Defect Repair in the Rat Mandible With Bone Morphogenic Protein 5 and Prostaglandin E₁

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Objective: To compare the osteogenic abilities of 2 growth factors (bone morphogenic protein 5 [BMP-5] and prostaglandin E₁ [PGE₁]) and 2 carriers (collagen/polylactic acid [PLA] and collagen/calcium hydroxyapatite cement [HAC]) in the repair of a rat mandibular body defect.

Design: Prospective controlled trial.

Subjects: Twenty-nine Sprague-Dawley rats.

Interventions: Critical size defects were created in the bilateral mandibular bodies of the rats. Each hemimandible was assigned to an experimental group. The defects were filled with PLA (group 1), PLA with BMP-5 (group 2), PLA with PGE₁ (group 3), HAC (group 4), HAC with BMP-5 (group 5), or HAC with PGE₁ (group 6). The control group (group 7) had unfilled defects. The animals were killed after 12 weeks, and the nonmineralized specimens were processed histologically. Stereologic techniques were used to determine the volume fractions of new bone, osteoid, marrow, remaining implant, and fibrous tissue in each defect.

Results: The HAC/BMP-5 group (group 5) contained significantly more new bone than the PLA/BMP-5 group (group 2) (P = .02), the HAC and HAC/PGE₁ groups (groups 4 and 6) (P = .002), and the control group (group 7) (P < .01). The HAC/BMP-5 group also had less fibrous tissue than the HAC group and the HAC/PGE₁ group (P < .001). Groups 5 and 6 had less fibrous tissue than group 7 (P < .01). The groups containing PGE₁ demonstrated significantly more osteoid development than the other experimental groups (P < .001).

Conclusions: Inclusion of BMP-5 in an implant with calcium hydroxyapatite cement resulted in the formation of significantly larger fractions of new bone and less fibrous tissue ingrowth than occurred in the other experimental groups. The presence of PGE₁ resulted in larger amounts of osteoid deposition, suggesting the potential for delayed bone healing.


The repair of craniofacial bony defects is surgically challenging because of the delicate and complex anatomy of the facial skeleton. The reconstruction of segmental mandibular defects following trauma or ablative surgery remains controversial because no single method results in the replacement of tissue that precisely matches the quantity or structural qualities of autogenous mandibular bone. In addition, all current methods of reconstruction have their attendant risks.

Refinements in tissue engineering techniques over the past decade have enabled the in vivo regeneration of living bone in many animal models. The ideal synthetic implant for bone reconstruction combines bone growth factors with a material that has enough plasticity to allow in situ molding. The bone morphogenic proteins (BMPs) are growth factors that have demonstrated ability to induce orthotopic and heterotopic new bone formation.¹⁻⁹ Prostaglandin E₁ (PGE₁) is also capable of bone growth induction but has not been studied in the repair of critical size defects in the mandible.¹⁰⁻¹⁵ Calcium phosphate–based synthetic implants are thought to be ideal carriers for bone growth factors, not only because of their biocompatibility and osteoconductive and osseointegrative properties, but also because of their ability to maintain the volume of regenerated bone.¹⁶⁻¹⁷ Other carriers studied for the repair of mandibular defects with BMPs include bioabsorbable synthetic polymers, which have not been compared with the calcium phosphate–based implants in terms of regenerated bone volume.¹⁻³,¹⁶ Both calcium hydroxyapatite and polylactic acid implants are widely used for craniofacial, dental, and orthopedic bone reconstruction.

In this study, we compared the osteogenic potential of 2 growth factors,
BMP-5 and PGE₁, in 2 carriers, collagen/polyactic acid (PLA) and collagen/calcium hydroxyapatite cement (HAC). Our objective was the development of a cost-effective synthetic bone substitute that would yield the best new bone deposition and effect repair of a critical-size rat mandibular defect.

Institutional guidelines for the humane use of laboratory animals were followed, and the institutional animal care and use committee of the University of Kentucky at Lexington approved the study. Twenty-nine Sprague-Dawley retired male breeder rats with a mean ± SD weight of 447.8 ± 41.2 g were housed in the Department of Laboratory Animal Resources at the University of Kentucky Medical Center at Lexington under a constant temperature of 24.5°C for 1 week prior to surgery. The animals were fed commercial rat chow and had access to food and water ad libitum. Each hemimandible was assigned to 1 of 7 experimental groups: (1) those filled with PLA (PLA; n = 8); (2) those filled with PLA containing BMP-5 (PLA/BMP-5; n = 8); (3) those filled with PLA containing PGE₁ (Sigma Chemical Company, St Louis, Mo) (PLA/PGE₁; n = 8); (4) those filled with HAC (HAC; n = 9); (5) those filled with HAC matrix with BMP-5 (HAC/BMP-5; n = 8); and (6) those filled with HAC matrix with PGE₁ (HAC/PGE₁; n = 9). The control group (7) consisted of hemimandibles with unfilled defects (n = 8) (Table 1).

PREPARATION OF IMPLANTS

The amount of each carrier used had been determined previously by the volume needed to fill the critical size defect. Twenty-five milligrams of the porous polyactic acid pellets (Drilac; Kensey Nash Corporation, Exton, Pa) and collagen/calcium hydroxyapatite cement (Bone Source; Stryker Leibinger, Kalamazoo, Mich) with either 100 µL of sterile isotonic sodium chloride solution (saline) (group 1), 100 µL of BMP-5 solution (group 2), or 100 µL of PGE₁ solution (group 3). This volume of liquid was found to be appropriate for filling the porous PLA implants without excess. The HAC bone substitute was prepared in a sterile fashion with 25 mg of collagen and 100 mg of calcium hydroxyapatite cement (Bone Source; Stryker Leibinger, Kalamazoo, Mich) with either 100 µL of sterile saline (group 4), 100 µL of BMP-5 solution (group 5), or 100 µL of PGE₁ solution (group 6). The BMP-5 (Sigma Chemical Company) was suspended in a buffer (5mM sodium glutamate, 2.5% glycine, 0.5% sucrose, and 0.01% Tween 80, pH 4.5) at a concentration of 0.02 mg/mL. The PGE₁ was suspended in sterile saline, pH 4.6, at a concentration of 2 mg/mL.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Implant Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Collagen/polyactic acid implant</td>
</tr>
<tr>
<td>2</td>
<td>Collagen/polyactic acid implant with BMP-5</td>
</tr>
<tr>
<td>3</td>
<td>Collagen/polyactic acid implant with PGE₁</td>
</tr>
<tr>
<td>4</td>
<td>Collagen/calcium hydroxyapatite cement implant</td>
</tr>
<tr>
<td>5</td>
<td>Collagen/calcium hydroxyapatite cement implant with BMP-5</td>
</tr>
<tr>
<td>6</td>
<td>Collagen/calcium hydroxyapatite cement implant with PGE₁</td>
</tr>
<tr>
<td>7</td>
<td>Unfilled defect (control)</td>
</tr>
</tbody>
</table>

Abbreviations: BMP-5, bone morphogenic protein 5; PGE₁, prostaglandin E₁.

METHODS

The rats were anesthetized with a standard anesthetic cocktail consisting of ketamine hydrochloride (60 mg/kg) and xylazine (5 mg/kg) administered intraperitoneally. Surgery was performed using an aseptic technique. A linear incision was made through the skin, subcutaneous tissues, and masseter muscle paralleling the inferior border of the mandible. The buccal and lingual surfaces of the mandible were exposed with an elevator, and a 5 × 5-mm full-thickness defect was created in the body of the mandible posterior to the root of the incisor. This ostectomy was performed with a high-speed drill and irrigation, and did not interrupt mandibular continuity at the alveolus. The resulting defects were filled as previously described. In those animals implanted with the HAC matrix (groups 4-6), the material was allowed to set in situ for 15 minutes. The surgical wounds were closed in 2 layers (periosteum/muscle layer and skin layer) with 4-0 polyglycolic suture. Calcein at a dose of 10 mg/kg was administered for fluorochrome labeling of the edges of the defect. The animals were allowed to recover from anesthesia and then returned to the Department of Laboratory Animal Resources for postoperative care where veterinarians supervised them. Buprenorphine (0.1 mg/kg) was administered subcutaneously twice a day for the first 3 days postoperatively, and the rats were maintained on a diet of ground rat chow and water, to which they had access ad libitum.

One rat (groups 4 and 5) died of asphyxiation on rat chips postoperatively. Another rat developed a postoperative wound infection that was effectively treated with drainage and antibiotics. One other animal developed a seroma that was drained uneventfully. Most of these animals continued to gain weight postoperatively, and this weight gain was significant (preoperative mean ± SD weight, 447.8 ± 41.2 g; postoperative mean weight, 511.3 ± 37.9 g; P < .001). The surviving animals were killed 12 weeks postoperatively by lethal injection of 150 mg/kg of pentobarbital sodium intraperitoneally, and hemimandibles were harvested.

HISTOLOGIC ANALYSIS

The hemimandibles were fixed in 10% neutral buffered formalin (Sigma Chemical Company) for 3 days. The nondeamineralized hemimandibles were dehydrated in graded ethanol and acetone under continuous negative pressure. Twenty-one of the hemimandibles had to be excluded from further analysis owing to histologic processing errors. These 21 specimens included 2 hemimandibles from group 1; 2 from group 2; 2 from group 3; 6 from group 4; 5 from group 5; 2 from group 6; and 2 from group 7.

The remaining 31 specimens were infiltrated with and embedded in polymethylmethacrylate. Sectioning was performed with a rotating diamond wafering saw (Buehler, Lake Bluff, Ill). The saw excision was 900 µm for each section, with approximately half of this thickness being absorbed by the blade width. The sections were then mounted on plastic slides (Watsatch Histology Consultants, Winnemucca, Nev), ground to a thickness of 100 µm or less, and polished. The sections were stained with Sanderson rapid bone stain (Surgipath Medical Industries, Richmond, Ill) and counterstained with acid fuchsin. This staining combination afforded sufficient contrast to distinguish bone, which stained pink, from cement (beige), osteoid (deep blue), and fibrous tissue (light blue). The remaining PLA implant stained a gray-blue. Fluorescence microscopy was used to identify the limits of the defect.

STEREOLOGIC ESTIMATES

The volume fractions of osteoid, remaining implant, mature bone, new bone, marrow, and fibrous tissue were determined.
for the entire defect using design-based stereologic techniques. These techniques provide a statistically unbiased, quantitative estimate of the 3-dimensional composition of the defect and do not depend on any assumption regarding the tissue geometry. At least 1 section was analyzed for each specimen (mean±SD number of specimens analyzed, 4.3±1.9), with the number of fields and field size varying with the size and orientation of the defect.

Data collection was performed in a blinded fashion using a X4 objective. A Nikon Eclipse E600 fluorescent microscope (Melville, NY) with a Spot Insight color digital camera (Openco, Dulles, Va) was interfaced to a Dell Dimension L866r computer (Austin, Tex) and used to project images onto a 15-inch Dell monitor. The stereologic data were collected with custom software using a uniform random sampling protocol with a stage micrometer. The sample fields were distributed in a regular lattice pattern that was randomly positioned with respect to the tissue and were defined by a graphic overlay that included a regular point-counting lattice consisting of 140 points, with a distance of 0.198 mm between points. The volume fraction estimates were made using test point counting:

\[
V_{Y,ref} = \frac{\sum_{i=1}^{n} P_Y}{\sum_{i=1}^{n} P_{ref}}
\]

where \(P_Y\) is the number of lattice points hitting phase Y and \(P_{ref}\) is the number of points hitting the reference space.

STATISTICAL ANALYSIS

Data analysis was performed with Statview statistical software (SAS Institute, Cary, NC). Two-way analyses of variance (ANOVA) were used to identify differences in mean values for the defect volume fractions of osteoid, cement, fibrous tissue, and new bone. The first factor of the ANOVA tested differences attributable to the carrier (PLA or HAC matrix). The second factor tested for the 2 growth factors (BMP-5 or PGE1). When there was significant interaction between these factors, an analysis of simple effects using a 1-way ANOVA was performed to determine the exact pattern of differences.

The Games/Howell post hoc test was used to identify significant pairwise differences. The importance of significant differences (effect size) was estimated by a Hayes \(\omega^2\) statistic. The effect size indicates the percentage of the total variances that is explained by the independent variable (0.01<\(\omega^2\)<0.05 indicates a small effect; 0.06<\(\omega^2\)<0.13 indicates a medium effect; and \(\omega^2\)≥0.14 indicates a large effect). The experimental means were compared with the control (group 7) using the Dunnett\(\tau\) statistic test. A P value of .05 or less was considered significant for all comparisons. The principal investigator (O.A.A.) had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

RESULTS

The specimens containing BMP-5 and PGE1 were generally characterized by incomplete defect healing with mature bone around the circumference of the defect that was contiguous with the cut edges of the native mandibular bone. This bony shell enclosed a cavity that was filled with varying amounts of fatty marrow, new bone, and remaining implant (Figure 1). Complete defect healing occurred in only 3 specimens (1 in the PLA group and 2 in the PLA/PGE1 group). Groups without growth factors demonstrated limited new bone growth at the defect edges, with collapse of adjacent musculature into the defect. The defects in these groups contained abundant fibrous tissue with various amounts of remaining implant (Figure 2).

NEW BONE GROWTH AND OSTEOID VOLUME FRACTIONS

The 2-way ANOVA indicated that the specimens containing BMP-5 had significantly more new bone growth than the groups containing PGE1 or no growth factor (P=.01; Table 2). However, because there was an interaction among the variables, an analysis of simple effects was performed. This analysis revealed that the HAC/BMP-5 group contained more new bone than the PLA/BMP-5 group (P=.02; \(\omega^2=0.05\); small effect) and the HAC and HAC/
Table 2. Group Mean Volume Fractions for Selected Measurements*

<table>
<thead>
<tr>
<th>Group</th>
<th>New Bone</th>
<th>Osteoid</th>
<th>Marrow</th>
<th>Remaining Implant</th>
<th>Fibrous Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.445 ± 0.363</td>
<td>0.053 ± 0.112</td>
<td>0.052 ± 0.087</td>
<td>0.116 ± 0.198</td>
<td>0.332 ± 0.363</td>
</tr>
<tr>
<td>2</td>
<td>0.450 ± 0.361</td>
<td>0.040 ± 0.082</td>
<td>0.060 ± 0.118</td>
<td>0.086 ± 0.148</td>
<td>0.356 ± 0.387</td>
</tr>
<tr>
<td>3</td>
<td>0.429 ± 0.368</td>
<td>0.085 ± 0.137</td>
<td>0.059 ± 0.116</td>
<td>0.078 ± 0.162</td>
<td>0.348 ± 0.406</td>
</tr>
<tr>
<td>4</td>
<td>0.316 ± 0.372</td>
<td>0.014 ± 0.047</td>
<td>0.034 ± 0.063</td>
<td>0.094 ± 0.184</td>
<td>0.542 ± 0.395</td>
</tr>
<tr>
<td>5</td>
<td>0.646 ± 0.300</td>
<td>0.018 ± 0.039</td>
<td>0.088 ± 0.104</td>
<td>0.133 ± 0.207</td>
<td>0.135 ± 0.179</td>
</tr>
<tr>
<td>6</td>
<td>0.457 ± 0.349</td>
<td>0.083 ± 0.125</td>
<td>0.042 ± 0.076</td>
<td>0.125 ± 0.213</td>
<td>0.277 ± 0.333</td>
</tr>
<tr>
<td>7</td>
<td>0.353 ± 0.365</td>
<td>0.017 ± 0.038</td>
<td>0.051 ± 0.102</td>
<td>None</td>
<td>0.490 ± 0.401</td>
</tr>
</tbody>
</table>

*See Table 1 for group descriptions.

Removal of implant volume fractions did not differ significantly between the groups (P = .26 for the effect of the carrier; P = .95 for the effect of the growth factor). The 2-way ANOVA indicated that the groups containing BMP-5 had significantly less fibrous tissue ingrowth than the other experimental groups (P = .001), though there was again an interaction among the variables. The carrier did not seem to have an effect on fibrous tissue ingrowth (P = .31). The analysis of simple effects revealed that the HAC/BMP-5 group had significantly less fibrous tissue than the HAC group and the HAC/PGE1 group. Also, the HAC/PGE1 group had less fibrous tissue ingrowth than the HAC group (P < .001; ω² = 0.14; large effect). The HAC/BMP-5 and HAC/PGE1 groups also had significantly less fibrous tissue than the control group (t = 3.847 for group 5 vs group 7; t = 3.156 for group 6 vs group 7; P < .01). The PLA group had less fibrous tissue than the HAC group (P = .004; ω² = 0.05; small effect) and the control group (t = 2.368; .05 > P > .01). However, the PLA/BMP-5 group had more fibrous tissue ingrowth than the HAC/BMP-5 group (P = .01; ω² = 0.06; medium effect). The PLA/BMP-5, PLA/PGE1, and HAC groups did not differ significantly from the control group.

Figure 3 is a graphic depiction of the volume fractions of new bone, osteoid, marrow, remaining implant, and fibrous tissue in each experimental group.

**REMAINING IMPLANT AND FIBROUS TISSUE VOLUME FRACTIONS**

Removal of implant volume fractions did not differ significantly between the groups (P = .26 for the effect of the carrier; P = .95 for the effect of the growth factor). The 2-way ANOVA indicated that the groups containing BMP-5 had significantly less fibrous tissue ingrowth than the other experimental groups (P = .001), though there was again an interaction among the variables. The carrier did not seem to have an effect on fibrous tissue ingrowth (P = .31). The analysis of simple effects revealed that the HAC/BMP-5 group had significantly less fibrous tissue than the HAC group and the HAC/PGE1 group. Also, the HAC/PGE1 group had less fibrous tissue ingrowth than the HAC group (P < .001; ω² = 0.14; large effect). The HAC/BMP-5 and HAC/PGE1 groups also had significantly less fibrous tissue than the control group (t = 3.847 for group 5 vs group 7; t = 3.156 for group 6 vs group 7; P < .01). The PLA group had less fibrous tissue than the HAC group (P = .004; ω² = 0.05; small effect) and the control group (t = 2.368; .05 > P > .01). However, the PLA/BMP-5 group had more fibrous tissue ingrowth than the HAC/BMP-5 group (P = .01; ω² = 0.06; medium effect). The PLA/BMP-5, PLA/PGE1, and HAC groups did not differ significantly from the control group.

**Figure 3** is a graphic depiction of the volume fractions of new bone, osteoid, marrow, remaining implant, and fibrous tissue in each experimental group.

**COMMENT**

Reconstruction of craniofacial bony defects following trauma and ablative oncologic procedures or in the repair of congenital anomalies is a frequent surgical challenge. Recent advances in tissue engineering technology give the promise of osteogenic, osteointegrative implants that will combine the advantages of in situ molding with rapid yet controlled resorption so that bone formation occurs in a predictable manner. Numerous biosynthetic materials have been used as bone graft substitutes, including collagen, extracellular matrix protein gels, inorganic calcium-based ceramics, and absorbable synthetic polymers.19,20 The biocompatibility and osteoconductive and osseointegrative properties of calcium-based alloplasts have been recognized for many years.16 Alloplasts have been recognized for many years.16 Alloplasts have been recognized for many years.16 Alloplasts have been recognized for many years.16 Alloplasts have been recognized for many years.16 Alloplasts have been recognized for many years.16
placed by bone, the process of in vivo replacement of calcium phosphate–based alloplasts has been reported to have been accelerated by the addition of bone growth factors to these materials.16,17

Biodegradable synthetic polymers, notably the poly-α-hydroxy acids (polyactic acid and polyglycolic acid), have been used for over 30 years as suture materials.21 Poly-α-hydroxy acid implants have been used as carriers for BMPs in several studies involving mandibular regeneration in rat mandibular critical size defect models.1,3 A poly-α-hydroxy acid polymer loaded with BMP-2 has been proven effective for repair of a full-thickness defect in the canine mandible model.9,10

The incorporation of bone-specific growth factors delivered in a suitable carrier is a requisite for an osteogenic implant. The BMPs (except for BMP-1) are members of the transforming growth factor β superfamily of polypeptide growth factors.22 Recombinant BMP have been shown to induce new bone formation in critical size mandibular defects in canine alveolar bone and rat mandibular angle models. We have been able to demonstrate the effectiveness of a synthetic bone substitute consisting of calcium hydroxyapatite cement, collagen, and a bone growth factor mixture containing BMP-3 through -7, as well as transforming growth factors β1 and β3, and fibroblast growth factor 1 for repair of a critical size defect in the rat mandibular body model.23

The prostaglandins have also been implicated in the regulation of bone metabolism. The prostaglandins are 20-carbon unsaturated fatty acid derivatives of arachidonic acid with a cyclopentane or cyclohexane ring structure. In the early 1980s, the intravenous administration of PGE, to infants with congenital cyanotic heart disease was found to result in periosteal bone proliferation.10,11 Similarly, systemic administration of PGE to animals has been shown to induce osteogenesis.12,13 Recently, local administration of PGE, to animals has been shown to result in new bone formation. The mandibular defect models used by Marks and Miller14,15 consisted of partial thickness ostectomy made in the lateral cortices of beagle mandibles. Repair was effected with local infusion of PGE, by osmotic pump and local implantation of controlled-release tablets.

The present study is the first report of the use of BMP-5 for defect repair in the mandible model. In this study, it is interesting that BMP-5 was effective in inducing significantly more bone growth than occurred in controls and PGE,-containing implants, but only when combined with the HAC implant (volume fraction of new bone, 64.6% ± 30.6% of defect area in the HAC/BMP-5 group compared with 45.5% ± 36.1% in the PLA/BMP-5 group, and 35.3% ± 36.5% in the control group). This effect could not be attributed to the HAC implant alone because the volume fraction of new bone in the HAC/BMP-5 group also differed significantly from the volume fractions of the other HAC-containing groups. Also, the HAC group did not differ significantly from the PLA group (31.6% ± 37.2% vs 44.5% ± 36.3%), and the groups containing PGE, also did not differ significantly (45.7% ± 34.9% for group 6 vs 42.9% ± 36.8% for group 3). In addition, the presence of BMP-5 alone could not explain this effect, as the PLA/BMP-5 group did not differ significantly from the other PLA groups, suggesting an interaction of the BMP-5 with the calcium hydroxyapatite cement. Other authors have demonstrated increased bone growth in the rabbit spinal fusion model using calcium-containing carriers in comparison with a collagen carrier.24 The results of the present study are significant in that the osteogenic potential of BMP-5 for mandibular regeneration has been previously unknown. All of the groups demonstrated some new bone growth, and this may reflect the potential for increased bone growth in this animal model in that bone growth plates do not fuse in the rat.25,26

The groups containing PGE, did have increased osteoid content compared with the other experimental groups and the control group, and this may suggest potential for delayed bone healing. The decreased osteogenic potential of PGE, compared with BMP-5 in the present study may in part be due to the lability of the prostaglandins at physiologic pH and body temperature.14,15

Complete defect filling occurred in only 3 of the specimens. It is interesting that none of these specimens were in the HAC/BMP-5 group. This contrasts to other studies using BMP-2 for rat mandibular defect healing. Linde and Hedner27 demonstrated complete bony bridging of a similar defect in the rat mandible after 12 days. This would suggest decreased osteogenic potential of BMP-5 in comparison with BMP-2.

Most of the specimens in our experimental groups contained relatively small amounts of remaining implant and marrow spaces, and these volume fractions were relatively consistent between groups (Table 2). It is interesting that the HAC implant was as effectively resorbed as the PLA implant, and this may reflect the lengthy healing time in this study. However, fibrous tissue infiltration of the defects was not uniform. The PLA groups demonstrated fairly consistent fibrous tissue volume fractions, and they showed limited fibrous tissue ingrowth in comparison with the HAC group (group 4) and the control. Yet the presence of a growth factor combined with the HAC implant (groups 5 and 6) seemed to have a greater effect on limiting fibrous tissue ingrowth, and this may again reflect the increased osteogenic potential of the HAC/bone growth factor combination implant.

In conclusion, this is the first published study comparing new bone growth in mandibular defects implanted with bone substitutes containing BMP-5 and PGE,. We found that BMP-5 in the presence of a calcium hydroxyapatite cement–containing implant resulted in the formation of significantly larger volume fractions of new bone and less fibrous tissue ingrowth than other experimental groups. The groups containing PGE, demonstrated more osteoid development than the other experimental groups, suggesting the potential for delayed bone healing.

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REFERENCES