Poster Presentations

Animal models

51 PRECLINICAL ANIMAL MODELS IN SINGLE SITE CARTILAGE DEFECT TESTING: A SYSTEMATIC REVIEW

B.J. Ahern[1], J. Parvizi[2], R. Boston[1], T.P. Schaer[1]. [1]*University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA,* [2]*Rothman Institute, Philadelphia, PA, USA*

Purpose: The goal of translational research is to transform biologic knowledge into new treatments for human disease. Although preclinical models replicate some of the features of naturally occurring disease, they invariably fail to reproduce the complexity of the degenerative disorder, and by their experimental nature, they are readily manipulated to maximize evidence of efficacy. The result is that successful translation from preclinical models to clinically effective therapy is uncommon, and that clinical trials are often undertaken without a comprehensive and realistic preclinical portfolio of studies to optimize their design. This review is an attempt to evaluate preclinical studies in the literature using criteria more reflective of the human clinical picture in single site joint resurfacing.
Methods: A systematic review was conducted from 1990 to 2007. Only studies with single site defects using the stifle joints were included. The following variables were tabulated and analyzed: species, age, animal number and defect dimensions. For all animal studies and six large representative human studies the total defect volume and chondral/subchondral components were calculated and subsequently compared using cluster analysis. This method of evaluation allowed species to be objectively assembled into sub-strata comprising 'groups' of 'similar' species. The suitability of each species used for single site defect research correlating to clinical human practice was discussed.

Table 1

Species	Medial femoral condyle cartilage thickness (mm)	No. of studies		No. of animals utilized	Volume (mm^3) Total	Cartilage	Subchondral
Rodent	0.1	5	Mean	30	2.17	0.12	2.05
			SD	15.88	2.85	0.06	2.81
			Mode	30	5.3	0.18	0
Rabbit	0.3	39	Mean	18.86	53	7.15	45.86
			SD	11.57	54.64	13.35	52.78
			Mode	16	21.21	2.12	19.09
Canine	0.95	16	Mean	34.82	82.39	18.43	63.86
			SD	46.85	197.94	17.4	181.9
			Mode	8	11.94	11.94	0
Ovine	0.45	13	Mean	23.69	359.54	18.03	341.51
			SD	15.74	683.35	19.97	663.79
			Mode	20	n/a	12.5	0
Caprine	1.1	13	Mean	30.55	251.65	45.71	63.67
			SD	20.39	448.46	35.1	78.9
			Mode	50	31.1	17.49	0
Porcine	1.5	10	Mean	9.56	107.43	43.76	63.67
			SD	2.35	87.87	24.05	78.9
			Mode	10	183.22	34.35	0
Equine	1.75	17	Mean	9	334.73	192.67	142.06
			SD	2.03	237.87	94.21	213.08
			Mode	8	137.44	137.44	0
Human	2.35	n/a	Mean	n/a	552.25	552.25	0

Results: 113 studies relating to single site defects were reviewed and tabulated. The mean, mode and standard deviation of the number of animals used per study, total defect volume, cartilage volume and subchondral volume is reported in table 1. The mean defect volumes ranged from smallest in rodents at 2.2 mm^3 to largest in horses at 334.7 mm^3. All animal models with the exception of the horse utilized defects with greater subchondral bone volume in comparison to cartilage. Cluster analysis included 101 studies and placed the rodent, rabbit, ovine, canine, porcine and caprine models in group one. Group two contained ovine, canine, porcine, caprine and equine models. Group three contained only equine models and commonly reported human defects. These statistics demonstrate horses to be the most comparable to humans with respect to cartilage defect dimensions used in research, followed by the animals in group 2. Animal models in group 1 bear more significant limitations as preclinical models as they do not mimic the clinical picture in the human patient.
Conclusions: The rabbit is the most utilized animal model but its small skeletal anatomy and cartilage morphology have significant limitations. The goat is a model suitable for small defect research. The horse is a suitable model for single site defect research. Its joint anatomy by scale and the cartilage morphology is most comparable to the clinical scenario of single site defects in human patients. Standardization of study design and its outcome parameters would greatly help to compare different studies evaluating various novel therapeutic concepts. Considering the clinical counterpart for single site defects in the human patient during species selection and preclinical study design may help increase the predictive value of clinical outcome. Thus it could be a major improvement in the process of developing novel efficacious therapies.

Table 2

Group		Mean	SD	Range
1	Thickness	0.66	0.44	1.65
	Diameter	4.3	1.59	7.15
	Volume	12.37	12.5	57.67
2	Thickness	1.46	0.39	1.3
	Diameter	10.11	1.64	7
	Volume	114.31	35.49	127.94
3	Thickness	2.05	0.31	0.6
	Diameter	16.79	2.85	9.98
	Volume	496.15	254.5	842.25
Total	Thickness	0.97	0.66	2.25
	Diameter	6.82	4.64	24.13
	Volume	88.02	177.83	1151.44

52 STUDY OF MOXIBUSTION STIMULATION FOR EXPERIMENTAL ARTHRITIS MODEL

S. Kanai. *Kansai University of Health Science, Osaka, JAPAN*

Purpose: Recently, moxibustion have been widely from the perspective of the effects for pain. In clinical studies, the efficacy of moxibustion has been reported for various orthopedic diseases, including shoulder pain and low back pain. Rat adjuvant arthritis (AA) is acute periarticular proliferative synovitis which is induced by single-dose intradermal injection of tubercle bacillus to the rat posterior foot. As one of experimental arthritis models, rat AA is used for evaluating the efficacy of drugs, especially anti-inflammatory drugs, at a large number of institutions. In the present study, rats AA were left untreated for a long time to prepare chronic pain models. In order to examine the effectiveness of moxibustion stimulation for pain relief, we performed the study on rat AA.
Methods: *Experimental methods*: Sprague-Dawley (SD) rats (6 weeks old, 160 g of body weight) were divided into 2 groups. In groups I, Mycobacterium butyrium suspended in paraffin oil, was injected into the left hind leg of 0.6 mg/0.05 ml to induce adjuvant arthritis (AA). These animals were then left untreated for 24 weeks to prepare a chronic pain model.
Measurement of locomotor activity before and after moxibustion in each group: Before and after moxibustion stimulation, daily locomotor activity was measured using a metabolism measuring system in groups I and II. In the metabolism measuring system, infrared rays are spread horizontally

(lengthwise and crosswise) at 5-mm intervals, and the number of infrared rays blocked by the animal was counted. The total locomotor activity in every 30 min was shown graphically and used as a daily behavioral pattern. The total locomotor activity over 24 hours was measured as daily locomotor activity.
Measurement of tail surface temperature before and after moxibustion in each group: The tail surface temperature was measured using a thermograph in group I and II. To avoid the influence of haircoat, the tail temperature which is used as an indicator for peripheral circulation was measured. Before and after, after 15 min of acclimation to the environment, the tail surface temperature was measured in conscious animals in a windless room maintained at a temperature of 15±1ºC and a relative humidity of 60±10%. Temperature of the rat tails was measured at a distance of 1m from the thermgraphy device. Rats were acclimated before the experiment.
Results: *Changes in locomotor activity before and after moxibustion stimulation*: Before moxibustion stimulation, in group I, the pattern of locomotor activity was regular. In the group I, there was no clear diference in the pattern of locomotor activity between the active and resting phases and the pattern of locomotor activity was irregular. The daily level of locomotor activity at 24 hours was significantly higher in the group I than in group II. After moxibustion stimulation of the group I, the pattern of locomotor activity became diphasic with clear active and resting phases, similar to that observed in group II. Moreover, the daily level of locomotor activity at 24 hours in group I was significantly higher than that in group II. *Changes in tail surface temperature before and after before moxibustion stimulation*: Before moxibustion stimulation, the tail surface temperature was significantly lower in group I than in group II. After moxibustion stimulation, there was no significant difference in the tail surface temperature between group I and group II.
Conclusions: The increased locomotor activity of rat AA was presumably ascribable to the removal of blood circulation reduced blood flow in the peripheral circulation rather than the induction of stress by moxibustion. These results demonstrate that moxibustion stimulation is effective for the treatment of pain relief.

53 TOWARDS AN ANIMAL MODEL FOR JOINT REGENERATION IN OSTEOARTHRITIS

R. Dinser[1], M. Geyer[1], A. Wietelmann[2], C. Schreiyäck[1], C. Fiehn[3], C. Müller[4], U. Müller-Ladner[1], T. Borchardt[2]. *[1]Kerckhoff-Klinik, Justus-Liebig-University of Giessen, Bad Nauheim, GERMANY, [2]Max Planck Institute for Heart and Lung Research, Bad Nauheim, GERMANY, [3]Centre for Rheumatic Diseases, Baden-Baden, GERMANY, [4]Kerckhoff-Klinik, Bad Nauheim, GERMANY.*

Purpose: Osteoarthritis is a disease of progressive joint failure, leading to pain and disability. Only symptomatic treatment options exist. A key problem is the absence of endogenous repair capacity of adult mammalian joint structures. Amphibians are able to regenerate lost limbs, thus we hypothesized that endogenous repair may be possible in amphibians after local joint injury.
Methods: Knee joints of adult newts (notophtalmus viridescens viridescens) were treated intraarticularly with collagenase in analogy to murine models of osteoarthritis. The clinical and histological course was analysed.
Results: Clear cut joint injury was observed by inspection, with a clinical score (incorporating swelling, spontaneous joint use, deformity, and range of motion), and with histologic analysis after treatment. The severity of joint damage increased over the first three weeks and then abated. Disruption of joint anatomy with cartilage loss was confirmed by magnetic resonance imageing. Histologically, loss of proteoglycan and collagen II staining was observed in addition to thinning of cartilage. Chondrocytes of the femoral and tibial joint underwent cell death. Beginning after three and five weeks, evidence for mesenchymal progenitor cell recruitment with chondrogenic differentiation was observed. Clinical use of joints was normal within five weeks, histological healing still continues after 12 weeks of observation. Currently, the observation period is extended and molecular factors involved are determined by RT-PCR of joint extracts.
Conclusions: Joint injury can be induced in the newt with approaches that lead to osteoarthritis in murine models. In contrast to murine models, newts are able to recruit progenitor cells in order to induce the regeneration of joint structures. Further studies will help to elucidate cellular and molecular mechanisms.

54 A NOVEL AGE- AND STRAIN-DEPENDENT IN VIVO MODEL OF ARTICULAR CARTILAGE HEALING IN MICE

N.M. Eltawil[1], C. De Bari[2], P. Achan[1], C. Pitzalis[1], **F. Dell'Accio[1]**. *[1]Barts and The London School of Medicine and Dentistry, London, UNITED KINGDOM, [2]University of Aberdeen, Aberdeen, UNITED KINGDOM*

Purpose: To optimize and validate an in vivo model of mechanical cartilage injury and regeneration in mice.
Chondral injuries are frequent, and can either improve spontaneously or acquire a chronic symptomatic course, which may require surgical intervention, and may lead to OA. Research in the molecular mechanisms controlling joint surface healing or evolution into OA has been so far limited by the lack of mouse models of joint surface regeneration which would allow the use of mouse genetics to study molecular function. In this study we have validated a mouse model of joint surface injury and repair with a strain and age-dependent outcome.
Methods: Full thickness defects were generated in the patellar groove of adult C57BL/6 and DBA/1 mice by microsurgery. Control knees were either sham-operated or non operated. Outcome was evaluated by histological scoring systems. Apoptosis and proliferation were studied using TUNEL and Phospho-Histone H3 staining. Type II collagen neodeposition and degradation were evaluated by immunostaining using antibodies to the CII telopeptide and C1,2C (Col2−3/4Cshort) respectively. Aggrecanase and MMPs activity were assessed by immunostaining for TEGE373 and VDIPEN neoepitope.
Results: Eight weeks following surgery, adult eight weeks old DBA/1 mice displayed consistent repair of the defects with safranin-O positive cartilage tissue. Age matched C57BL/6 mice repaired poorly and developed osteoarthritic (OA) features. Cartilage injury induced apoptosis and matrix remodeling in both strains. However, compared to C57BL/6, DBA1 mice displayed a progressive decline of chondrocyte apoptosis, persistent cell proliferation within the repair tissue, persistent type II collagen neodeposition, less type II collagen degradation, less aggrecanases-induced aggrecan degradation, and more MMP-induced aggrecan degradation. Aged eight months old DBA/1 mice failed to repair, but, contrarily to age-matched C57BL/6 mice, developed no signs of OA.
Conclusions: We have generated a murine model of cartilage regeneration in which the outcome of joint surface injury is strain and age dependent. This model will allow testing the function of different molecules in the context of joint surface regeneration in adult mammals using genetic models.

55 VERY RAPID CLEARANCE AFTER A JOINT BLEED KNEE CAN NOT PREVENT ADVERSE EFFECTS ON CARTILAGE AND SYNOVIAL TISSUE; A CANINE *IN VIVO* STUDY

N.W. Jansen[1], G. Roosendaal[1], M.J. Wenting[1], J.W. Bijlsma[1], H.A. Hazewinkel[2], F.P. Lafeber[1]. *[1]University Medical Center Utrecht, Utrecht, NETHERLANDS, [2]Utrecht University Veterinary Medicine, Utrecht, NETHERLANDS*

Purpose: Joint bleeds lead to joint destruction. *In vitro* exposure of human and canine cartilage to blood results in long lasting severe adverse changes in cartilage. An *in vivo* joint haemorrhage in the canine knee joint demonstrates similar adverse effects although less outspoken and long-lasting. We investigated the clearance rate of blood from canine knee joints as a possible explanation for this discrepancy.
Methods: Blood was injected into the knee joint of Beagle dogs, either 48 h, 24 h or 15 m before termination. The amount of red and white blood cells present in the joint cavity was determined. Chondrocyte activity and cartilage matrix integrity as well as cartilage destructive activity of synovial tissue were determined biochemically. Additionally, synovial tissue was analyzed by use of histochemistry.
Results: Fifteen minutes after the injection of autologous blood, the red blood cell count was 5.7×10^{12}/L, comparable to the amount present in whole blood, and gradually decreased (1.6×10^{12}/L at 24 hours) to 0.2×10^{12}/L within 48 hours (less than 5%). The amount of white blood cells increased in the first 24 hours, and was still increased after 48 hours, although less than after 24 hours.
The proteoglycan synthesis rate and -release were adversely affected already within 24 hours (−22% and +24% respectively), and these effects were more severe 48 hours post-injection (−34% and +53% resp.).
Synovial tissue culture supernatants demonstrate cartilage destructive properties as expressed by an increased release, a decreased synthesis rate, and decreased content of cartilage proteoglycans; increasing with time after the experimental haemorrhage (+207%/+247%; −58%/−62%; −8%/−28% respectively, for 24/48 hours).