control and experimental groups were compared at 21 days of evaluation, it was observed that all animals in the control group revealed a hypodense stroke, which is suggestive of lack of calcification. In the experimental group, 3 of the 4 animals had no hyperdense material, suggesting that the material was resorbed by the organism. In one of them the displacement of fracture fragments was still evident, as well as hyperdense spots and osteolytic areas with edema and hyperdense spots in the soft tissue. These hyperdense areas are visible when barium sulfate is added to Portland cement in order to permit its radiographic visualization.

Histological inflammation was detected in four of the six experimental rats at euthanasia after seven days, which did not occur in the control group. This initial evidence of inflammatory changes is positive in nature and may signal the ability of Portland cemet in relation to its osteoinductive action. This action has been observed previously by Abdullah *et al*.[12] to test Portland cement for cell culture.

At the same time, the absence of granulation tissue in the histological cross-sections of this study also suggests that the inflammatory response is transitory and sustains the non-aggressive nature of the implanted material. This emphasizes what was previously observed by Ribeiro *et al*.[13], whose material was tested *in vitro*.

In only one animal of the experimental group was an inflammatory reaction observed. There was no foreign body reaction in either group. These elements characteristically enhance the biocompatibility of the cement, according to the literature[4].

The experimental group also presented a higher percentage of osteoid and mature bone, which suggests that the material is also biocompatible and osteoinductive. Osteoinduction is due to the fact the material promotes an alkaline pH and a release of calcium and phosphate elements that stimulate the calcification process and account for the basic mechanisms of physico-chemical regeneration of hard tissue[14].

On the basis of these properties all specimens in the experimental group showed bone formation. This suggests that Portland cement is able to promote bone remodeling.

In the control group two animals had cartilage formation, which was not observed in the experimental group, characterizing a pseudarthrosis. We suggest that further research should be conducted observing the Portland cement at longer intervals of 30, 60 and 90 days in order to obtain more information on this material when used in the conditions chosen for this study. It is noteworthy that none of the specimens in the experimental group displayed any infection caused by nonsterile material. This may be due to the fact that the material is produced at a high temperature and results in a highly alkaline product incompatible with microbial reproduction, as evidenced in the study of Duarte *et al*.[15].

It is important in traumatology to use models other than the one adopted here to achieve better results in terms of bone fixation, thereby leading to a more effective treatment of the fractures. Such studies represent a challenge in the attempt to evaluate the behavior of a material in a mobile bone area, the results of which in animals may not be reproducible in humans because of the bioethical requirements.

Conclusion

The Portland cement served to promote bone healing.

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