Comp Clin Pathol (2006) 15: 98–102
DOI 10.1007/s00580-006-0616-4

ORIGINAL ARTICLE

K. Sardari · A. Mirshahi · M. Maleki ·
M. R. Aslani · M. Najar Barjasteh

Effects of topical allicin on second-intention wound healing in dogs (histological aspects)

Received: 18 January 2006 / Accepted: 2 April 2006 / Published online: 29 April 2006

Abstract Alllicin is one of the pharmacologically active garlic sulfur compounds that have antimicrobial (antibacterial, antiviral, antifungal and antiparasitic) and vasodilating effects. Five normal, male, mixed-breed dogs were selected to investigate the effects of allicin (5 mg/ml in methyl cellulose gel) as a topical treatment for full-thickness, excisional wounds. The dogs were approximately 3 years old. The histological aspects of second-intention wound healing were studied. Eight full-thickness skin wounds (20×20 mm) were created on the back of each dog. On days 0, 7, 14 and 21, each dog received two wounds, symmetrically, and were assigned to one of two groups: control (methyl cellulose gel) or test (allicin 5 mg/ml methyl cellulose gel). Wounds were treated once daily for a week. Left-side wounds were treated with allicfin (test group) and right-side wounds were treated with methylcellulose gel (control group). At day 28 (4 weeks) after initial wounding, biopsies were taken from wounds for histological examination. The density of inflammatory cells in the center of the day 7 wounds was significantly lower in test group (P=0.041), but the density of fibrocytes and fibroblasts in the center of day 7 wounds was significantly higher in the test group (P=0.042). No significant differences were observed in the amount of collagen and fibrin between the test and control wounds (P>0.05).

K. Sardari (✉) · A. Mirshahi ·
M. R. Aslani
Department of Clinical Sciences,
Faculty of Veterinary Medicine,
Ferdowsi University of Mashhad,
P.O. Box 91775-1793, Mashhad, Iran
e-mail: sardari@ferdowsi.um.ac.ir
Fax: +98-511-6620166

M. Maleki
Department of Pathobiology, Faculty of Veterinary Medicine,
Ferdowsi University of Mashhad,
P.O. Box 91775-1793, Mashhad, Iran

Present address:
M. N. Barjasteh
Private Veterinarian,
Mashhad, Iran

Keywords Allicin · Healing · Dog

Introduction

Second-intention wound healing is often necessary for management of wounds in veterinary practice. Second-intention wound healing is the healing of an open wound by contraction and epithelialisation. Wound contraction is a major component of second-intention wound healing, and the pivotal feature for contraction is granulation tissue (Swaim et al. 2001). Second-intention wound healing is generally used to treat wounds that are large, have extensive tissue damage and are contaminated or infected. In many cases, this practical and economical method can be used to attain wound closure as long as adequate wound care is provided (Swaim et al. 2001). Treatment methods that allow wounds to heal faster and minimise cosmetic defects and the production of exuberant granulation tissue are desired. Many topically applied agents have been used to treat open wounds but most products investigated in domestic animals either do not affect wound healing or inhibit rather than enhance it (Lee et al. 1986, 1988; Swaim and Lee 1987; Swaim and Henderson 1990).

The use of topical medications in healing wounds is a controversial subject (Harari 1993). The effect of anti-inflammatory agents is most pronounced when they are used during the inflammatory stage of healing. The inflammatory process is necessary for the production of mediators that initiate the repair stage. Controlling these effects may be beneficial in wounds with an exaggerated inflammatory response (Harari 1993). Garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids and minerals such as selenium (Newall et al. 1996). The pharmacologic activity of garlic involves a variety of organosulfur compounds. The most notable of these is allicin, which is responsible for the characteristic garlic odor (Katzung 2001). Allicin has demonstrable effects on several risk factors for atherosclerotic disease—hyperlipidemia, hypertension and platelet aggregation (antilipemic, antihypertensive, anti-atherosclerotic and antiplatelet).

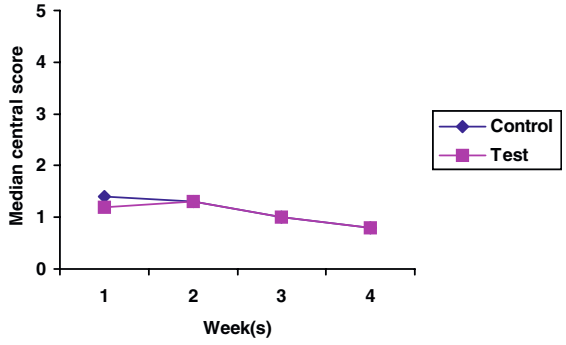

Fig. 1 Density of inflammatory cells in the center of the wounds

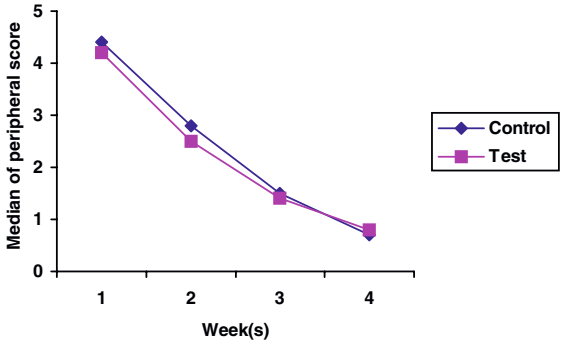

Fig. 3 Density of inflammatory cells in the periphery of the wounds

Garlic is nicknamed Russian penicillin for its widespread use as a topical and systemic antimicrobial agent (Adetumbi and Lau 1983; Agarwal 1996; Farbman et al. 1993).

Allicin has antimicrobial effects in vitro against many viruses, bacteria, fungi and parasites (Katzung 2001; Elnima et al. 1983). Allicin also exerts an inhibitory immunomodulatory effect on intestinal epithelial cells, which suggests that allicin may have the potential to attenuate intestinal inflammation (Lang et al. 2004).

To our knowledge, the use of topically applied allicin has not been evaluated in animals. The present study was undertaken to test the healing effect of allicin as a topical medication in full-thickness wound in dogs.

Materials and methods

Five male, mixed breed dogs (30±5 kg) were used in the study. They were approximately 3 years old. The dogs were housed in kennels, fed a maintenance ration twice daily and had free access to water. They were given rabies vaccine and antiparasitic drugs (praziquantel 5 mg/kg and piperazine 100 mg/kg, p.o.). All dogs were also sprayed with Neguvon 2 weeks before wounding. For investigation of animal's health, clinical examination, complete blood count and blood serum biochemical analysis (blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, cholesterol and glucose) were carried out. Skin preparation protocol was hair clipping and povidone iodine scrubbing. Under general anesthesia (using acepromazine 0.05 mg/kg, i.m., and ketamine 20 mg/kg, i.m.), eight full-thickness skin wounds (20×20 mm) were created on the back of each dog during the study period. On days 0, 7, 14 and 21, each dog received two wounds, symmetrically, and was assigned to one of two groups: control (methyl cellulose gel) or test (allicin 5 mg/ml methyl cellulose gel). Bleeding from the wound bed was significantly reduced by direct swab pressure. The wounds were numbered 1 to 4 so that wound 1 was 4 weeks old and wound 4 was one week old. The wounds were covered with a sterile bandage and the back was bandaged with an absorbent layer of cotton wool and elastic tape.

Left-side wounds (test group) and right-side wounds (control group) were treated topically with allicin 0.5% (Nopex Company, UK) in methyl cellulose gel (Kruse Company, Denmark) and methyl cellulose gel, respectively. Treatment was started 24 h after wounding. The bandages were changed once daily.

On day 28 (4 weeks) after initial wounding, under general anesthesia, biopsies were taken for histological examination. A 5-mm-diameter biopsy was taken from the center and peripheral site of each wound using a scalpel. The biopsies were fixed in neutral-buffered 10% formalin, embedded in paraffin wax and cut into 5-μm sections. These were stained with haematoxylin and eosin (H&E) for evaluation of inflammatory cells and fibroblasts–fibrocytes, phosphotungstic acid hematoxylin for evaluation of fibrin fibers and with Masson's trichrome and Van Gieson

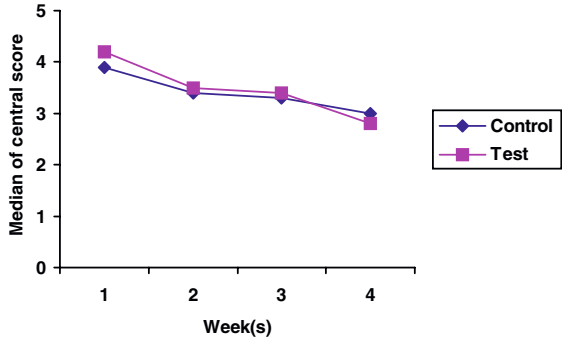

Fig. 2 Density of fibrocytes and fibroblasts in the center of the wounds

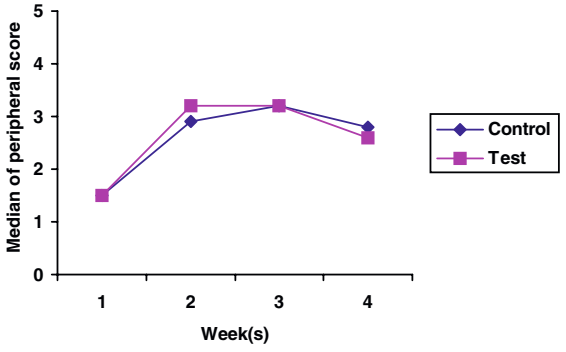

Fig. 4 Density of fibrocytes and fibroblasts in the periphery of the wounds

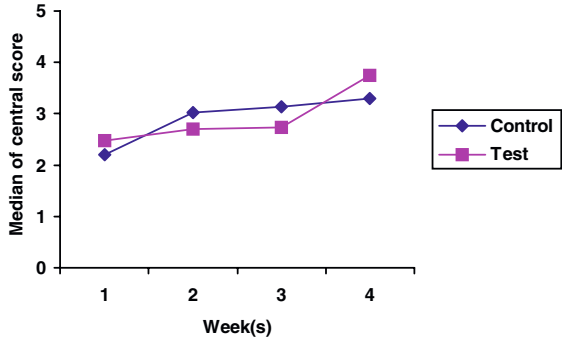

Fig. 5 Amount of collagen in the center of the wounds

for evaluation of collagen fibers. Each section was divided into ten horizontal fields of the peripheral aspect of the wound and ten horizontal fields of the central aspect of the wound (the fields were of equal depth). Using this method, fiber and cell evaluation was carried out. Inflammatory cells and fibroblasts–fibrocytes were evaluated at a magnification of ×400. The density of inflammatory cells and fibroblasts–fibrocytes was scored from 0 (no cell) to 5 (the highest density). Collagen and fibrin were evaluated at a magnification of ×200. Collagen was scored from 0 (no collagen visible) to 5 (only collagen visible). Fibrin was determined semi-quantitatively on a scale from 0 (no fibrin) to 5 (deep crypts filled with fibrin). The pathologist who analysed the biopsies and excised wound specimens was unaware of the treatment group.

Statistical analysis was performed using the SPSS 9 program for windows (SPSS, Chicago, IL, USA). The means of the groups were compared using Wilcoxon signed rank tests. Differences were considered statistically significant when $P<0.05$.

Results

In the first week after wounding, the density of inflammatory cells was increased. However, the density of inflammatory cells (Figs. 1, 7 and 8) in the center of day 7 wounds was relatively significantly lower in the test group ($P=0.041$). The density of fibrocytes and fibroblasts (Figs. 2, 9 and 10) in the center of day 7 wounds was also significantly higher in the test group ($P=0.042$). The

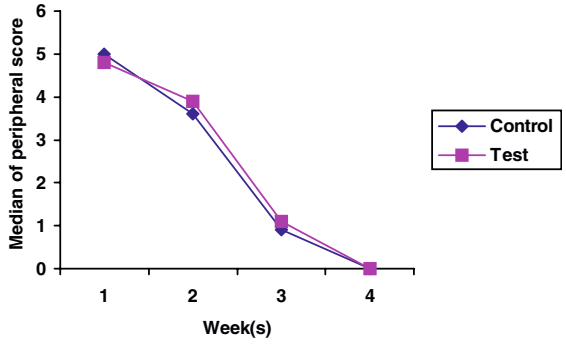

Fig. 6 Amount of fibrin in the periphery of the wounds

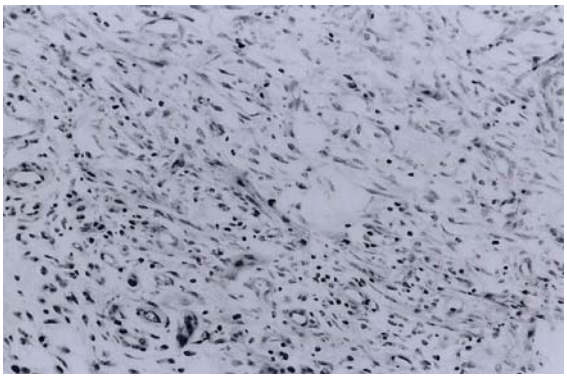

Fig. 7 Density of inflammatory cells in the center of first-week wounds (control group). H&E, ×200

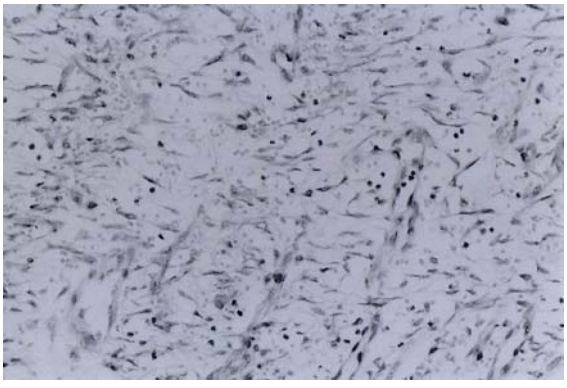

Fig. 8 Density of inflammatory cells in the center of first-week wounds (test group). H&E, ×200

difference in density of inflammatory cells (Fig. 3) in the periphery of day 7 wounds was not significant between control and test groups ($P>0.05$). The difference in density of fibrocytes and fibroblasts (Fig. 4) in the periphery of day 7 wounds was not significant between control and test groups ($P>0.05$). No significant differences were observed in the amount of collagen (Fig. 5) and fibrin (Fig. 6) between the test and control wounds ($P>0.05$).

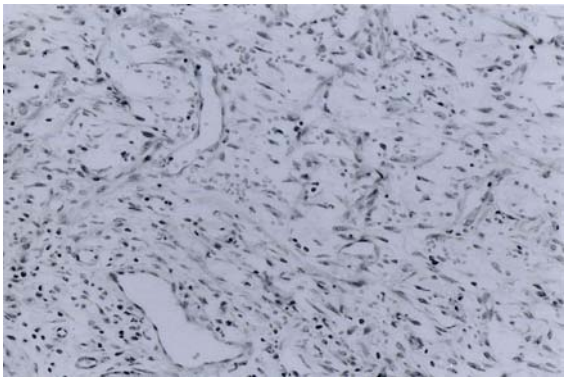

Fig. 9 Density of fibrocytes and fibroblasts in the center of first-week wounds (control group). H&E, ×200

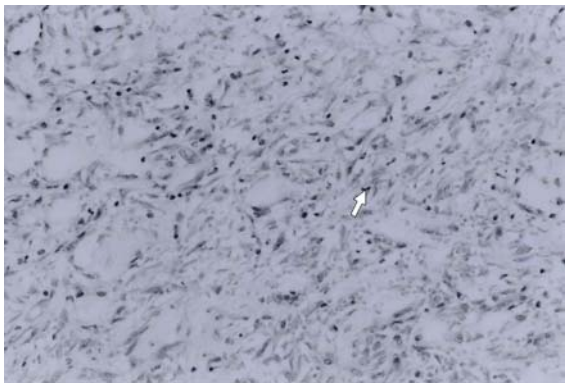

Fig. 10 Density of fibrocytes and fibroblasts in the center of first-week wounds (test group). Division of the fibroblast nucleus (*arrow*). H&E, ×200

Discussion

Many topically applied agents have been used to treat open wounds but allicin has not yet been fully assessed. The excellent ability of allicin to permeate through phospholipid membranes may contribute to its biological activity (Miron et al. 2000). Some studies have indicated that allicin exerts an inhibitory immunomodulatory effect on intestinal epithelial cells and have suggested that allicin may have the potential to attenuate intestinal inflammation (Lang et al. 2004). In the study reported here, the densities of inflammatory cells in the center of day 7 wounds were significantly lower in the test group (P=0.041; Figs. 7 and 8). This effect seems to be related to the anti-inflammatory effect of allicin. Although the initial inflammatory response is essential for adequate wound healing, chronic inflammation with persistent involvement of polymorphonuclear leucocytes (PMNs) may lead to cell destruction and an altered composition of the extracellular matrix, with subsequent failure of epithelialisation (Cotran et al. 1994; Knottenbelt 1997). The specific role of PMNs has been studied in vivo (Page and Good 1958; Simpson and Ross 1972). Their primary role is the defence against microorganisms, but PMNs also attract mononuclear cells and account for some phagocytosis of fibrin and debris. Lysosomal enzymes released by PMNs contribute to debris breakdown (Stashak 1991).

Inflammation is essential in the early phases of wound healing. However, the persistence of inflammation leads to inhibition of healing in the later phases (Wilmink et al. 1999). In the study reported here, the densities of fibrocytes and fibroblasts in the center of day 7 wounds were significantly higher in the test group (P=0.042) but no significant increase was observed in the amount of collagen (P>0.05; Figs. 9 and 10).

The inhibitory effects of adenosine and 16 quantitatively determined organosulfur compounds derived from garlic cloves or commercial garlic preparations on collagen-stimulated in vitro platelet aggregation in whole blood have previously been determined (Lawson et al. 1992). Fibroblasts in wounds originate from undifferentiated mesenchymal cells in adjacent connective tissue, primarily from the adventitia of small blood vessels. Under the influence of cytokines released by platelets and macrophages, they usually appear about the third or fourth day after injury and advance into a wound by using the fibrin of the clot as scaffold along which to grow. The fibroblasts deposit new collagen after they first secrete fibronectin and proteoglycans, which constitute an amorphous milieu of ground substance in the wound. The early type III collagen in wounds is quickly replaced by more mature type I collagen. As inter- and intramolecular bonding of collagen molecules continue, wound strength increases, and as the collagen content of the wound increases, the ground substance and fibroblasts content decrease (Swaim et al. 2001). In the study reported here, allicin attracted fibroblasts but no significant increase was observed in the amount of collagen. It is possible that allicin exhibits an inhibitory effect on collagen synthesis by fibroblasts.

References

Adetumbi MA, Lau BH (1983) Allium sativum (garlic)—a natural antibiotic. Med Hypotheses 12:227–237

Agarwal KC (1996) Therapeutic actions of garlic constituents. Med Res Rev 16:111–124

Cotran SC, Kumar V, Robbins SL (1994) Cellular growth and differentiation: normal regulation and adaptations. Inflammation and repair. In: Schoen FJ (ed) Robin's pathologic basis of disease, 5th edn. Saunders, Philadelphia, pp 35–92

Elnima EI, Ahmed SA, Mekkawi AG, Mossa JS (1983) The antimicrobial activity of garlic and onion extracts. Pharmazie 38:747–748

Farbman KS, Barnett ED, Bolduc GR, Klein JO (1993) Antibacterial activity of garlic and onions: a historical perspective. Pediatr Infect Dis J 12:613–614

Harari J (1993) Surgical complications and wound healing in the small animal practice. Saunders, pp 78–79

Katzung BG (2001) Basic and clinical pharmacology, 8th edn. McGraw-Hill, pp 1092–1093

Knottenbelt DC (1997) Equine wound management: are there significant differences in healing at different sites on the body? Vet Dermatol 8:273–290

Lang A, Lahav M, Sakhnini E, Barshack I, Fidder HH, Avida, B, Bardan E, Hershkoviz R, Barmeir S, Chowers Y (2004) Allicin inhibits spontaneous and TNF-α induced secretion of proinflammatory cytokines and chemokines from intestinal epithelial cells. Clin Nutr 23(5):1199–1208

Lawson LD, Ransom DK, Hughes BG (1992) Inhibition of whole blood platelet-aggregation by compounds in garlic clove extracts and commercial garlic products. Thromb Res 65: 141–156

Lee AH, Swaim SF, Yang ST (1986) The effects of petrolatum, polyethylene glycol, nitrofurazone, and a hydroactive dressing on open wound healing. J Am Anim Hosp Assoc 22:443–451

Lee AH, Swaim SF, McGuire JA (1988) Effects of chlorhexidine diacetate,povidone iodine, and polyhydroxydine on wound healing in dogs. J Am Anim Hosp Assoc 24:77–84

Miron T, Rabinkov A, Wilchek M, Mirelman D, Weiner L (2000) The mode of action of allicin: its ready permeability throughphospholipid membranes may contribute to its biological activity. Biochim Biophys Acta 1463:20–30

Newall CA, Anderson LA, Phillipson JD (1996) Herbal medicines: a guide for health-care professionals, vol. 9. Pharmaceutical, London, pp 296

Page AR, Good RA (1958) A clinical and experimental study of the function of neutrophils in the inflammatory response. Am J Pathol 34:645–669

Simpson DM, Ross R (1972) The neutrophilic leucocyte in wound repair. A study with antineutrophil serum. J Clin Invest 51:2009–2023

Stashak TS (1991) Equine wound management. Lea and Febiger, Pennsylvania, pp 1–18

Swaim SF, Lee AH (1987) Topical wound medications: a review. J Am Vet Med Assoc 190:1588–1593

Swaim SF, Henderson RA (1990) Wound dressing materials and topical medication. In: Swaim SF, Henderson RA (eds) Small animal wound management. Lea & Febiger, Philadelphia, pp 34–52

Swaim SF, Hinkle SH, Bradley DM (2001) Wound contraction: basic and clinical factors. Compendium 23:20–24

Wilmink JM, Stolk PWT, van Weeren PR, Barneveld A (1999) Differences in second-intention wound healing between horses and ponies: macroscopical aspects. Equine Vet J 31:53–60