PHYTOTHERAPY RESEARCH
Phytother. Res. **25**: 49–52 (2011)
Published online 8 July 2010 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.3199

Bromelain in the Early Phase of Healing in Acute Crush Achilles Tendon Injury

A. I. Aiyegbusi,[1]* F. I. O. Duru,[1] C. C. Anunobi,[2] C. C. Noronha[1] and A. O. Okanlawon[1]

[1]Department of Anatomy College of Medicine, University of Lagos, Nigeria
[2]Department of Morbid Anatomy, College of Medicine, University of Lagos, Nigeria

Bromelain, an enzyme extracted from the stem of the pineapple plant has been proposed as a treatment for reducing pain and swelling following acute muscle injuries but studies are yet to be done on its effect on tendon healing. This study therefore investigated the effects of bromelain on tenocyte proliferation and the tendon malondialdehyde (MDA) level in the early stage of healing in a crush injury to the Achilles tendon of Sprague-Dawley rats.

Twenty four male rats were divided randomly into three groups; groups 2 and 3 had induced crush injury to the left Achilles tendon. Group 1; nil injury and nil treatment, Group 2; nil treatment, Group 3; oral bromelain treatment. Bromelain was given at a dosage of 7 mg/kg body weight daily over the first 14 days post-injury. On day 15 post injury, the animals were killed and the tendons excised and processed for histological study and MDA assay.

The results showed a significant increase in the tenocyte population in the bromelain group; $p < 0.05$. There was, however, no significant difference in the MDA level.

Conclusion: Based on this study, 600 GDU bromelain given once daily in acute tendon injury at a dosage of 7 mg/kg promoted healing by stimulating tenocyte proliferation.

Keywords: bromelain; tenocyte proliferation; tendon injury; crush injury; MDA.

INTRODUCTION

The management of acute tendon injuries has always posed a serious challenge to clinicians because of its poor vascularity and slow rate of healing. Tendons respond to injury and micro-trauma by exhibiting an inflammatory response; a condition known as tendinitis. A failed acute inflammation in the early stage of healing results in tendon degeneration known as tendinosis (Leadbetter,1992); a vicious cycle of failed healing and re-injury which is characterized by tenocyte death, tendon degeneration, chronic pain, disability and deformity (Kulig *et al.*, 2009). Tendon cells are specialized fibroblasts known as tenoblasts and tenocytes which constitute about 90–95% of cellular elements in the tendon (Sharma and Maffulli, 2005). These specialized cells synthesize collagen molecules in response to injury. Tenoblasts are round, immature proliferating cells regarded as the activated form of tenocytes during intrinsic tendon healing (Davidson *et al.*, 1997; Chuen *et al.*, 2004). As healing progresses, tenoblasts mature and are transformed into the elongated tenocytes (Kannus *et al.*, 2000).

Tendinosis also partly occurs secondary to apoptosis induced by oxidative stress which results from an increase production of reactive oxygen species (ROS) by inflammatory cells and proliferating tenocytes in response to cytokines and growth factors (Banes *et al.*, 1995; Yuan *et al.*, 2002; Kehrer and Smith, 2007). ROS have been reported to be beneficial in healing in low physiological levels by stimulating fibroblast proliferation (Murrell *et al.*, 1990). In large doses, however, ROS are toxic resulting in lipid peroxidation and apoptosis (Murrell *et al.*, 1997). Lipid peroxidation can be evaluated by the thiobarbituric acid reactive substance method (TBARS) which evaluates the oxidative stress assayed for malondialdehyde, the last product of lipid breakdown caused by oxidative stress (Omodeo-Sale *et al.*, 1998).

Studies have shown that highly concentrated proteolytic enzymes can be used to treat tendinitis due to their antiinflammatory properties (Lopez *et al.*, 1994). Bromelain, a general name for a family of sulfhydryl proteolytic enzymes obtained from *Ananas comosus*, the pineapple plant, has been reported to be helpful in healing minor injuries, especially strains and sprains, muscle injuries, tendonitis and bruising by reducing swelling, healing time and pain (Tassman *et al.*, 1965). Bromelain's action is in part a result of inhibiting the generation of bradykinin at the inflammatory site via depletion of the plasma kallikrein system, as well as limiting the formation of fibrin by reduction of clotting cascade intermediates. These actions result in significant reduction in pain and oedema, as well as enhanced circulation to the injured site (Kelly, 1996). Bromelain has been shown to exert beneficial effects, some of which are antiinflammatory in acute injuries and prevent platelet aggregation at doses as low as 160 mg/day (Blonstein, 1960). The general consensus among researchers, however, is that the best results occur when bromelain is given at doses above 500 mg per day and

* Correspondence to: A. I. Aiyegbusi, Department of Anatomy, College of Medicine, University of Lagos, Lagos, Nigeria.
E-mail: bogphysio@yahoo.com

Received 06 October 2009
Revised 19 March 2010
Accepted 22 March 2010

that result improves in a dose-dependent manner with higher levels of bromelain supplementation (Kumakura *et al.*, 1988). Bromelain is considered to have very low toxicity, with an LD_{50} greater than 10 g/kg (Taussig and Batkin, 1988) and can be absorbed in the gut without losing its biological properties (Uhlig and Seifert, 1981).

Rather than blocking the arachidonic acid cascade at the enzyme cyclooxygenase, like NSAIDs, bromelain may selectively decrease thromboxane generation and change the ratio of thromboxane/prostacyclin (PGI2) in favor of prostacyclin (Taussig and Batkin, 1988) and unlike NSAIDs which have deleterious effects with long term use, it appears to be safe at high doses and when taken long term (Miller *et al.*, 2004).

As suggested by Marsolais *et al.* (2003), it is pertinent to investigate other treatment modalities that may stimulate the tenocytes to proliferate and increase the biosynthesis of extracellular matrix and collagen which are crucial stages for the return of normal tendon strength (Davidson *et al.*, 1997; Dahlgren *et al.*, 2002). From the foregoing, bromelain could be an alternative to consider in the treatment of acute tendon injuries if found to induce the proliferation of tenocytes in addition to its reported effect on the reduction of pain and swelling. This study is designed to determine the effect of a daily administration of bromelain on tenocyte proliferation and the oxidative stress level in a crush injury model of the rat's Achilles tendon.

MATERIALS AND METHODS

Materials. Twenty four 8-week old male Sprague-Dawley albino rats weighing 120–160 g were used for the study. The animals were kept in the animal room of the Department of Anatomy, College of Medicine, University of Lagos where the study was carried out. They were kept under standard conditions of 12-h light and 12-h darkness photoperiodicity. The rats were fed on commercial rat chow and water *ad libitum.*

Bromelain. Bromelain tablets 500 mg, 600 GDU manufactured by ™ Holland and Barrett, USA were used for this study. The tablet was constituted into syrup form by adding 500 mL of water to 500 mg of the crushed tablet, with 1 mL of the syrup containing 1 mg bromelain.

Experimental protocol. The animals were randomly assigned into three groups of eight rats per group. All the rats in groups 2 and 3 had a crush injury to the left Achilles tendon. The animals were treated as follows:

Group 1 (normal control): The rats did not undergo a crush injury and had no treatment
Group 2 (experimental control): No treatment was administered
Group 3: The rats had oral bromelain once daily.

The rats in each group were further randomly subdivided into groups A and B with four rats in each group.

Subgroup A – Tendons processed for histology.
Subgroup B – Tendons assayed for MDA evaluation.

Injury procedure. The pre-injury circumference of the left hind limb for each rat was taken 2 cm above the calcaneal insertion of the Achilles tendon by means of a flexible inextensible marked cord. The Achilles tendon of the left limbs was clamped with Number 1 artery forceps to the forceps maximum closure for 60 s to induce a crush injury (Paulo *et al.*, 2006). Twenty-four hours post-injury, the post-injury circumference of the hind limbs was taken again at the same marked point to ascertain if there was oedema as a sign of inflammation.

Bromelain administration. Oral bromelain administration commenced 24 h post-injury by gavage at a dosage of 7 mg/kg of body weight using a calibrated oro-pharyngeal metal cannula. The animals were given between 0.8–1.1 mL of syrup depending on the body weight. Treatment was given once a day for the next 14 days. Bromelain was given for six consecutive days with a day interval in between. Only the rats in group 3 were treated with bromelain.

Animal killing and tendon harvest process. On day 15 post-injury, the animals were killed and the Achilles tendons excised. The tendons in subgroup A were processed for histology using the technique of stereology described by Young and Dyson (1990) while those in subgroup B were assayed for MDA evaluation.

Stereological analysis. The slides were observed under the light microscope fitted with an ocular test grid at a magnification of ×100 using the method of Cruz-orive and Weibel (1990). The tenocyte profile identified was the nucleus. Forty random values (10 per animal) were obtained for each group. The numerical density (NA) is the number of tenocyte profiles per unit area of field (Gundersen, 1986). This is estimated as the profile number of tenocytes (N) within the frame of the test grid (A). N was determined by counting all the tenocyte profiles partially or totally within the frame area that did not intersect the forbidden lines, which are the top and left margins of the test grid (Cruz-orive and Weibel, 1990). The tenoblasts were further differentiated from the typical tenocytes by their shapes. The elongated cells were identified as tenocytes while the ovoid cells were identified as tenoblasts (Chuen *et al.*, 2004).

Determination of tendon malondialdehyde. Tendon malondialdehyde (MDA) levels were determined using the modified thiobarbituric acid (TBA) method of Buege and Aust (1978). The excised tendon was homogenized in 2 mL of normal saline and the homogenate centrifuged at 1000 × **g** for 10 min in a Uniscope laboratory centrifuge and the supernatant assayed for MDA evaluation. The concentration of malondialdehyde was calculated using the molar absorptivity coefficient of malondialdehyde which is 1.56×10^5 M^{-1} cm^{-1}

Statistical analysis. The data obtained from the stereological evaluation of the tenocyte population and the MDA values were subjected to statistical analysis and expressed as mean ± SD. Differences between groups were compared using analysis of variance (ANOVA). In order to eliminate the possibility of the non-significant values masking the effect of the significant values as a result of the large sample sizes, paired samples were further analysed using a Students' *t*-test. The null hypothesis was set at a significant level of 0.05 or 5%.

Table 1. Comparison of tenocyte population among the different groups

	Group	
Mean ± SD of tenocyte count/unit area	N/control 24.3 ± 16.5 Expt. control 22.0 ± 12.9 Bromelain 27.7 ± 7.8	Expt control 22.0 ± 12.9 Bromelain 27.7 ± 7.8
p	0.21	0.03[a]

[a] Significant at $p < 0.05$.
Group 1, normal control; nil injury, nil treatment.
Group 2, experimental control; injury, nil treatment.
Group 3, bromelain.

Table 2. Comparison of MDA level among the different groups

	Group	
Mean ± SD of MDA level µmol/mg protein	N/control 0.84 ± 0.17 Expt. control 0.99 ± 0.14 Bromelain 0.98 ± 0.10	Expt control 0.99 ± 0.14 Bromelain 0.98 ± 0.10
p	0.33	0.97

Group 1, normal control; nil injury, nil treatment.
Group 2, experimental control; injury, nil treatment.
Group 3, bromelain.

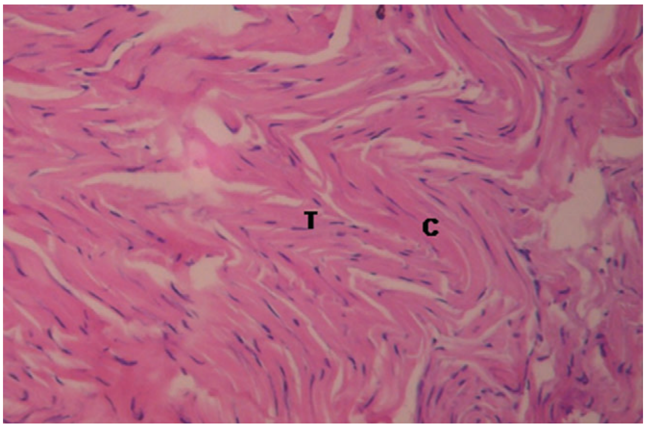

Figure 1. L/S of tendon of rat treated with 7 mg/kg, 600 GDU oral bromelain daily showing coarse, dense, well laid out collagen fibers (C) and mature tenocytes (T). H & E × 100.

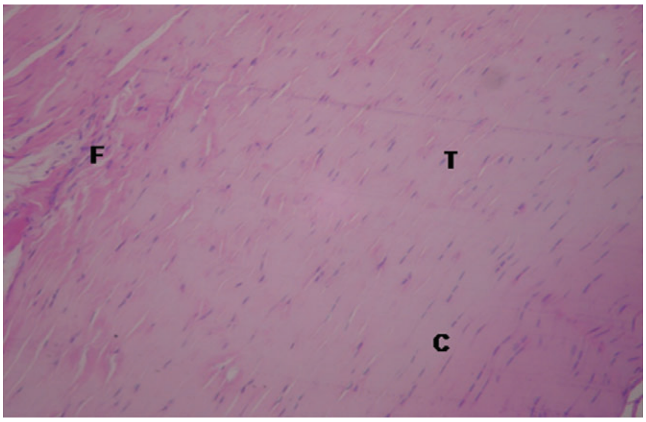

Figure 2. L/S of tendon of rat in the experimental control group showing proliferating, plump immature tenocytes (T), infiltration of mononuclear inflammatory cells (F) and relative absence of well formed collagen fibers (C). (H & E) × 100.

RESULTS

The injury protocol adopted in this study evidently elicited a crush injury as seen in the observable hyperaemia over the skin and soft tissue of the crush-injured tendons 24 h post-injury. There was also a mean circumference increase of 0.2 ± 0.01 cm in the affected limbs indicating the presence of oedema.

The results of the tenocyte count showed tenocyte proliferation in the bromelain treated group compared with the control groups (Table 1). There was a statistically significant difference in the means of the experimental control and bromelain groups ($p = 0.03$). However, bromelain had no significant effect on the reduction of MDA levels compared with the injured untreated group (Table 2).

Histological sections of the bromelain treated tendon showed bundles of coarse, dense and regularly arranged collagen fibers among which were scattered tenocytes with elongated nuclei (Fig. 1). In the experimental control group, sections showed proliferating tenocytes with relatively large and rounded nuclei. There are focal areas of mononuclear inflammatory cell infiltrates and a relative absence of well formed collagen fibers (Fig. 2)

DISCUSSION

With the increase participation in both competitive and recreational sports in recent times, there has been an upsurge in the incidence of acute tendon injuries. The management of these injuries has remained a challenge to clinicians because of a poor healing response which predisposes the affected tendon to tendinosis, risk of re-injury and eventual rupture. Any treatment modality that will induce tenocyte proliferation will accelerate tendon healing and prevent tendinosis (Caring Medical, 2007).

Bromelain, reported to be effective in reducing pain and swelling following acute injuries is shown by this study to cause a significant proliferation of tenoblasts when administered once daily at a dosage of 7 mg/kg, 600 GDU though it just marginally reduced the MDA level. Compared with the injured untreated tendon, bromelain did not significantly lower the MDA level yet resulted in significant proliferation of tenoblasts. That bromelain was able to cause a significant proliferation of tenoblasts at about the same level of oxidative stress with the injured untreated tendon may suggest a role for ROS in bromelain's effect on the proliferation of tenoblasts. Alternatively, it may be attributed to the fact that bromelain increases the expression of PGI_2 and PAF through a modulation of the cytokine system. This in turn results in an increase proliferation of tenoblasts and collagen synthesis (Kelly, 1996). The increased population of tenocytes thus generates ROS in response to cytokines and growth factors in addition to the fact that the pro-proliferative action of growth factors may be mediated through H_2O_2 production (Banes *et al.*, 1995; Sundaresan *et al.*, 1995). Thus, the high level of MDA in the bromelain treated tendon may be secondary to its proliferative effects on the tenoblasts.

Further studies are needed to determine the effects of bromelain on the generation of different types of ROS particularly nitric oxide (NO) which has been reported to enhance tenocyte proliferation and tendon healing (Murrell *et al.*, 1990, 1997).

In addition, the profound positive effects of bromelain on the morphology of the healing tendon just 2 weeks post-injury as indicated by the presence of mature tenocytes and well-aligned collagen bands suggests that this extract of the pineapple plant may play a significant role in the healing of acute tendon injuries. This effect may also be due to the fact that proteolytic enzyme formulas have demonstrated the ability to remove toxic waste and excess free radicals in the tendinitis areas that inhibit recovery from injury (Lopez *et al.*, 1994). This study has thus shown that 7 mg/kg, 600 GDU bromelain administered once daily over the first 14 days following acute tendon injury augmented healing compared with the group that was allowed to heal naturally. From the foregoing, it is pertinent to study the effects of a higher dosage of this enzyme since its effect has been reported to be dose dependent and non-toxic even at doses as high as 10 g/kg (Taussig *et al.*, 1975). It is also important to undertake clinical trials on human subjects as well.

In conclusion, this study suggests that bromelain may be an option to consider in the management of acute tendon injuries.

Declaration

No part of this work has been submitted to any other journal for publication and there are no conflicting financial interest.

Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

Banes AJ, Tsuzaki M, Yamamoto J. 1995. Mechanoreception at the cellular level: the detection, interpretation, and diversity of responses to mechanical signals. *Biochem Cell Biol* **73**: 349–365.

Blonstein J. 1960. Control of swelling in boxing injuries. *Practitioner* **203**: 206.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. In *Methods in Enzymology,* Fleischer S, Packer L (eds). Academy Press: New York, L11; 302–330.

Caring Medical. 2007. NSAIDs: Why we do not recommend them. *Sports Injury Med* **97**: 472–476.

Chuen FS, Chuk CY, Ping WY, Nar WW, Kim HL, Ming CK. 2004. Immunohistochemical characterization of cells in adult human patella tendons. *J Histochem Cytochem* **52**: 1151–1157.

Cruz-orive LM, Weibel ER. 1990. Recent stereological methods for cell biology brief survey. *Am J Physiol* **258**: L148–L156.

Dahlgren LA, van der Meulen MCH, Berram JEA, Starrak GS, Nixon AJO. 2002. Insulin- like growth factor-I improves cellular and molecular aspects of healing in a collagenase-induced model of flexor tendinitis. *J Orthop Res* **20**: 910–919.

Davidson CJ, Ganion LR, Gehlsen GM, Verhoestra B, Roepke JE, Sevier TL 1997. Rat tendon morphologic and functional changes resulting from soft tissue mobilization. *Med Sci Sports Exerc* **29**: 313–319.

Gundersen HJG 1986. Stereology of arbitrary particle: a review of unbiased number and size estimators and the presentation of new ones; in memory of William R Thompson. *J Microsc* **143**: 3–45.

Kannus P, Jozsa L, Jarvinnen M. 2000. Basic science of tendons. In *Principles and Practice of Orthopaedic Sports Medicine*, Garrett WE JR, Speer KP, Kirkendall DT, (eds). Lippincott Williams and Wilkins: Philadelphia, 21–37.

Kehrer J, Smith CV. 1994. Free radicals in biology: sources, reactivities, and roles in the etiology of human diseases. In *Natural Antioxidants in Health and Disease,* Frei B (ed.). Academic Press: San Diego, Ch 2, 25–62.

Kelly GS. 1996. Bromelain: a literature review and discussion of its therapeutic applications. *Altern Med Rev 1996* **1**(4): 243–257.

Kulig K, Reischl SF, Pomrantz AB *et al.* 2009. Nonsurgical management of posterior tibial tendon dysfunction with orthoses and resistive exercise: a randomized controlled trial. *Phys Ther* **89**: 26–37.

Kumakura S, Yamashita M, Tsurufuji S. 1988. Effect of bromelain on kaolin-induced inflammation in rats. *Eur J Pharmacol* **150**: 295–301.

Leadbetter WB. 1992. Cell-matrix response in tendon injury. *Clin Sports Med* **11**: 533–578.

Lopez DA, Williams RM, Miehlke M. 1994. *Enzymes: The Fountain of Life*. The Neville Press: Charleston.

Marsolais D, Cote CH, Frenette J. 2003. Nonsteroidal anti-inflammatory drug reduces neutrophil and macrophage accumulation but does not improve tendon regeneration. *Lab Invest* **83**: 991–999.

Miller PC, Bailey SP, Barnes ME, Janelle Derr S, Hall EE 2004. The effects of protease supplementation of skeletal muscle function and DOMS following downhill running. *J Sports Sci* **22**: 365–372.

Murrell GAC, Francis MJO, Bromley L. 1990. Modulation of fibroblast proliferation by oxygen free radicals. *Biochem J* 265: 659–665.

Murrell GA, Szabo C, Hannafin JA *et al.* 1997. Modulation of tendon healing by nitric oxide. *Inflamm Res* **46**: 19–27.

Omodeo-Salè F, Basilico N, Folini M, Olliaro P, Taramelli D. 1998. Macrophage populations of different origins have distinct susceptibilities to lipid peroxidation induced by β-haematin (malaria pigment). *FEBS Lett* 433: 215–218.

Paulo de Tarso C-C, Iandara SS, Filipe AR *et al.* 2006. Histological study of tendon healing in malnourished Wistar rats treated with ultrasound therapy. *Acta Cir Bras* **21** suppl. 4 São Paulo.

Sharma P, Maffulli N. 2005. Tendon injury and tendinopathy: healing and repair. *J Bone Joint Surg Am* **87**: 187–202.

Sundaresan M, Yu Z-X, Ferrans VJ. 1995. Requirement for generation of H_2O_2 for platelet derived growth factor signal transduction. *Science* **270**: 296–299.

Tassman G, Zafran J, Zayon G. 1965. A double-blind crossover study of a plant proteolytic enzyme in oral surgery. *J Dent Med* **20**: 51–54.

Taussig SJ, Batkin S. 1988. Bromelain, the enzyme complex of pineapple (*Ananas comosus*) and its clinical application. An update. *J Ethnopharmacol* **22**: 191–203.

Taussig SJ, Yokoyama MM, Chinen N. 1975. Bromelain: A proteolytic enzyme and its clinical application. *Hir J Med Sci* **24**: 185–193.

Uhlig G, Seifert J. 1981. The effect of proteolytic enzymes (traumanase) on post traumatic edema. *Fortschr Med* **99**: 554–556.

Young SR, Dyson M. 1990. The effect of therapeutic ultrasound on the healing of full thickness excised skin lesions. *Ultrasonics* **28**: 175–180.

Yuan J, Murrell GAC, Wei A, Wang M. 2002. Apoptosis in rotator cuff tendonopathy. *J Orthop Res* **20**: 1372–1379.

