The objective of this study was to investigate bone response after implantation of MTA (Mineral Trioxide Aggregate) in the rabbit mandible. Experiments were carried out on the right mandibular body of 8 adult male rabbits. The animals were divided into two groups (control group and test group). In this in vivo study, MTA was used as an interpositional graft material in critical-size bone defects of rabbit mandibles. The animals were sacrificed on day 30 after surgery. The samples obtained from the mandibles were subjected to histological procedures, which permitted the collection of sections with a thickness of 60±10µm. The sections were stained with Haematoxylin and Eosin and Goldner Trichrome stain and examined under a light microscope.

No important inflammatory reactions were detected in any of the samples of the treated group. The results confirm the excellent biocompatibility of MTA. The implantation of MTA in bone defects led to bone regeneration 4 weeks after surgery. However, the growth rate was not significant and the amount of newly formed bone was limited with the use of MTA in this specific application. Sample examination did not suggest complete evidence of new bone growth from either an inductive or conductive perspective.

Key words: Mineral Trioxide Aggregate (MTA) – Dental materials – Oral surgery techniques – Animal experiments – Histological studies.
**Materials and Methods**

**MTA (Mineral Trioxide Aggregate)**
MTA (ProRoot™; Dentsply/ Tulsa Dental™, Tulsa OK, USA) powder consists of fine hydrophilic particles and its principle compounds are tricalcium silicate, tricalcium oxide, and silicate oxide. Additionally, there are small amounts of other mineral oxides that are responsible for the chemical and physical properties of this material (Torabinejad et al., 1995b). The presence of bismuth oxide powder makes the material radiopaque and when the material is hydrated it becomes a colloidal gel (Schwarz et al., 1999).

**Surgical Technique**

The studies on animals were carried out in accordance with the guidelines laid down by the European Communities Directives (86/609/EEC-24/11/1986). At all stages of animal manipulation, special care was taken to minimize the injury, pain and discomfort of the animals.

Eight healthy 3-month old adult male New Zealand rabbits weighing approximately 2.5 +/- 0.5 Kg were used. The animals were divided equally into two groups according to the treatment received. The hair on the skin around the mandible and neck regions of the rabbits was shaved and the skin scrubbed with Betadine™ (Viatris Pharmaceuticals™ S.A.U.) to induce asepsis and disinfection. The animals were anaesthetized with an intraperitoneal injection of 10mg/2ml Diazepam™ (Lab. Roche™ S.A., Spain) and Ketamine™ (Parke Davis™, Lab., Spain) at a dose of 1.5 mg/Kg per body weight, supplemented with local infiltration of 2% Lignocaine with 1:50,000 adrenaline. Subsequently, specimens were dehydrated in a graded series of alcohol solutions for 48 hours, with a final soaking in 100% ethanol for six days. The samples were embedded in hard methyl methacrylate resin and were cut perpendicular to the external mandibular surface. Blocks of resin were sectioned using hard-tissue cutting equipment (Accutom™, Struers) to a thickness of 180±30 μm, and ground in a grinding machine (DIAP-8™, Struers) to a thickness of 60±10 μm. Then, the samples were stained with Hematoxylin (Harris Hematoxylin Acidified Shandon Sci™, Ltd, UK) and Eosin (Eosin Y, Shandon Sci™. Ltd, UK), and Goldner Trichrome for histological examination under the light microscope (Leica™ LB30T, Germany) which was connected to a video camera (Don-pisha™ 3CCD, Sony™, Japan) through a computer-based system (Leica™ Q500IW, Germany).

**RESULTS**

None of the samples studied presented signs of severe adverse reactions. The bone defect control demonstrated normal healing, with little new bone formation (Figs. 1 and 2). In the treated group, histological examination revealed the presence of a dense fibrous connective layer around the material (Fig. 3). Almost none or a slight inflammatory reaction was noticed in the adjacent tissues. The bone-defects implanted with MTA were full of particles, although some particles had migrated into the masseter muscle. It was common to find some particles of the material in the defect. Figure 4 illustrates an area where early osteoid formation can be seen to be associated with MTA particles. Histological examination revealed that the grafts were surrounded by a thin fibrous layer in all cases.
DISCUSSION

The model for testing bone repair or regeneration materials has frequently been the tibia of small animals (Olsen et al., 1994; Olsson et al., 1981). The mandible bone has been tested less (Bhambhani and Bolanos, 1993) despite being recommended for testing endodontic materials since root-filling cements are placed in intimate contact with periradicular tissues (Torabinejad et al., 1995c; 1998). MTA is a new material developed for endodontic use that has significant improvements over other materials used for procedures in bone tissues (Schwarz et al., 1999). In addition to endodontic applications, MTA may also have other clinical uses. The present study is a preliminary report on the use of MTA as a possible bone substitute and further studies are needed to ascertain this possible application. An interesting clinical question is whether MTA can be used together with bone-grafting techniques: for example, if a tooth perforation has caused an infra-osseous periodontal defect, the perforation could be repaired with MTA while assuming treatment of the bone defect.

Another aspect we considered in this study was the type of bone implantation used. Displacement of the MTA to the surrounding tissues occurs with this type of intraosseous implantation since it is a complex and time-consuming technique (Friend and Browne, 1969). We did not use Teflon tubes or silicone tubes as a vehicle to carry MTA into the prepared bone defects because the presence of inflammatory cells in the histological preparations is caused more by the properties of the vehicle material than the cytotoxicity of the material tested (Olsson et al., 1981; Bhambhani and Bolanos, 1993). Despite the technical difficulties, we used this technique in mandibles since it represents a more realistic approach. The only premise to test a new material must be that it must be in contact with osseous tissue. Previous histological findings revealed that the application of MTA as a apical filling material does not prevent the regeneration of dental and osseous tissues and may produce cementoid formation on MTA (Torabinejad et al., 1995a). However, one of the most interesting aspects of this study was the failure to note any significant increase in the amount of bone associated with MTA. With current emphasis on the...
importance of cortical penetration to stimulate bone formation or at least to facilitate osteoconduction (Majzoub et al., 1999), the failure to note more widespread osteogenesis in any animals of our series suggests that additional criteria are necessary to support the osteoconductivity of MTA, which might include longer-term studies. There are a number of factors that must be considered prior to drawing any definitive conclusions from the results reported. Essentially, in order to conclude that the particles exhibited osteoconductive potential, the characteristic histological presentation of osteoblastic and osteoclastic activity should have been present.

MTA has been shown to be biocompatible in several reports, including the present study (Bhambhani and Bolanos, 1993; Kettering, 1995; Torabinejad et al., 1995d). The ideal bone substitute should be biocompatible, osteoinductive or at least osteoconductive, and should have satisfactory mechanical properties. Among inorganic materials, calcium salts were the first implants to be successfully used in clinical practice. They have the advantage of simulating the human mineral bone phase and they do not elicit any immune response because they do not contain living cells or any organic matrix. Calcium and phosphorous ions are the main ions present in the composition of MTA. MTA may prove to be biocompatible when used in contact with osseous cells and hard tissues because these ions are also the principal components of these tissues (Koh et al., 1998; Mitchell et al., 1999). Thus, when MTA was used as a apical filling material, fibrous connective tissue and thin layers of hard tissue formed in direct contact with it (Regan et al., 2002; Economides et al., 2003). The formation of cementum and periodontal ligament fibres was also observed on its surface (Torabinejad et al., 1995a; 1997). Although MTA has been used as an apical plug (Torabinejad et al., 1999), an extensive search of literature performed for this study no evidence was found relating MTA to a bone substitute. In addition, MTA has proned to be useful in many clinical situations (Schwarz et al., 1999). In fact, the pattern of healing adjacent to MTA, characterized by the almost complete absence of inflammation, together with a greater incidence of hard tissue formation against the MTA implants, supports the investigation of this material as a bone repair material (Bhambhani and Bolanos, 1993). Our histological study demonstrated none or minimal inflammatory reaction of MTA implanted in bone. This factor may be an advantage in the clinical use of MTA as a bone substitute in small bone defects, despite the fact that its handling properties are not ideal. MTA can be difficult to place and compact in bone defects. In practical terms, it does not present the ideal handling properties essential in a bone repair material.

The results of the present study confirm the excellent tissue biocompatibility of MTA allowing us to conclude that the implantation of this material, in critical-size bone defects, results in bone regeneration, although not at a considerable growth rate. The results of this study also demonstrate the osteoinductive capacities of MTA.

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REFERENCES


The osteoinductive potential of MTA (Mineral Trioxide Aggregate): a histologic study in rabbits