Background: Fresh or frozen nonvascularized osteotendinous joint allografts (OTJA) have not been used previously, clinically or experimentally, for metacarpophalangeal joint reconstruction. Therefore, we evaluated the viability of OTJA for metatarsophalangeal joint (MTJ) reconstruction in rats.

Methods: In the experimental group of 12 Lewis rats, we reconstructed the MTJ of the third digit of the hindlimb with a fresh, nonvascularized OTJA obtained from the same digit from 12 donor rats. In the control group of 6 Lewis rats, an autologous composite osteotendinous graft of the MTJ of the same digit was obtained and repositioned in situ as an auto-transplant. Weight, pain, edema, dehiscence, and wound infection were evaluated every 24 hours for 30 days postoperatively. At the end of 30 days, we evaluated digit position, flexion and extension, passive mobility, radiological bone healing, and histological grades of rejection.

Results: We found no statistically different changes in weight, edema, pain, digit position, or radiological bone healing in either group. No wound dehiscence or infection was seen in any of the rats. Ten degrees of flexion and extension mobility were lost in the control group; the experimental group lost up to 30 degrees ($P = 0.009$). Histologically, 9 of the experimental group rats (9/12, 75%) showed rejection reactions compared with none of the controls (0%) ($P = 0.009$).

Conclusions: Fresh nonvascularized OTJA caused an immune reaction without exposure of the graft, but with bone resorption. However, the rats maintained digital form and alignment with decreased passive flexion and extension of 10–30 degrees.

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sor apparatus, tendon insertions of the intrinsic muscles, and flexor tendon pulleys. The OTJA was transplanted fresh, nonvascularized, and without immunosuppressive therapy.

The objectives of this study were to: (1) evaluate the clinical feasibility of OTJA; (2) histologically evaluate the rejection reaction to the OTJA; (3) evaluate radiological bone consolidation with the OTJA; and (4) evaluate the final mobility of the MTJ and digit.

MATERIALS AND METHODS

This experimental, longitudinal, prospective, and blinded study was approved by the Ethical Review Board of our institution.

The experimental group consisted of 12 Lewis rats, weighing 400–500 g. The MTJ of the third digit of the left hind leg was reconstructed with a fresh, nonvascularized MTJ OTJA, harvested from the third digit of the left hind leg of 12 donor rats. The donor rats were Lewis rats of the same weight as the recipient rats. The control group consisted of 6 Lewis rats, weighing 300 g. Control rats received an MTJ autograft from the third digit of the left hind leg, which was subsequently placed in situ to reconstruct the same MTJ.

Technique

Anesthesia was induced with an intraperitoneal injection of a mixture of ketamine hydrochloride (80 mg/kg) and propanidid (20 mg/kg), and the rats were maintained on 2% isoflurane by face mask. Preoperatively, aseptic skin preparation was performed with chlorhexidine solution. Ceftriaxone 20 mg/kg intramuscularly was also administered, prophylactically. In both groups, surgery was performed with the sterile technique and with magnification at 16×–24×. In the experimental group, one surgical team worked with the recipient, and another surgical team recovered the MTJ OTJA from the donor rat. In the control group, the same surgical team obtained and implanted the MTJ autograft.

In the experimental group, the defect in the recipient rat was created as follows: the extensor communis tendon, central extensor tendon, and interosseous tendons were transected at the level of their insertion into the MTJ. A hole was drilled in the third metatarsal bone 7 mm proximal to the MTJ in the dorso-plantar direction with a number 15 surgical bur. A similar hole was drilled in the proximal phalanx, 7 mm distal to the MTJ. Approximately 5 mm proximal to the MTJ, an osteotomy of the third metatarsal bone was then performed, followed by an osteotomy of the proximal phalanx 5 mm distal to the MTJ. Finally, the flexor sheath was sectioned longitudinally and the MTJ graft was withdrawn. Flexor tendon integrity was preserved.

In the donor rats, recovery of the MTJ graft was similar to that in the recipient rats, with the following differences: (1) The extensor communis tendon and interosseous tendons were sectioned 1 cm proximal to the MTJ, and the central extensor tendon was sectioned 1 cm distal to the MTJ. (2) The hole in the third metatarsal bone was made 3 mm distal to the MTJ. (3) An approximately 2-cm-long tendon graft was harvested from the extensor communis tendon of the second digit. (4) Finally, the donor rat was killed with 1 mL of intracardiac pentobarbital (Fig. 1).

In the control group, the osteotendinous MTJ autograft was harvested using a similar technique, with the following differences: (1) The extensor communis tendon and interosseous tendons were sectioned 1 cm proximal to the MTJ, and the central extensor tendon was sectioned 1 cm distal to the MTJ. (2) Two holes were drilled in the third metatarsal bone: one hole at 7 mm and another at

![Fig. 1. The OTJA. (1) The extensor communis tendon. (2) The central extensor tendon. (3) Sagittal bands. (4) Interosseous tendons. (5) Interosseous muscles.](image-url)
prophylaxis, ceftriaxone was administered intramuscularly every 12 hours for the first 3 days. For infection (b) Pain: evaluated according to the parameters reported (a) Body weight: recorded daily.

(3) Two holes were drilled in the proximal phalanx: one at 7 mm and another at 3 mm distal to the MTJ. Osteotomy of the proximal phalanx was performed 5 mm distal to the MTJ. The remainder of the harvesting technique was similar to the other groups.

Implantation of the MTJ OTJA in the experimental group and implantation of the osteotendinous autograft in the control group began with placing the recipient flexor tendons into the donor tendon pulley system. The tendon pulley system was repaired using 8-0 silk sutures. The tendon graft was used to reinforce the repair of the tendon sheath and was sutured with simple interrupted 8-0 nylon. We continued with proximal and distal bone fixation with 0.23-mm cerclage wire. Latero-lateral tenorrhaphies using 7-0 and 9-0 nylon sutures were performed to repair the extensor communis tendon, central extensor tendon, and interosseous tendons. Before closing the skin, a single dose of buprenorphine hydrochloride 0.15 mL/100 g (0.324 mg buprenorphine hydrochloride/mL) was injected subcutaneously. The skin was sutured with simple interrupted 5-0 nylon sutures. Finally, we placed an Elizabethan collar made of tape.

Postoperative Care

The splint was maintained until the fifth postoperative day, at which point the limb was immersed in a solution of quinine twice daily to prevent self-mutilation. Buprenorphine hydrochloride 0.1 mL/100 g (0.324 mg buprenorphine hydrochloride/mL) was injected subcutaneously every 12 hours for the first 3 days. For infection prophylaxis, ceftriaxone was administered intramuscularly at 20 mg/kg/d for 5 days.

Clinical Progression

The rats were observed for 30 days, and evaluated and ranked every 24 hours for the following variables:

(a) Body weight: recorded daily.
(b) Pain: evaluated according to the parameters reported by the Advisory Ethics Committee for Animal Experimentation of the University of Zaragoza. This scale was also used to determine euthanasia.
(c) Limb edema: evaluated as grade 1 when the edema was local in the wound; grade 2 when the edema was generalized to the entire foot; and grade 3 when the edema was generalized to the entire leg.
(d) Surgical wound dehiscence: evaluated by the presence or absence of wound dehiscence.
(e) Infection of the surgical wound: assessed by the presence of 2 or more of the following factors: hyperemia, presence of discharge, and/or surgical wound dehiscence.

At postoperative 30 days, we assessed:

(a) Position of the third digit: classified as grade 1 when the digit was in good alignment, or with diversion in any direction <15 degrees; grade 2 when the digit was deviated ≥15 degrees but <30 degrees; and grade 3 when the deviation was ≥30 degrees.

(b) Radiological bone healing: based on plain radiographs of the limb including dorso-plantar and lateral views. Radiological consolidation was evaluated according to the modified scale described by Weiland et al. (Table 1).

Movement Angles

The movement angles of the MTJ were assessed before implantation and after euthanasia. A 10-mg load was applied on the proximal phalanx to evaluate movement during flexion and extension. Mobility was evaluated in degrees, with loss of mobility in flexion and extension classified as grade 1 when the loss was <10 degrees; grade 2 when the loss was ≥10 and <30 degrees; and grade 3 when the loss was ≥30 degrees.

At the end of the monitoring period, we surgically removed the grafts and the rats were euthanized with 1 mL of intracardiac pentobarbital.

Histological Evaluation

Surgical specimens were fixed in 10% formalin and stained with hematoxylin and eosin to evaluate the degree of rejection, according to previously published methods (Table 2).

Statistical Analysis

Quantitative variables including weight gain and maximum pain were analyzed using the rank-sum test or Mann-Whitney test. Results were expressed as mean ± standard deviation. Fisher’s exact test was used for categorical variables (edema, dehiscence, infection, digital deviation, loss of mobility, osteosynthesis, and rejection), and variables were expressed as a number and a percentage. A difference in any analysis was considered statistically significant at P < 0.05.

RESULTS

None of the rats in either the control group or experimental group died suddenly or required euthanasia before the end of the study. Body weight increased in the control group by an average of 27 ± 5 g, compared with an average body weight increase in the experimental group of 23 ± 3 g. The maximum average pain score in the control group was 2.1, which gradually decreased until it stabilized on postoperative day 5. The maximum average pain score in the experimental group was 2.8 ± 0.8, which
 gradu ally decreased until no pain was detected on postoperative day 8. The maximum edema score in both the control group and the experimental group was 2 ± 0.4. Edema resolved on postoperative day 6 in the control group and on postoperative day 8 in the experimental group. Maximum pain and edema in both groups occurred 24 hours after surgery, and no dehiscence or infection of the surgical wound was seen in any group. All rats in the control group had grade 1 digital deviation. Digital deviation in the experimental group was grade 1 in 10 rats and grade 2 in 2 rats. The differences between the aforementioned variables were not statistically significant. The results of the radiological evaluation are shown in Table 3; there were no statistically significant differences.

In the control group, a <10-degree loss of flexion was present in 100% of the rats compared with only 33% of the rats in the experimental group (P = 0.013). A 10-degree loss of extension was present in 100% of the control group rats and in 25% of the experimental group rats (P = 0.009) (Table 3).

In the control group, histology revealed mineralized osteoid matrix containing osteocytes and osteoblasts at the periphery of the osteotendinous autografts, with no signs of rejection, in 100% of the rats (Fig. 2). In the experimental group, tissue necrosis was observed histologically as the rejection reaction in 50% of the rats. Another 25% presented with grade 2 rejection reactions characterized mainly by infiltrated periosteum and irregular cortical bone, but with signs of revascularization (Fig. 3). The remaining 25% showed no signs of rejection reaction, which was similar to the control group (P = 0.009) (Table 3).

**DISCUSSION**

Swanson-type joint implants and nonvascularized auto-osteocondral grafts harvested from other damaged fingers or the MTJs have both been used to reconstruct MPJs; however, these techniques have all resulted in articular cartilage necrosis. To avoid such necrosis, vascularized autografts have been transferred instead with good success owing to the restoration of form and preservation of intra-articular space and function. Based on our experience, the same results can be obtained in the reconstruction of phalangeal and interphalangeal joints with fresh nonvascularized OTJAs. We performed this study to evaluate the feasibility and safety of this procedure.

**Table 2. Rejection Scale**

| Grade | Characteristics                  |
|-------|----------------------------------|
| 0     | Normal                           |
| 1     | Infiltrated periosteal and rejection |
| 2     | Intratrabecular space not vascularized, irregular cortical bone, local tissue is not viable |
| 3     | Edema, vasculitis, and necrosis. |

Reprinted from De Achauer BM. Rejection of the component tissues of limb allografts in rats immunosuppressed with FK-506 and cyclosporine-Discussion. *Plast Reconstr Surg* 1996;97:149–151.

**Table 3. Clinic, Radiologic, and Histologic Outcomes**

| Variable                              | Control Group (n = 6) | Experimental Group (n = 12) | P     |
|---------------------------------------|-----------------------|----------------------------|-------|
| Weight gain, g (mean ± DS)            | 27±5                  | 23±3                       | 0.099 |
| Maximum pain, scale 0–4 (mean ± DS)   | 2.2±0.4               | 2.8±0.8                    | 0.095 |
| Digital deviation, no. (%)            |                       |                            |       |
| 0° to <15°                            | 6 (100)               | 10 (83.3)                  | 0.529 |
| ≥15° to <30°                          | 0 (0)                 | 2 (16.6)                   | 0.529 |
| ≥30°                                  | 0 (0)                 | 0 (0)                      | —     |
| Loss of mobility (flexion), no. (%)   |                       |                            |       |
| <10°                                  | 6 (100)               | 4 (33.3)                   | 0.013 |
| ≥10° to <30°                          | 0 (0)                 | 7 (58.3)                   | 0.038 |
| ≥30°                                  | 0 (0)                 | 1 (8.3)                    | 1.0   |
| Loss of mobility (extension), no. (%)  |                       |                            |       |
| <10°                                  | 6 (100)               | 3 (25)                     | 0.009 |
| ≥10° to <30°                          | 0 (0)                 | 7 (58.3)                   | 0.038 |
| ≥30°                                  | 0 (0)                 | 2 (16.6)                   | 0.529 |
| Proximal osteosynthesis, no. (%)      |                       |                            |       |
| Grade 0                               | 0 (0)                 | 4 (33.3)                   | 0.245 |
| Grade 1                               | 0 (0)                 | 0 (0)                      | —     |
| Grade 2                               | 0 (0)                 | 1 (8.3)                    | 1.0   |
| Grade 3                               | 0 (0)                 | 1 (8.3)                    | 1.0   |
| Grade 4                               | 6 (100)               | 6 (50)                     | 0.054 |
| Grade 5                               | 0 (0)                 | 0 (0)                      | —     |
| Grade 6                               | 0 (0)                 | 0 (0)                      | —     |
| Distal osteosynthesis, no. (%)         |                       |                            |       |
| Grade 0                               | 0 (0)                 | 3 (25)                     | 0.515 |
| Grade 1                               | 0 (0)                 | 0 (0)                      | —     |
| Grade 2                               | 0 (0)                 | 2 (16.6)                   | 0.329 |
| Grade 3                               | 0 (0)                 | 4 (33.3)                   | 0.245 |
| Grade 4                               | 6 (100)               | 6 (50)                     | 0.054 |
| Grade 5                               | 0 (0)                 | 0 (0)                      | —     |
| Grade 6                               | 0 (0)                 | 0 (0)                      | —     |
| Body of graft osteosynthesis, no. (%)  |                       |                            |       |
| Grade 0                               | 0 (0)                 | 0 (0)                      | —     |
| Grade 1                               | 0 (0)                 | 0 (0)                      | —     |
| Grade 2                               | 0 (0)                 | 2 (16.6)                   | 0.329 |
| Grade 3                               | 0 (0)                 | 4 (33.3)                   | 0.245 |
| Grade 4                               | 6 (100)               | 6 (50)                     | 0.054 |
| Grade 5                               | 0 (0)                 | 0 (0)                      | —     |
| Grade 6                               | 0 (0)                 | 0 (0)                      | —     |
| Rejection, no. (%)                    |                       |                            |       |
| Grade 0                               | 6 (100)               | 3 (25)                     | 0.009 |
| Grade 1                               | 0 (0)                 | 0 (0)                      | —     |
| Grade 2                               | 0 (0)                 | 3 (25)                     | 0.515 |
| Grade 3                               | 0 (0)                 | 6 (50)                     | 0.054 |

*Statistically significant
The OTJAs used in this study were fresh, and only local inflammatory processes with no systemic repercussions were observed in the rats in the experimental group. Postoperative rat behavior, pain, and weight gain were similar between the experimental group and the control group. None of the data indicated severe acute rejection, as shown by the lack of wound dehiscence or graft exposure in the experimental group. This likely resulted from the quality of the skin cover, a good microsurgical environment, and the small amount of transplanted allogeneic tissue, which avoided exposure of the OTJA. Based on these factors, our results substantiate the possible clinical application of this technique.

In our protocol, the OTJA was nonvascularized. However, at the end of the study, bone healing in the control group was grade 4 in the metatarsal union and the proximal phalanx in 100% and 83.3% of the rats, respectively. This likely resulted from good contact between the donor and recipient bone grafts, and from the small size of the graft, which permitted early revascularization mainly by osteoconduction. A longer follow-up in our study might have shown better grades of radiological consolidation based on Weiland’s scale. According to Wend et al., early consolidation leads to strong bony union and good remodeling, which in turn confer long-term bone viability. These findings indicate the possible clinical utility of osteotendinous joint autografts without the need for revascularization surgery for reconstructing MPJs. In the experimental group in our study, bone consolidation of grade 4, or early union, at the metatarsal level was achieved in only 50% of the rats, and it was achieved at the level of the proximal phalanx in 66.6% of the rats. Complete bone resorption (grade 0) occurred at the level of the metatarsal bone in 33.3% of the rats and at the proximal phalanx in 25% of the rats. However, none of these differences were statistically significant. In the OTJAs with complete bone resorption (grade 0), grade 2 (mild resorption), or grade 3 (resembling postoperative), or poor or no revascularization, the weak allogeneic effect of the OTJAs was most likely the main cause of these results.

Despite the changes in bone consolidation, 10 treated digits (83.3%) in the experimental group had grade 1 digital deviation, and only 2 (16.6%) had grade 2 digital deviation. In comparison, 100% of the digits in the control group had grade 1 deviation. Grade 1 deviation is considered functional; therefore, it is possible that despite the mild to severe bone resorption (nonunion) that was present in the experimental group, the low-level deviation kept the digits in functional alignment. These OTJAs likely worked as spacers similar to spacer prostheses.

Experimental and clinical studies have used OTJAs primarily in large bones such as the radius and femur. The metatarsal bone and proximal phalanges used in this study were smaller, and therefore revascularization was satisfactory in 100% of cases in the control group and in 50%
of cases in the experimental group, based on the quality of the radiological consolidation. Bone grafts are known to cause rejection reactions, and the presence or absence of rejection reactions may be related to the intensity of the immune response, and/or a result of a graft–host mismatch. Histologically, our experimental group showed rejection reactions in 75% of the rats (grade 3 reactions in 50% and grade 2 reactions in 25%). Twenty-five percent of the rats in the experimental group showed no signs of rejection and none of the control rats developed rejection reactions. This finding was statistically significant. Therefore, to consider using this procedure clinically, the technique would have to guarantee greater allograft revascularization and/or require short-term immunosuppression.

Changes in passive flexion and extension mobility at the end of the study were different with statistical significance. In the control group, passive flexion and extension mobility were grade 1 in 100% of the rats compared with the experimental group, in which loss of flexion mobility was grade 1 in only 4 rats (33%) and the loss of extension mobility was grade 1 in 3 rats (25%). The majority of the rats had a loss of flexion and extension mobility of up to 30 degrees. Our mobility results for the OTJA are similar to those obtained with spacer implants used in MPJ reconstruction.11,12 Despite this loss of mobility, we consider the OTJA used in our protocol to confer the advantage of being an anatomical reconstruction that maintains the correct axis for the flexor mechanism because tendon pulleys A1 and A2 are transplanted, which thereby improves movement.

CONCLUSIONS

Our control group results support the hypothesis that fresh nonvascularized OTJ autografts can be successfully revascularized and provide distal and proximal bone healing, as well as good digital alignment. However, the technique can result in decreased mobility in flexion and extension of up to 10 degrees. Fresh nonvascularized OTJA caused an immune reaction that did not lead to exposure of the graft or systemic problems. Although this immunological reaction did lead to bone resorption in the majority of the rats, digital form and alignment were maintained with decreased passive flexion and extension of 10–30 degrees. Our results indicate that the functionality obtained using OTJAs could be similar to that reported in the reconstruction of MPJs with silicone joint implants.11–16 However, the evaluation period in our study was short, and a longer follow-up in a larger number of rats is required to evaluate the ultimate utility of OTJAs in MTJ reconstruction.

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