6-14-2013

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Recommended Citation

Parkes, MD, William J.; Greywoode, MD, Jewel; O'Hara, MD, Brian J.; Heffelfinger, MD, Ryan N.; and Krein, MD, PhD, Howard, "A Preliminary Report of Percutaneous Craniofacial Osteoplasty in a Rat Calvarium" (2013). *Department of Otolaryngology - Head and Neck Surgery Faculty, Presentations and Grand Rounds*. Presentation 16.

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A Preliminary Report of Percutaneous Craniofacial Osteoplasty in a Rat Calvarium

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ABSTRACT

Objective: To evaluate the potential for injectable, permanent bone augmentation by assessing the biocompatibility and bioactivity of subperiosteal hydroxylapatite (Radiesse) deposition in a rat model. Methods: Fourteen adult Sprague Dawley rats were injected in the parietal skull with 0.2 ml of HA (n=10) or a carrier gel control (n=4), using a subperiosteal injection technique on the right and a subcutaneous injection technique on the left. At 1, 3, and 6 months, 3 rats (1 negative control, 2 variables) were sacrificed. At 12 months, the remaining 5 rats were sacrificed. After each harvest, the skulls were examined under both light and polarized microscopy. Results: The inflammatory response was limited in all specimens. Injectables were still present 12 months after the injection. New bone formation was only seen when the injection was located deep to a disrupted peristeme. The odds of new bone formation was 49.94 times higher (95% CI (2.637, 3759.961), p = 0.002) with subperiosteal hydroxylapatite injection group than the subcutaneous injection group. Animals were subsequently sacrificed at 4 time points (1, 3, 6, and 12 months after the initial injections) and calvaria were harvested for histologic analysis. Each of the first 3 harvests included 2 rats from the HA group and 1 negative control from the carrier gel group. At the 12 month harvest again included 4 rats from the HA group and 2 negative controls from the carrier gel group. All specimens were fixed, decalcified, and dehydrated. Under power magnification, the injectables were located and the surrounding tissue was examined. Proper identification of the injectables was confirmed by examining and comparing separate samples of the HA and carrier gel ex vivo (Figure 1). Polarest microscopy was used to distinguish new (woven) bone from mature (lamellar) bone. Odds ratios, p values and 95% confidence intervals (CI) were calculated using Fisher’s conditional maximum likelihood estimation. P values < 0.05 were considered significant. Discussion: This preliminary report of percutaneous craniofacial osteoplasty in a rat calvarium suggests the potential for new bone formation when HA is injected in a subperiosteal plane. Because prior studies have shown that bone formation is most evident in specimens with an intact peristeme, care should be taken to ensure that the peristeme remains intact during injection. Furthermore, any specimen without an identifiable injectable (subcutaneous injection site in specimen 12 and both sites in specimen 13) were excluded. The odds of new bone formation in the subperiosteal HA injection group (49.94 times higher than the other 3 combinations in aggregate (95% CI (2.637, 3759.961), p = 0.002). The marginal effect of subperiosteal injection was also significant, but a discrete odds ratio could not be computed due to the zero-counts in the subperiosteal groups (95% CI (0.068, infinity), p < 0.001). The marginal effect of HA, however, was not significant. Minor deformities of the craniofacial skeleton can be quite bothersome aesthetically to patients. Radiesse provides an easy alternative to the current gold standard of autologous bone as a main biologic constituent. HA, has been used for over 2 decades in various open craniofacial reconstructions.4,15 This study adds further supporting evidence for subperiosteal augmentation.4,14 Over the years, various studies have confirmed its safety, longevity and bioactivity (specifically the degradable plastic elastomer) injected subperiosteally.5,7 Not surprisingly, off label uses of Radiesse have arisen as well.11,12 To our knowledge, no one to date has examined the histologic effects of Radiesse injection at the bone calvarium. We have now shown that Radiesse is biocompatible and long-lasting subperiosteally. In designing the study, we did consider the fact that the trauma of peristeme disruption could trigger osteoclasty and therefore confound results. We attempted to control for this with the carrier only injections; hypothesizing that new bone formation would be either absent or less pronounced without HA. Unfortunately, our technique for peristeme disruption was only successful 54% of the time. Consequently, the numbers for truly subperiosteal HA and carrier injections were simply too low to demonstrate a statistically significant difference in the rate of new bone formation between the two. Notably, though, the odds of new bone formation in the subperiosteal HA injection group was significantly higher than the aggregate of all other combinations of injection plane and injectable. We were also able to show that the plane of injection seems to be critical in any effort to induce osteoclasty as none of the subcutaneous injections resulted in new bone formation. Our findings suggest that, regardless of the mechanism triggering new bone formation, injectable HA can be osteointegrated. Refinements in the technique for subperiosteal injection would allow for larger scale studies which would allow for a larger scale to be warranted to better elucidate the stimulus for the osteoactivity we observed historically.REFERENCES

INTRODUCTION

Currently, calcium phosphate cements (CPC), such as hydroxylapatite (HA), are commonly used for the augmentation of bony defects. Favorable characteristics of CPC include customizability, isothermic setting, biocompatibility, and bioactivity (resorption is accompanied by bone-like new bone formation). However, the chemical properties pertaining to setting, open exposure is required to allow for adequate bone deposition. Hydroxylapatite application of CPC is reserved for large defects, such as those that result from tumor extirpation or extensive trauma. However, injectable agents are often faced with smaller craniofacial deformities that are of aesthetic concern to the patient but do not warrant the morbidity of open surgery. Examples would include relatively minor augmentation.4,15 Presently, calcium phosphate cements (CPC), such as hydroxylapatite (HA), are commonly used for the augmentation of bony defects. Favorable characteristics of CPC include customizability, isothermic setting, biocompatibility, and bioactivity (resorption is accompanied by bone-like new bone formation). However, the chemical properties pertaining to setting, open exposure is required to allow for adequate bone deposition. Hydroxylapatite application of CPC is reserved for large defects, such as those that result from tumor extirpation or extensive trauma. However, injectable agents are often faced with smaller craniofacial deformities that are of aesthetic concern to the patient but do not warrant the morbidity of open surgery. Examples would include relatively minor augmentation.4,15 Presently, calcium phosphate cements (CPC), such as hydroxylapatite (HA), are commonly used for the augmentation of bony defects. Favorable characteristics of CPC include customizability, isothermic setting, biocompatibility, and bioactivity (resorption is accompanied by bone-like new bone formation). However, the chemical properties pertaining to setting, open exposure is required to allow for adequate bone deposition. Hydroxylapatite application of CPC is reserved for large defects, such as those that result from tumor extirpation or extensive trauma. However, injectable agents are often faced with smaller craniofacial deformities that are of aesthetic concern to the patient but do not warrant the morbidity of open surgery. Examples would include relatively minor augmentation.4,15... Methods: Fourteen adult Sprague Dawley rats were injected in the parietal skull with 0.2 ml of HA (n=10) or a carrier gel control (n=4), using a subperiosteal injection technique on the right and a subcutaneous injection technique on the left. At 1, 3, and 6 months, 3 rats (1 negative control, 2 variables) were sacrificed. At 12 months, the remaining 5 rats were sacrificed. After each harvest, the skulls were examined under both light and polarized microscopy. Results: The inflammatory response was limited in all specimens. Injectables were still present 12 months after the injection. New bone formation was only seen when the injection was located deep to a disrupted peristeme. The odds of new bone formation was 49.94 times higher (95% CI (2.637, 3759.961), p = 0.002) with subperiosteal hydroxylapatite injection group than the subcutaneous injection group. Animals were subsequently sacrificed at 4 time points (1, 3, 6, and 12 months after the initial injections) and calvaria were harvested for histologic analysis. Each of the first 3 harvests included 2 rats from the HA group and 1 negative control from the carrier gel group. At the 12 month harvest again included 4 rats from the HA group and 2 negative controls from the carrier gel group. All specimens were fixed, decalcified, and dehydrated. Under power magnification, the injectables were located and the surrounding tissue was examined. Proper identification of the injectables was confirmed by examining and comparing separate samples of the HA and carrier gel ex vivo (Figure 1). Polarest microscopy was used to distinguish new (woven) bone from mature (lamellar) bone. Odds ratios, p values and 95% confidence intervals (CI) were calculated using Fisher’s conditional maximum likelihood estimation. P values < 0.05 were considered significant. Discussion: This preliminary report of percutaneous craniofacial osteoplasty in a rat calvarium suggests the potential for new bone formation.