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ORIGINAL STUDY

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Oral L-arginine supplementation for fracture healing: a systematic review of preclinical studies

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Introduction: Approximately 5 to 10% of all patients with fractures experience deficient fracture healing that results in fracture nonunions. Previous studies have shown that nitric oxide production from arginine could improve fracture healing by improving local blood supply, supplementing growth factors, and improving collagen synthesis. Apart from its simple oral mode of administration, this amino acid provides a non-toxic and inexpensive option for fracture healing. To date, no systematic reviews regarding oral L-arginine supplementation for fracture healing are available. We present the first systematic review of oral L-arginine supplementation for fracture healing.
Methods: A systematic literature search was carried out using PubMed, Google Scholar, and ScienceDirect until February 1, 2021 using a combination of text words. No date limits were set. Studies investigating the use of oral L-arginine supplementation for fracture healing were included. Reference lists of relevant publications were assessed for additional references. In addition, bibliographies from other reviews were searched.
Results: Four studies were included. Of these, 3 were animal studies, and the other one was an in vitro study. Animals that were given oral L-arginine supplementation had significantly increased angiogenesis, reduced defect area, higher osteoblasts and osteoclasts, and higher rate of bone formation compared to controls.
Conclusions: The available preclinical studies suggest that oral L-arginine supplementation is a potential new therapy for fracture healing. This amino acid supplement is not only affordable and non-toxic; it is also simple. Further clinical studies are required to investigate the optimal dose of oral L-arginine supplementation for fracture healing in human subjects.

Keywords : arginine; supplementation; fracture healing; non-union; delayed union.

INTRODUCTION

The number of fractures will continue to increase in the future due to rapid ageing of the population and the associated incidence of osteoporosis *(1)*. It is estimated that the risk of a person for in the general population on sustaining a fracture is 1 in 100 persons annually *(2)*, with risks of suffering an osteoporotic fracture varied from 13 to 50% *(3,4)*. Approximately 5 to 10% of all patients with fractures developed fracture nonunions, which will continue to increase

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in the future due to the increasing incidences of fractures. Such condition often cause pain, swelling, stiffness, and inability to bear weight, all of which result in quality of life impairment. Moreover, it requires multiple surgeries, which may lead to high socioeconomic cost.

Fracture healing is a complex process. Numerous factors are known to affect such process, and even when reduction and/or fixation is stable, many fractures still fail to unite. Poor fracture healing can be overcome by the supplementation of certain factors which can promote fracture healing. Arginine, a non-essential amino acid, has been reported to stimulate fracture healing in experimental animal models *(5-9)*. Apart from its simple oral mode of administration, this amino acid provides a non-toxic and inexpensive option for enhancing fracture healing *(8)*. Thus, such supplementation would have considerable benefits, particularly for patients who sustain multiple fractures *(9)*. To date, no systematic reviews regarding oral L-arginine supplementation for fracture healing are available. We present the first systematic review of oral L-arginine supplementation for fracture healing.

METHODS

This systematic review is conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidance.

Eligibility criteria

This systematic review included studies involving animals (rats, guinea pigs and rabbits) with self- made fracture regardless of location and given the oral L-arginine diet. The intervention was oral L-arginine with any route, dose or frequency. The outcomes included surface defect area histologically. All study designs including in vitro, animal study, randomised blinded clinical trial, or prospective blinded animal study were included in the study. Case reports and case series were excluded.

Information sources

We conducted a systematic literature search at PubMed, ScienceDirect, and Google Scholar with no language and time restrictions. In addition, a manual search of all the bibliographies of the retrieved articles and relative review were conducted to further identify potentially eligible trials. The first search was performed on January 31, 2019. The second and the last searches were conducted on September 30, 2019 and February 1, 2022, respectively.

Search strategy

A combination of both free words and MeSH terms were used: ‘arginine’, ‘fracture healing’, ‘delayed union’ and ‘non-union’. Boolean operators including AND, OR, and NOT were used (Table I).

Evidence quality appraisal

Quality of the included studies was appraised by using Oxford Centre for evidence-based medicine levels of evidence. Clinical evidence was grossly divided to five levels, ranging from I to V. Level Ia represents the highest quality evidence and V is the lowest.

RESULTS

Literature search

A total 9,480 publication were initially retrieved. Of these, 9,464 were excluded during abstract screening, and 4 articles were finally included. Of the 4 articles, 3 were animal studies and 1 was in vitro study. All the studies were published from 2001 to 2016. All of the studies demonstrated that arginine improves fracture healing in animal models (Table II).

Table I. – Search strategy

Database	Search Strategy
PubMed	(arginine) AND (fracture healing OR delayed union OR non-union)
ScienceDirect	(arginine) AND (fracture healing OR delayed union OR non-union)
Google Scholar	(arginine) AND (fracture healing OR delayed union OR non-union)

Table II. – Characteristics of the included studies

Authors	Year	Study Design	Subjects	Mean Weight	Control Group	Test Group	Defects	Treatment	Follow-up (weeks)	Fracture healing by X-ray	Fracture healing by histological exam
Torricelli et al[10]	2001	In vitro	12 (rats)	360 ± 30 g	12 femora in BGJb medium	12 femora in BGJb medium	Defects on distal femoral condyle with a stainless-steel drill.	Test group : L-arginine (Arg) and L-lysine (Lys) 0.625mg/ml/day and 0.587mg/ml/day. Control group: no amino acid treatment	3	Not reported	After 21 days: the surface of the defect area in the Arg-Lys group was smaller than in the control group, with a significant difference in the healing rate (26.3% in Arg-Lys vs 7.9 % in controls).
Kdolsky et al[9]	2005	Prospective blinded animal study	44 (Guinea-pig model)	>300 g	14	high dose 2 weeks: 15 and high dose 4 weeks : 15	7-mm diaphyseal and periosteal defect was produced in the right femur and splinted intramedullary with a 1.4-mm K-wire	Test group: L-arginine 100mg/kg + aquabidest for 2 and 4 weeks. Control group : NaCl 0.9%	4	2 weeks: 10/15; 4 weeks: 11/15) than in the control group (3/14)	Histology and histomorphometry showed significantly increased coverage of nonvascularized bone fragments with newly formed bone in the treatment groups
Sinha et al[11]	2009	Randomised blinded control trial	40 (rabbits)	approx. 1.5 kg	14	26	Transverse osteotomy of the midshaft of ulna	lysine at the rate of 47 mg/kg body weight (body wt) and arginine at the rate of 50 mg/kg body wt	Control group : each 2 animals at 1, 2, 3, 4, 8, 12, and 20 weeks. Test group: each 2 animals at 1, 2, and 3 weeks and five each at 4, 8, 12, and 20 weeks.	At 3 weeks, 20 rabbits in the test group and 4 in the control group showed partial healing which corresponds to XRG = 1	Vascularity, Haversian system formation, and cortical and medullary repair between the two groups, with HPG being better in the test group.
Yaman et al[12]	2016	Animal study	42 rats	230–280 g	21	21	Critical-size defect on calvarial bones	Test group : diet containing 1.81 g/kg of ASI for 12 weeks; after the first 8 weeks, a calvarial critical-sized defect was created, and the rats were sacrificed 7, 14, and 28 days later. Control group: normal dietary	4	Not reported	1. Osteoblasts and osteoclasts were detected at higher levels in the ASI group compared with the control group at days 7, 14, and 28 of the calvarial defect (P,0.05) 2. New bone formation was detected at higher levels in the ASI group compared with the controls at day 28 (P<0.05).

Fracture Healing

In the present review, 89 of 150 subjects had histologically improved bone tissue. The follow-up ranged from 3 to 4 weeks. No side effects occurred during the follow-up period.

Adverse events

All the included studies reported no adverse effect.

DISCUSSION

In the healing process of 5-10% of all fractures, difficulties occur, resulting in non-union. Nutrition has a major influence on fracture healing, with observed fracture-healing impairment in the malnourished and undernourished population. Non-essential amino acids including arginine possess beneficial anabolic properties which are essential during fracture healing *(1)*.

Arginine is metabolised by nitric oxide syntase (NOS) to form nitric oxide (NO) and citrulline. There are three kinds of NOS: inducible NOS(iNOS), endothelial NOS(eNOS), and neuronal NOS(bNOS). iNOS is a calcium independent enzyme and both eNOS and bNOS are calcium-dependent enzymes *(14)*. NO influences vascular activity and stimulates bone cells to regulate bone remodelling. The conversion of arginine into citrulline and NO is a prerequisite for a normal inflammatory phase of fracture healing which is important for normal fracture healing. But in conditions of stress, such as wound healing and inflammation, arginine availability is limited which may lead to insufficient callus formation. This is caused by the increased activity of arginase, which e arginine into ornitine and urea resulting arginine concentration in plasma decreased. Furthermore, there is decreased endogenous conversion of citrulline into arginine in the kidney *(15)*. Thus, arginine bioavailability may become a rate-limiting factor for NO production.

L-argine also plays an active role in numerous physiological processes. It may lead to increased collagen accumulation and wound-breaking strength, anti-aging and antioxidant activities, and immunity. It also enhanced biological crosslinking of natural polymers, and stimulate mesenchymal stem cell growth and differentiation *(16-18)*. Type II collagen plays an essential role in enchondral healing and soft callus formation, leading to fracture healing. This shows that L-arginine may be a potential supplement for enhancing fracture healing *(13)*.

Disturbances in the conversion of arginine into NO and citrulline have already been associated with an impaired fracture healing resulting in non-union in humans. This finding also reported by Wijnands et al that showed arginine, citrulline, and ornitine concentration in callus were significantly lower in atrophic non-union patients than in healed fracture patients. In hypertrophic nonunions, arginine was significantly higher and ornithine was lower than in healed fractures. Plasma arginine concentrations were significantly lower in patients with hypertrophic nonunions (62 lmol/L; $P < 0.001$) and acute-fracture patients (41 lmol/L; $P < 0.001$) but not in atrophic-nonunion patients. Plasma ornithine concentrations were lower in all groups than in acute-fracture patients *(19)*.

Diwan et al stated that NOS is presence in all phases of human fracture healing and iNOS is the first enzyme that occurs in these phases *(20)*. We conclude that NOSs have been found to be involved in fracture healing. It is in line with findings from Meesters et al *(21)* and Baldik et al *(22)*. who found that the deficiency of iNOS and eNOS results in diminished bone formation and delayed union and non-union development. They suggested that the arginine-NO metabolism may play a role in the prevention of delayed unions and nonunions.

Furthermore, Wang et al *(23)* found that NO also modulates the vascular endothelial growth factor (VEGF) gene transcription in human endothelial cells. VEGF is an important regulator for angiogenesis and endochondral bone formation by inducing endothelial cell migration, proliferation, and capillary permeability.Click or tap here to enter text. This finding also supported by Tomlinson et al *(24)*, who found that in their animal study, NOS inhibitor was given to suppress NO generation resulting in significant decreases in early blood flow rate and bone formation.

All these findings showed that NO, derived from arginine, plays many roles in fracture healing

and prevents delayed unions and non-union. In experimental animal studies of fracture healing, the improved availability of arginine and associated NO were shown to have beneficial effects on the fracture healing process *(25)*.

Hughes et al *(26)* observed an enhanced fracture and soft tissue healing, in rats where a closed femoral midshaft fracture was induced with subsequent intramedullary nailing and afterwards received anabolic dietary supplementation, consisting of proteins and the conditionally essential amino acids glutamine, arginine, and taurine. Groups with high concentrations of glutamine and arginine (among others) showed increased muscle mass and bone mineral density in the fracture callus after a healing period of six weeks when compared with animals that were fed a diet with low concentrations of proteins.

Torricelli et al *(10)* conducted an in vitro study involving cultured femora of 24 rats in BGJb medium (12 as test group 12 as controls). Defects were created on femoral condyle with a stainless-steel drill. The test group was given arginine and lysine 0.625 mg/day and 0.587 mg/day, respectively. The controls had no amino acid treatment. After 21 days, the surface of the defect area in the test group was smaller than in the control group, with a significant difference in the healing rate (26.3% vs 7.9%). They also found higher ALP and NO levels in test group at 21 days.

Kdolsky *(9)* treated 44 guinea pigs in three groups. two treatment groups received high doses of L-arginine (one group for 2 weeks and the other for 4 weeks). A control group received vehicle only. Radiographs showed significantly more healings in the treatment groups (2 weeks: 10/15; 4 weeks: 11/15) than in the control group (3/14). Histology and histomorphometry showed significantly increased coverage of nonvascularized bone fragments with newly formed bone in the treatment groups ($p < 0.05$). Sinha et al8 performed an experimental study on 40 rabbits were subjected to ulnar osteotomy. The test group ($n = 26$) was fed with a diet rich in lysine and arginine. 14 served as controls. There was better healing of osteotomy in terms of better vascularisation, callus formation, and mineralisation in the test group. The time of healing in the test group was reduced by 2 weeks.

Yaman et al *(27)* performed an experimental study on 42 rats. Two groups of 21 rats each; after first 8 weeks, calvarial critical-sized defects were created. The test group received 1.81 g/kg arginine silicate inositol complex (ASI) or 12 weeks, and the controls were fed a standard diet. They found that osteoblasts and osteoclasts were detected at higher levels in the ASI group at days 7, 14, and 28 ($P<0.05$). New bone formation was detected at higher levels in the ASI group at day 28 ($P<0.05$)

Sinha et al *(8)* found that the better fracture healing in test rabbits at 3 weeks postoperatively were mainly because of increased vascularity and better angiogenesis which had occurred due to increased NO synthesis from arginine supplementation. NO is expressed during fracture healing, and suppression of NOS impairs fracture healing

In this study, we found that all included studies demonstrated that oral L-arginine supplementation improves fracture healing, which could be attributed to NO production. This supplementation influences bone formation by enhancing local insulin-like growth factor-1 production, NO, and angiogenesis. NO, derived from arginine, is involved in tissue injuries and repair, inflammatory and immunological tissue injury, DNA synthesis, cell growth, and collagen production *(8)*.

This review demonstrates that oral L-arginine may be beneficial for enhancing fracture healing. However, human studies are needed to investigate further as there are no such available studies. Therefore, the optimal dose of oral L-arginine supplementation for treating non-union in humans is still unknown.

We conclude that TJA can play an important role in the treatment of hypersensitivity, but strong evidence is present regarding the role of sensitized pain in chronic pain after TJA. Therefore, further research on the use of the earlier mentioned centrally acting agents is justified.

CONCLUSION

The available preclinical studies suggest that oral L-arginine supplementation is a potential

new therapy for fracture healing.This amino acid supplement is not only affordable and nontoxic; it is also simple.Further clinical studies are required to investigate the optimal dose of oral L-arginine supplementation for fracture healing in human subjects.

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