β-Carotene affects antioxidant status in non-insulin-dependent diabetes mellitus

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Abstract

Oxidative injury by reactive oxygen species (ROS) has been suggested to explain excess prevalence of vascular complications in diabetes mellitus. ROS are normally trapped by antioxidants such as β-carotene, vitamins C and E and glutathione. The consumption of reducing equivalents in the form of NADPH may interfere with the regeneration of glutathione and ascorbic acid in diabetes mellitus. We have compared 20 patients with non-insulin-dependent diabetes mellitus (NIDDM) with no vascular complications to age and sex matched healthy control subjects, and later treated them with natural (Dunaliella) β-carotene, 60 mg daily for 3 weeks. Compared with control patients there was a significant decrease in plasma glutathione by 50% (P < 0.01) and in erythrocyte glutathione peroxidase by 30% (P < 0.01) in the patients with NIDDM. Upon β-carotene treatment there was a 3.3-fold increase in plasma β-carotene as measured by high-performance liquid chromatography (HPLC; from 0.296 ± 0.072 to 0.968 ± 0.133 μg/ml, P < 0.001). Plasma glutathione increased by 77%, erythrocyte glutathione increased by 39% (P < 0.05) and glutathione peroxidase increased by 21% (P < 0.01). Thus, basal plasma glutathione, erythrocyte glutathione and erythrocyte glutathione peroxidase levels are higher in age matched healthy controls than in NIDDM patients. Natural β-carotene affects the glutathione and part of its redox cycle enzymes by potential augmentation of its regeneration. Restoring antioxidant status may slow the rate of development of vascular complications in diabetes mellitus. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Antioxidants; β-Carotene; Glutathione redox cycle; Non-insulin-dependent diabetes mellitus (NIDDM)

1. Introduction

Diabetes mellitus is associated with accelerated development of vascular complications which exceeds that expected from the classic risk factors [1]. Recent observations suggest that the damage which occurs when plasma and tissue constituents are exposed to hyperglycemia is mediated by auto-oxidation and glycation which leads to the production of reactive oxygen species (ROS) [2]. ROS, if not inactivated by antioxidative enzymes (catalase, superoxide dismutase and glutathione peroxidase) and nutrients (carotenoids, vitamins C and E, glutathione and lipoic acid) cause peroxidation of membrane lipids and induce oxidative modification of low-density lipoprotein (LDL) which results in vascular dysfunction and foam cell accumulation [3,4]. High requirement for chain-breaking antioxidants and the augmentation of the polyol pathway (during the conversion of glucose to sorbitol) increases the consumption of reducing equivalents in the form of NADPH and may impair the regeneration of glutathione and ascorbic acid [5]. Indeed, a variety of defects in antioxidative status have been previously reported in experimental and in diabetic patients when compared with a control population [6,7]. Consequently, there is some evidence about the benefit from supplementation of antioxidants (vitamins C and E, probucol and lipoic acid) to patients with diabetes mellitus [8–10]. The crucial importance of this topic has been recently presented and discussed at a workshop on antioxidative treatment in diabetes mellitus [11].

β-Carotene is a potent antioxidant in biological systems and incorporates into LDL and other lipid struc-
Glutathione transferase (GST) is a family of enzymes that play a crucial role in the detoxification of various toxic substances, including reactive oxygen species (ROS). In diabetes mellitus, we have proposed that the supplementation of ROS in diabetes mellitus, we have proposed that the utilization of a β-carotene preparation rich in the natural occurring 9-cis isomer results in preferred antioxidative activity both in vitro and ex-vivo. Thus, this natural stereo-isomer necessarily behave in a different way compared with the all-trans synthetic β-carotene which has been traditionally used in most studies [19,20]. Considering the excessive generation of ROS in diabetes mellitus, we have proposed that the supplementation of β-carotene to these patients may oppose the defect in their antioxidative status. The suggested regeneration of antioxidative nutrients (glutathione, ascorbic acid and vitamin E) is maintained by a synergistic process between them [21].

2. Materials and methods

Blood was taken from 20 patients with non-insulin-dependent mellitus (NIDDM) with a 7 ± 3-year duration of disease, a mean age of 52 ± 8 years, fasting blood glucose of 200 ± 60 mg/dl, who were in general good health and uncomplicated by vascular, renal or neural complications. Patients were not taking any food supplements and were not smoking. The patients were compared to an age and sex matched healthy control group. β-Carotene was provided at a dose of 60 mg/day for 3 weeks as Dunaliella preparation composed of 50:50 all-trans:9-cis isomers ratio [14–16] three times per day with meals. Plasma was separated after centrifugation at 4°C for 20 min at room temperature.

For erythrocyte enzymes, heparinized blood was kept in ice. Erythrocytes were separated using ficoll buffer and centrifuged at 4°C for 20 min at 1700 rpm. Erythrocytes were rewashed for measurement of enzymes.

Plasma β-carotene was analyzed by a three-dimensional HPLC system [16]. Plasma and erythrocyte glutathione was measured by DTNB glutathione reductase method [22]. Plasma and erythrocyte glutathione peroxidase was measured by the method of Kokatnur and Jelling [23]. Erythrocyte glutathione reductase was analyzed by Beutler’s method [24] and erythrocyte glutathione S-transferase was assayed by the method of Habig et al. [25].

2.1. Statistics

All the measurements were done in triplicates. Statistical analysis comparing baseline to 3 weeks β-carotene treatment used paired Student’s t-test. Statease program (version 1.00 Dataplus System, NY) was used for computations. All the results represent mean ± SEM.

3. Results

Compared with healthy control subjects, there was a 50% decrease in plasma glutathione (P < 0.01) and a 13% decrease in plasma glutathione peroxidase. There was a 30% decrease in erythrocyte glutathione peroxidase (P < 0.01), a 27% decrease in erythrocyte glutathione transferase (P < 0.01) and a decrease in glutathione reductase by 46% without and by 39% with FAD (Table 1).

β-Carotene treatment was well-tolerated by all the patients without any side effects. No skin carotenemia was noted. Patients kept their normal life activity and diet, with stable weight and no change in routine blood tests (including renal, liver and electrolytes) and in diabetic metabolic control. (Fasting blood glucose and lipids were not altered upon β-carotene treatment.)

Regarding β-carotene plasma concentration, upon 3 weeks treatment there was a significant 3.3-fold increase in plasma β-carotene (all-trans isomer) from 0.296 ± 0.072 to 0.968 ± 0.133 μg/ml (P < 0.001) (Fig. 1A).

Plasma glutathione increased by 77% (Fig. 1B), whereas erythrocyte glutathione increased by 39% (P < 0.05) (Fig. 1C). Upon β-carotene treatment, plasma glutathione peroxidase increased from 0.095 ± 0.006 to 0.115 ± 0.017 U/ml plasma.

Concerning erythrocyte antioxidative enzymes, glutathione peroxidase increased by 21% (P < 0.01) (Fig. 2A). Glutathione transferase dropped by 75% (P < 0.01) (Fig. 2B). Glutathione reductase did not change (Fig. 2C) upon β-carotene treatment nor when FAD was added to the reaction (data not shown). Thus, β-carotene treatment resulted in a significant elevation of glutathione and part of its enzymes.

Table 1
Glutathione redox cycle in patients with non-insulin-dependent diabetes mellitus

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 20)</th>
<th>Patients (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (nmol/ml)</td>
<td>1.240 ± 0.191</td>
<td>0.614 ± 0.091**</td>
</tr>
<tr>
<td>Erythrocytes (nmol/grHb)</td>
<td>4.768 ± 0.355</td>
<td>4.502 ± 0.707</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (U/ml)</td>
<td>0.109 ± 0.007</td>
<td>0.095 ± 0.006</td>
</tr>
<tr>
<td>Erythrocytes (U/grHb)</td>
<td>32.654 ± 0.953</td>
<td>22.737 ± 1.692**</td>
</tr>
<tr>
<td>Glutathione transferase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(U/grHb)</td>
<td>51.416 ± 3.706</td>
<td>37.413 ± 4.783*</td>
</tr>
</tbody>
</table>

* All results are mean ± S.E.M.
* P < 0.05.
** P < 0.01.
4. Discussion

The glutathione plasma and erythrocyte antioxidative enzymes were lower in patients with NIDDM, compared to control subjects. There is evidence that hyperglycemia results in auto-glycooxidation of sugars, lipoproteins and proteins. This protein generates free radicals [26]. Also, the polyol pathway results in a decrease in NADPH/NADP in tissues exposed to hyperglycemia (due to increased aldose reductase activity) with resulting decrease in glutathione and its redox cycle enzymes [5,27].

Glutathione is required for the generation of vitamin C from dehydroascorbic acid by the enzyme dehydroascorbate reductase. This later reaction also requires the regeneration of reduced glutathione from oxidized glutathione [27]. Ascorbic acid is linked to the genera-

Fig. 1. Plasma (B) and erythrocyte (C) glutathione before (empty bar) and upon 3 weeks β-carotene supplementation (60 mg daily) (black bars) to 20 patients with NIDDM (*P < 0.01). All-trans β-carotene was measured by high performance liquid chromatography (A).

Fig. 2. The glutathione redox cycle enzymes, glutathione peroxidase (A), glutathione transferase (B) and glutathione reductase (C) before (empty bars) and upon 3 weeks β-carotene supplementation (60 mg daily) (black bars) to 20 patients with NIDDM (*P < 0.01). All results mean ± S.E.M.
Glutathione peroxidase inactivates lipid peroxides in a reaction which depends on the provision of glutathione and selenium from dietary resources. Apparently, the supplementation of β-carotene may involve the same mechanisms which protect against glutathione consumption, thus enhancing low antioxidant capacity in the patients with diabetes mellitus.

Recent investigations have attracted attention to the role of glutathione S-transferase in detoxification mechanisms [31]. It is believed that this enzyme protects the DNA in general, especially in heavy smokers, against the damaging effects of free radicals. The significant drop in the activity of this enzyme upon β-carotene treatment in diabetes has not been documented before, and its cause and consequence are not clear. We are left with the speculation that due to the operation of another antioxidant (β-carotene) against free radicals, the activity of the enzyme is reduced. Also, reduction in glutathione transferase activity may explain the paradoxical results of studies showing a harmful effect of β-carotene supplementation [19,20]. An alternative explanation to these results involves the oxidation of β-carotene by free radicals (such as in heavy smokers). In support of this data, we have observed a significant increase in the generation of oxidation products in plasma which was exposed to a free radical generating system AAPH from subjects supplemented with β-carotene (unpublished data). It has to be emphasized that natural 9-cis β-carotene has not been previously employed in such studies [32]. Excess morbidity, suggested to be related to β-carotene auto-oxidation has not been related to a high intake from natural (dietary) resources (fruits and vegetables). Further studies are essential for the elaboration of the role of glutathione S-transferase in patients with diabetes as compared to other conditions (mostly related to cancer development) where the potential importance of this enzyme is being elucidated.

In conclusion, the hypothesis that antioxidants may protect patients with NIDDM against complications is promising and supported by our data showing the augmentation of the glutathione and part of its redox cycle enzyme activity upon β-carotene supplementation.

References