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The effects of vitamin E and selenium on blood flow to experimental skin burns in rats using the ^{133}Xe clearance technique

Research Article

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Abstract: The aim of the present experimental study was to determine and to compare the effects of vitamin E and of selenium on the blood flow to a wound region in rats, using the xenon-133 (^{133}Xe) clearance technique. Burn wounds were made on the right thighs of rats followed by oral administration of vitamin E or selenium for a period of 10 days. The effect of vitamin E and of selenium individually on blood flow in the wound region was assessed before and after oral administration using the ^{133}Xe clearance technique. Both vitamin E and selenium significantly increased the blood flow in the wound region ($P<0.05$). Our results in rats suggest that both vitamin E and selenium have beneficial effects on the skin wound healing process by increasing the skin blood flow.

Keywords: *Vitamin E • Selenium • Wound healing • Blood flow • Xenon-133*

1. Introduction

Vitamin E, the term for a group of tocopherols and tocotrienols, is the major lipid soluble antioxidant in skin; it protects cell membranes from peroxidative damage [1]. It has been used to treat almost every type of skin lesion, and is frequently used by the general population to treat burns, surgical scars, and other wounds [2]. Studies have shown that vitamin E also enhances healing of irradiated rat skin and increases the healing rate of patients with chronic post-thrombotic leg ulcers [3,4].

Selenium is an essential micronutrient; small amounts of it are necessary for tissue oxygenation and for protection against lipid peroxidation. It is involved in one of the main antioxidant defense systems of the body in the form of glutathione peroxidase (GSH-Px), which is the main plasma selenoprotein. It consists of four identical subunits, each of which contains one covalently bound Se atom [5]. The beneficial effect of selenium has been shown in the treatment of burned patients and in the healing process in patients with leg ulcers [6-9]. It has also been shown that selenium accelerates the healing of experimental skin wounds [10].

The Sejrsen xenon-133 clearance technique is the most thoroughly studied and most frequently used blood flow technique [11-13]. The isotope xenon-133 (^{133}Xe) is an inert lipophilic gas that can easily cross cellular membranes. The basic principle of the Sejrsen technique is that the clearance of a freely diffusible tracer from a tissue is determined only by the blood supply to the tissue, and therefore reflects the level of local blood flow [14]. The clearance technique has been used in many experimental and clinical investigations of blood flow changes [15-18].

The aim of the present study was to evaluate and compare the effect of vitamin E and of selenium on the blood flow to experimental wounds in rats as part of the healing process, using the ^{133}Xe clearance technique.

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2. Material and Methods

2.1. Animals

The 30 male Sprague-Dawley rats used in this study (225±25g each) were fed with standard laboratory chow and water; they were randomly divided into groups A (vitamin E, n=10), B (selenium, n=10) and C (control, n=10). The experiments were performed in an ethically proper way by following guidelines set by the Ethical Committee of the University in accord with international standards of experimental work on animals.

2.2. Experimental protocol

Full-skin-thickness burns were produced by the Arons' burn model method [19]. Under light ether anesthesia, rats were injected intraperitoneally with ketamin-HCl (20 mg/kg, Ketalar®). After carefully shaving the surface of the right thigh, each rat was exposed to a heated metal apparatus with a diameter of 1 cm (90 °C, 20 s). By this means, a second-degree burn was formed in the right thigh.

Vitamin E (Ephynal 300 capsule, Roche, France) dissolved in corn oil (30 mg/mL) was administered orally through a stomach tube for 10 days following creation of the wound, (approximately 100 mg/kg/day) to group A, and selenium (sodium selenite anhydrous, 44-46% Se, Acros Organics, Belgium) dissolved in tap water (8.88 mg/L) was administered orally to group B. Animals in group C were not treated.

2.3. Measurement of blood flow

Separate blood flow measurements were carried out in the anaesthetized rats before and after administration of vitamin E and selenium; blood flow was also measured in the control group. A sterile saline solution containing 1.85 MBq ^{133}Xe (DuPont Pharma SA, Brussels, Belgium) was injected intradermally at the wound margin of the right thigh and the left thigh skin. Immediately after ^{133}Xe injection, the anesthetized rats were placed under a single-head gamma camera with a parallel-hole, low-energy, high-resolution collimator (GE-Starcam 4000 XR/T, St Albans, Hertfordshire, UK). A dynamic image series was acquired in a frame mode of a 64x64 matrix at one frame per 30 s for 10 min. Time-activity curves were generated from the regions of interest (ROI) at the sites of injection. The clearance half-time of ^{133}Xe ($T½$) was measured from these curves. Blood flow, f, was calculated by the relation

$$f = (\lambda \times \ln 2 \times 100 \text{ g tissue}) / T½$$

where f is cutaneous blood flow in mL/100 g tissue/min and λ is the partition coefficient of ^{133}Xe between tissue and blood (0.7 mL/g) [20].

Table 1. The blood flow in wound regions in the right thigh before and after vitamin E and selenium administrations and in the control group. Data is presented as mean ± standard deviation (SD).

Groups	Before	After	P
A: Vitamin E	0.08±0.01	0.26±0.04	<0.05
B: Selenium	0.08±0.02	0.18±0.03	<0.05
C: Control	0.09±0.01	0.13±0.02	>0.05

2.4. Statistical analysis

The mean value of the two blood flow measurements from each wound in the right thigh was calculated. Statistical analysis for blood flow values between groups, before and after administration, was done using the Wilcoxon signed rank test and the Mann-Whitney U test. Differences with a P value less than 0.05 were accepted as significant, with data in the text presented as mean±standard deviation (SD). The SPSS version 11.5 (SPSS Inc., Chicago, IL) software program was used for statistical analysis.

3. Results

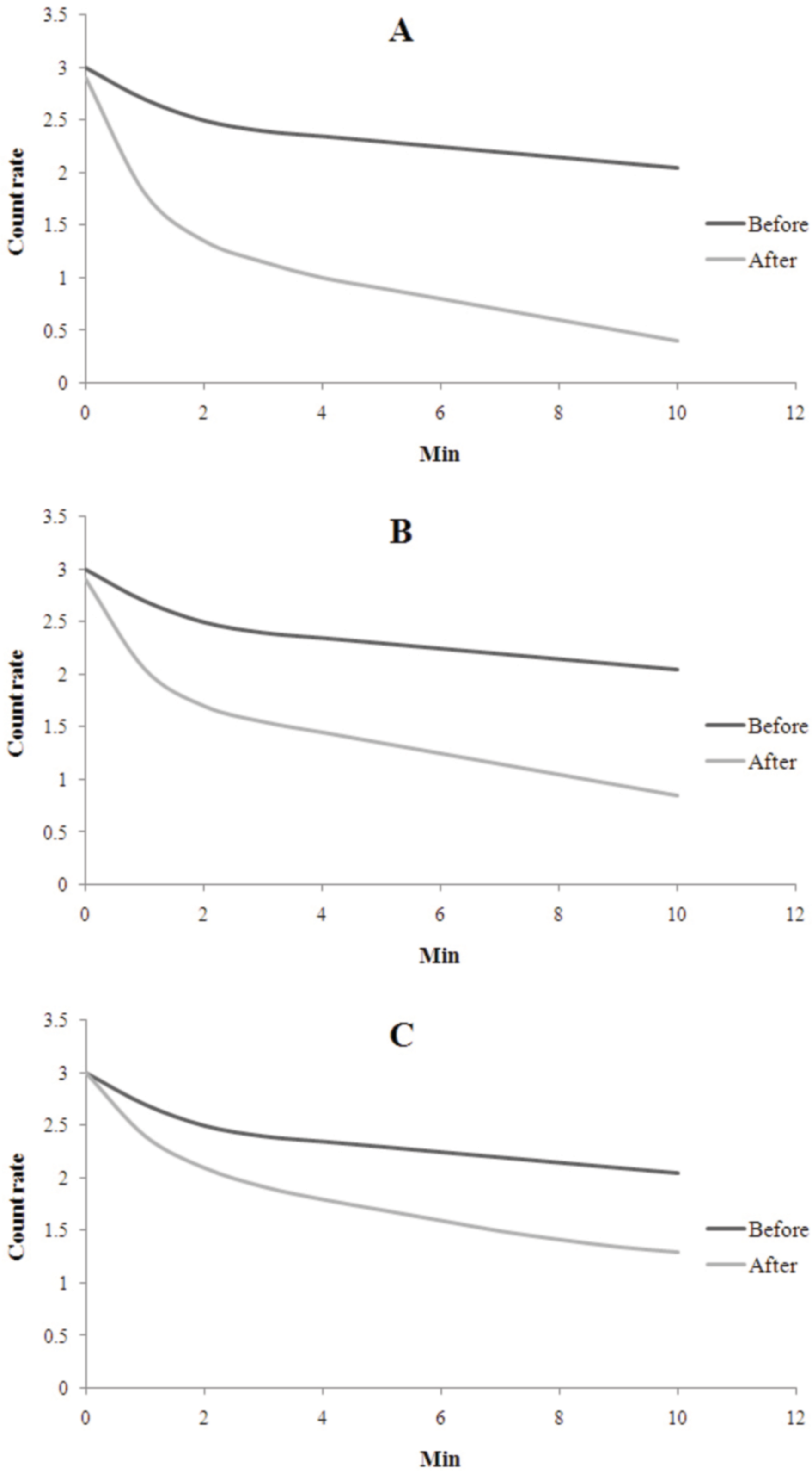
The clearance curves of ^{133}Xe from the wound region in the right thigh before and after vitamin E (Group A) and selenium administrations (Group B) and in the control group are shown in Figures 1a–1c. Table 1 shows the detailed statistical results. The blood flow in wound regions before administration was the similar in all groups (0.08±0.01, 0.08±0.02 and 0.09±0.01 mL/100 g/min for vitamin E, selenium, and control groups, respectively). After administration, the blood flow in wound regions was 0.26±0.04, 0.18±0.03 and 0.13±0.02 mL/100 g/min for vitamin E, selenium, and control groups, respectively. These values for vitamin E and selenium were significant ($P<0.05$). However, no significant difference was found in the control group ($P>0.05$).

Before administration, there was no significant difference in blood flow among all groups ($P>0.05$). After administration, no significant difference was found for the blood flow of wound regions between the vitamin E and selenium groups ($P>0.05$). However, the blood flow in wound regions significantly increased in the vitamin E and selenium groups compared to that of the control group ($P<0.05$).

4. Discussion

Wound healing consists of an orderly progression of events that re-establish the integrity of the damaged tissue. The process of wound healing is essential to

Figure 1. ^{133}Xe clearance curves from wound regions in the right thighs before and after administrations of vitamin E (A) and selenium (B) and in the untreated control group (C).



prevent the invasion of damaged tissue by pathogens, and to partially or completely reform the damaged tissue. The healing involves different phases, including inflammation, granulation, fibrogenesis, neo-vascularization, wound contraction, and epithelialization [21]. Successful wound healing requires adequate blood and nutrients to be supplied to the site of the damage; the overall health and nutritional status of the patient influences the outcome of the damaged tissue as well [22].

Vitamin E is an antioxidant that protects the body against the deleterious effects of free radicals [3,23]. Selenium is an essential component of GSH-Px. Selenium and selenium-containing amino acids appear to function as free radical scavengers, maintaining the stability of lipid-containing biologic membranes [24]. To date, no study has reported the effects of vitamin E and selenium on blood flow in the wound region. However, it is well known that both substances have beneficial effects on wound healing, as illustrated by the following studies. It has been shown that vitamin E enhances healing in irradiated rat skin and increases the healing rate of patients with chronic post-thrombotic leg ulcers [3,4]. Musalmah et al. showed that vitamin E accelerated the rate of wound closure in rats [25]. Also, a faster healing process was observed in experimental wounds where vitamin E was used in animal studies [26,27]. Gümüştekin et al. showed that supplementation of selenium accelerates wound healing by preventing nicotine-induced oxidative stress [10]. Haberal et al. reported that selenium as a membrane-stabilizing agent may help in the treatment of burned patients [8]. The promotive effect of selenium was also shown in the healing process of experimental wounds [26].

In conclusion, our results in rats suggest that both vitamin E and selenium promote wound healing activity by increasing the blood flow in the wound region. Therefore, supplementation with vitamin E and selenium at appropriate doses may have beneficial effects on wound healing. The ^{133}Xe clearance technique can be used in measurement of the blood flow changes around various skin wounds experimentally.

References

[1] Suleyman H., Gumustekin K., Taysi S., Keles S., Oztasan N., Aktas O., et al., Beneficial effects of Hippophae rhamnoides L. on nicotine induced oxidative stress in rat blood compared with vitamin E, Biol. Pharm. Bull., 2002, 25, 1133-1136

[2] Baumann L.S., Spencer J., The effects of topical vitamin E on the cosmetic appearance of scars, Dermatol. Surg., 1999, 25, 311-315

[3] Rojas A.I., Phillips T.J., Patients with chronic leg ulcers show diminished levels of vitamins A and E, carotenes, and zinc, Dermatol. Surg., 1999, 25, 601-604

[4] Taren D.L., Chvapil M., Weber C.W., Increasing the breaking strength of wounds exposed to preoperative irradiation using vitamin E supplementation, Int. J. Vitam. Nutr. Res., 1987, 57, 133-137

[5] Gül M., Temoçin S., Hänninen O., Selenium supplementation sensitizes renca cells to tert butylhydroperoxide induced loss of viability, Indian J. Exp. Biol., 2000, 38, 1020-1025

[6] Gartner R., Albrich W., Angstwurm M.W., The effect of a selenium supplementation on the outcome of patients with severe systemic inflammation, burn and trauma, Biofactors, 2001, 14, 199-204

[7] Borner J., Zimmermann T., Albrecht S., Roesner D., Selenium administration in severe inflammatory surgical diseases and burns in childhood, Med. Klin. Suppl., 1997, 15, 17-19

[8] Haberal M., Mavi V., Oner G., The stabilizing effect of vitamin E, selenium and zinc on leucocyte membrane permeability: a study in vitro, Burns Incl. Therm. Inj., 1987, 13, 118-122

[9] Agren M.S., Stromberg H.E., Rindby A., Hallmans G., Selenium, zinc, iron and copper levels in serum of patients with arterial and venous leg ulcers, Acta Derm. Venereol., 1986, 66, 237-240

[10] Gumustekin K., Seven B., Karabulut N., Aktas O., Gursan N., Aslan S., et al., Effects of sleep deprivation, nicotine, and selenium on wound healing in rats, Int. J. Neurosci., 2004, 114, 1433-1442

[11] Sejrsen P., Atraumatic local labeling of skin by inert gas: epicutaneous application of xenon 133, J. Appl. Physiol., 1968, 24, 570-572

[12] Sejrsen P., Blood flow in cutaneous tissue in man studied by washout of radioactive xenon, Circ. Res., 1969, 25, 215-229

[13] Marcus F.S., Cho S.I., Kountz S.L., Powell M.R., Measurement of blood flow in skin with an intact vascular pedicle, Int. J. Dermatol., 1976, 15, 131-135

[14] Quirinia A., Jensen F.T., Viidik A., Ischemia in wound healing. I: Design of a flap model--changes in blood flow, Scand. J. Plast. Reconstr. Surg. Hand. Surg., 1992, 26, 21-28

[15] Andrade S.P., Fan T.P., Lewis G.P., Quantitative

in-vivo studies on angiogenesis in a rat sponge model, Br. J. Exp. Pathol., 1987, 68, 755-766

[16] Hughes S., Brain S., Williams G., Williams T., Assessment of blood flow changes at multiple sites in rabbit skin using a ^{133}Xenon clearance technique, J. Pharmacol. Toxicol. Methods, 1994, 32, 41-47

[17] Lin W.Y., Kao C.H., Hsu C.Y., Liao S.Q., Wang S.J., Yeh S.H., Evaluation of tissue perfusion by the ^{133}Xe washout method in lower limbs of patients with noninsulin-dependent diabetes mellitus, Clin. Nucl. Med., 1995, 20, 449-452

[18] Güler R., Ercan M.T., Ulutunçel N., Devrim H., Uran N., Measurement of blood flow by the ^{133}Xe clearance technique to grafts of amnion used in vestibuloplasty, Br. J. Oral Maxillofac. Surg., 1997, 35, 280-283

[19] Arons M.S., Burn wound infection--a review, Conn. Med., 1965, 29, 718-722

[20] Andersen A.M., Ladefoged J., Partition coefficient of ^{133}xenon between various tissues and blood in vivo, Scand. J. Clin. Lab. Invest., 1967, 19, 72-78

[21] Clark R.A.F., Wound repair: Overview and general considerations, In: Clark R.A. and Henson P.M. (Eds.), The Molecular and Cellular Biology of Wound Repair, Plenum Press, New York, 1996

[22] MacKay D., Miller A.L., Nutritional support for wound healing, Altern. Med. Rev., 2003, 8, 359-377

[23] Johnson E.J., Russell R.M., Vitamin A., In: Hartz S.C., Russell R.M., Rosenberg I.H. (Eds.), Nutrition in the Elderly: the Boston nutritional status survey, Smith, Gordon and Co., London, 1992

[24] Tappel A.L., Free radical peroxidation damage and its inhibition by vitamin E and selenium, Fed. Proc., 1965, 24, 73-78

[25] Musalmah M., Fairuz A.H., Gapor M.T., Wan Ngah W.Z., Effect of vitamin E on plasma malondialdehyde, antioxidant enzyme levels and the rates of wound closures during wound healing in normal and diabetic rats, Asia Pac. J. Clin. Nutr. Suppl., 2002, 11, 448-451

[26] Sieradzki E., Olejarz E., Strauss K., Marzec A., Mieszkowska M., Kaluzny J., The effect of selenium and vitamin E on the healing process of experimental corneal lesions in the eye of the rabbit, Klin. Oczna., 1998, 100, 85-88

[27] Kim J.E., Shklar G., The effect of vitamin E on the healing of gingival wounds in rats, J. Periodontol., 1983, 54, 305-308