Evaluation of the activity of intraarticular hyaluronic acid in the repair of experimentally induced osteochondral defects of the stifle joint in dogs

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ABSTRACT: The present study examined the results of using hyaluronic acid with autogenetic cancellous grafts in the treatment of experimentally induced osteochondral defects in the stifle joints of dogs. In this study, 10 mature dogs of different breeds, weights and of both sexes were used. General anesthesia and usual operation procedures were followed. A 10 mm deep defect was created on the femoral sulcus of the trochlea with a drill tip of 8 mm in diameter. The defects in the right and left legs were filled with autogenic cancellous grafts taken from the metaphysial region of the tibia. The left legs constituted the experimental group while the right legs served as control group. In the experimental group, 2 mg/kg intraarticular hyaluronic acid was twice administered into the stifle joint, i.e., immediately subsequent to the operation and 1 month afterwards. Parenteral antibiotics were prescribed postoperatively for ten days. Five animals were sacrificed at the third and sixth month after surgery. Macroscopic and microscopic findings obtained from each case were evaluated. On macroscopical examination, trochlear defects were determined to be incompletely filled at the third month in both control and experimental groups. On histopathologic examination, a loose fibrovascular formation in the area where the graft was applied was observed to be present in both control and experimental groups. However, in the experimental group this formation was more superficial, ossification activity was greater and trabeculous bone formation had been initiated. Macroscopical examination carried out in the sixth month determined that in the control group the defect surface did not fill up to the trochlear sulcus level. In the histopathologic examination, in control groups it was found that fibrocartilageous structures were developing in the fibrovascular space even though ossification was incomplete. The macroscopic examination showed that in the experimental group, the defect surface reached the trochlear sulcus level of defects in this month. The histopatologic examination revealed that fibrous tissue comprised a thin layer, under which ossification processes were complete and bone trabeculates fully formed. It was concluded that the usage of autogenic cancellous graft along with hyaluronic acid may be useful in the repair of large osteochondral defects.

Keywords: osteochondral defect; hyaluronic acid; dog

Joint cartilage is a highly differentiated tissue and as a structure it is frequently injured but has limited chances of recovery. Synovial fluid provides joints with the required degree of friction, lubrication and friability needed for mobility and permanent lubrication without pain. At the same time, it absorbs mechanical shock waves and distributes pressure that is applied to bones beneath it so that stress placed on subchondral bones is minimized (Endre and Janet, 1993; Mahadev et al., 2001; Bilgili et al., 2006).

Joint cartilage defects are one of the most frequently encountered problems in dogs in recent years. Injuries to both cartilage and subchondral bones are called osteochondral defects. Osteochondral and chondral defects generally occur post-traumatically. The structure of joint cartilage does not contain veins and so it has a limited chance of rehabilitation (Outerbridge et al., 1995; Messner and Wei, 1998; Avki et al., 2003; Tins et al., 2005; Kang et al., 2008). Joint cartilage injuries that do not involve the subchondral bones

progress in a manner that causes joint cartilage degeneration. Although there is limited mitotic activity around the cartilage defect, this activity is not sufficient for recovery. However, if a cartilage defect reaches a subchondral bone that has vascular access, recovery can be enhanced. Small defects can be corrected with fibro cartilage tissue stemming from the subchondral locale but the recovery of large defects is relatively difficult (Messner and Wei, 1998; Makino et al., 2002; Arican et al., 2006; Gunes et al., 2006).

Although many methods have been developed for the treatment of cartilage defects, a satisfactory long-term solution has not been found and, in the long-term, existing treatments seem subject to degenerative problems. The main problem in applied treatment methods is the deficient biomechanical properties of new regeneration tissue. So far, studies (Tsai et al., 1992; Mahadev et al., 2001; Tins et al., 2005) have failed to describe the explicit regeneration of cartilage defects. Pain and loss of function stemming from joint cartilage defects necessitate the development of methods for the simplification and improvement of cartilage rehabilitation (Breinan et al., 1997; Gunay et al., 2005; Bilgili et al., 2006; Gunes et al., 2006).

Many methods have been tried for the rehabilitation of joint cartilage defects. Methods such as the drilling of subchondral bone (Sgaglione et al., 2002), micro fracture (Steadman et al., 2003), lavage and debridement (O'Driscoll, 1998) are surgical attempts to increase the intrinsic capacity of joint cartilage. Periosteal arthroplasty (Tsai et al., 1992; Gunes et al., 2006), perichondral arthroplasty (Amiel et al., 1985; O'Driscoll, 1998), autologous osteochondral transplantation (Outerbridge et al., 1995), autologous chondrocyte transplantation (Grande et al., 1989; Brittberg et al., 1996; Breinan et al., 1997; Tins et al., 2005), autogenetic cancellous grafts (Van Dyk et al., 1998; Gunay et al., 2005) and tendon autografts (Turhan et al., 1999) are methods which aim to form a new chondral surface.

The treatment of defects on the cartilage surfaces of joints should be cheap; furthermore, treatment should prevent osteoarthritis in the long term (Makino et al., 2002; Sgaglione et al., 2002). Cartilage rehabilitation has two main objectives: the first is to eliminate pain. This can be achieved by the rehabilitation of defects through the formation of hyaline cartilage which sustains the biomechanical features of joints. It should be resistant to daily stress and should stay healthy in the long term. The second aim is to prevent, or delay the onset of osteoarthritis (Outerbridge et al., 1995; O'Driscoll, 1998; Avki et al., 2003; Frisbie et al., 2003; Reinholz et al., 2004).

In controlled clinical studies, it has been reported that intraarticular hyaluronic acid (HA) injections in osteoarthritis cases are well-tolerated and provide effective relief from pain and recovery of function (Frizziero et al., 1998; Dougados, 2000; Kirwan, 2001; Amiel et al., 2003; Sen et al., 2004). Hyaluronic acid has been used in many studies because it prevents formation of anti-inflammatory, analgesic, anabolic, antioxidant substances, and fibrin, and inhibits proliferation. The observations of its protective effects on cartilage, because of its higher viscosity and its high concentration in synovial fluid, are reasons for its frequent usage in studies (Watterson and Esdaile, 2000; Brandt et al., 2001; Kilic et al., 2001; Tunay et al., 2002; Williams et al., 2003; Arican et al., 2006).

It has been reported that the empirical application of HA to rabbits by partial meniscectomy or after severing the anterior cruciate ligament has a potential protective effect on cartilage and delays progression of osteoarthritis (Yoshioka et al., 1997; Shimizu et al., 1998; Elmali et al., 1999; Tunay et al., 2002). While some previous researchers (Amiel et al., 2003; Sen et al., 2004) suggest that cartilage has protective features, some other studies maintain that there is insufficient evidence to prove this (Watterson and Esdaile, 2000; Brandt et al., 2001; Williams et al., 2003).

The present study examined the results of using hyaluronic acid with autogenetic cancellous grafts in the treatment of empirically formed osteochondral defects in the stifle joints of dogs.

MATERIAL AND METHODS

The present study was carried out after securing the approval of the Ethics Committee, Veterinary School, Firat University (numbered 2007/3). Ten adult dogs of both genders, and whose weight ranged from 17 to 30 kilos were used. Dogs were anesthetized with an intramuscular application of 20 mg ketamine hydrochloride after pre-medication with an injection of 2 mg xylazin hydrochloride. After anesthesis, both legs were shaved and disinfected widely. Animals were laid in a lateral position on the operation table. The lower and upper parts of the two legs were covered with a sterile cover-

ing and the stifle joints were exposed. In order to reveal the stifle joint, an S shaped dermal incision was made from the distal femur, turning to the medial genu and finishing at the proximal tibia. After cutting through the hypodermic collagenous tissue along the same line, an incision was made to the fascia lata on the caudal border of the quadriceps femoris muscle as far as the fascia lata. A parapatellar incision was made to the joint capsule and the patella was deviated to the medial by bringing the joints to extension position (Aslanbey, 2002). The defect was formed to a depth of 10 mm on the exposed femur's trochlear sulcus using an 8 mm diameter drill (Van Dyk et al., 1998). Defects that were formed on the right and left legs after drilling the proximal metaphysis locale of the tibia were tightly filled with autogenetic cancellous grafts taken with a curette. (Figure 1A, B) Subsequently, the locale was covered using standard procedures. Animals whose right legs were operated on were classified as the control group and those whose left legs were operated on constituted the experimental group. 2 mg/kg intraarticular hyaluronic acid (Orthovisc, Anika Therapeutics Inc., USA Sodium Hyaluronate 15 mg/ml) were injected. Parenteral antibiotics (Iecilline 800 000 IU, I.E. Uluagay Ilac Sanayii Turk AS (Turkish Pharmaceutical Company Co.)) were applied post-operatively for 15 days and stitches were removed on the tenth day. At one month postoperatively, the same doses of intraarticular HA were injected into the legs of the experimental group.

After operation, five animals were euthanized in the third and sixth months. Bone tissue samples taken from defect locales were analyzed in 10% formalin solution and then decalcified with nitric acid. After standard procedures were applied, paraffin blocks were prepared and cut to a 5 µ width, painted with haematoxylon-eosin and examined under a light microscope (H.E. × 40) (Luna, 1968).

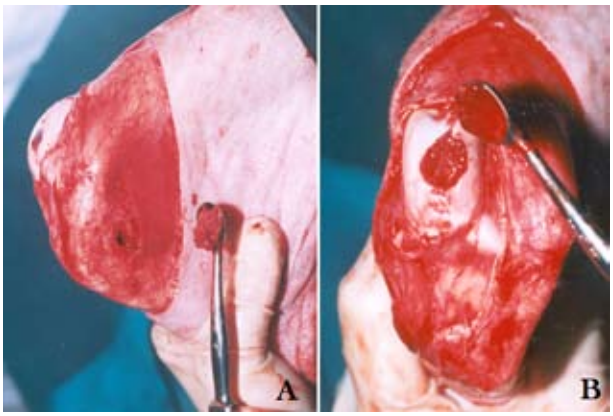

Figure 1. (A) Cancellous grafts taken with a curette (black arrow) after the proximal metaphyser part of tibia was drilled. (B) Filling of osteochondral defect with autogenetic cancellous grafts

RESULTS

No complications were observed during and after operation. In all cases gradually decreasing lameness was observed until 10–15 days post-operation. By 25–30 days post-operation lameness had disappeared completely.

Macroscopic examination showed that throchlear defects had not been filled in both control (Figure 2, small picture) and experimental (Figure 3, small picture) groups by the 3rd month. In histopathologi-

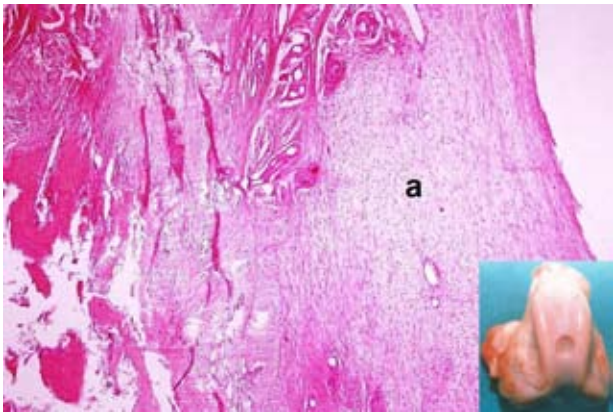

Figure 2. Macroscopic (small picture) and histopathological appearance in control group at 3rd month postoperatively. (a) Fibro vascular histogenesis; H.E. × 40

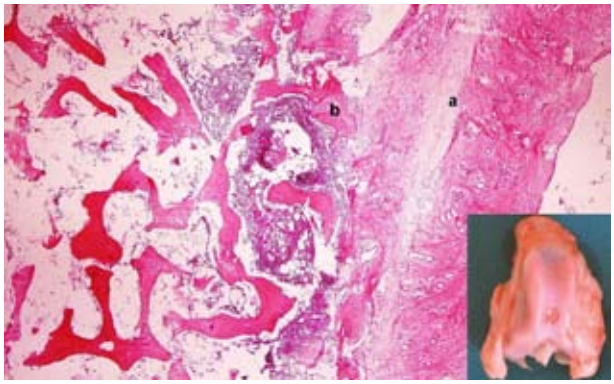

Figure 3. Macroscopic (small picture) and histopathological appearance in experimental group at 3rd month postoperatively. (a) Fibro vascular histogenesis, (b) bone trabeculas; H.E. × 40

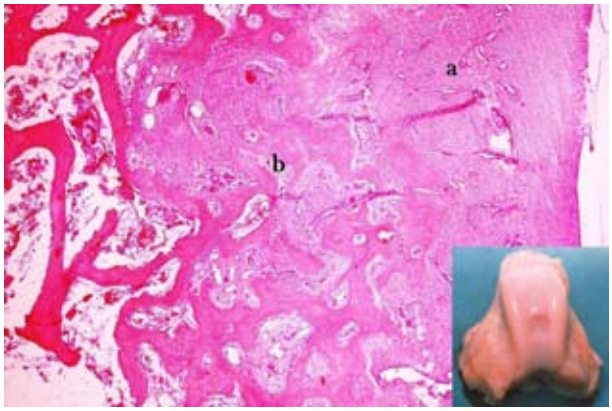

Figure 4. Macroscopic (small picture) and histopathological appearance in control group at 6th month postoperatively. (a) Fibro vascular histogenesis, (b) fibrocartilaginous formations; H.E. × 40

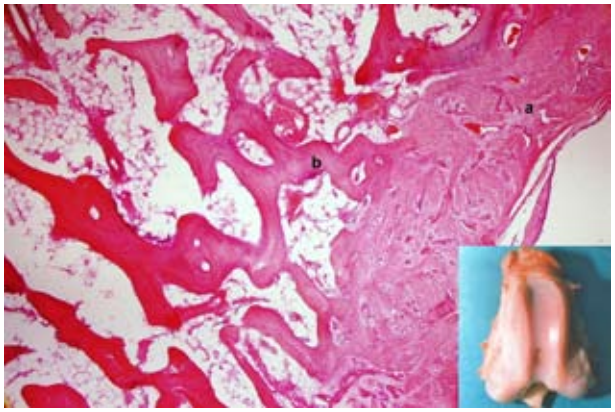

Figure 5. Macroscopic (small picture) and histopathological appearance in experimental group at 6th month postoperatively. (a) Fibro vascular histogenesis, (b) bone trabeculas H.E. × 40

cal examinations, regeneration and reparation were detected in defect locales in control and experimental groups. It was detected that in both groups, grafts applied at locales had a loose fibrovascular structure (Figure 2a, Figure 3a), although in the experimental group this structure was superficial and ossification was more intense. Trabecular bone was observed to be taking shape (Figure 3a, b).

In the 6th month macroscopic examinations detected that the defect surface was not filled to the trochlear channel level in control groups (Figure 4, small picture). Histopathological examination of fibrovascular structure showed that focal fibrocartilaginous structures were developed but ossification had not proceeded to completion (Figure 4a, b). In that month, macroscopic examination of these defects reached the trochlear channel level (Figure 5, small picture).

Tissue filling defects were highlighted in the same bright color with the surrounding tissues. In histopathological examinations, fibrocartilaginous tissue was detected as a thin layer; beneath this structure ossification was complete and trabecular bone was taking shape (Figure 5a, b).

Analysis of the histopathological findings suggested that a higher rate of recovery was observed in the experimental group.

DISCUSSION

Joint cartilage injuries are one of the most frequently encountered problems in orthopaedics. Cartilage injuries to leg joints can cause lesions (chondral) limited to cartilage tissue and can also cause osteochondral lesions containing cartilage and subchondral bone (Outerbridge et al., 1995; Avki et al., 2003; Gunay et al., 2005; Bilgili et al., 2006). Local joint cartilage lesions generally stem from traumatic chondral injuries, osteochondritis dissecans or osteonecrosis. The recovery processes of osteochondral defects are not known in detail but if they are not treated, complications will arise in the healing of lesions. In such cases which encompass a large part of the bone surface, damage may progress to degeneration in joints (Makino et al., 2002; Arican et al., 2006; Gunes et al., 2006). Although many methods have been developed for the treatment of chondral and osteochondral defects, none have been observed to result in the formation of normal cartilage. As many and varied conclusions have been drawn from recent studies (Avki et al., 2003; Tins et al., 2005; Bilgili et al., 2006), the best treatment method for focal defects remains unknown.

In the present study, empirically-formed osteochondral defects were filled with autogenetic cancellous grafts and the efficiency of hyaluronic acid in treatment was investigated. In macroscopic and histopathological observations at the 3rd and 6th months postoperatively, experimental group animals were observed to recover swiftly compared with the control group.

In the treatment of osteoarthritis patients, it is extremely important to prevent stifle pain, or to stop or slow its progress providing for an increase in the quality of life. The preservation of cartilage is therefore extremely important. The main function

of hyaluronic acid is to ensure elasticity and viscosity of leg joints (Endre and Janet, 1993; Peyron, 1993; Scale et al., 1994; Frizziero et al., 1998; Kirwan, 2001). These effects were assessed according to the amount of stress applied to the legs. The elasticity of HA increases and viscosity decreases in accordance with high stress forces applied to the legs. In slow actions, the reverse is possible (Watterson and Esdaile, 2000; Kirwan, 2001). Intraarticular hyaluronic acid application has anti inflammatory, anabolic, analgesic, and cartilage protective effects besides its physical effects (Watterson and Esdaile, 2000; Brandt et al., 2001; Tunay et al., 2002; Williams et al., 2003). Kirwan (2001) refers to the injection of HA into joints as viscosupplementation. Takahashi et al. (2001) stated that hyaluronic acid produces therapeutic effects by suppressing the production of nitric oxide. Intraarticular injection of HA increases lubrication in synovial joints and increases friability which is lowered in empirically formed osteoarthritis. Furthermore, it decreases the concentration of fibronolitic factors whose numbers rise during the progress of osteoarthritis and it causes a rise in glycosaminoglycan levels that form the inner structure of the cartilage matrix (Obara et al., 1997). However, there are some who suggest that further research is required on the cartilage-protective effects of hyaluronic acid (Watterson and Esdaile, 2000).

In the present study, empirically formed osteochondral defects were filled with autogenetic cancellous grafts which are easily obtained and applied. The effect of hyaluronic acid on pain reduction in joint cartilage lesions and rehabilitation of hyaline cartilage were investigated. HA (hyaluronic acid), a polysaccharide chain, is composed of recurring disaccharide units of N-acetylglucosamine and glucuronic acids. It is synthesised and secreted to joints by synoviocyte and synovial fibroblasts (Watterson and Esdaile, 2000; Kirwan, 2001). In accordance with previous studies (Endre and Janet, 1993; Peyron, 1993; Scale et al., 1994; Yoshioka et al., 1997; Shimizu et al., 1998; Dougados, 2000), it was observed that HA injections are generally well-tolerated and that they apparently aid recovery from pain and restoration of function.

One of the most anticipated effects from methods of treating joint cartilage defects is the delay of prosthesis replacement in the long term (Sumen et al., 1995; Reinholz et al., 2004). There are many factors affecting rehabilitation of cartilage in the long term. However, rehabilitation tissue should cling to the defect locale and the continued presence of tissue is critically important (Amiel et al., 1985; Outerbridge et al., 1995; Frisbie et al., 2003; Tins et al., 2005). Periosteal grafts are widely used in the treatment of osteochondral defects because they are easily accessible, abundant and include mesenchymial progenitor cells (Rubak, 1982; Tsai et al., 1992; Gunes et al., 2006). Tsai et al. (1992) stated that, in their study on rabbits, complications such as graft instability, graft degeneration or allergic reactions can be observed due to the inadequate fixation of periosteal grafts stemming from chondrocyte loss. In such cases the application of grafts with fibrin adhesive can result in more successful outcomes. In this study autogenetic cancellous grafts were applied to defects with finger pressure. The higher part which comes to joint surface was flattened by applying pressure with a rounded tool. Due to the rough and uneven structure of grafts, it has been observed that they can cling to their position without the need for a tissue adhesive and do not become displaced due to movement in the cartilage during operation.

Restoration of joint surface and defect rehabilitation procedures are based primarily on two strategies: the first is the enhancement of the intrinsic capacity of cartilage and subchondral bone for recovery; the second is the formation of new joint surfaces by transplantation of chondrogenic cells and chondrocytes which have the potential to form new cartilage (O'Driscoll, 1998; Mahadev et al., 2001; Avki et al., 2003; Frisbie et al., 2003; Reinholz et al., 2004). All applied methods for the rehabilitation of joint defects can aid recovery from clinical symptoms such as pain, locked stifle and panicula, but they cannot rehabilitate hyaline joint cartilage. Fibro cartilage is the rehabilitation tissue irritated by these procedures and it does not have the biochemical and biomechanical features of joint cartilage.

Some researchers (Amiel et al., 1985; Namzek et al., 1994; Sumen et al., 1995) have claimed that meniscal and osteochondral allografts can be used in the rehabilitation of osteochondral defects. However, they emphasized that these applications can transmit diseases and can lead to complications such as a low incidence of survival at the applied areas. It has been proposed that osteochondral autografts are a safe source of grafts and also that histoincompatibility is impossible. However, the use of this procedure in reconstruction of extensive defects can present difficulties in obtaining the necessary autografts.

In the present study, autogenetic cancellous grafts were applied and postoperative complications were not observed. Histopathological examination showed that elements of regeneration and reparation were developed in both the operative and control groups. In the 3rd post-operative month, it was observed that graft locales had a loose fibrovascular structure in both control and experimental groups. However, in the experimental group this structure was more superficial, ossification activities were more intense, and trabecular bone had started to take shape. Examination of the control group during the 6th post-operative month showed that local fibro cartilaginous structures developed in the fibro vascular structure but that ossification was lacking. In the experimental group, fibro cartilaginous tissue formed a thin layer, ossification beneath this structure and the shaping of trabecular bone were complete.

Recent studies on the rehabilitation of osteochondral defects studies have examined the role of autologous chondrocyte transplantation (Grande et al., 1989; Brittberg et al., 1996; Tins et al., 2005; Bilgili et al., 2006). Although satisfactory results were reported, drawbacks were identified, as this technique needs financial support and further detailed laboratory studies. Grande et al. (1989) used prepared autologous chondrocytes for patella cartilage defects of rabbits and noted that the recovery of experimental group animals was more rapid than control group animals. However, similar studies on dogs could not replicate these results (Breinan et al., 1997). The method used in this study for the rehabilitation of wide osteochondral defects was simple, cheap and successful. These features are regarded as the key positive results of the present study.

Structural lesions typically develop on joint cartilage during the progress of osteoarthritis. The process begins with the loss of proteoglycans from the extracellular matrix and failure of the collagenous fiber network and continues with cell metaplasia and cell loss (Hunziker, 2001). In this phase the application of intraarticular exogenous HA, increases both HA synthesis and proteoglycan synthesis. Also, the oscillation of enzymes leading to cartilage catabolism has been observed to be inhibited (Shimizu et al., 1998; Elmali et al., 1999). Tunay et al. (2002) used 2 mg/kg HA twice every other month in the rehabilitation of empirically formed osteoarthritis. These results suggested that the usage of intraarticular HA prevents inflammation and catabolic degeneration in cartilage and synovial tissues. Kang et al. (2008) applied HA after an empirically-formed micro fracture. In this study, intraarticular injection of hyaluronic acid was applied twice with autogenetic cancellous grafts for rehabilitation of empirically formed osteochondral defects.

It is concluded that the application of autogenetic cancellous grafts in the rehabilitation of wide osteochondral defects is beneficial due to a number of advantages. These include the simplicity of the surgical method; fast recovery of the experimental group compared with the control group; applicability in the treatment of osteochondral defects which usually result in big financial losses, and the low cost of application. A biomechanical evaluation of the cartilage tissue formed on the defect was not undertaken at the end of the experiment; this is considered to be a shortcoming of the methodology. It is suggested that future research may include the testing of the biomechanical characteristics of cartilage formed on joint defects and that long term monitoring for the risk of osteoarthritis progression is necessary.

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