The effect of thranechron and autograft bone on bone defect healing in rabbit model

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Abstract

Background Nowadays, bone grafting is used in both human and veterinary orthopedics to stimulate fracture healing, accelerate joint union, and to restore bone defects. In such procedures, orthopedic surgeons are searching to favorable substitute for autograft bone. The present study seeks to investigate the use of this extract in bone defect healing process. 20 native rabbits were divided into 4 groups of five, and bone fragments were removed from their radius bones. In the first group, Theranekron 1mg/kg was injected into bone defect. Normal saline was injected into the same site of the five rabbits in the second group (saline treated controls). As for the third group (the untreated controls), nothing was injected. In the fourth group (autograft group), the removed bone was put in place and muscle and skin were sutured. Post op lateral x-rays were prepared on days 14, 28, 42, and 56. Bone biopsy for histopathological study was performed after 8 weeks. Radiographs and samples were analyzed statistically in terms of union, osteogenesis activity, and bone Remodeling.

Results Radiological evaluation showed that autograft group was significantly superior to both untreated and saline treated controls ($p=0.02$, $p=0.04$, respectively). Also, Theranekron group proved better than untreated controls ($p=0.04$). Histopathological evaluation showed trabecular bone and bone marrow formations in Theranekron and also autograft groups. In the untreated and saline treated controls, most part of defect was filled with fibrous tissue.

Conclusion Theranekron treated group showed almost similarly to autograft counterpart, but better than normal saline and untreated controls.

Background

Bone graft is currently used to treat fractures, accelerate joint union, and restore the bone defects. Autograft is considered the gold standard in such operative procedures, because it contains repair stimulating substances and the cells within does not provoke immune responses nor cause the transmission of contagious diseases [9]. In small animals, autograft bone is harvested from the iliac crest, the inner surface of the upper end of the tibia as well as humerus; while in humans iliac crest bone is used. Complications such as pain, infection, fracture, blood loss and need for further operative procedures are associated with autograft bone harvesting while there is a limited amount of bone for that purpose [8]. Given such problems associated with autografting bone, the use of non-autograft bones including allograft and xenograft is more favored in current orthopedics for some reasons as follows: there is no limit to these non-autograft bones and they contain cells and proteins which enhance healing; a supportive scaffold is formed within the huge gaps when tumors are removed and bone tissue is lost [6]. However, there is the risk of contagious diseases transmission when using allograft. Tarantula cubensis is an extract widely used in the treatment of tumors, abscesses, septicemia and toxemic conditions [10, 15, 20]. Reports indicate that it is helpful to bovine wound healing on day 14, leading to reduced inflammation and epithelialization [2, 19]. Moreover, the extract reportedly reduces a wide range of infections [5, 12]. Tarantula cubensis come from mygalomorph order/genus and is referred to as

brown, hairy spider or mygalocubensis [17]. It is native to south Carolina, Texas and Cuba. Tarantula is brown and hairy and of less toxicity, compared to Tarantula hispanica. The basic composition of the extract is prepared from the whole spider, with arachnid as its main element. In doing so, the whole spider is initially crushed and an alcoholic extract with trade name Theranekron is produced [17]. Investigated the effects of lavamisole and theranekron on skin papilloma and concluded that the former was more effective [5]. Gultiken, Vural, 2007 demonstrated that Theranekron was effective against breast benign tumors in dogs [10]. Albay Ahinduran et al, 2010 investigated the effects of this agent on the mouth ulcers due to bluetongue in cattle and revealed that the treatment improved food intake and appetite, reduced oral inflammation and decreased leukocyte and lymphocyte counts in CBC within 24 hours. Nonetheless, no change was evident after 10 days in the clinical findings and leukocyte counts and only a significant change was observed in lymphocyte count [2].

Sardari and colleagues in 2011 carried out a study on the effect of Theranekron on horse serum hematological and biochemical properties and concluded that most of the observed changes in hematological and biochemical parameters were statistically significant. Also, Theranekron treatment in the experimental horses yielded no adverse reaction [19].

Oryan et al, 2012, investigated the effect of Theranekron on the damaged tendon and showed an improvement in biomechanical parameters in the treatment group [16]. Furthermore, Adib hashemi et al, 2015 investigated the anti-inflammatory effects of Theranekron on the healing of peritoneal lesion in rats and reported that histopathological examination revealed enhanced angiogenesis, re-epithelialization and declined inflammatory reaction in the experimental group, compared to the controls, as well as the emergence of mature parallel dense collagen fibers after 29 days [1].

In the present study attempts to investigate the healing potential of Theranekron was evaluated in a rabbit critical-sized radial defect model.

Methods

In total, 20 native rabbits weighing approximately 2 kg and aged 1.5-2 years were selected for surgical operations. The rabbits were purchased from local private farm. They were placed in a special cage for 15 days to let them adjust to the new conditions and were fed with special foods. Conditions and feeding were the same for all the rabbits. Ivermectin, an antiparasitic drug was injected subcutaneously 0.2mg/kg to remove internal and external parasites of ringworm type. All animals received humane care in compliance with the Guide for Care and use of Laboratory Animals (NIH publication No. 85-23, revised 1985). The study was approved by the local Ethics Committee of our Veterinary School. They were killed by intra-cardiac injection of 1 mg/kg Gallamine triethiodide (Specia Co., Paris, France).

The rabbits were divided into four groups of five and Theranekron diluted 1mg/ml in normal saline 0.9% was injected 1mg/kg into the lesion sites of the five rabbits. Injections were performed on days 3,5,7 following the operations [16]. The 5 rabbits in normal saline treated control group received saline with theranekron dose on the above mentioned days.

In the untreated control group of rabbits, no injection was performed. Finally, in the autograft group, the harvested bone was put in place and the skin and muscle were sutured. All the rabbits were kept in the same condition. The rabbits were sedated with acepromazine 2mg/kg and anesthetized with ketamine (30 mg/kg IM, xylazine 5mg/kg IM) and diazepam (4mg/kg, IM). Their right forelimbs were shaved and prepared for the operation. After towelings, skin incision was made on the anterior-medial surface of the radius bone so that the bone was exposed and a piece of radius bone about 10 mm in size, (twice as long as the width), was accessed from medial site. The skin was then sutured with 2-0 nylon suture. The rabbits were left in the cage without external fixation till full recovery. To prevent infection after surgery, Enrofloxacin 5%, 5mg/kg was injected IM into the rabbits on daily basis for 3 days.

All animals received humane care in compliance with the Guide for Care and use of Laboratory Animals (NIH publication No. 85-23, revised 1985). The study was approved by the local Ethics Committee of our Veterinary School. They were killed by intra-cardiac injection of 1 mg/kg Gallamine triethiodide (Specia Co., Paris, France).

Clinical Evaluation

To evaluate their clinical status, rabbits were examined twice a day and the operation site was checked regularly as well as using their hands, weight gaining, topical wound healing, inflammation and infections.

Radiological Evaluation

Lateral hand x-ray was prepared post operation on days 14, 28, 42, and 56. It is worth mentioning that X-ray source and film were 70 cm apart and the X-ray machine was adjusted on 45kv and 20mAs. Lane and Sandhu modified scoring system was used for the evaluation and grading of the obtained radiographs [13].

Histopathological Evaluation

On day 56, all the rabbits were euthanized using anesthetic induction with a high level of magnesium sulphate cardiac injection. Then, the radius bone in all the rabbits was removed and the surrounding soft tissues were separated. Bones with defect were placed in Formalin 10% (Merk, Germany) and sent to the lab for histopathological examination. Samples were stored in the formalin 10% for one day, then formalin was replaced and samples again stored in it till fixation. To demineralize the bones, they were stored in nitric acid (%10) for 4 days, followed by washing with water and analyzed by an autotechnicon. To prepare microscopic slides, they were sent to histopathology lab to be examined by light microscopy. Emery histopathological scoring system was used for analysis [3].

Statistical Analysis

To analyze the obtained data, SPSS , and Kruskal-Wallis non parametric ANOVA were used. In case of $P < 0.05$, Mann-Whitney was used to reanalyze the data. Level of significance was set at $P < 0.05$. Statistical

analyses were performed by SPSS software, version 16.0 (SPSS, Inc, Chicago, USA).

Results

Observations revealed no surgical wound infection. Radiographic evaluation of bone regeneration in rabbits was performed on days 14, 28, 42, and 56 in the four groups of rabbits, i.e., autograft, Theranekron, untreated controls, and normal saline treated controls. Grading was performed for bone formation, upper and lower union rate and bone re-modeling (Table 1).

In the second week post operation, autograft group was significantly superior to both untreated and saline treated controls ($p=0.02$, $p=0.04$, respectively). Also, Theranekron group proved better than untreated controls ($p=0.04$), (table1, Fig. 1)

In the 4th week, Theranekron group was significantly superior to both untreated and saline treated controls ($p=0.03$, $p=0.02$, respectively) (table 1, Fig. 1)

Similar to the 4th week, in the 6th week, Theranekron proved better ($p=0.006$, $p=0.008$, respectively) while autograft group was superior to saline treated controls ($p=0.01$) (Table 1, fig. 1)

In the 8th week, autograft group was superior to saline, untreated controls and Theranekron group ($p=0.009$, $p=0.006$, $p=0.006$, respectively)

As revealed, no more between group significant differences was observed, other than the above mentioned ones.

Fifty six days after surgical operations, the radial bone was removed and the separated samples were transferred to histopathology lab for evaluation and grading based on Emery [3]. No sign of inflammation or infection was revealed in histopathological examination.

In addition, terabecular bone and bone marrow formations were evident in Theranekron group. In the untreated and saline treated controls, most part of defect was filled with fibrous tissue and in autograft group, formation of both thick terabecular bone and bone marrow was seen clearly.

Similar to radiological results, histopathological results were also statistically analyzed using kruskal-wallis non parametric ANOVA followed by Mann Whitney U test. As shown, a significant difference was only between untreated controls and two groups of Theranekron and autograft. Accordingly, untreated controls was superior to autograft group ($p=0.04$), but inferior to Theranekron group (table 2, Fig 2).

Discussion

It is suggested that investigation of bone defect healing be conducted on rabbits rather than rats, because long bone in rabbits are very similar to those of humans; therefore, rabbits can serve as a good model for the studies addressing fracture healing [15].

In this study, bone autografting and theranekron injection (in defected site) were carried out using rabbit animal model. The selected bone for comparison of grafts in the rabbit was radius bone because the removal of a part of this bone does not require fixation and bone defect healing can be evaluated without interfere of fixation instruments. The reason is related to joining of radius and ulna in the rabbit model [18]. The defect was made on the mid radius bone with the harvested bone length being twice the width of bone diaphysis to produce the critical nonunion bone model [4]. Autograft group in the present study performed the best in terms of healing pace, compared to other groups and Theranekron group functioned similarly to autograft counterpart.

Autograft is considered the gold standard in bone regenerating procedures, because it contains repair stimulating substances and the cells within does not provoke immune responses nor cause the transmission of contagious diseases [9]. In small animals, autograft bone is harvested from the iliac crest, the inner surface of the upper end of the tibia as well as humerus, while in humans iliac crest bone is used. Complications such as pain, infection, fracture, blood loss and need for further operative procedures are associated with autograft bone harvesting while there is a limited amount of bone for that purpose [8].

Autograft transplant is considered as a positive control group in the research studies and in the current study it functioned properly. Theranekron group was almost the same as autograft group but better in comparison with normal saline treated and untreated control groups. The beneficial effect theranekron on bone regeneration could be explained by regulating role of theranekron in inflammatory conditioned and pot op necrosis as well as regulating fibroplasias stage of repairing process [7].

Oryan, Moshiri et al, 2012 was demonstrated positive effects of theranekron on tendon repairs, compared to the control group, including weight gaining, mobility, reduced tissue inflammation during the treatment [16]. Adib-Hashemi, Farahmand et al, 2015 reported significant reduced inflammatory cells in Theranekron treated group, compared to the controls, while a significant increase in collagen content was evident [1]. Antiinflammatory effect is a prominent feature of theranekron, as reported in the studies. In healing process, inflammation is an inevitable phenomenon for mediator formation which in turn triggers reconstruction process [11]. The anti-inflammatory effect of Theranekron could be attributed to its capability of inhibiting extra inflammation and consequently enhanced tissue repair [16]. Chronic inflammation may cause cell deterioration due to stabilized PMNs which function as supportive against microorganisms. Other functions include mononuclear cell absorption and phytogosis of fibrins and debris. It is worth mentioning that lysosomal enzymes released by PMNs are influential in debris elimination [19]. It is also suggested that Theranekron causes the release of peptides which characterize necrotized tissues [14]. Demarcative and necrotizing effects of Theranekron can help differentiate between necrotic and viable tissues at cellular level [16].

Conclusion

As radiographs and histopathology results indicated in the present study, the bone defect was filled with fibrous tissue in untreated and saline treated controls, which inhibits bone regeneration. Theranekron treated group showed almost similarly to autograft counterpart, but better than normal saline and untreated controls.

Declarations

Abbreviations Not applicable

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Authors' contributions

A Bigham-Sadegh, Siavash Sharifi and Yasamin Alavi carried out the operation. Ahmad Oryan conceived of the study design. Ahmad Oryan, A Bigham-Sadegh, Siavash Sharifi and Yasamin Alavi interpreted the data and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

All animals received humane care in compliance with the Guide for Care and use of Laboratory Animals (NIH publication No. 85-23, revised 1985). The study was approved by the local Ethics Committee of our Veterinary School. They were killed by intra-cardiac injection of 1 mg/kg Gallamine triethiodide (Specia Co., Paris, France).

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Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication Not applicable

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Tables

Table1: Radiographical findings for bone healing at various post-operative intervals

*P	(Med (min~max				Postoperative Weeks
	Untreated control group	Autograft group	Normal saline group	Thranekron group	
0.02	0(1-0)	5(5-1) ⁺	1(1-0)	1(2-0) [¥]	2
0.01	1(6-1)	5(9-5) [¶]	1(5-0)	5(9-2) [§]	4
0.004	1(6-1)	5(10-5) ^{**}	1(5-0)	9(9-8) ^{¥¶}	6
0.005	4(9-1)	10(10-9) ^{¶¶}	1(8-0)	8(8-1)	8

* Kruskal- Wallis non parametric ANOVA, when P value was less than 0.05 complementary MannWhitney U test was done and P value less than 0.05 was considered statistically significant

⁺ a significant difference between autograft group and the two groups of untreated and saline treated controls (P=0.02, p=0.04, respectively)

[¥] a significant difference between Theranekron group and untreated controls (p=0.04)

[¶] a significant difference between autograft group and saline treated group (p=0.01)

- a significant difference between Theranekron group and two groups of untreated and saline treated controls (p=0.02, p=0.03, respectively)

^{**} a significant difference between autograft and saline treated groups (p=0.01)

¶¶a significant difference between Theranekron group and two groups of untreated and saline treated controls (p= 0.006, p=0.008, respectively)

¶¶autograft group significantly outperformed untreated, saline treated controls and Theranekron groups (p=0.009, p=0.006, p=0.006, respectively)

Table2: Histopathological findings (sum of histopathological criteria) for bone healing at various groups

*P	(Med (min~max				
	Untreated group	Autograft group	Normal saline group	Thranekron group	
0.05	1 (3-0)¶	6(6-2)	1 (7-1)	5(6-2)	Sum of histopathological criteria

* Kruskal- Wallis non parametric ANOVA, when P value was less than 0.05 complementary MannWhitney U test was done and P value less than 0.05 was considered statistically significant

¶ untreated group was significantly inferior to autograft and Theranekron groups (p=0.004)

Figures

Figure 1

Radiographs through second week to eighth week: (a)Theranekron, (b) autograft, (c) normal saline treated controls, (d) untreated controls

Figure 2

Histopathological signs in Theranekron group, terabecule bone formation, in normal saline treated and untreated groups, fibrous tissue formation and in autograft group, bone marrow regeneration, thick trabecular formation were observed. H & E (a: 4x, b: 10x)

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