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ORIGINAL ARTICLE

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Effect of vitamin C and E supplementation in modulating the peripheral nerve conduction following cold exposure in humans

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Abstract Exposure to an extremely cold environment without proper protection leading to hypothermia is an emergency, one of the several complications of which is impairment in nerve conduction. Our previous work in the rat model has shown the beneficial effect of vitamin C in modulating the effect of hypothermia on nerve conduction. The present study aimed to evaluate the effect of vitamins C and E, administered alone or in combination, in modulating the effect of mild hypothermia on human ulnar nerve conduction. The study was carried out on 26 volunteers divided into three groups: group I received vitamin C supplementation (2000 mg/day in a single dose and 1,000 mg/day for the next 6 days), group II received vitamins C and E in combination (1,000 mg and 800 mg respectively in a single dose and 500 mg and 400 mg respectively for the next 6 days) and group III received vitamin E (800 mg in a single dose and the same for the next 6 days). The recordings were carried out before and after single and weekly supplementation in each group. There was a fall in ulnar nerve conduction velocity with a reduction in the oral temperature of 2–2.5 °C. Vitamin C administered alone and in combination with vitamin E reduced the fall in ulnar nerve conduction velocity. Prior supplementation with vitamin C and E could help ameliorate the impairment in human ulnar nerve conduction due to hypothermia.

Keywords Cold exposure · Nerve conduction · Vitamin C · Vitamin E · Supplementation of vitamins

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Introduction

Defense personnel in our country work in high altitude and polar environments, where extreme cold conditions and sub-zero ambient temperatures and prevalent. In such conditions, exposure to extreme cold without adequate protection could lead to a situation of hypothermia. One of the several complications of hypothermia is an impairment in nerve conduction. It is well known that nerve conduction is altered by changes in skin temperature (Doiszhghy and Stalberg 1992; Franssen and Wieneke 1994; Trojaborg et al. 1992). However, there are hardly any reports in the literature on the effects of experimentally induced hypothermia on peripheral nerve conduction in human volunteers.

An important consideration would be protection against accidental hypothermia. If prior supplementation with a natural or synthetic compound could give some protection against the impairment in nerve conduction due to hypothermia, it could help by acting as a prophylactic and could be used before the expected cold exposure. In view of this, we thought it worthwhile to study the effect of antioxidant vitamin supplementation. Our previous work (Panjwani et al. 1996) had already demonstrated that vitamin C supplementation in a "mega" dose modulates the hypothermic influence on nerve conduction in the rat model. The present study aimed to assess the possible beneficial effect of vitamin C and E supplementation, given alone or in combination in a single or weekly dosages, on the impairment in ulnar nerve conduction due to mild hypothermia in human volunteers.

In addition, there is generation of free radicals during hypothermia (Bhaumik et al. 1995). Vitamin C increases the peripheral blood flow by vasodilatation (Mathew et al. 1981). It accelerates thermogenesis and attenuates sympathetic hyper-reactivity (Beaton 1967; Leblanc 1975; Wilson 1974). Antioxidant enzymes as well as antioxidant vitamins scavenge these. In cell membranes the most important antioxidant is vitamin E, which acts as a chain-breaking antioxidant intercepting lipid peroxyl radicals

and terminating lipid peroxidation (Brigelius-Flohe and Traber 1999; Itoh et al. 2000). The major water-soluble free-radical scavenger is vitamin C, which also regenerates vitamin E from tocopherol radical (Niki et al. 1995). A combined supplementation of vitamin C and E is reported to be more beneficial for lipoprotein oxidation in Alzheimer's disease (Kontush et al. 2001). On the other hand, a recent study found no beneficial effect of antioxidant vitamin supplementation on exercise-induced oxidative stress (Meijer et al. 2001). Studies in our Institute have shown that supplementation with these vitamins helps reduce hypoxia-induced oxidative stress (Ilavazahagan et al. 2001). In view of these reports, the present study hypothesized that antioxidant vitamins C and E give protection against the impairment in peripheral ulnar nerve conduction due to hypothermia.

Materials and methods

Twenty-six normal healthy male volunteers participated the study. The study was carried out double blind. The volunteers were 20–30 years old, mean age 23.3 ± 1.3 years (±SEM). The procedure was explained to them and an informed, written consent was obtained from each volunteer. The clearance from the Ethics Committee of the Institute was obtained. The tenets of the Declaration of Helsinki were followed.

The recordings were obtained prior to cold exposure, after hypothermia without vitamin supplementation and after hypothermia with vitamin supplementation in each participant. The basal recordings were carried out in an air-conditioned laboratory, room temperature 25 ± 1 °C. The participants were bare bodied except for a pair of shorts. The effects of hypothermia was studied in a cold chamber maintained at a temperature of 6 ± 2 °C. The participants entered the chamber in groups of three. They were allowed to move freely within the chamber and either stood or sat on a stool. A table fan was placed at one end of the chamber for air circulation. The subjects remained in the cold chamber for approximately 1 h, which was enough to lower the temperature to the desired level. After the oral temperature had been lowered to 2–2.5 °C, the recordings were carried out. The vitamin supplementation was administered every day at 0900 hours and the recordings were carried out at 1200 ± 0.5 hours. The blood samples for vitamin supplementation were drawn immediately prior to the recordings.

The effect of vitamin supplementation was studied for single and weekly dosages. The subjects were randomly assigned into three groups. Group I (n = 10) received vitamin C supplements taken orally in a single dose of 2,000 mg. In the same group, vitamin C was administered at 1,000 mg.day for the next 6 days to obtain the weekly supplementation dosage. Group II (n = 8) was given vitamins C and E combined in a single dose of 1,000 mg and 800 mg respectively. In the same group, the weekly dosage was obtained by administering 500 mg and 400 mg daily for the following 6 days. Group III (n = 8) was given vitamin E alone in a dosage of 800 mg/day in a single dose and the same amount was given for the next 6 days to achieve the weekly dosage.

The recordings of nerve conduction velocity (NCV) were carried out using a physiological recording device (BIOPAC systems, Santa Barbara, California USA). The subject was seated comfortably. The stimulating electrodes (BIOPAC systems, Santa Barbara, California USA EL500 dual electrodes) were pre-gelled connected pairs 1.6 cm apart (center to center). These were placed lengthwise along the ulnar nerve directly over the elbow, on the bottom inside the posterior side of the arm, in between the two bony projections, The stimulating electrodes were connected with the negative polarity towards the torso to the stimulus isolation unit (STIMISOl; BIOPAC Systems, Santa Barbara, California USA) via a pair of LEAD 100S (BIOPAC Systems, Santa Barbara, California USA) electrode leads. The recording electrodes were EL500 (BIOPAC Systems, Santa Barbara, California USA) dual electrodes placed lengthwise along the ulnar nerve at the inside of the wrist, slightly towards the little finger with the negative polarity towards the hand. The electrodes were connected to an EMG 100A (BIOPAC Systems, Santa Barbara, California USA) amplifier via two electrode leads (LEAD 100S). The amplifier was grounded using LEAD 100 (BIOPAC Systems, Santa Barbara, California USA) and an electrode (EL503) placed on the opposite arm. The ulnar nerve was stimulated using a stimulator (STM 100A). The latent period was the isoelectric line measured as the time interval between the positive peak of the stimulus artifact to the start of the compound action potential. The distance between the midpoint of the stimulating and recording electrodes was measured. The conduction velocity (m/s) was obtained by dividing this distance by the latent period (ms). The oral temperature was recorded by a temperature probe connected to the recording system (TSD 120A fast response time thermometer probe, BIOPAC Systems, Santa Barbara, California USA). It was not posible to monitor the rectal temperature for ethical reasons. Hence we had to use the oral temperature as a measure of the core temperature. In order to ensure that the oral temperature did not fluctuate in response to air currents inside the chamber, due care was taken to ensure that the subjects did not speak and kept their mouths closed throughout the duration of the experiment.

The oral temperature, when measured accurately, is a good indicator of core temperature. The average normal temperature is generally considered to be between 36.7 °C and 37.0 °C when measured orally and approximately 0.6 °C higher when measured rectally (Ganong 1999)

Blood samples were drawn for the estimation of plasma levels of vitamins C and E, the baseline level, after hypothermia without vitamin supplementation and after hypothermia with vitamin supplementation. Vitamin C was estimated by a method that is based upon the measurement of the colour of the product formed by the coupling of 2,4 dinitrophenylhydrazine with the ketonic group of oxidized ascorbic acid (Rae and Kuetter 1943). Vitamin E was also estimated; the proteins in the plasma were precipitated and the mixture was subjected to extraction by n-heptane. Addition of ferric chloride reagent produced the colour obtained by the Emmeric-Erigel procedure, which was measured at 510 nm (Hashim and Schuttringee 1966).

Statistical analysis was carried out using repeated-measures analysis of variance with the Student Newman Keuls multiple-comparison test.

Results

As shown in Table 1, reduction of the oral temperature by between 2 °C and 2.5 °C significantly reduced the ulnar nerve conduction in all groups. In group I subjects, a 2,000 mg single oral dose of vitamin C significantly improved the NCV during hypothermic exposure. The NCV values after oral administration of 2,000 mg vitamin C were almost similar to basal values. On the other hand, a 1,000 mg/day oral dose of ascorbic acid for 7 days did not produce any significant effect on ulnar nerve conduction during hypothermic exposure. Comparison with basal values of the results from subjects who had received single-dose supplements and undergone hypothermia showed no significant differences. Comparisons between values obtained after supplementation showed significant differences, the former being significantly higher ($P < 0.01$). In group II subjects, a single oral dose of vitamin C (1,000 mg/day) and vitamin E (800 mg/day) combined significantly improved ulnar nerve conduction

Table 1 Effect of vitamin C and E supplementation on human ulnar nerve conduction velocity before and after hypothermic exposure. Values are means ± SEM

Group	Ulnar nerve conduction velocity (m/s)			
	Basal (*A*)	HT (*B*)	HT + SD (*C*)	HT + WD (*D*)
I	53.85 ± 1.76	43.01[*1] ± 1.06	53.49[*4] ± 1.83	45.53[*3,*5] ± 1.46
II	51.23 ± 1.76	39.57[*1] ± 1.73	50.87[*4] ± 1.80	45.49[*3,*5] ± 1.58
III[*6,*7]	53.54 ± 1.80	40.58[*1] ± 1.71	40.49[*2] ± 2.01	42.95[*3] ± 1.76

[*1] *A* versus *B* $P < 0.01$; [*2] *A* versus *C* $P < 0.01$; [*3] *A* versus *D* $P < 0.01$; [*4] *B* versus *C* and *B* versus *D* $P < 0.01$; [*5] *C* versus *D* $P < 0.01$; [*6] overall I versus III $P < 0.01$; [*7] overall II versus III $P < 0.01$
HT Hypothermia, *SD* single dose of vitamin, *WD* weekly dose of vitamin.

Table 2 Effect of oral supplementation of vitamins C and E on plasma leves. Values are means ± SEM

Group	Vitamin	Plasma level of vitamin (μg/ml)		
		Basal (*A*)	SD (*B*)	WD (*C*)
I	C	0.89 ± 0.06	3.02[*1] ± 0.09	1.09[*2,*4] ± 0.06
II	C	0.36 ± 0.10	2.22[*1] ± 0.14	1.31[*3,*4] ± 0.18
	E	21.82 ± 3.95	50.64[*1] ± 3.64	69.13[*3,*5] ± 7.07
III	E	15.74 ± 2.80	77.08[*1] ± 6.74	104.97[*3,*4] ± 7.96

[*1] *A* versus *B* $P < 0.01$; [*2] *A* versus *C* $P < 0.01$; [*3] *A* versus *C* $P < 0.01$; [*4] *B* versus *C* $P < 0.01$; [*5] *B* versus *C* $P < 0.01$

and the NCV was significantly ($P < 0.01$) higher after supplementation than values following hypothermia with no supplementation. A weekly dose of vitamin C (500 mg/day) and vitamin E (400 mg/day) in combination also improved nerve conduction significantly. However, single-dose supplementtation values did not differ significantly from basal values but weekly-dose supplementation values were significantly lower ($P < 0.01$) than basal values, indicating that single-dose supplementation brought values close to normal baseline levels but this was not the case with weekly supplementation. Comparison of single-dose and weekly supplementation values showed significant differences ($P < 0.01$), the values being significantly higher following single-dose supplementation. In group III subjects, vitamin E supplementation of 800 mg/day in single and weekly dosages did not reduce the impairment in ulnar nerve conduction due to hypothermia. Comparison between groups I, II and III of ulnar NCV after vitamin supplementation in single dosage showed significant differences (Table 1). The values in groups I and II were significantly higher than those of group III ($P < 0.01$ for both). However, comparison between the ulnar NCV reading of the three groups after weekly supplementation with vitamins showed no significant differences.

Plasma levels showed significant elevation following supplementation. As shown in Table 2, in group I subjects the mean plasma levels of vitamin C were significantly elevated following single-dose supplementation with a 2,000-mg single dose in vitamin C. Comparison of single and weekly supplementation showed significant differences, the levels being significantly higher following single-dose supplementation. In group II subjects, the plasma levels of vitamins C and E were significantly higher than basal levels following single- and weekly-dose supplementation. Comparison of single- and weekly-dose supplementation of vitamin C (vitamins C and E combined as in group II) showed significantly higher levels following the single dose ($P < 0.01$) but, in the case of vitamin E, significantly higher levels ($P < 0.05$) were found following weekly-dose supplementation. Group III subjects also showed significant changes in plasma levels following vitamin E supplementation. Comparison of single and weekly dosages showed significantly greater elevation in plasma levels in the latter case ($P < 0.01$). Comparisons between groups of plasma levels of vitamins after supplementation revealed some interesting observations. In groups I and II there was no significant differences in vitamin C levels after single and weekly dosages although the dosages were much higher in group I. Similar findings were obtained in groups II and III after vitamin E supplementation in single and weekly dosages (Table 2).

Discussion

Vitamin C supplementation in a single oral dose of 2,000 mg/day appeared to help protect against the reduction in ulnar NCV that occurred when the temperature fell by 2–2.5 °C, the NCV values after supplementation being almost similar to basal values. A weekly supplementation with 1,000 mg/day did not give any beneficial effect. The vitamin C and E combination used for group II subjects, on the other hand, was also found to be beneficial. Here again, a higher single dose appeared to be more effective since the NCV values after weekly-dose supplementation remained significantly lower than the basal values whereas, after single-dose supplementation the NCV values did not differ significantly from basal values. In group III subjects, 800 mg/day vitamin E taken orally in a single dose and a weekly dose did not have any beneficial effect on the amelioration in NCV due to hypothermia. NCV values after single dosage also did not

register any difference from those after weekly doses. We did not measure the regional temperature and blood flow; we were more interested in addressing the effect of a fall in core temperature. However, this is a limitation of the study. The fall in regional temperature and blood flow due to peripheral vasoconstriction because of local cooling would contribute to the fall in NCV. This factor may be responsible for the greater fall in NCV/°C lowering of oral temperature as against the expected lesser fall in NCV that was obtained in our earlier study (Panjwani et al. 1996).

Reduction in nerve blood flow precedes the slowing of NCV. This is accompanied by an increase in levels of free radicals (Otani et al. 1999; Coopey et al. 2000) For this reason the effect of antioxidants in improving diabetes-induced peripheral neuropathy is being studied (Coopey et al. 2001a). Antioxidant α-lipoic acid has been found to improve nerve blood flow and NCV (Ford et al. 2001; Coopey et al. 2001b). Similarly, pentoxyfylline, which improves nerve blood flow, is reported to improved NCV (Flint et al. 2000). On the other hand, only minor benefits of α-lipoic acid on nerve blood flow and oxidative state were reported in another study (Van Dam et al. 2001). In the present study. vitamin C could act by increasing the peripheral blood flow by vasodilatation (Mathew et al. 1981). It accelerates thermogenesis (Beaton 1967; Leblanc 1975; Wilson 1974) and attenuates sympathetic hyper-reactivity (Itoh et al. 2000). It is reasonable to speculate that the reduction in ulnar nerve blood flow due to hypothermia would be ameliorated by vitamin C. It may also help in restoring the intracellular substances as it can rapidly enter cells particularly leukocytes. The physiological functions of ascorbic acid also include the formation of collagen and intracellular cement substances. In view of these important actions of vitamin C, it is not surprising to find that vitamin C supplementation has beneficial effects on the deterioration in ulnar nerve conduction due to hypothermia.

During mild hypothermia, as in the persent study, it is expected that there is a generation of free radicals (Bhaumik et al. 1995). Oxidative stress is also a feature of high-altitude hypoxia (Simon-Schnass 1994). Vitamin C is a well-known free-radical scavenger (Stone 1972). It is a major water-soluble antioxidant, which acts as the first line defense of against oxidative injury (Itoh et al. 2000) and in a recent study it has been found to prevent oxidative damage at high altitude (Ilavazhagan et al. 2001). Ascorbic acid and α-tocopherol act as potent and probably the most important hydrophilic and lipophilic antioxidants respectively. They function at their own site and act synergistically (Meijer et al. 2001). In line with the findings of the present study, a related study (Purkayastha et al. 1999) also found an improvement in peripheral vascular response to local cold stimulus at high altitude following vitamin C supplementation, with single supplementation showing the best response and no additional benefit evident on administration of vitamin C and E in combination.

A single dose of 2,000 mg vitamin C gave the same protection as a combination of 1,000 mg ascorbic acid and 800 mg tocopherol given in a single dose. The interaction of vitamins C and E is well documented. Vitamin C interacts with membrane-bound vitamin E by reducing the tocopherol radical back to tocopherol in vitro (Niki et al. 1995). Although the nature of the interaction is controversial (Brigelius-Flohe and Traber 1999) a sparing effect of vitamin C in vivo has been reported, suggesting that, in vivo, vitamin C and E interact and each can exert sparing effects in the absence of the other (Tanaka et al. 1997). In this case the effect could be possibly explained by an enhanced oxidative stress in the membrane region being transferred to the aqueous phase in the cell, leading to a utilization of vitamin C (Ganong 1999). The interaction with vitamin E may explain out finding of reduced vitamin C dosages in the vitamin C and E combination having the same effect as a larger single vitamin C dosage.

Vitamin E did not give additional protection against impairment in nerve conduction due to hypothermia. This supports the suggestion vitamin E supplementation is beneficial only to certain groups of population and not in volunteers maintained on a nutritionally adequate diet where there is no additional benefit (Chow 1991). This may be true for the present study where the volunteers were army personnel receiving a nutritionally balanced diet. In our experiment relatively high doses of vitamins, especially vitamin C, have been found to be beneficial. The optimal dosage of the vitamin may be dependent upon the body's requirement or the level of stress. Some reports have highlighted the beneficial effect of taking mega doses of ascorbic acid for a wide range of stresses. Stress is accompanied by lower levels of vitamin C in particular tissues, e.g. in leukocytes (Pauling 1970). Experimental animals that synthesize vitamin C endogenously (e.g. rodents) show elevated levels in various situations of stress, including cold stress. Studies on cold acclimatization have shown beneficial effects with increased concentrations in specific tissues (Dugal 1961).

The plasma level of the vitamins showed significant elevations following oral supplementation. Interestingly comparison between groups of plasma levels (Table 2) showed no significant differences between vitamin C in group I and group II whereas the dosages of vitamin C in group II were half of those in group I. A similar observation was made in vitamin E levels in group II and III subjects. It has been reported that plasma tocopherol levels are greater in individuals receiving vitamin C supplementation. However, increased intake of supplemented vitamin E was weakly correlated with plasma ascorbic acid (Jacques et al. 1983). We did not estimate the vitamin C levels in volunteers on vitamin E supplementation or vitamin E levels in people on vitamin C supplementation. Such estimations may help in evaluating the interaction between these vitamins after supplementation. The present study suggests that vitamin C supplemention may help ameliorate the impairment in ulnar

nerve conduction due to experimentally induced hypothermic exposure.

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