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Mechanism of Anti-Inflammatory and Anti-Thermal Burn Action of Aloe Arborescens Mill. Var. Natalensis Berger

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ABSTRACT

Carboxypeptidase (CPase) was partially purified from Kidachi aloe (Aloe Arborescens Mill. var natalensis Berger) by FPLC system, and was administered intravenously to female ICR mice with inflammation. The enzyme preparation revealed significant effects on alleviation of pain and inhibition of vascular permeability in abdominal region. It also revealed an anti-thermal burn action on rat's hind paws, when it was administered to female Wister rat intravenously.

Key Words: aloe, anti-inflammatory action, anti-thermal burn action, carboxypetidase, bradykinin, vascular permeability.

INTRODUCTION

Aloe species have been used as folk medicine since ancient times (Morton, 1961; Crosswhite and Crosswhite, 1984). They have still been used for the treatment of radiation injuries (Collins and Collins, 1935), skin diseases (Zawahry et al., 1973), and gastro-intestinal disorders (Blitz et al., 1963). Anti-inflammatory action of aloe has been described regarding different types of

inflammation (Davis et al., 1986, 1987, 1988, and 1989), and therapeutic use of aloe in thermal burn was also reported (Ashley et al., 1957; Cera et al., 1980 and 1982, Bigas et al., 1987). Today, more than 100 species of aloes are now cultured in Japan. One of them, Aloe Arborescens Mill. var natalensis Berger is called Kidachi aloe in Japanese, and it is the most popular one in Japan.

Bradykininase activity was found in high molecular (Mr) components of Kidachi aloe (Fujita et al., 1976), and the enzyme activity was estimated by biological assay one the guinea pig ileum. This enzyme was characterized as serine carboxypeptidase which also hydrolyzed angiotensin I, a vassopressor in vitro (Fujita et al., 1979; Yagi et al., 1986). Anti-inflammatory action of rat serum carboxypeptidase N was reported (Rybak et al., 1978), and bradykininase activity was also found in other species of aloe (Yagi et al., 1982). We focused on the anti-inflammatory activity of aloe CPase as one of the key factors involved in pharmaceutical effectiveness of aloe. This paper reports the anti-inflammatory action of aloe CPase and discuss of the possible mechanism.

MATERIALS AND METHODS

Fresh aloe leaves (4-5 years old) were harvested at the herb garden of Pharmacognosy, Fujita Health University, Hisai, Mie, Japan. Leaf skin was separated from pulp, and was homogenized by a food mixer MX-V350 (National electric Co. Osaka, Japan) with the same volume of 0.05 M acetate buffer (pH 5.0). Leaf skin homogenates were filtrated by Whatman GF/A glass microfiber filters (Whatman International Ltd., Maidstone, U.K.). Filtrates were mixed with 2 fold of cold acetone (-20℃), and low Mr. phenolic substances were removed from high Mr protein fractions. Precipitates which contained CPase were then lyophilized (Kyowa, lyophilizer RLE-206, Tokyo, Japan). Lyophilized powder of Kidachi aloe was dissolved into 0.05 M acetate buffer (pH 5.0), and size fractionated by a Sephadex G-25 column (Ø 11.5 x 50cm, Pharmacia LKB Biotechnology, Uppsala, Sweden) with 0.05 M acetate buffer (pH 5.0 as an eluant). High Mr fractions from Sephadex G-25 were applied to a DEAE Sephadex A-50 ion exchange column (Ø 11.5 x 10cm, Pharmacia LKB Biotechnology, Uppsala, Sweden), and CPase was eluded by 0.05 M acetate buffer with 0.25 M Nacl. CPase active fractions from DEAE Sephadex A-50 were precipitated by 80% saturation of $(NH_4)_2SO_4$, and stayed at 4℃ overnight. Precipitates were obtained by centrifugation (Ultra centrifuge 18PR-3, Hitachi Ltd., Tokyo, Japan), and dissolved into 0.05 M acetate buffer (pH 5.0). CPase fraction was then applied to a sephacryl S-200 column(Ø 2.5 X 65 cm, Pharmacia LKB Biotechnology, Uppsala, Sweden), and size fractionated again with 0.05 M acetate buffer (pH 5.0). Fractions of CPase were then separated by a Mono Q HR 5/5 ion exchange column on FPLC system (Pharmacia LKB Biotechnology, uppsala, Sweden) as a single peak, and dialyzed against 0.005 M acetate buffer (pH 5.0) overnight before administration to animals.

Enzyme activity was measured, incubating at 37℃ for 1 hour with bradykinin (Peptide Institute Inc. Osaka, Japan) as a substrate. One unit of enzyme cleaves 1μ mole of bradykinin in 1 minute.

Anti-inflammation experiment:

Anti-inflammation experiment was performed following the study of (Whittle, 1964; Brown, 1968, and Fujimura, 1968) 0.1ml/10g body weight of aloe CPase (containing about 0.05 units of enzyme) was administered to 7-8 week old female ICR mice (20-30g) intravenously. 0.1ml/10g body weight of phosphate buffered saline (PBS, pH 7.4) alone was administered to the control mice. Seventeen μg/10g body weight of Bromelain (Mochida Phamaceutical Co. Ltd., Tokyo, Japan, approximately 0.05 units) and 4.2μg /10g body weight of Indomethacin (Shioe Pharmaceutical Co. Ltd. Kyoto, Japan) were administered as positive controls. One hour after the dosage, xylol treatment was performed on ears of all mice, and 0.1ml/10g body weight of 0.5% brilliant blue (Nakarai tesque Inc., Kyoto, Japan) and 1% acetic acid were injected intravenously or intraperitoneally, respectively. Five minutes after acetic acid injection, writhing response of mice was observed for 15 minutes. Number of animal writhing was compared with that of controls, and statistically examined by Student's t-test. Twenty minutes after dye injection, ears were removed from mice, and the blue dye was extracted from ears with 30% pyridine, and measured calorimetrically. Dye concentration extracted from ears was compared with that of control, and statistically examined by Student's t-test. Thirty minutes after acetic acid injection, all mice were sacrificed by drawing the whole blood from vein, abdominal dropsy was collected, and the extracted blue dye was measured calorimetrically. Dye concentration extracted from abdomen was compared with that of control, and statistically examined by Student's t-test.

Anti-thermal burn experiment:

Anti-thermal burn experiment was performed as follows. 0.5ml of aloe CPase (approximately 0.25 units) was administered to female Wistar rats (130-150g) intravenously (Arrigori-Marteli et al., 1969). Control group was only administered the same volume of PBS. Initial paw volume was measured before thermal burn, dipping the paws in the glass bottle full of water, and the water volume overflown was estimated as paw volume. Thirty minutes before and after the dosage, thermal burn was made on the hind paws of rats by dipping the paws in hot water (54 ℃) for 20 seconds. Rates of edema and edema inhibition rates were measured at 2 and 4 hours after the thermal burn and compared with that of control. All values were statistically examined by Student's t-test.

RESULTS

Aloe CPase revealed no significant difference on xylol inflammation on ears (Fig. 1). Neither Bromelain nor Indomethacin revealed any significant difference. However, pain relief of aloe CPase was striking (Fig. 2). It revealed almost the same effect with Bromelain ($p<0.02$).

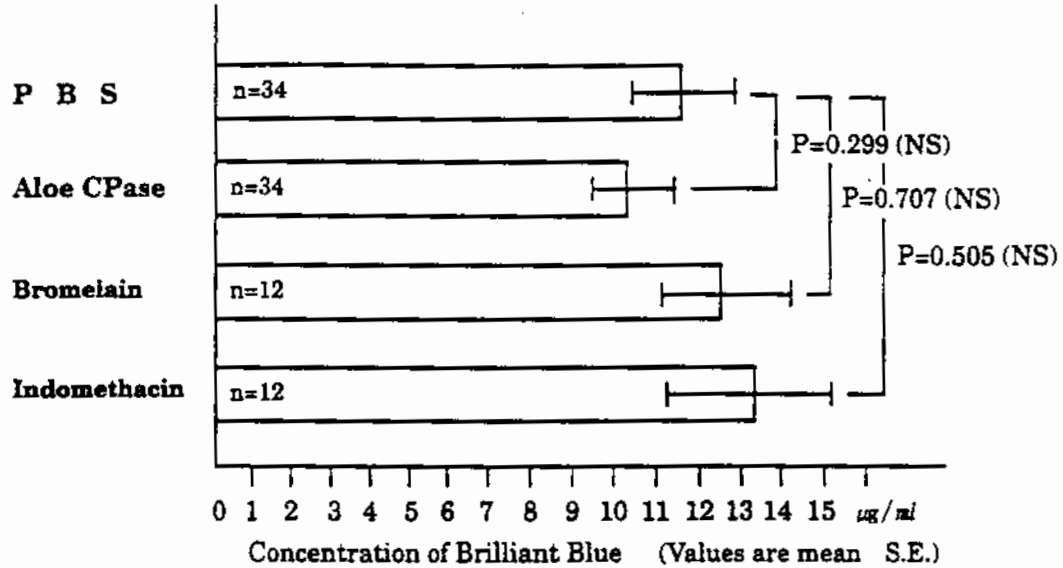

Fig 1. Dye concentration extracted from ears after xylol inflammation.

PBS: 0.1ml/10g b.w. Aloe CPase: 0.1ml/10g b.w. (approx. 0.05units)

Bromelain: 17μg/10g.b.w. (approx. 0.05units) Indomethacin: 4.4μg/10g b.w.

NS: not significant S.E.:standard errow

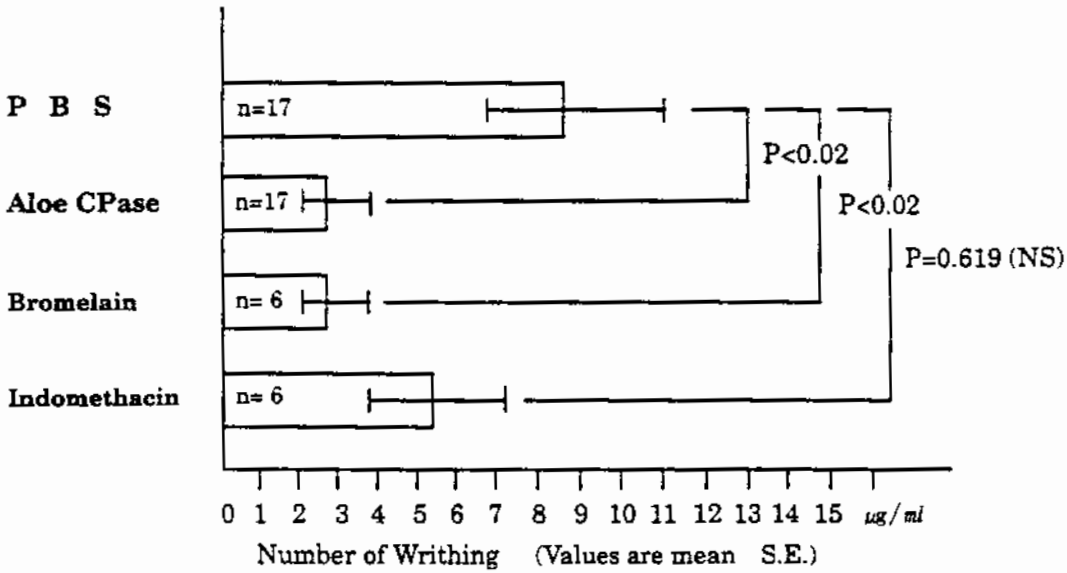

Fig 2. Writhing responce of mice after acetic acid information.

PBS: 0.1ml/10g b.w. Aloe CPase: 0.1ml/10g b.w.(approx. 0.05units)

Bromelain: 0.1ml/10g b.w.(approx 0.05units) Indomethacin: 4.2μg/10g b.w.

NS: not significant S.E.standard errow.

Aloe CPase also revealed significant difference on inhibitory effect against acceleration of vascular permeability in abdominal region (Fig. 3, $p<0.01$). Anti-thermal burn action of aloe CPase was significantly effective on both pre-and post-dosage (Fig. 4).

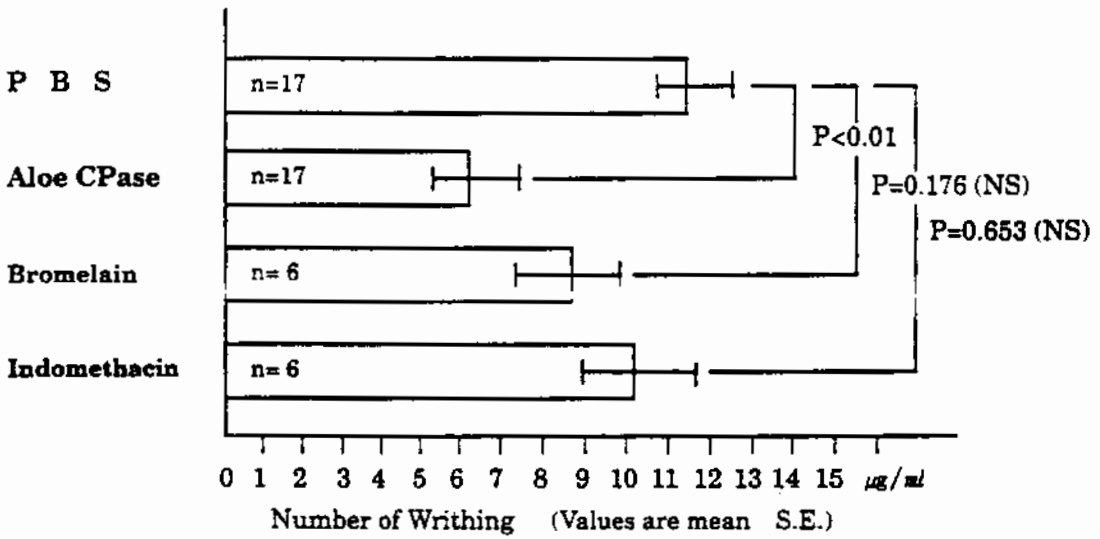

Fig 3. Dye concentration extracted from abdomen after acetic acid inflammation.

PBS: 0.1ml/10g b.w. Aloe CPase: 0.1ml/10g b.w.(approx. 0.05units)

Bromelain: 17μg/10g b.w.(approx. 0.05units) Indomethacin:4.2μg/10g b.w.

NS:not significant SE.standard errow

Two hours after the thermal burn, post-dosage group revealed no significant difference from the control group, while pre-dosage group revealed a significant difference from the control group in inhibition of edema rates ($p<0.01$). Four hours after the thermal burn, both pre and post-dosage groups revealed a significant anti-edema effect on rat's hind paws ($p<0.01$). However, it is quite apparent that pre-dosage of aloe CPase is more effective in inhibition of thermal edema than post-dosage.

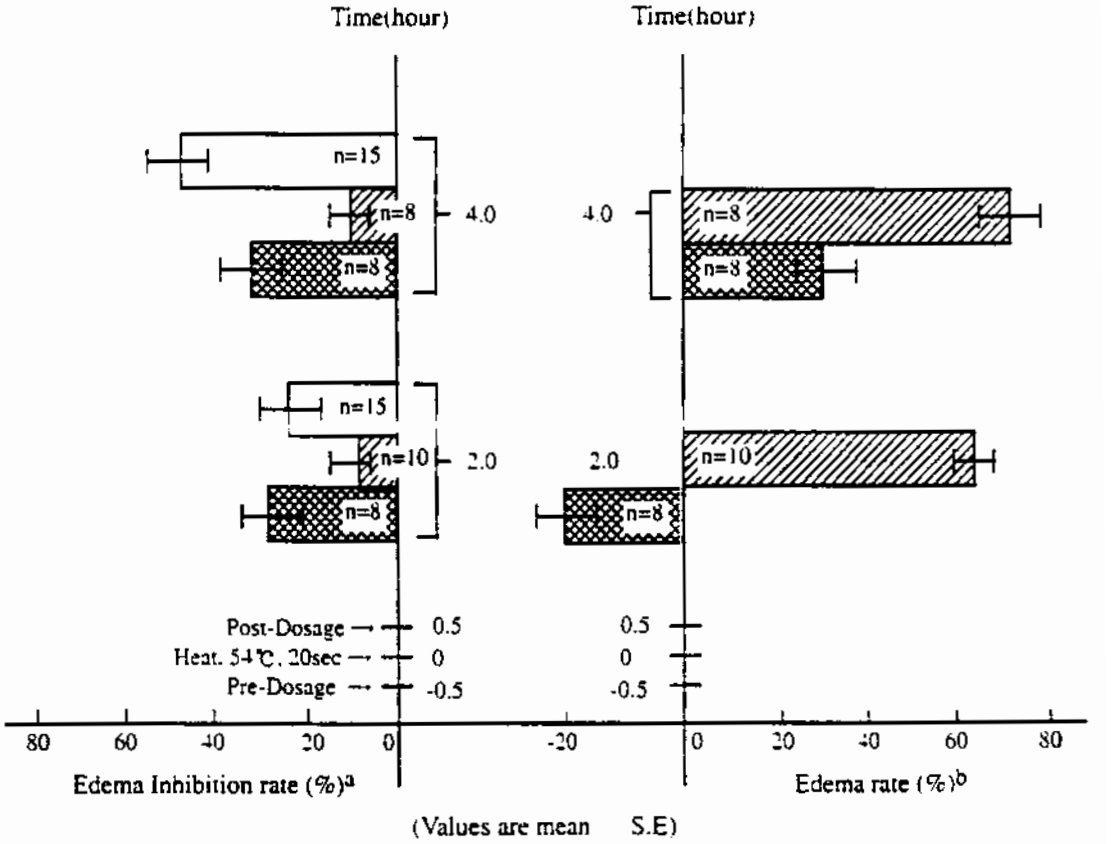

Fig 4. Anti-thermal edema effect of aloe CPase.

Control Pre-Dosage Post-Dosage

Control:PBS, 0.5ml/rat

Pre and Post-Dosage:aloe CPase 0.5ml/rat (approx. 0.25units)

a: Edema rate = $\frac{Vt-Vn}{Vn} \times 100\ (\%)$

Vn: Paw volume before thermal burn (ml)

Vt: Paw volume after thermal burn (ml)

Measurement of paw volume is described in the text.

b: Edema inhibition rate = $\frac{Ec-Et}{Ec} \times 100\ (\%)$

EC: Edema rate of control group (%)

Et: Edema rate of aloe group (%)

Negative value(-, minus) means swelling.

S.E.standard error $^{**}p<0.01$

DISCUSSION

When partially purified aloe CPase was administered to rats with thermal edema, and to mice with abdominal acetic acid inflammation, it revealed significant healing and preventive effects. The present results indicate that prophylactic use of aloe CPase is much more effective than post dosage trealinent. Inflammatory reaction is the process of self defence, and it is initiated by effusion, followed by migration of neutrophils. In an early stage of acute inflammation, chemical mediators, such as bradykinin and histamine etc. are produced in the topical region of inflammation. Bradykinin is one of the most potent pain-producing agents. So, the degradation of bradykinin will relieve pain from inflammation. The application of fresh aloe leaf to thermal burn has been empirically known to ameliorate the symptoms. High Mr components of Kidachi aloe revealed significant healing effects, when they were applied to the skins of rats and rabbits after the production of thermal burn on their backs (Fujita et al., 1980). Crude extracts of Kidachi aloe (Mr > 10,000) also inhibited the thermal edema on rat's paws, when they were administered orally (Beppu et al., 1980).

They also revealed anti-inflammatory effects in xylol and acetic acid inflammation. When partially purified aloe CPase was administered to ICR mice, it revealed significant alleviation of pain and inhibited the effusion of abdominal dropsy, which suggests that aloe CPase hydrolyzes bradykinin in vivo and relieved mice from pain. It also inhibited the acceleration of vascular permeability in abdominal region. This mechanism may be due to its vasopressor activity, such as hydrolysis of angiotensin I and production of angiotensin II.

Contrary to the data of crude extracts of Kidachi aloe, partially purified aloe CPase did not inhibit the acceleration of vascular permeability of xylol inflammation in ears, which means there exist some other anti-inflammatory agents in aloe. There are several such anti-inflammatory agents in aloe. Magnesium lactate in aloe gel inhibits histidine decarboxylase, and reduces the production of histamine (Hirata T, Suga T, 1977; Rubel, 1983). Thus, magnesium lactate may be effective against inflammation. Ascorbic acid in aloe is also anti-inflammatory, because of its enhancing activity of collagen synthesis (Pierce, 1983). Aloe also contains salicylic acid, which is both analgesic and anti-inflammatory due to the inhibition of prostaglandin synthesis (Where is the reference et al., 1982). Salicylic acid in aloe could be broken down from anthraquinone-type components, such as emodin and barbaloin. Lectin, Aloctin A were also separated from Kidachi aloe, which is active against edema and adjuvant arthritis in rats (Saito et al., 1982).

Aloe CPase should be a main anti-inflammatory agent, but some other anti-inflammatory agents could also contribute to the anti-inflammatory effects of crude aloe extracts.

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