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Is CAPE a Therapeutic Agent for Wound Healing?

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Abstract: Wound healing involves a number of processes, including an acute inflammatory response to production of Reactive Oxygen Species (ROS) and depression in antioxidant status. The aim of the study is to examine the therapeutic effects of Caffeic Acid Phenethyl Ester (CAPE), an antioxidant agent, on cutaneous wound healing in a rat model. There was a significant decrease in serum, wound and liver tissue TSA and serum LBSA levels in the CAPE group than in the control group. Also there was a significant decrease in serum and wound tissue TAC levels of the control group when compared to the CAPE group. But there was no difference between the groups in serum ADA activity, PBSA levels and liver tissue TAC levels. In conclusion, the present study showed the CAPE's efficacy in the treatment of cutaneous wounds by its antioxidant and ROS-scavenging capabilities.

Key words: Adenosine deaminase, CAPE, cutaneous wound, rat, sialic acids, total antioxidant capacity

INTRODUCTION

Wound healing involves a number of processes, including wounding, remodeling, collagenization and acquisition of wound strength (Gupta *et al*., 2002). Reactive Oxygene Species (ROS) are produced in response to cutaneous injury (Serarslan *et al*., 2007). ROS increase lipid peroxidation and thus, impair cell structure and functions.

Antioxidants are used to eliminate the negative effects of ROS on wound healing (Degim *et al*., 2002). Caffeic Acid Phenethyl Ester (CAPE) is an active component of honeybee propolis (Sud'ina *et al*., 1993). CAPE has anti-inflammatory (Orban *et al*., 2000), immunomodulatory (Natarajan *et al*., 1996) and antioxidant effects (Pascual *et al*., 1994) and it also suppresses lipid peroxidation (Sud'ina *et al*., 1993). There is limited study on CAPE's antioxidant effect in cutaneous wound healing. In this study, we aimed to examine the effect of CAPE on cutaneous wound healing by determining the changes in adenosine deaminase (ADA) activity, Sialic Acid (SA) and Total Antioxidant Capacity (TAC) levels.

MATERIALS AND METHODS

This study was performed with 40 male *Wistar albino* rats weighing 200-250 g. Rats were housed in individual cages and they were maintained in a 12 h light/12 h dark cycle at room temperature (25±3°C). Food and water were provided *ad libitum* to the animals. Animal protocols were approved by the ethics committee for care and use of laboratory animals at Mustafa Kemal University.

Rats were divided into 2 groups. The animals were anesthetized with xylazine hydrochloride (2-5 mg kg^{-1}) and ketamine hydrochloride (40-50 mg kg^{-1}). A linear full thickness incision 8 cm in length was made on the back of each rat. Twenty rats (group I: control) were received saline (Fig. 1) and the other 20 rats (group II: CAPE-treated) were received CAPE (10, from 25 μmol mL^{-1} solution, i.e.) once a day (Fig. 2).

On 1, 3, 7 and 14 days, wound tissue, liver tissue and blood samples were taken from the animals. The levels of Total Sialic Acid (TSA), Lipid Bond Sialic Acid (LBSA), Protein Bond Sialic Acid (PBSA), TAC and ADA activity were measured in serum of the rats in both groups. Additionally TSA and TAC levels were measured in the wound and liver tissues.

Sample preparation: Blood samples were stored for 1 h at room temperature, then were centrifugated at 2000 rpm for 10 min and then were kept at -20°C until analyzed. For tissue TSA and TAC analysis, a 10% homogenate was made in ice-cold Potassium Phosphate Buffer (PBS,

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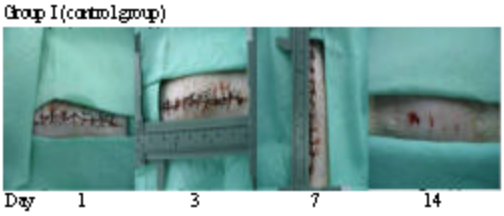

Fig. 1: Healing process in Group I (control group) on 1.3, 7 and 14 day in rats with cutaneous wound

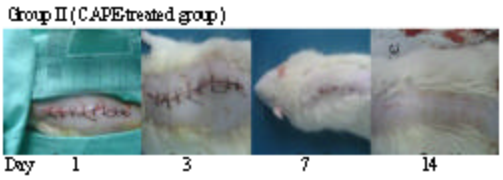

Fig. 2: Healing process in Group II (CAPE-treated group) on 1.3, 7 and 14 day in rats with cuteneous wound

pH 7.4) containing 5 mM EDTA, using a glass homogenizer. The homogenate was centrifugated at 15,000 rpm for 10 min at 4°C.

Sialic acids determination: TSA analyses for serum and tissue samples were carried out according to the method reported by Sydows *et al.* (1988). Briefly, 400 μL of serum were treated with 3 mL of 5% perchloric acid for 5 min at 100°C and centrifuged at 1400 g for 4 min. The supernatant (2 mL) was mixed with 400 μL of Echrlich reagent (5 g pdimethylaminobenzaldehyde/50 mL, Hcl/ 50 mL distilled water). After incubation at 100°C for 15 min, a spectrophotometer (Shimadzu, UV-1601) was used to read the optical density at 525 nm. LBSA concentrations were measured colorimetrically on a spectrophotometer (UV-1201, Shimadzu, Japan), as described by Katopodis and Stock (1980). PBSA values were calculated by subtracting of LBSA from TSA levels.

Adenosine deaminase activity determination: Serum adenosine deaminase (ADA; EC 3.5.4.4.) activity were determined with Giusti (1974) method. It is a colourimetric method based on the principle of measuring absorbance of the coloured indophenole complex at 628 nm.

Total antioxidant capacity determination: Serum Total Antioxidant Capacity (TAC) was determined by the method of Koracevic *et al.* (2001). The assay measured the capacity of the samples to inhibit the production of Thiobarbituric Acid Reactive Substances (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction. This reaction can be measured spectrophotometrically and the inhibition of colour development defined as the TAC. A solution of 1 mmol L^{-1} uric acid was used as standard.

Statistical analysis: Results were expressed as means±SE. Data were analyzed with SPSS software (SPSS Inc., Chicago, IL, USA). Independent samples t-tests were performed to examine the difference between the CAPE and controls on 1, 3, 7 and 14 days. Comparisions between days within group that present significant values were evaluated with one-way Analysis of Variance (ANOVA), $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Serum, wound and liver tissue TSA concentrations in Group I and II are given in Table 1. Serum TSA concentrations were significantly lower in Group II than in group I on 7 and 14 days ($p<0.05$). In the present study, there was a statistically significant decrease in wound and liver tissue TSA levels in group II beginning on 3 day when compared to group I (Table 1).

CAPE was shown to reduce serum LBSA concentrations in group II and it was statistically significant on 7 day ($p<0.01$) and 14 ($p<0.001$) when compared with group I (Table 2). Moreover, there was a decrease in PBSA concentrations in both groups on 14 day, but there was no significance between the groups (Table 2). There was no significant change in serum ADA activity between the 2 groups in the present study. But there was an increase in serum ADA activity within the both group on 3 day (Table 2).

Serum, wound and liver tissue TAC concentrations in wound healing in Group I and II are given in Table 3. In the present study, we have determined a significant decrease ($p<0.05$) in serum TAC levels of Group I beginning on 3 day when compared to Group II. And also wound tissue TAC levels of Group I decreased significantly ($p<0.01$) beginning on 7 day, while the wound tissue of Group II were increasing (Table 3). There was a decrease in liver tissue TAC levels beginning on 3 day in both groups, but there was no significance between the groups.

Caffeic Acid Phenetyl Ester (CAPE) is an active component of honeybee propolis (Sud'ina *et al*., 1993). Although, CAPE has antioxidant effects (Russo *et al*., 2002), its effect on cutaneous wound healing has been investigated limited to date. In this study, we aimed to examine the changes in SA, TAC and ADA levels in response to CAPE in wound healing.

SA concentration increases in various diseases of humans and animals (i.e., cattle, dogs and horses)

Table 1: Serum (mg dL^{-1}), wound tissue (mg g^{-1} wet tissue) and liver tissue (mg g^{-1} wet tissue) TSA concentrations in Group I (control) and Group II (CAPE-treated)

		Day			
TSA concentrations	Group	1	3	7	14
Serum TSA	I	101.27±2.63^{a}	107.10±1.69^{ab}	119.85±9.20^{b}	93.00±4.76^{a}
	II	103.88±2.38^{a}	108.73±2.23^{a}	113.16±5.45^{a*}	84.92±7.76^{b*}
Wound tissue TSA	I	0.95±0.12^{a}	1.32±0.04^{b}	1.17±0.09^{ab}	1.03±0.03^{a}
	II	1.09±0.06^{a}	1.03±0.05^{a*}	1.01±0.05^{a*}	0.89±0.04^{b*}
Liver tissue TSA	I	2.12±0.14^{a}	3.41±0.30^{b}	2.35±0.20^{a}	2.50±0.06^{a}
	II	1.87±0.20	2.46±0.11*	2.06±0.11	2.38±0.18

Table 2: Serum LBSA, PBSA concentrations and ADA activity in Group I (control) and Group II (CAPE-treated)

		Day			
	Group	1	3	7	14
LBSA (mg dL^{-1})	I	27.01±0.67^{b}	26.20±1.37^{b}	33.45±1.38^{a}	32.35±0.55^{a}
	II	25.90±1.84	25.01±1.41	26.05±1.30*	22.95±1.23*
PBSA (mg dL^{-1})	I	74.26±2.62^{ab}	80.90±1.97^{a}	86.40±10.04^{a}	60.65±4.65^{b}
	II	77.98±3.61^{ab}	83.72±2.50^{a}	87.11±5.70^{a}	61.98±8.02^{b}
ADA (IU L^{-1})	I	7.32±0.55^{b}	11.44±0.52^{a}	7.22±0.62^{b}	7.19±0.31^{b}
	II	7.46±0.56^{b}	11.85±0.69^{a}	7.13±0.51^{b}	7.12±0.15^{b}

Table 3: Serum (mg dL^{-1}), wound tissue (mg g^{-1} wet tissue) and liver tissue (mg g^{-1} wet tissue) TAC concentrations in Group I (control) and Group II (CAPE-treated)

		Day			
TAC concentrations	Group	1	3	7	14
Serum TAC	I	1.33±0.10^{a}	0.59±0.08^{b}	0.64±0.06^{b}	0.53±0.19^{b}
	II	1.24±0.24^{a}	1.19±0.02^{a*}	0.97±0.08^{b*}	0.83±0.17^{b*}
Wound tissue TAC	I	1.15±0.28^{a}	1.17±0.37^{a}	0.97±0.08^{b}	0.83±0.17^{b}
	II	1.19±0.03^{a}	1.31±0.21^{b*}	1.55±0.21^{b*}	1.40±0.09^{b*}
Liver tissue TAC	I	1.29±0.24^{a}	1.20±0.14^{b}	0.99±0.43^{b}	1.17±0.25^{b}
	II	1.30±0.04^{a}	1.16±0.03^{b}	1.13±0.05^{b}	1.19±0.03^{b}

^{a,b}Differences are statistically significant in groups marked with different letters in the same row ($p<0.05$); In Groups marked with * arestatistically significant in the same vertical column ($p<0.05$)

(Gunes *et al*., 2004). But there have been a limited number of studies reporting SA levels in wound healing (Yamamoto *et al*., 1995). Recent studies have shown that serum SA is increased in wound healing (Yamamoto *et al*., 1998; Uslu *et al*., 2002). Immediately after a surgical incision, a number of epithelial cells and connective tissue cells die and the basement membrane is disrupted. This initiates an acute phase response. In the acute phase reaction, hepatocytes in the liver increase the levels of acute phase reactants, like SA (Yamamoto *et al*., 1998).

In many inflammatory conditions, including burns, wounding, etc. the serum SA level has been shown to rise as an acute responder (French *et al*., 2002; Uslu *et al*., 2004), as it was shown in the present study. A rise in TSA levels in wound healing might be associated with destruction and damage in cell membranes, which cause lipid peroxidation. In a sunburn rat model, Yamomoto *et al*. (1995) showed that TSA levels increased and reached the maximum 3 days after irradiation. This level was maintained for about 2 days, eventually returning to normal within about one week. In the present study, we observed that serum TSA concentrations were significantly lower in group II than in group I on 7 and 14 days ($p<0.05$). And on day 3, there was a decrease in the wound and liver tissue TSA levels in group II. This early decrease in wound and liver tissue TSA levels in group II was related with the CAPE's antioxidant effect and the start of the healing process.

The majority of sialic acid in serum is protein-and lipid-bound with little occurring in the free form. Elevations in the serum sialic acid concentration have been reported for both the protein-and lipid-bound fractions (Millar, 2001). In the present study, serum TSA and LBSA levels were significantly lower in Group II than in group I on 7 and 14 days, but there was no difference in serum PBSA levels between the groups. Higher LBSA levels in group I may be a result of secretion of sialic acids from the cell membrane surface due to cutaneous wound. Therefore, the removal of sialic acid residues in serum or from the cell-membrane surfaces may result with elevations in LBSA and TSA levels in group I. Lipid peroxidation is an important cause of destruction and damage to cell membrans and SA is a component of cell membranes (Motoi *et al*., 1984), any damage to cell membranes will result in an increase in the level of SAs, especially LBSA. The decrease in serum TSA and LBSA levels of group II might have been the result of antioxidant and suppressive effects of CAPE on lipid peroxidation.

Oxidative stress occurs when there is either an over production of ROS or a decrease of antioxidant defences (Ozyurt *et al*., 2001). Antioxidant status of tissues can be described by the analysis of single components in defence systems against ROS as well as by the determination of TAC. Although, TAC measurement does not represent the sum of activities of single antioxidants, it can be of clinical use, because, it is easy and not time consuming procedure (Kankofer *et al*., 2005).

In the present study, we have determined a significant decrease ($p<0.05$) in serum TAC levels of Group I beginning on 3 day when compared to Group II. And wound tissue TAC levels of group I decreased significantly ($p<0.01$) beginning on 7 day, while the wound tissue TAC levels of group II were increasing (Table 3). Also there was a decrease in liver tissue TAC levels beginning on 3 day in both groups, but there was no significance between the groups. The less decrease determined in serum TAC levels compared with group I and the increase determined in wound tissue TAC levels in Group II in the study, can be a result of antioxidant and suppressive effects of CAPE on lipid peroxidation. The protective effect of CAPE against lipid peroxidation has been demonstrated in rat plasma after treatment for burns (Hosnuter *et al*., 2004), ischaemia-reperfusion injury (Ozyurt *et al*., 2001) and dermal wound (Serarslan *et al*., 2007).

Lipid peroxidation is an important cause of destruction and damage to cell membrans and SA is a component of cell membranes (Motoi *et al*., 1984), any damage to cell membranes will result in an increase in the level of SAs, especially LBSA. The results of present study displayed significant negative correlation between TAC and TSA levels in rats with cutaneous wound, demonstrating an association between TAC and TSA levels.

ADA is an enzyme that is present in all cells. ADA enzyme levels are high in many diseases where cellular immunity is stimulated (Ustundag *et al*., 1999). Wound healing involves a number of processes, including an acute inflammatory process in response to the wounding and regeneration (Gupta *et al*., 2002). In the present study, there is no significant change in serum ADA activity between the two groups. But there was an increase in serum ADA activity within the both group on 3 day, having resulted from the cell damage caused by cutaneous wound.

CONCLUSION

The present study showed that CAPE enhances wound healing by its antioxidant effects and that it could be useful in the treatment of cutaneous wounds. This is supported by the measurement of ADA activity, TAC and SA levels in serum, wound and liver tissue.

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