Effect of recombinant human bone morphogenetic protein–2 on bone regeneration in large defects of the growing canine skull after dura mater replacement with a dura mater substitute

Laboratory investigation

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Object. This study was designed to evaluate the bone regeneration potential of the dura mater and dura mater substitute (Durepair) in the presence of recombinant human bone morphogenetic protein–2 (rhBMP-2) delivered in a collagen sponge–collagen-ceramic matrix (CCM; MasterGraft Matrix) in a large skull defect in growing canines.

Methods. Forty immature male beagles were used to create two 2.5 × 4-cm cranial defects on each side of the sagittal suture. The dura mater on the left side was cut to make a 1 × 3-cm defect and replaced with bovine skin collagen (Durepair). The dura mater on the right side remained intact. Different doses of rhBMP-2 (none [8 animals], 0.11 mg/ml [4 animals], 0.21 mg/ml [4 animals], and 0.43 mg/ml [8 animals]) were infused on 2 Type I bovine absorbable collagen sponge (ACS) strips. The strips were layered with the CCM (15% hydroxyapatite [HA]/85% tricalcium phosphate [TCP]) to reconstruct both cranial defects. In a fifth group (8 animals), 0.43 mg/ml rhBMP-2 was directly infused into the CCM. Demineralized canine cancellous freeze-dried demineralized bone matrix (DBM; 8 animals) was used as a control in a sixth group. All materials were fixed under 2 resorbable protective sheets (MacroPore). Skulls were resected 16 weeks after operation. Histological and histomorphometric analyses on the percentage of the defect spanned by bone, and the percentage of residual HA-TCP granules and collagen were analyzed.

Results. Calcified seroma was the only complication observed and only occurred in the 0.43-mg/ml rhBMP-2 groups (Groups 4 and 5). Dura mater repair appeared complete at 4 months in all animals. New bone was formed sporadically throughout the skull defect in the ACS+CCM and DBM groups without rhBMP-2. In all rhBMP-2 groups, mature new bone (compact and trabecular) was uniformly formed across the defect on both the repaired and intact dura mater sides. There was significant new compact bone formation on top of the repaired dura mater, which did not appear in the ACS+CCM and DBM groups lacking rhBMP-2. Greater HA-TCP and collagen scaffold resorption was noted in rhBMP-2 groups compared with non–rhBMP-2 groups. Statistical analysis showed there was a significantly lower percentage of bone spanning the defect in the ACS+CCM group compared with groups with rhBMP-2, with more residual HA-TCP and collagen on the repaired dura mater side than the intact dura mater side (p < 0.05). In all rhBMP-2 groups, there were no significant differences in new bone formation between the repaired and intact dura mater sides (p > 0.05).

Conclusions. The ACS+CCM combination had an effect similar to demineralized bone-on-bone regeneration in craniofacial reconstruction. The addition of rhBMP-2 to CCM directly or with ACS induces mature new bone formation in large cranial defects both in the presence of intact dura mater and repaired dura mater.

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Key Words • bone generation • dura mater repair • large cranial defect • recombinant human bone morphogenetic protein–2

Abbreviations used in this paper: ACS = absorbable collagen strips; CCM = collagen-ceramic matrix; DBM = demineralized bone matrix; HA = hydroxyapatite; rhBMP-2 = recombinant human bone morphogenetic protein–2; TCP = tricalcium phosphate.
ally, including allograft products such as demineralized bone, xenograft products, and alloplast materials such as calcium phosphates. Each material possesses its own advantages and disadvantages. Demineralized perforated bone has been reported as an effective alternative to autogenous bone in the treatment of large skull defects in children, but it may result in delayed wound healing and large areas of nonvital bone. Alloplast materials, such as calcium phosphates, have proven to be reasonable bone substitutes for cranioplasty. Hydroxyapatite, in particular, has been demonstrated to integrate into surrounding native bone; however, several studies have shown complication rates ranging from 5 to 30%. With these shortcomings, the need for better bone graft options persists. Recently a new product, CCM (Master-Graft Matrix) composed of 15% HA/85% TCP granules impregnated in collagen, has been used for spinal fusion in animal experiments. This CCM may elicit significant new bone formation due to its rigid space-maintaining scaffold and a pore size optimal for vascular ingrowth.

Challenging clinical scenarios amplify the need for improved bone graft substitutes. In craniofacial surgery, such challenges arise when the dura mater is damaged. The dura mater has been shown to have or enhance osteogenic potential in cranial bone reconstruction. Cranial defects created with the underlying areas denuded of dura mater show poor bone regeneration and heal primarily with cartilaginous tissue. The potential for bone regeneration by the dura mater appears to be greatest in immature dura mater, with little bone regeneration occurring from using mature dura mater. Inlay grafts isolated from dura mater by a barrier have decreased revascularization, interface healing, and cross-sectional areas of bone compared with those in contact with the dura mater. Unfortunately in many craniofacial procedures, the dura mater may be damaged or destroyed, or removed along with the overlying bone. The ideal bone graft material for craniofacial reconstruction should be safe, easy to acquire, resorbable, and capable of predictably inducing bone formation in difficult clinical procedures, such as those that contain compromised dura mater.

Recombinant human BMP-2 is a potent osteoinductive molecule. In combination with an ACS carrier, rhBMP-2 is currently used in multiple bone regenerative applications, including certain dental regenerative and spinal fusion procedures as well as open tibia fractures. This study was designed to determine if the use of rhBMP-2 to repair large cranial defects could overcome the negative impact a compromised or absent dura mater has on bone healing. As the mechanical properties of the ACS may not be ideal for load-bearing craniofacial reconstructions, rhBMP-2 was tested in 2 configurations. The first configuration involved the use of ACS layered with CCM (ACS+CCM) with rhBMP-2 added to the ACS. The second configuration used the CCM as the carrier for rhBMP-2 with no ACS.

The hypotheses for this study were that rhBMP-2/ACS will heal large cranial defects in immature canines in a rhBMP-2 dose-dependent manner, that rhBMP-2 applied directly to the CCM will heal large cranial defects similar to application via ACS, and that both of these rhBMP-2 configurations will promote healing in defects with repaired dura mater. All hypotheses were tested in a bilateral, critical-sized cranial defect model in immature beagles.

Methods

Surgical Procedure and Group Design

Forty immature (~5–6 months) male beagles weighing 7–13 kg were used in the study. All animals were allowed to acclimate prior to the surgeries, following the rules and regulations of the Medical City Animal Care and Use Committee, in compliance with guidelines of the National Institutes of Health. The preanesthesia medications given were subcutaneous or intramuscular 0.01 mg/kg glycopyrrolate, 0.2 mg/kg midazolam, 0.2 mg/kg hydromorphone, and 6 mg/kg gentamicin. Anesthesia was induced by sevoflurane and oxygen through a mask. Each dog was intubated and maintained on isoflurane (1.5–3.5%) and oxygen. Perioperatively, 30 mg/kg intravenous methylprednisolone was given as the calvarial defects were made. Postoperatively, the dogs received 15 mg/kg intravenous methylprednisolone, 0.2 mg/kg hydro-morphone 4–6 times hourly and then as needed, 0.1–0.3 mg/kg/hr midazolam for 2 hours, and subcutaneous 6 mg/kg gentamicin for 5 days. In veterinary medicine, glucocorticoids are used to reduce CSF pressure, lower seizure threshold, and reduce inflammation due to trauma induced during surgery.

Each dog was placed prone. The head was shaved, aseptically prepared, and draped. A midline incision with layered closure over the reconstructed areas was made to reapproximate the muscle and decrease the available skin dissection. The deep temporal fascia and temporalis muscle were reflected on each side of the sagittal crest to reveal the cranium. Two 2.5 × 4.0–cm cranial defects were marked 5 mm away from the sagittal crest. By epidural dissection, the bone was elevated and resected using a craniotome without injuring the dura mater. A 1.5 × 3–mm screw was placed at each corner to mark the edges of the defect. A surgical drill was used to make 4 holes at each corner as a window for the osteotomy. By epidural dissection, the bone was elevated and resected using a craniotome without injuring the dura mater. On the left side, the dura mater was cut to make a 1 × 3–cm defect. A bovine skin collagen dural substitute (Durepair, Medtronic Neurosurgery) was sutured in place to repair the defect. By epidural dissection, the bone was elevated and resected using a craniotome without injuring the dura mater. On the left side, the dura mater was cut to make a 1 × 3–cm defect. A bovine skin collagen dural substitute (Durepair, Medtronic Neurosurgery) was sutured in place to repair the defect (Fig. 1A). The dura mater on the right side was left intact. In Groups 1–4, a piece of CCM (MasterGraft Matrix, Medtronic Sofamor Danek) measuring 2.5 × 4.0 × 0.4 cm was used with the rhBMP-2 carrier (ACS) to reconstruct each side of the cranial defect. In Group 5, a slightly larger piece of matrix was used (2.5 × 4.0 × 0.5 cm).

Different doses of rhBMP-2 were infused into 2 ACS strips and then layered with the CCM (Fig. 1B, Table 1). The infusion of rhBMP-2 into the ACS was performed for ≥15 minutes but no longer than 2 hours prior to implantation, according to the supplier’s instructions. Group 1 (8 dogs) served as an ACS+CCM control with no rhBMP-2, Group 2 (4 dogs) had 0.11 mg/ml, Group 3 (4 dogs) 0.21 mg/ml, and Group 4 (8 dogs) had 0.43 mg/ml rhBMP-2 added to the ACS. Group 5 (8 dogs)
had 0.43 mg/ml rhBMP-2 infused throughout the larger CCM (Fig. 1C). Because no ACS was used in this group, Surgicel (Johnson & Johnson) was used to separate the CCM from the brain. The defects in the 8 dogs in Group 6 were repaired with DBM (Osteo-Allograft, Veterinary Transplant Services, Inc.) and served as a standardized control (Fig. 1D). All materials were fixed under 2 resorbable protective sheets (MacroPore, Medtronic, Inc.) with 2 titanium screws in the middle (Fig. 1C–D). This fixation was considered necessary because mobility can result in failure of bone formation, especially at the interface between the material and the host bone.

### TABLE 1: Group designations and calculations of total dose and bulk concentrations of rhBMP-2 used in experiments

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Carrier</th>
<th>Solution Conc of rhBMP-2 (mg/ml)</th>
<th>Vol of Carrier (ml)</th>
<th>Total rhBMP-2 Dose (mg)</th>
<th>Bulk Conc of rhBMP-2 (mg/ml)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>ACS+CCM</td>
<td>0.0</td>
<td>2.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>ACS+CCM</td>
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<td>2.8</td>
<td>0.31</td>
<td>0.11</td>
</tr>
<tr>
<td>3</td>
<td>ACS+CCM</td>
<td>0.21</td>
<td>2.8</td>
<td>0.59</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>ACS+CCM</td>
<td>0.43</td>
<td>2.8</td>
<td>1.2</td>
<td>0.43</td>
</tr>
<tr>
<td>5</td>
<td>CCM</td>
<td>0.86</td>
<td>5</td>
<td>2.15</td>
<td>0.43</td>
</tr>
<tr>
<td>6</td>
<td>DBM</td>
<td>0.0</td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Conc = concentration.
All animals were killed 16 weeks after surgery with an overdose of intravenous sodium pentobarbital (4 ml of 390 mg/ml). The skulls were resected, and specimens were obtained for histological and histomorphometric evaluation.

Sample Collection and Preparation

The resected calvariae were photographed, and gross observations were recorded. The specimens were put into 10% buffered formalin; after 2 weeks of fixation each calvaria was cut in half from the sagittal plane, and each half was then cut into 4 equally sized blocks (5 mm thick) with 5 mm of native bone preserved on the anterior and posterior edges of the defects. Two specimens were taken from the center of the defect on each side. One specimen was embedded in methylmethacrylate for undecalcified sectioning, sectioned at 75 μm, and stained with Stevenell Blue and van Gieson picrofuchsin to reveal mineralized tissue. The other specimen was decalcified in EDTA and embedded in paraffin, sectioned, and stained with H & E.

Histological and Histomorphometric Analysis

Undecalciﬁed sections were scanned on a Hewlett Packard scanner at high resolution. The digital images were opened in Metamorph software (version 6.2r2) for histomorphometric measurement. The defect area was outlined and measured, as was new bone spanning the defect area. These measurements were used to calculate the percentage of the defect spanned by bone and total new bone within the defect.

Digital photos of H & E–stained decalcified sections were taken at × 2.5 using Spot software (version 4.0.8) with a Kodak color videocamera mounted on a Zeiss Axioplan microscope. Area measurements were taken using Metamorph software on an average of 10 ﬁelds per animal, spanning the region of the reconstructed defect. The area covered by residual HA-TCP granules and collagen was measured and the percentage area calculated.

Statistical Analysis

Statistical analysis was carried out using SPSS software version 12 (SPSS, Inc.). The Student paired t-test was used for establishing intragroup (repaired dura mater and intact dura mater) signiﬁcance and 1-way analysis of variance; the Tukey post hoc tests were used for establishing intergroup signiﬁcance. An α level of ≤ 0.05 or less was considered signiﬁcant.

Results

Clinical Observations and Gross Morphology

Surgery was successful in all animals and no infection was noted for the duration of the study. The only complication was seroma formation, which was observed ~ 7 days postoperatively (Fig. 2). To avoid an increased risk of infection, needle drainage of seromas was not attempted. Some seromas resorbed, but calcified seromas were noted in 6 of 8 dogs in Group 4 (0.43 mg/ml rh-BMP-2/ACS+CCM) and 4 of 8 dogs in Group 5 (0.43 mg/ml rhBMP-2/CCM). The seromas were hollow inside and contained a brown liquid, surrounded by eggshell-like bone. Gross analysis of the craniectomies at 4 months after surgery showed integration of the newly formed bone to the edges of the defect. There were no apparent gross morphological differences between groups (Fig. 3A–F). Dural repair appeared complete in all animals. The resorbable mesh was nonmalleable in all cases.

Histological Analysis of All Groups

In all groups, the dural repair material was still visible at the center of each defect (Fig. 4).

In Group 1 (ACS+CCM), new bone was present within the skull defect, with CCM material visible within the new mineralized tissue (Fig. 4A). There were gaps in the bone in the defect with repaired dura mater (Fig. 4A). Little resorption of either the collagen or HA-TCP scaffold was noted, as HA-TCP granules appeared largely intact, with little cellular activity associated with them (Fig. 5A and G). Parts of the collagen scaffold appeared to become remodeled into osteon-like structures (Fig. 5G).

In Groups 2–5 (ACS+CCM/rhBMP-2 groups), new bone bridged the defect on both the repaired and intact dura mater sides (Fig. 4). A thin layer of compact bone was noted on the periosteal and dural aspects of the defects, with trabecular bone forming between these 2 layers of bone (Fig. 4B–E). At higher magnifications, compact bone was conﬁrmed adjacent to the dural repair material in all rhBMP-2 groups (Fig. 5B–E). The spaces between the bone trabeculae were ﬁlled with adipocytes, and small arterioles and venules (Fig. 5H).

In Group 6 (DBM), bone was present throughout the skull defects, and there appeared to be less new bone on the repaired dura mater side than the side with intact dura mater (Fig. 4F and F'). Bone fragments were scattered throughout the skull defect, surrounded by ﬁbrous tissue (Fig. 5F). Some new bone was formed against the DBM chips, and there was little to no evidence that the DBM was undergoing remodeling, based on the absence of osteoclasts and osteoblasts (Fig. 5I).
Histomorphometric Findings

Percentage of Bone Spanning the Defect. In Group 1, significantly (p < 0.05) less bone spanned the defect on the repaired dura mater side (40.73%) than the side with the intact dura mater (73.34%; Fig. 6A). In the rhBMP-2–treated groups (Groups 2–5), no significant differences in bone spanning the defect were noted between the repaired dura mater and intact dura mater sides. In Group 6, significantly (p < 0.05) less bone spanned the defect on the repaired dura mater side (55.20%) than the side with the intact dura mater (71.67%). There was no significant difference between Groups 1 and 6 in bone spanning the defect, either on the repaired or intact dura mater sides.

The repaired dura mater side in Group 1 had significantly less bone than the repaired dura mater sides in Groups 2–5 (p < 0.05). The intact dura mater side of Groups 1 and 6 had significantly less bone spanning the defect than the intact dura mater side of Groups 2–5 (p < 0.05).

Total New Bone Formed in the rhBMP-2 Groups. The amount of new bone formed inside the defect site was evaluated to compare the effects on new bone formation of rhBMP-2 concentration and delivery modes. On the intact dura side, new bone values increased from 25.05 ± 8.39 to 49.44 ± 14.82% with increasing concentrations of rhBMP-2; however, no significant differences were noted between groups (data not shown). On the repaired dura side, new bone values ranged from 31.15 ± 16.88 to 52.74 ± 14.8% with no relation to rhBMP-2 dose, and there were no significant differences between groups in the total amount of bone formed in the defect (data not shown). No significant differences were found when comparing the percentage of new bone formed on the repaired versus intact dura mater sides.

Residual HA-TCP Granules and Collagen From the CCM. In Group 1, there was significantly more residual HA-TCP (20.27 vs 17.51%; p < 0.05) (Fig. 6B) and collagen (59.27 vs 26.66%; p < 0.05) (Fig. 6C) on the repaired dura mater side than the side with intact dura mater (p < 0.05). Groups 2–5 (rhBMP-2 groups) had significantly less residual HA-TCP than Group 1 on both the repaired and intact dura mater sides (p < 0.05).

On the repaired dura mater side, Group 1 had significantly more residual collagen than Groups 2–5 (that is, the groups containing rhBMP-2, p < 0.05). On the intact dura mater side, Group 1 had significantly more residual collagen than Groups 4 (0.43 mg/ml rhBMP-2/ACS+CCM) and 5 (0.43 mg/ml rhBMP-2/CCM) (p < 0.05). No significant differences in the amount of residual HA-TCP or collagen were noted among Groups 2–5 (p > 0.05) on both the repaired and intact dura mater sides.

Discussion

Cranioplasty for large skull defects requires a considerable amount of bone, with limited availability from autograft, especially in children. Demineralized bone matrix is the most widely used bone substitute and has been investigated thoroughly in craniofacial surgery. Previous studies have shown that the addition of rhBMP-2 with DBM could accelerate new bone formation in large cranial defects. Synthetic materials like HA and TCP do not normally provide a risk of disease transmission and do not usually induce an inflammatory response. These materials have a good history of biocompatibility with no reported foreign body reactions, and they can incorporate well with the adjacent bone. The disadvantage of ceramics is the low resorption rate; resorption may not occur for up to 10 years. Previous studies showed that sintered HA is osteoconductive, but it
is relatively insoluble at a neutral pH. However, we showed some resorption of this material by 1 year in an earlier study.\textsuperscript{17} Granules of β-TCP are osteoconductive and they undergo dissolution more rapidly than highly crystalline HA,\textsuperscript{3} with substantial dissolution after 1 year.\textsuperscript{41} A new osteoconductive material, which is a matrix of cross-linked collagen fibers impregnated by HA-TCP granules (CCM) was tested for bone healing in a cranial defect model. This matrix appeared to serve as a good carrier for rhBMP-2 during spinal fusions, and it may provide a favorable environment for bone regeneration in the craniofacial region.\textsuperscript{2,18} The current study showed that this new material could be used for craniofacial reconstruction as effectively as DBM, as the histological appearance of the materials was comparable at the end of the study. In the absence of rhBMP-2, no resorption of DBM or HA-TCP granules was noted at 16 weeks after surgery, and few osteoclasts were seen around them. Furthermore, the results showed that the amount of bone regeneration in the presence of CCM was not significantly different from that seen with DBM.

Alam et al.\textsuperscript{1} investigated the use of calcium phosphate ceramic pellets for carrying rhBMP-2 by placing the pellets under the pericranium of Wistar rats. They found that pellets impregnated with rhBMP-2 exhibited new bone formation in an rhBMP-2 dose-dependent manner. Lafargue et al.\textsuperscript{33} examined the effects of TCP in combination with rhBMP-2 placed in femoral condyle defects created in rabbits. They established that TCP loaded with rhBMP-2 showed histologically more osteoid surface than the controls without BMP. Several groups have also shown the safety and efficacy of this carrier for rhBMP-2 in cranial and spinal applications.\textsuperscript{24,35,48} Because these studies demonstrated the efficacy of new bone formation in the presence of HA-TCP and rhBMP-2 and it is known that rhBMP-2 improved bone generation in DBM,\textsuperscript{12} the effect of rhBMP-2 on cranial bone formation in the presence of the new CCM formulation was examined. The results of this study showed that the addition of rhBMP-2 to ACS+CCM significantly improved the amount of bone regeneration compared with ACS+CCM alone. The complete bone bridging observed in the rhBMP-2 groups provides a solution for the important need to get early, complete coverage of the defects with new bone. This early coverage of the defects is encouraging, and the findings suggest that the rhBMP-2 treatment might result in accelerated load bearing compared with ACS+CCM and DBM alone so that it could be used to enhance the protection of intracranial contents.\textsuperscript{10,19,25} The findings with ACS+CCM are similar to those of previous studies with DBM.\textsuperscript{12} Compared with defects with no rhBMP-2 added, the addition of rhBMP-2 resulted in a rapid removal of the CCM and in the appearance of a 3-layered (compact bone-trabecular bone-compact bone) architecture, similar to the compact bone-diploe-compact bone arrangement of the skull.

In craniofacial surgery, it is often difficult or impossible to preserve the dura mater during tumor resection, or when the dura mater is damaged after trauma. Duraplasty is then needed with bone grafting. It has been well documented that the dura mater is the main source of osteogenic cells during active growth.\textsuperscript{21} After skeletal maturation, the dura loses much of its osteogenic ability.

\begin{figure}[h!]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Photographs of undecalcified sections through defects in all groups. Sections through the repaired dura mater defects, with the pale-staining repair material indicated by arrows (A–F). Sections through the intact dura mater defects (A’–F’). A and A’: Group 1 (ACS+CCM). B and B’: Group 2 (ACS+CCM/0.11 mg/ml rhBMP-2). C and C’: Group 3 (ACS+CCM/0.21 mg/ml rhBMP-2). D and D’: Group 4 (ACS+CCM/0.43 mg/ml rhBMP-2). E and E’: Group 5 (CCM/0.43 mg/ml rhBMP-2). F and F’: Group 6 (DBM). In Groups 1 and 6, islands of bone are visible throughout the defect, and unmineralized gaps can be seen punctuating the bone span across the defect. In Groups 2–5, 2 thin cortical bands of bone form a continuous span across the defect. This bone appears as 2 cortical seams on the dural and periosteal surfaces of the defects (arrowheads). Stevenell Blue and van Gieson picrofuchsin, original magnification ×2.5.}
\end{figure}
Bone regeneration with rhBMP-2 after dura repair

and the main source of osteogenesis becomes the peri-
cranium. In the skeletally immature animals used in
this study, replacing the dura with Duraplasty has the
potential of depriving the defect of its main regenerative
tissue. When dura is repaired with a dural substitute, similar
amounts of bone healing were noted in the presence of
CCM, as with DBM, with no evidence of an inflamma-
tory response. Because rhBMP-2 was previously shown
to be the single most important factor accelerating new
bone formation in repaired cranial defects, this study
tested the effectiveness of several different concentrations
in inducing bone repair, both in the presence of an intact
dura mater and with a repaired dura mater. The addition
of rhBMP-2 significantly improved the bone regeneration
on the repaired dura mater side, so that no differences in
the percentage of bone were found compared with the in-
tact dura mater side. These data are similar to the findings
of Chang et al., who showed that BMP-2 adenovirus-
infected cells infused into an alginate-based matrix in-
duced bone formation in cranial defects in the absence of
a dura mater. These data are also similar to the findings
of Shi et al., who showed that large cranial defects with
fascia lata reconstruction of the dura mater showed im-
proved bone healing with the addition of BMP. Within the

Fig. 5. Photomicrographs of decalcified sections through repaired dura mater (asterisk) defects in all groups. A: A Group 1 defect with irregular HA-TCP chips (arrows) interspersed with pale pink collagen sponge. Little new bone can be seen in the defect. B: A Group 2 defect with reduced numbers of HA-TCP chips (arrows) compared with Group 1. New bone trabeculae (arrowhead) can be seen forming against the HA-TCP chips. Pale pink collagen sponge remnants are also visible. C: A Group 3 defect with remnants of HA-TCP chips (arrows) similar to Group 2 and bone trabeculae (arrowhead) forming. D: A Group 4 defect with small HA-TCP chips (arrows) and bone trabeculae (arrowhead) similar to Groups 2 and 3. E: A Group 5 defect with little residual HA-TCP (arrow) and many small bone trabeculae (arrowhead). F: A Group 6 defect, with small demineralized bone chips (arrows) surrounded by fibrous tissue. G: A high-power image through a representative example of an rhBMP-2-treated defect. The HA-TCP chips (arrows) can be seen surrounded by new bone formation (arrowhead), while in the center of the image an HA-TCP chip can be seen surrounded by cells. In this image, the spaces visible at low power can be seen filled with adipocytes, interspersed with arterioles, venules, and sinusoids reminiscent of bone marrow. H: A high-power image through a Group 1 defect. The HA-TCP chips (arrows) appear intact, with few visible osteoblasts and osteoclasts. The collagen sponge appears to have some modeling activity producing osteon-like structures. I: A high-power image through a Group 6 defect in which demineralized bone chips (arrows) with empty osteocyte lacunae can be seen. The large chip on the left can be seen surrounded by new bone to the upper right. H & E, original magnification ×15 (A–F) and ×60 (G–I).
limitations imposed on this study by the different healing rates and differential growth rates of beagles and humans, the current findings indicate that the CCM is an appropriate rhBMP-2 carrier when dural repair is indicated.

The unique aspect of the current study is the testing of several different concentrations of rhBMP-2 in repairing cranial defects. Interestingly, all concentrations of rhBMP-2 led to marked bone formation, and while no significant dose-dependent effect of rhBMP-2 was noted both with repaired and intact dura mater, there was a trend to more bone generated within defects and increased resorption of the CCM. This trend was more marked with intact dura mater. Interestingly, the addition of rhBMP-2 directly to CCM appeared to produce the most marked resorption of the CCM, both with repaired and intact dura mater. Because ACS alone has a shorter retention time for rhBMP-2 than CCM, it is possible that the longer availability of rhBMP-2 in the defect allowed for greater recruitment of osteoclasts. This effect would appear not to hold for osteoblasts, because the amount of bone spanning the defect was similar with application of rhBMP-2 in ACS versus directly on CCM.

Importantly, the appearance of calcified seromas as a complication of addition of rhBMP-2 appeared to be a dose-dependent phenomenon. The lowest 2 doses of rhBMP-2 did not result in seroma formation and appear to be appropriate concentrations for use in repairing cranial defects in this model. To avert seroma formation in further animals, a U-shaped coronal incision through the skin was made between the 2 ears. The superficial temporal muscle fascia was dissected and reflected to totally cover the skull and the materials, and the muscle fascia was sutured to the subcutaneous tissue. It must be noted, however, that seroma formation may be averted by placing a temporary shunt, an option that was not attempted in this dog study due to the high risk of infection associated with draining in animals.

Conclusions

A new collagen fiber, HA-TCP matrix developed for use in spinal fusions is an appropriate carrier for rhBMP-2 in healing large cranial bone defects, both with repaired and intact dura mater. When delivered on ACS and combined with the matrix or when applied directly to the matrix, rhBMP-2 can induce bone formation in the presence of repaired dura mater comparable to that achieved when the dura mater remains intact. With an intact dura mater, this material is capable of healing large cranial defects similar to DBM in the absence of rhBMP-2. The significant additional bone healing and CCM resorption noted in the presence of rhBMP-2, especially with repaired dura mater, appears to support the use of CCM with rhBMP-2 for maximum effect.

Disclosure

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