GAVIN PUBLISHERS

Journal of Surgery

Lei J, et al. J Surg 2017. J117.
DOI: 10.29011/JSUR-117.000017

Case Report

Heterotopic Ossification of Laparotomy Scars in Nonhuman Primate

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Citation: Lei1 J, Markmann JF, McCoy CS, Luo H, Harrington EP, Rosales I, Poznansky MC (2017) Heterotopic Ossification of Laparotomy Scars in Nonhuman Primate. J Surg 2017. J117. DOI: 10.29011/JSUR-117.000017

Received Date: 18 January, 2017; **Accepted Date:** 26 January, 2017; **Published Date:** 31 January, 2017

Abstract

Heterotopic ossification is a common sequela of abdominal surgical incisions which has been reported extensively in humans. It is usually an incidental finding, presenting with no apparent clinical signs. Nonhuman primates (NHPs) are frequently used as models in preclinical research. Despite the relative frequency of abdominal surgery performed in this animal model, no previous incidences of heterotopic ossification have been reported in this species. This case study examines the finding of heterotopic ossification lesions in two NHPs (Macaca fascicularis). Both animals had lesions in the midline abdominal scars with structure grossly resembling bone and histology showed cortex and myeloid cells present in the medulla of the tissue.

Keywords: Post-Laparotomy Heterotopic Ossification in Non-Human Primates.

Abbreviations: Fibrodysplasia Ossificans Progressive (FOP), Heterotopic Ossification (HO).

Introduction

Heterotopic ossification (HO) is the formation of bone tissue outside of the skeletal system. It has been noted to develop at sites of trauma, burns, or surgery. Formation of HO in midline abdominal surgical incisions is a relatively common sequela which has been reported in humans [1-3]. It is usually an incidental finding, presenting with no apparent clinical signs, although it may cause mild to moderate chronic pain and on rare occasion can become fractured by acute trauma. Cynomolgus macaques (Nonhuman primates [NHPs]) are often used in preclinical research as models for humans, especially in transplant surgery research [4,5]. Despite the relative frequency of abdominal surgery performed in this animal model, no previous incidences of heterotopic ossification have been reported in cynomolgus macaques. This case study examines the finding of heterotopic ossification lesions in two different NHPs six months after abdominal surgery.

Animals

Studies involving animal subjects were performed under a protocol approved and monitored by the Massachusetts General Hospital (MGH) Institutional Animal Care and Use Committee. Animals were housed at the Center for Comparative Medicine and Laboratory Animal Services at MGH. Two naive male NHPs, aged 7.9 and 7.3 years, were obtained from Charles River Laboratories (Houston, TX) and were specific pathogen free. Animals tested negative for Mycobacterium, Salmonella, Shigella, Yersinia, Simian Type D Retrovirus, Simian T-Lymphotropic Virus, Simian Immunodeficiency Virus, and Herpes B Virus. Pair-housed monkeys were supplied with water ad libitum, environmental enrichment, and fed a balanced primate diet three times daily. MGH holds Animal Welfare Assurance A3596-01, effective 22 December 2014, with the Office of Laboratory Animal Welfare, National Institutes of Health, and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Procedures

Both animals were recipients of pancreatic islets encapsulated within a calcium-alginate matrix, and received no immunosuppressive drugs [6]. Encapsulated islets were suspended in normal saline to a volume of approximately 20 mL, and gently infused over the surface of the omentum during midline laparotomy. Abdominal closure was in three standard layers with absorbable suture. The animals received prophylactic antibiotic and multimodal analgesia perioperatively. Surgical biopsies of the encapsulated islets were taken via midline laparotomy at 4 weeks and necropsy was performed at 6 months. The first animal received autologous islets transplant, which were isolated and encapsulated after partial pancreatectomy. This entailed surgical removal of 60-70% of the distal pancreas and the spleen. The second animal received xenogeneic encapsulated porcine islets transplant. Animals were moni-

tored postoperatively with periodic sedated physical examination, urinalysis and blood work, including complete blood count and serum biochemical profile.

Results

Autotransplanted Animal (AA)

The autotransplanted animal had moderate inappetance for three days following partial pancreatectomy and transplant, but otherwise recovered well. Post-operative blood work showed mildly elevated GGT and alkaline phosphatase consistent with cholestasis, which resolved after two weeks. The animal developed an incisional infection after the 4-week biopsy which was treated effectively with a 7-day course of oral cefpodoxime. During the 4-week biopsy fat necrosis was noted, localized to the left cranial abdominal quadrant in the area of the pancreas. At the 6 months time point post transplant, the animal had gained over 3.5 kg and had become obese, weighing 13.0 kg. On necropsy, there were extensive abdominal adhesions between the abdominal wall and the greater omentum. A significant area of mineralization was present in the scar of a previous laparotomy, extending caudally 10 cm from just caudal to the xiphoid with an average width of 3 cm and depth of 1 cm. On cross-section, the mineralized area grossly resembled the structure of a long bone, with a thin cortex around the periphery, and trabeculae and red soft tissue in the center. Histology confirmed the presence of lamellar and cancellous bone with all three lineages of myeloid cells present in the medulla of the HO lesion as well as cartilaginous component along with the bone (Figure 1).

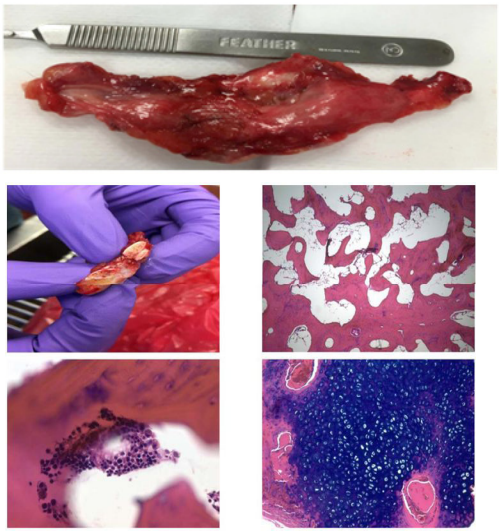

Figure 1: Gross structure and histology of the dissected area of mineralization of animal AA. (A) The tissue of the lesion is firm in texture with structure grossly resembling an irregular long bone. (B)Cross-section shows a thin cortex around the periphery, with trabeculae and red soft tissue in the center. (C&D) Histology confirms the presence of lamellar and cancellous bone with all three lineages of myeloid cells present in the medulla of the tissue (HE stain). (E) Shows chondrocytes from dissected cartilaginous tissue of the lesion (HE stain).

Xenotransplanted Animal (XA)

The xenotransplanted animal had a history of chronic alopecia treated with behavioral intervention. Routine diagnostics throughout the study revealed chronic mild azotemia, chronic proteinuria and hematuria. No abnormalities were found on abdominal ultrasound, and urine culture yielded no bacterial growth. On necropsy, there were minor adhesions between the omentum and the abdominal wall. The urinary bladder contained a 2 cm diameter calcium carbonate urolith with no nidus, and the bladder wall was thickened. The scar of a previous laparotomy contained an approximate 0.5 cm by 0.5 cm by 1 cm area of mineralization. Histologically, the lesion contained a small area of woven bone embedded in the abdominal muscle, with some evidence of maturation and a single pocket of hematopoietic cells (Figure 2).

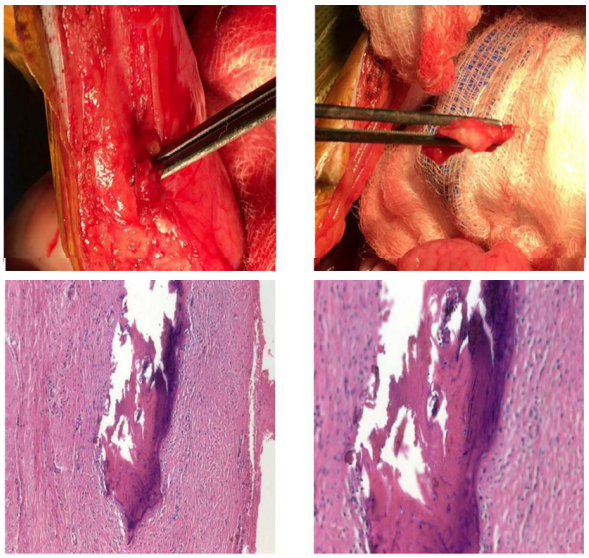

Figure 2: Gross structure and histology of the dissected area of mineralization of animal XA. (A&B) The tissue of the lesion is firm in texture with structure grossly resembling an irregular bone and cartilage. (C&D) Histology confirms the presence of spongy bone and hematopoietic cells in the dissected tissue of the lesion (HE stain).

Discussion

Two adult male NHPs in this case report were found to have mineralization of their midline abdominal scars six months after surgery. Histology confirmed both to be due to heterotopic ossification of the incisions. Animal AA's lesion was quite substantial in size, and exhibited the gross structure of a long bone diaphysis, including a central marrow cavity containing cells of all three myeloid lineages.

Heterotopic ossification is the formation of bone in soft tissue. It is thought to be an advanced form of dystrophic calcification, and is not associated with hypercalcemia as with metastatic calcification. Key factors in formation include: 1) an inciting event, usually trauma; 2) osteogenic signals from the site of injury; 3) supply of mesenchymal cells; and 4) environment conducive to continued production of bone [3]. There are two main theories put forth for the pathogenesis. One is that the incision site is "seeded" by bone fragments or cells (such as from the xiphoid process or the pelvic symphysis), which then proliferate and form bone in the healing incision. The presence of HO lesions in locations not apparently seeded by bone fragments (such as in burns, traumatic brain injury, non-midline surgical incisions, and mesentery) gives evidence that this mechanism is not necessary for the formation of HO. The second theory is that local humoral or hormonal factors stimulate pluripotent mesenchymal stem cells already present in the site to differentiate into osteoblasts or chondroblasts and subsequently induce bone formation at the site [3]. There is extensive research that supports the validity of the second theory [7]. No doubt, the repeated laparotomy in the reported two cases caused significant trauma as a contributing factor to initiate the HO.

There is a compelling case for the role of inflammation in the formation of HO lesions. The normal inflammatory response following bone injury leads to the release of a cascade of cytokines, which promote angiogenesis and induce osteoprogenitor cells to release BMPs and promote osteogenic differentiation. Progenitor cells differentiate into osteoblasts, which release IL-1, IL-6, and IL-11 to promote osteoclastogenesis. It is well known that macrophages and monocytes can differentiate into osteoclasts. Indeed, macrophages in mice forming HO were found to express high levels of osteogenic growth factors, including BMP4. In addition, removing macrophages from a mouse model significantly reduced HO formation [8].

A genetic disease in humans called fibrodysplasia ossificans progressiva (FOP) causes progressive widespread ectopic bone formation in soft tissues. It shares many similarities with other forms of HO, and is thought to share many of the same underlying mechanisms. The disease is caused by a recurrent activating genetic mutation in the BMP type 1 receptor gene ACVR1. Progression of the disease is preceded by inflammatory "flare-ups", which are generally precipitated by soft tissue injury, immunizations or viral infection [7,9,10]. Anecdotally, immune-suppression in one person with FOP appeared to inhibit flares for years, which recurred after immune-suppression was weaned [8]. Our cases also seem to support the implication of inflammation in the pathogenesis of HO, as the animal AA with adhesions throughout the abdomen also had a significantly larger HO lesion. The presence of hematopoietic cells in the HO lesion of this animal is consistent with the larger and more developed HO lesions containing hematopoietic cells that have been reported in humans [2,11].

In humans, HO lesions have a higher prevalence in adult males, and in upper midline abdominal incisions [3]. Our two cases fit this description, and it would be interesting to see if these demographics continue to be consistent in NHPs. Although neither animal showed obvious clinical signs associated with their HO lesions, we speculate that the animal AA with the larger lesion may have been experiencing some discomfort which led to decreased activity levels and significant weight gain. A retrospective study of CT scans in humans after abdominal surgery found heterotopic ossification occurred far more frequently than had previously been thought [1]. In that study, approximately 25% of the patients developed HO. Surprisingly only 5% of those patients with HO had surgery to remove it due to pain. Follow up CT scans showed regression of the lesions in the majority of the remaining patients.

In light of these findings and our own limited experience, we suspect HO may also occur more frequently in NHPs than previously thought. Finding a second smaller lesion so soon after we discovered the first seems to support this, although the timing of these two cases could be purely coincidental. Either way, it is possible we have overlooked previous minor HO lesions. If this type of lesion has a similar clinical course in NHPs, it is not hard to see how it could be overlooked, especially if it is not causing clinical signs and is potentially regressing over time. So far, much research has been done to understand the causes of HO formation. However, studies on the dynamic impacts to the immune system during the time of either HO formation or regression are warranted, especially in the context of transplantation.

Acknowledgements

This work was supported in part by JDRF grant: 2-SRA-2014-290-Q-R to Mark C. Poznansky/James F. Markmann at Massachusetts General Hospital

References

1. Kim J, Kim Y, Jeong WK, Song SY, Cho OK (2008) Heterotopic ossification developing in surgical incisions of the abdomen: analysis of its incidence and possible factors associated with its development. Journal of computer assisted tomography 32: 872-876.
2. Christofi T, Raptis DA, Kallis A, Ambasakoor F (2008) True trilineage haematopoiesis in excised heterotopic ossification from a laparotomy scar: report of a case and literature review. Annals of the Royal College of Surgeons of England 90: 12-14.
3. Koolen PG, Schreinemacher MH, Peppelenbosch AG (2010) Heterotopic ossifications in midline abdominal scars: a critical review of the literature. Eur J Vasc Endovasc Surg 40: 155-159.

4. Lei J, Kim JI, Shi S, Zhang X, Machaidze Z, et al (2015) Pilot Study Evaluating Regulatory T Cell-Promoting Immunosuppression and Nonimmunogenic Donor Antigen Delivery in a Nonhuman Primate Islet Allotransplantation Model. Am J Transplant 15: 2739-2749.
5. Oura T, Hotta K, Lei J, Markmann J, Rosales I, et al (2016) Immunosuppression With CD40 Costimulatory Blockade Plus Rapamycin for Simultaneous Islet-Kidney Transplantation in Nonhuman Primates. Am J Transplant 2016.
6. Chen T, Yuan J, Duncanson S, Hibert ML, Kodish BC,et al (2015) Alginate Encapsulant Incorporating CXCL12 Supports Long-Term Allo- and Xenoislet Transplantation Without Systemic Immune Suppression. Am J Transplant 15: 618-627.
7. Ramirez DM, Ramirez MR, Reginato AM, Medici D (2014) Molecular and cellular mechanisms of heterotopic ossification. Histology and histopathology 29: 1281-1285.
8. Kraft CT, Agarwal S, Ranganathan K, Wong VW, Loder S, et al (2016) Trauma-induced heterotopic bone formation and the role of the immune system: A review. The journal of trauma and acute care surgery 80: 156-165.
9. Pignolo RJ, Shore EM, Kaplan FS (2011) Fibrodysplasia ossificans progressiva: clinical and genetic aspects. Orphanet J Rare Dis 6: 80.
10. akenobu Katagiri KO, Sho Tsukamotoa, Mai Fujimotoa, Arei Miyamotoa, Takato Mizutaa (2014) Bone morphogenetic protein-induced heterotopic bone formation: What have we learned from the history of a half century?. Japanese Dental Science Review 51: 42-52.
11. Davis TA, Lazdun Y, Potter BK, Forsberg JA (2013) Ectopic bone formation in severely combat-injured orthopedic patients -- a hematopoietic niche. Bone 56: 119-126.