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# Antioxidant effect of beta-carotene on hypoxia induced oxidative stress in male albino rats

S.K.S. Sarada \*, P. Dipti, B. Anju, T. Pauline, A.K. Kain, M. Sairam, S.K. Sharma, G. Ilavazhagan, Devendra Kumar, W. Selvamurthy

Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, New Delhi 110054, India

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## Abstract

Hypoxia is known to induce oxidative stress in organisms leading to tissue injury. In the present study  $\beta$ -carotene (BC) given at 10 mg/kg body weight (BW) in reducing the oxidative stress induced by hypoxia was evaluated on male albino rats. Hypoxia exposure caused an increase in malondialdehyde (MDA) levels in plasma and tissues, a concurrent decrease in blood glutathione (GSH), glutathione peroxidase (GPx), plasma protein and plasma BC content. Hemoglobin concentration, Red blood corpuscles (RBC) and White blood corpuscles (WBC) count were also increased under hypoxia. BC supplementation reversed the trend, inducing a significant decrease ( $P < 0.05$ ) in MDA and subsequent increase in plasma and tissue GSH levels in animals exposed to hypoxia. Blood GPx and plasma protein also increased significantly in BC supplemented animals. BC supplementation did not alter the changes in Hb concentration, RBC and WBC count. BC has potent antioxidant activities in reducing the oxidative stress induced by hypobaric hypoxia. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Hypoxia; Oxidative stress; Antioxidants;  $\beta$ -carotene; High altitude

## 1. Introduction

High altitude (HA) studies have received lot of attention with regard to free radical (FR) production leading to oxidative damage. During initial stages of exposure to HA, hypoxia together with cold causes many adverse effects such as acute mountain sickness, pulmonary edema, monge's disease (Heath and Williams, 1977). Exposure to extreme cold at HA also causes problems like frostbite. These problems are mainly due to low partial pressure and reduced availability of oxygen. Decreased  $O_2$  availability at HA changes the body metabolism (Simon Schnass, 1996) thereby individual's mental and physical performance is altered. All these changes are probably due to an imbalance between the stress level and the antioxidant status of the body.

*Abbreviations:* BC,  $\beta$ -carotene; BW, body weight; FR, free radicals; HA, high altitude; GPx, glutathione peroxidase; GSH, reduced glutathione; HB%, hemoglobin content;  $O_2$ , molecular oxygen;  $^1O_2$ , singlet oxygen; OFR, oxygen free radicals; MDA, malondialdehyde; RBC, red blood corpuscles; WBC, white blood corpuscles.

\* Corresponding author.

E-mail address: sarada\_sagi@yahoo.com (S.K.S. Sarada).

The hypoxic cells are particularly susceptible to oxidative stress, a phenomenon commonly known as 'Oxygen Paradox' (Goldfarb and Sen, 1994) leading to FR generation. FRs are known to be directly involved in pathogenesis of various diseases and aging (Halliwell and Susanna, 1993; Jackson et al., 1998). The HA stress leads to lipid peroxidation resulting in membrane damage (Simon-Schnass, 1992). Various endogenous radical scavenging antioxidants are known. Some are hydrophilic (vitamin C, uric acid, bilirubin, albumin and thiols) and others are lipophilic (vitamin E, and ubiquinol) (Kraus et al., 1997). In recent years the function of carotenoids as radical scavenging antioxidants has also been extensively studied (Whittaker et al., 1996; Smith, 1998; Dugas et al., 1999).

$\beta$ -carotene (BC) a carotenoid pigment functions mainly as provitamin A in animals. It also acts as a powerful FR scavenger and chain breaking antioxidant (Smith, 1998). It is the most effective naturally occurring quencher of singlet oxygen ( $^1O_2$ ) and is also highly reactive and energized molecule (Foote et al., 1968). The peroxy trapping activity of BC and other carotenoids depends upon the partial oxygen pressure

(Simon Schnass, 1996). Vile and Winterbourn (1988) reported that BC inhibits the lipid peroxidation even better than vitamin E at HA. Earlier studies showed that vitamin E is the only significant lipid soluble chain breaking antioxidant (Burton et al., 1983) at HA. Several reports have revealed (Peto et al., 1981; Bendich et al., 2000; Desai et al., 1997; Carlos et al., 1997) that, by virtue of its antioxidant activity, BC enhances the immune response, reduces photoinduced neoplasm, inhibits mutagenesis, reduces tumor growth in vitro, prevents sister chromatid exchange and also reduces so called 'precancerous changes' such as leukoplacia and micronuclei prevalence in buccal epithelia. The antioxidant function of BC might complement the action of other antioxidants viz., vitamin C, vitamin E and selenium which are not very effective at low O<sub>2</sub> concentrations.

However, very little is known about the influence of dietary supplementation of antioxidants, especially BC during oxidative stress induced by hypoxia. The present study was undertaken to assess the effect of BC on lipid peroxidation and its antioxidant status in animals exposed to hypoxia.

## 2. Materials and methods

### 2.1. Animals

Experiments were carried out on male Sprague–Dawley rats weighing 200–250 g body weight (BW). These were maintained at 22 ± 2 °C with day and night cycles of 12 h each and given food and water ad libitum.

The effect of BC was studied in 24 rats, divided into four groups of six rats each. Group I ('Control') served as control receiving only vehicle (1 ml). Group II ('Hypoxia') received only vehicle and was exposed to hypoxic stress. Group III ('BC' or 'Normoxia') was supplemented orally with BC 10 mg/kg BW. Group IV ('Hypoxia + BC') was supplemented orally with BC 10 mg/kg BW and exposed to hypoxic stress.

### 2.2. Oxidative stress (exposure to hypoxia)

The rats were exposed to a simulated altitude of 25 000 ft (7620 m) in hypobaric chamber 6 h daily for 1 week. Throughout the study the temperature was maintained at 25 ± 1 °C and the air flow rate was 4 l/h. The barometric pressure of the hypoxic chamber was maintained at 280 mmHg.

At the end of the experiment, the animals were anaesthetized and the blood was collected by cardiac puncture. Suitable pieces of muscle and liver were collected for the biochemical analysis. Reduced glutathione (GSH; in plasma, blood, muscle and liver) was estimated by using the method of Kum-Talt and Tan (1974). Malondialdehyde (MDA) in plasma and blood was estimated by the method of Doussset et al. (1983) and in muscle and liver by the method of Ohkawa et al. (1979). Glutathione peroxidase (GPx) activity in the whole blood was determined using kit (RANDOX) following manufacturer's instructions. Hemoglobin, RBC and WBC was determined with the help of haematological analyzer. BC content in the plasma was determined by the method of Herman and Oscar (1968). Plasma protein level was estimated by using the method of Lowry et al., 1951. The data was subjected to statistical analysis applying Student's 't'-test. The significance level up to  $P < 0.05$  was considered as significant. This study was approved by the Institutional Ethic Committee of DIPAS, Delhi, India.

## 3. Results

MDA and reduced glutathione levels in various tissues and plasma of all experimental animals are shown in Tables 1 and 2. There was a significant increase in MDA levels in both plasma and tissues of animals exposed to hypoxia and 58 and 68.5% fall in GSH levels of plasma and liver, respectively, in animals exposed to hypoxia. Significant reduction of blood and muscle GSH values were also observed during hypoxia. Supplementation of 10 mg BC/kg BW under normoxia did not alter GSH levels in plasma and muscle. How-

Table 1  
Effect of BC supplementation on MDA levels in plasma and tissues of albino rats

Groups	Plasma (μmoles/ml)	Blood (μmoles/ml)	Muscle (μmoles/g)	Liver (μmoles/g)
Control	2.6 ± 0.4	3.6 ± 0.9	46.9 ± 7.2	141.8 ± 13.5
Hypoxia	4.4 ± 1.6 <sup>a</sup>	5.4 ± 1.2 <sup>a</sup>	60.0 ± 8.0 <sup>a</sup>	215.9 ± 61.0 <sup>a</sup>
BC	2.2 ± 0.5 <sup>b</sup>	3.96 ± 1.1 <sup>b</sup>	59.2 ± 19.8	133.9 ± 22.9 <sup>b</sup>
Hypoxia + BC	1.7 ± 0.1 <sup>a,b</sup>	3.78 ± 0.6 <sup>b</sup>	50.4 ± 14.3	142.7 ± 53.0 <sup>b</sup>

All values are mean ± S.D. ( $n = 6$ );  $P < 0.05$ ; BC, β-carotene.

<sup>a</sup> Versus control

<sup>b</sup> Versus hypoxia

Table 2  
Effect of BC supplementation on GSH levels in plasma and tissues of albino rats

Groups	Plasma ( $\mu\text{mole/ml}$ )	Blood ( $\mu\text{mole/ml}$ )	Muscle ( $\mu\text{mole/g}$ )	Liver ( $\mu\text{mole/g}$ )
Control	21.2 $\pm$ 10.0	109.0 $\pm$ 3.0	0.29 $\pm$ 0.08	3.05 $\pm$ 1.0
Hypoxia	12.4 $\pm$ 3.1 <sup>a</sup>	93.0 $\pm$ 12.0 <sup>a</sup>	0.22 $\pm$ 0.06 <sup>a</sup>	2.0 $\pm$ 0.6 <sup>a</sup>
BC	18.9 $\pm$ 8.6	102.0 $\pm$ 5.0 <sup>a</sup>	0.29 $\pm$ 0.09	2.9 $\pm$ 0.2 <sup>a</sup>
Hypoxia + BC	18.2 $\pm$ 7.8	122.0 $\pm$ 13.0 <sup>a,b</sup>	0.33 $\pm$ 0.06 <sup>b</sup>	3.3 $\pm$ 0.6 <sup>b</sup>

All values are mean  $\pm$  S.D. ( $n = 6$ );  $P < 0.05$ ; BC,  $\beta$ -carotene.

<sup>a</sup> Versus control

<sup>b</sup> Versus hypoxia

ever, the same dose under hypoxia showed a significant increase in muscle and liver GSH values.

The changes in GPx activity in whole blood are depicted in Fig. 1. Upon exposure to hypoxia, a significant fall in blood GPx levels was seen as compared with control. BC supplementation resulted in higher GPx levels during normoxia and it completely arrested the fall in GPx levels during hypoxia.

Plasma protein values were reduced during hypoxia, while BC supplementation significantly prevented the fall in their level (Fig. 2). As expected, the RBC, WBC and Hb% content was significantly increased in rats exposed to hypoxia (Table 3). However, BC supplementation had very little effect on these changes.

The BC content in plasma of various groups of animals studied is shown in Fig. 3. In animals exposed to hypoxia, the BC content was found significantly lower than the control. Whereas, the animals supplemented with BC showed increase in plasma BC content both under normoxia and hypoxia.

#### 4. Discussion

Hypoxia is well known to produce oxidative stress in organisms. Various oxyradicals if not neutralized, cause damage to bio-membranes which is reflected by lipid peroxidation. Supplementation of antioxidants like vitamin E, vitamin C and BC were found to inhibit the cellular damage induced by oxidative stress (Burton and Traber, 1990; Simon-Schnass, 1992; Palozza et al., 1997). BC has been reported to have a potent antioxidant effect, which is 100 times higher than that of vitamin E (Vile and Winterbourn, 1988).

In the present study the animals exposed to hypoxia showed a significant increase in plasma and tissue MDA levels. This increase may be attributed to the augmentation of FR production. Hypoxia is known to increase MDA levels significantly at HA and even in in-vitro conditions (Younes et al., 1992; Simon Schnass, 1996). When animals were supplemented with antioxidant i.e. BC the MDA level in plasma and tissues decreased significantly, which may be due to decreased oxidative stress. As reported by authors (Whittaker et

al., 1996), BC is a potent antioxidant that quenches the singlet oxygen and other FR. The present findings are in agreement with the results reported on the antioxidant effects of BC at HA by Palozza et al. (1997), Vile and Winterbourn (1988).

In the present study, levels of reduced glutathione (GSH) in blood, plasma, muscle and liver were decreased in animals exposed to hypoxia, while BC supplementation clearly enhanced the GSH levels. Interestingly, the normoxia group supplemented with BC showed unmodified liver GSH level, indicating that during the stress (Hypoxia), GSH synthesis is increased to cope up with the oxidative stress which is an adaptive phenomenon. There was a decrease in GPx activity in animals exposed to hypoxia, which could be due to the higher production of oxygen free radicals (OFR). In the presence of BC, GPx levels were restored back to control levels. This again confirms the fact that BC prevents the OFR formation, is also in accordance with the earlier findings (Desai et al., 1997).

Hypoxia resulted in reduction of plasma protein values. It was reported that in stressful environment (HA), protein catabolism takes place resulting in reduced protein levels (Klain and Hannan, 1970). Hypoxia also causes anorexia during the initial stages of acclimatiza-

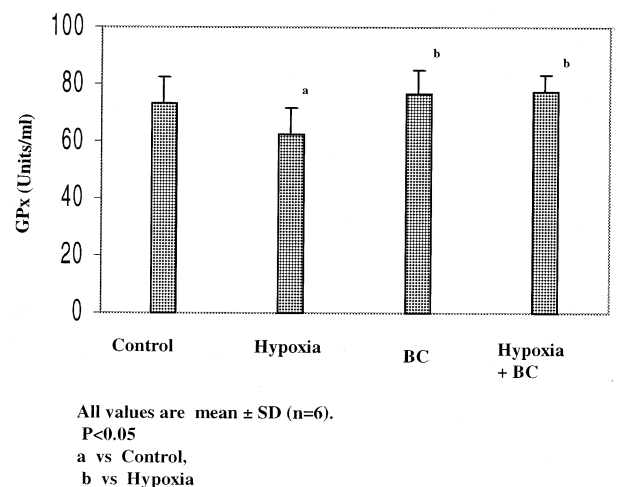
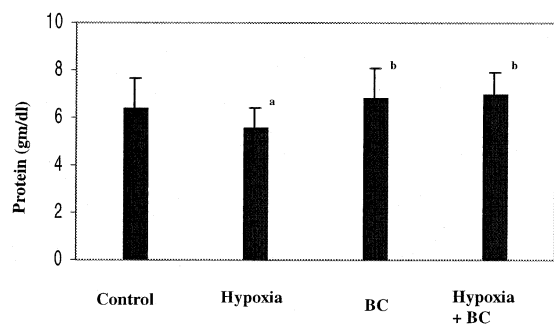


Fig. 1. Effect of BC supplementation on blood GPx levels in albino rats.



All values are mean  $\pm$  SD (n=6).

P<0.05

a vs Control

b vs Hypoxia

Fig. 2. Effect of BC supplementation on plasma protein levels in albino rats.

tion to HA (Kumar et al., 1999), leading to hypophagia, decrease in plasma protein, and loss of BW (Singh et al., 1996). BC supplementation under hypoxia showed significant increase in plasma protein value.

Increase in RBC count and Hb concentration is a result of long-term exposure to HA (Rodriguez et al., 2000). In the present study we have found that hypoxia increased the Hb% concentration, RBC and WBC count significantly. The rise in Hb% content is mainly bound to the increased haem-concentration (Picon-Reategui et al., 1970). It is likely that due to more oxygen demand in the tissue, a release of RBC from certain reservoirs in the body takes place. In the present study, BC supplementation did not change the rise in Hb% concentration, RBC and WBC count. This shows that BC acts as an antioxidant only in reducing the oxidative stress at HA and did not alter the body's adaptive phenomenon to stress. The fall of BC content in animals exposed to hypoxia indicates its utilization by tissues to neutralize the FRs. BC supplementation results in maintenance of higher BC levels in plasma which will be beneficial to protect the cells from oxidative damage.

The present findings reveal that BC has potent an-

Table 3

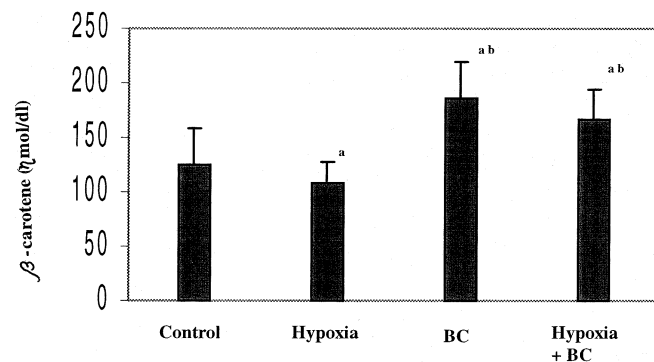
Effect of BC supplementation on haemoglobin concentration, RBC and WBC counts in albino rats

Groups	Hb (g/dl)	R.B.C ( $\times 10^{12}/l$ )	W.B.C $\times 10^9$ (l)
Control	14.6 $\pm$ 0.66	5.9 $\pm$ 0.5	4.35 $\pm$ 1.8
Hypoxia	17.6 $\pm$ 0.9 <sup>b</sup>	7.2 $\pm$ 0.3 <sup>a</sup>	7.3 $\pm$ 1.1 <sup>a</sup>
BC	15.5 $\pm$ 0.7 <sup>a,b</sup>	6.5 $\pm$ 0.5 <sup>a,b</sup>	3.8 $\pm$ 0.4 <sup>b</sup>
Hypoxia + BC	17.5 $\pm$ 0.5 <sup>a</sup>	7.7 $\pm$ 0.7 <sup>a</sup>	6.4 $\pm$ 1.2 <sup>a</sup>

All values are mean  $\pm$  S.D. (n = 6); P<0.05; BC,  $\beta$ -carotene.

<sup>a</sup> Versus control

<sup>b</sup> Versus hypoxia



All values are mean  $\pm$  SD (n=6).

P<0.05

a vs control

b vs hypoxia

Fig. 3. Effect of BC supplementation on plasma BC concentration in albino rats.

tioxidant effect in reducing the oxidative stress induced by hypobaric hypoxia. Further, these data provide information regarding the possible use of BC as a nutritional supplement in amelioration of hypoxia-induced oxidative stress at HA.

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