Effect of the pyridoindole antioxidant stobadine on development of experimental diabetic cataract and on lens protein oxidation in rats: comparison with vitamin E and BHT

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Purpose: The aim of this study was to investigate the effect of dietary supplementation with the pyridoindole antioxidant stobadine on the development of diabetic cataract in rats. The findings were compared with the effect of the natural antioxidant vitamin E and the well known phenolic synthetic antioxidant butylated hydroxytoluene.

Methods: Streptozotocin induced diabetic male Wistar rats were fed for 18 weeks a standard diet or a diet supplemented with stobadine (0.05% w/w), vitamin E (0.1% w/w), butylated hydroxytoluene (BHT, 0.4% w/w), or a mixture of stobadine (0.05% w/w) and vitamin E (0.1% w/w). The progress of cataract was monitored biweekly by ophthalmoscopic inspection. Plasma glucose and body weight were recorded regularly. At the end of the experiment, the content of free sulphhydril and carbonyl was determined in total lens proteins and in the stobadine group plasma levels of malondialdehyde were also measured.

Results: Long term treatment of diabetic animals with stobadine (STB), vitamin E, or BHT led to a marked delay in the development of advanced stages of cataract. At the end of the experiment, the visual cataract score was significantly decreased in the diabetic groups treated with stobadine or BHT, while vitamin E had no significant effect. Unexpectedly, combined treatment with STB+vitamin E advanced the progression of the higher stages of cataract, though without affecting the overall visual cataract score. Neither of the antioxidants exerted an effect on the glycemic state or body weight of the animals. Biochemical analyses of eye lens proteins showed significant diminution of sulphhydril groups and elevation of carbonyl groups in diabetic animals in comparison to healthy controls. Dietary supplementation with any of the antioxidants studied did not influence the levels of these biomarkers significantly. Nevertheless, in diabetic animals, stobadine supplementation significantly attenuated plasma levels of malondialdehyde, an index of systemic oxidative damage.

Conclusions: The results are in accordance with the postulated pro-oxidant role of chronic hyperglycemia, however, the direct oxidative free radical damage of eye lens proteins does not seem to be the key mechanism effective in the development of diabetic cataract. Sugar cataractogenesis appears to be a complex process, in which multiple mechanisms may be involved, including consequences of the overt oxidative stress in diabetes (e.g., protein modifying potential of toxic aldehydes generated as byproducts of carbohydrate autoxidation and lipid peroxidation). The ability of stobadine to attenuate lipoxidation reactions in diabetes may account, at least partly, for its observed anticataract action. Mechanisms involving reduction of mitochondrial damage by stobadine are also discussed.

Cataractogenesis is one of the earliest secondary complications of diabetes mellitus. Since extracellular glucose diffuses into the lens uncontrolled by the hormone insulin, the lens is one of the most affected body parts in diabetes mellitus. We believe that diabetic cataract is a disorder that requires a biochemical and pharmacological, rather than a surgical solution. First of all, tight metabolic control remains the primary intervention in prevention of lens opacification. However, pharmacological blockade of biochemical events triggered by disposal of excess glucose could be required.

The proteins of the lens are extremely long lived and there is virtually no protein turnover, which provides great opportunities for post-translational modification to occur. Multiple mechanisms have been implicated in the development of cataract in diabetes [1-3]. To date, the exact sequence of events which lead to opacification has not been clearly defined. Thus the relationship of the opacity to the initiating event may be obscure.

Free radical production is increased in the diabetic lens, while natural antioxidant defenses are compromised and thus increased oxidative stress results. Shifts in redox balances due to derangement in energy metabolism of carbohydrates and lipids also contribute to the overt oxidative stress in the diabetic individual. Human studies, in vitro, and in vivo animal experiments strongly suggest that there is an association between increased oxidative stress and the development of cataract [4-8]. Antioxidant supplementation was found to inhibit the development of cataract in experimentally induced diabetes.
tes in rats [9-13]. Several clinical studies pointed to a diminu-
tion of cataract incidence following adequate supply of anti-
oxidants in food [14-16].

Numerous studies proved stobadine (STB), a novel syn-
thetic pyridoindole, to be an efficient antioxidant [17]. Re-
cently, under conditions of an experimental glycation model
in vitro, stobadine was found to protect bovine serum albu-
min against glyco-oxidative damage [18]. Using a model of
streptozotocin-diabetic rats in vivo, stobadine was found to
attenuate pathological changes in the diabetic myocardium
[19,20] and kidneys [20,21], to decrease matrix collagen cross-
linking [21], and to reduce plasma cholesterol and triglycer-
ide levels in diabetic animals [19,22]. Stobadine treatment
normalized calcium homeostasis in the diabetic heart and liver
[22] and produced a beneficial effect on leukocyte function in
diabetic rats [23]. These findings, along with its high oral
bioavailability [24], efficient detoxification pathways [25,26],
and toxic safety [27,28] render stobadine a promising agent in
prevention of late diabetic complications.

Butylated hydroxytoluene (BHT), a well known synthetic
antioxidant, was found in numerous studies to delay or pre-
vent sugar cataractogenesis in rats [10,29-31]. In their clinical
studies, Robertson et al. [32,33] and Rouhiainen [34] showed
a significantly reduced risk of cataract after increased intake
of the natural antioxidant vitamin E. Equivocal effects of vita-
min E were described in its relation to sugar cataract develop-
ment in rodents; a significant prevention of cataractogenesis
[9,11,35] or an improvement of lens biochemical indices, yet
without affecting the visual cataract score [36,37].

The present study investigated the effect of dietary supple-
mentation with stobadine, vitamin E and butylated hydroxy-
toluene (BHT) on the progression of eye lens opacification in
streptozotocin-diabetic rats during a period of 18 weeks. The
effect of combined stobadine and vitamin E treatment was
also studied. At the end of the experiment, markers of lens
protein glyco-oxidation were determined and effects of the
antioxidants evaluated.

METHODS

Disease model: This investigation conforms with the Guide
for the Care and Use of Laboratory Animals. The study was
approved by the Ethics Committee of the Institute and per-
formed in accordance with the Principles of Laboratory Ani-
mal Care (NIH publication 83-25, revised 1985) and the Slo-
vak law regulating animal experiments (decree 289, part 139,
July 9, 2003). Male Wistar rats, 8-9 weeks old, weighing 200-
230 g, were used. The animals came from the Breeding Faci-

ty of the Institute of Experimental Pharmacology, Dobra Voda
(Slovak Republic). Experimental diabetes was induced by a
single intravenous dose of streptozotocin (STZ, 55 mg/kg).
STZ was dissolved in 0.1 mol/l citrate buffer, pH 4.5. The
animals were fasted overnight prior to STZ administration.
Control animals received 0.1 mol/l citrate buffer. Water and
food were available immediately after dosing. Ten days after
STZ administration, all animals having a plasma glucose level
>15 mmol/l were considered diabetic and were randomly di-
vided into the following experimental groups (n=8): untreated
diabetic rats (group D); diabetic rats treated with STB (0.05% w/w stobadine dipalmitate, group D+STB); diabetic rats treated
with vitamin E (0.1% w/w D-L-alpha-tocopheryl acetate, group
D+E); diabetic rats treated with BHT (0.4% w/w t-butyl hy-
droxytoluene, group D+BHT); and diabetic rats treated with
the mixture of STB (0.05% w/w stobadine dipalmitate) and
vitamin E (0.1% w/w D-L-alpha-tocopheryl acetate, group
D+STB+E). Five control groups (C, C+STB, C+E, C+BHT,
and C+STB+E) corresponding to the diabetic experimental
groups were created (n=4). The animals were treated for a
period of 18 weeks beginning 10 days after either vehicle or
STZ injection. During the experiment the animals were housed
in groups of two in cages of the type T4 Velaz (Prague, Czech
Republic) with bedding composed of wood shavings (changed
daily). Tap water and pelleted standard diet KKZ-P-M (Dobra
Voda, Slovak Republic) were available ad libitum. The ani-
mal room was air conditioned and the environment was con-
tinuously maintained at a temperature of 23±1 °C and a re-
lative humidity of 40-70%.

Blood measurement: Heparinized blood samples were
taken from rat tails after overnight fasting of the animals.
Plasma glucose levels were measured using the commercial
Glucose (Trinder) kit (Sigma, St. Louis, MO).

For detection of malondialdehyde (MDA) in plasma
samples, the modified 2,4-dinitrophenylhydrazine (DNPH)
derivatization method was used [38]. Plasma samples (250
µl) taken from heparinized blood were hydrolyzed for 30 min
with 0.5 µl 6 M NaOH at 60 °C. After subsequent precipita-
tion with 200 µl of 30% TCA, the samples were centrifuged
(7,000 rpm/10 min) and 300 µl of the supernatant derivatized
with 30 µl of 5 M DNPH in 12 M HCl at laboratory tempera-
ture for 10 min. Then the samples were extracted twice with 2
ml of n-hexane, evaporated to dryness and finally analyzed
by HPLC on a reversed phase C(18) column with UV detec-
tion (310 nm).

For stobadine determination, plasma samples (1 ml) were
diluted with isotonic saline up to 3 ml and precipitated with 1
ml of ice cold 30% TCA. After subsequent centrifugation
(3,000 rpm/15 min), the pH was adjusted to 10-11 with 5 M
NaOH. These alkaline solutions, after addition of a known
amount of the internal standard N-propyl analog of stobadine,
were extracted with chloroform (3x2.5 ml). These extracts
were treated with anhydrous sodium sulfate and then evaporated to
dryness under N2 at 37 °C. The residues were dissolved in 25
µl of methanol and analyzed by GC (GC HP 5890) using an
HP-5 (12 m x 0.2 mm x 0.33 μm) column and the HP 5970
Mass Selective Detector.

Evaluation of cataract development: The progress of cata-
ракt was monitored biweekly by a hand held ophthalmoscope
equipped with a slit lamp by an individual without prior knowl-
edge of affiliation of the animal to an experimental group.
Eye inspection was preceded by topical administration of 1% mydriacyl drops. Cataract formation was scored essentially
according to the classification of Ao et al. [39] or
Suryanarayana et al. [40] as follows: clear normal lens (O),
peripheral vesicles (I), peripheral vesicles and cortical opaci-
ties (II), diffuse central opacities (III), and mature cataract (IV).
Cataract formation was considered complete (grade IV) when the red fundus reflex was no longer visible through any part of the lens and the lens appeared dull white to the naked eye.

Lens preparation: At the end of week 18, the rats were killed and the eye globes were excised. The lenses were then dissected, rinsed with ice cold saline, and frozen in saline for preservation. Each pair of lenses was homogenized in a glass homogenizer with a teflon pestle in 1 ml of ice cold phosphate buffer (20 mM, pH 7.4) saturated with nitrogen. The total homogenate was used for further analyses.

Protein determination: Total protein concentration in the lens homogenate was determined by the Lowry method [41] using bovine serum albumin as a standard.

Carbonyl determination: The content of free carbonyl in the total lens proteins was determined by the procedure of Levine et al. [42] using the 2,4-dinitrophenylhydrazine (DNPH) reagent. Two 0.2-ml aliquots of lens homogenate containing approximately 3 mg of proteins were precipitated with equal volume of 10% trichloroacetic acid (TCA), and after centrifugation, the pellets were treated with 0.5 ml of 10 mM DNPH dissolved in 2 M HCl as a sample or with 0.5 ml of 2 M HCl as a control blank. The reaction mixtures were allowed to stand for 1 h at room temperature, with stirring at 10 min intervals. Next, 0.5 ml of ice cold 20% TCA was added and the sample was left on ice for 15 min. The precipitated proteins were subsequently washed three times with 1 ml of ethanol-ethyl acetate (1:1). The washed pellets were dissolved overnight in 1.8 ml of 6 M guanidine. Any insoluble material was removed by centrifugation at 3,000 rpm for 15 min. The difference spectrum of the DNPH derivatized samples compared to HCl controls was scanned at 322-370 nm on a Hewlett Packard 8452A Diode Array Spectrophotometer. Carbonyl content was calculated from the absorbance readings, using 22,000 M$^{-1}$ cm$^{-1}$ as the molar absorption coefficient. The final values were normalized to actual protein amount determined on the basis of absorbance readings at 280 nm of parallel HCl treated control blanks.

Sulfhydryl determination: The content of sulfhydryl groups in lens proteins was determined using Ellman’s procedure as modified by Altomare et al. [6]. Aliquots (0.2 ml) of total lens homogenate of approximately 3 mg of proteins were precipitated with an equal volume of 4% sulfosalicylic acid (SSA). The pellets obtained after centrifugation were washed with 1 ml of 2% SSA to remove free thiols. The washed pellets were dissolved in 0.2 ml of 6 M guanidine (pH 7.4) and read spectrophotometrically at 412 nm and 530 nm, before and after 30 min incubation in the dark with 50 µl of 10 mM DTNB (5,5’-dithiobis[2-nitrobenzoic acid]). The content of protein sulfhydryls was calculated using a calibration curve prepared with reduced glutathione.

Reagents: Streptozotocin (STZ), 2,4-dinitrophenylhydrazine (DNPH), and DTNB (5,5’-dithiobis[2-nitrobenzoic acid]) were obtained from Sigma. Other chemicals were purchased from local commercial sources and were of analytical grade quality.

Statistical analysis: Statistical analysis of the data was done using the unpaired Student’s t-test and the Mann-Whitney U-test.

**RESULTS**

**Blood glucose and body weight:** The average blood glucose levels of the control and diabetic experimental groups at the beginning and at the end of the 18 week experiment are given in Table 1. Persistent hyperglycemia, on average over 15 mmol/l, was recorded in diabetic animals throughout the whole experiment. At the end of the experiment, the weights of diabetic rats were significantly lower as compared with those in control rats. The experimental groups are: control rats on standard diet (C); control rats treated with stobadine (0.05% w/w stobadine dipalmitate; C+STB); control rats treated with vitamin E (0.1% w/w DL-alpha-tocopheryl acetate; C+E); control rats treated with t-butyl hydroxytoluene (0.4% w/w t-butyl hydroxyltoluene; C+BHT); control rats treated with a mixture of stobadine (0.05% w/w stobadine dipalmitate) and vitamin E (0.1% w/w DL-alpha-tocopheryl acetate; C+STB+E); untreated diabetic rats (D); diabetic rats treated with stobadine (0.05% w/w stobadine dipalmitate; D+STB); diabetic rats treated with vitamin E (0.1% w/w DL-alpha-tocopheryl acetate; D+E); diabetic rats treated with t-butyl hydroxyltoluene (0.4% w/w t-butyl hydroxyltoluene; D+BHT); and diabetic rats treated with a mixture of stobadine (0.05% w/w stobadine dipalmitate) and vitamin E (0.1% w/w DL-alpha-tocopheryl acetate; D+STB+E).

<table>
<thead>
<tr>
<th>Table 1. Blood glucose levels (mmol/l) of rats in the 18 week experiment</th>
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<tr>
<td><strong>Control groups</strong></td>
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<td>Treatment</td>
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<tr>
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</tr>
<tr>
<td>C</td>
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<td>C+STB</td>
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<td>C+E</td>
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<td>C+BHT</td>
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<td>C+STB+E</td>
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<table>
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<th>Table 2. Body weight (g) of rats in the 18 week experiment</th>
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<tr>
<td><strong>Control groups</strong></td>
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<tr>
<td>Treatment</td>
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<tr>
<td>C</td>
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<td>C+STB</td>
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<td>C+E</td>
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<td>C+BHT</td>
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Figure 1. Cataract progression in STZ-diabetic rats treated with antioxidants based on biweekly scoring of lens opacity. Cataract formation was scored biweekly according to the following classification: clear normal lens (O), peripheral vesicles (I), peripheral vesicles and cortical opacities (II), diffuse central opacities (III), mature cataract (IV). A: Untreated diabetic rats (D; red). B: Diabetic rats treated with stobadine (0.05% w/w stobadine dipalmitate, D+STB; blue). C: Diabetic rats treated with vitamin E (0.1% w/w D,L-alpha-tocopheryl acetate, D+E; green). D: Diabetic rats treated with BHT (0.4% w/w t-butyl hydroxytoluene, D+BHT; violet). Statistical analysis of the data was done using the Mann-Whitney U-test. The single asterisk indicates a p<0.05, the double asterisk indicates a p<0.01, and the triple asterisk indicates a p<0.001 compared to untreated diabetic rats (D; red).
the control group (Table 2). Administration of the antioxidants did not significantly affect blood glucose and body weights either in control or diabetic animals.

**Cataract formation:** As shown in Figure 1A, in untreated diabetic rats, appearance of advanced stages of cataract (stage III and IV) became apparent after 10 weeks. In stobadine treated diabetic animals (Figure 1B), the advanced stages of cataract formation were postponed by 6 weeks, while a 4 week delay was observed in the diabetic groups administered vitamin E (Figure 1C) or BHT (Figure 1D). The values of the average cataract score were significantly (Mann-Whitney U-test) lower in treated than untreated diabetic groups (1) for vitamin E (Figure 1B) and vitamin E and BHT treated diabetic animals (Figure 1D) respectively (p<0.05 for D+STB compared to D; not significant for D+E compared to D (p=0.1120); p<0.01 for D+BHT compared to D, Mann-Whitney U-test.)

Unexpectedly, in diabetic animals treated with the combination of STB and vitamin E, advanced stages of cataract III and IV appeared as early as in week 12 (Figure 2). In spite of the fact that in this experimental group and in untreated diabetic animals progression of the average cataract score values was very close through weeks 12 to 18 (see Table 3), development of the final stage IV was apparently faster in the diabetic rats treated with the combination of STB and vitamin E.

None of the control rats untreated or administered the antioxidants studied developed lens opacity.

**Changes in free carboxyls and sulfhydryls of lens proteins:** The diabetic state led to an increase in free carbonyl and sulfhydryl groups of lens proteins as measured by absorbance of DNPH bound to total lens proteins (Figure 3A). At week 18 of the experiment, significantly higher values of protein free carbonyl were observed in the lenses of diabetic animals compared to healthy controls (p<0.05). The increase in DNPH reactive carbonyl was accompanied by a decrease in protein free sulfhydryl groups titratable by DTNB (Figure 3B, p<0.001). The individually administered antioxidants studied did not significantly affect lens protein levels of free carbonyl and sulfhydryl, either in diabetic (Figure 3) or control (data not shown) animals. However, administration of the mixture STB+E to diabetic rats significantly stimulated depletion of free sulfhydryl groups in the total eye lens proteins (p<0.05, Student’s t-test).

**Plasma levels of MDA and stobadine:** At the end of the experiment (week 18), significantly (p<0.05) elevated plasma levels of MDA were observed in diabetic rats in comparison with healthy controls (Figure 4). Stobadine supplementation to diabetic animals resulted in decrease of plasma MDA level from 3.1±0.3 to 2.2±0.2 µmol/l. (p<0.05). Effects of other antioxidants were not evaluated.

At the end of week 18, average levels of stobadine in deproteinized plasma in diabetic animals treated with food

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**Table 3. AVERAGE CATARACT SCORES OF DIABETIC RATS IN THE 18 WEEK EXPERIMENT**

<table>
<thead>
<tr>
<th>Week</th>
<th>D</th>
<th>D+STB</th>
<th>D+E</th>
<th>D+BHT</th>
<th>D+STB+E</th>
</tr>
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<tbody>
<tr>
<td>8</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>10</td>
<td>1.1±0.5</td>
<td>0.0±0.0</td>
<td>0.2±0.1</td>
<td>0.1±0.1</td>
<td>0.8±0.4</td>
</tr>
<tr>
<td>12</td>
<td>1.9±0.4</td>
<td>0.1±0.1</td>
<td>0.8±0.3</td>
<td>0.5±0.3</td>
<td>2.1±0.6</td>
</tr>
<tr>
<td>14</td>
<td>2.5±0.5</td>
<td>0.8±0.2</td>
<td>1.5±0.6</td>
<td>1.0±0.5</td>
<td>2.4±0.6</td>
</tr>
<tr>
<td>16</td>
<td>2.9±0.5</td>
<td>2.3±0.4</td>
<td>2.7±0.4</td>
<td>1.5±0.5</td>
<td>2.6±0.6</td>
</tr>
<tr>
<td>18</td>
<td>3.5±0.2</td>
<td>2.8±0.3</td>
<td>3.1±0.3</td>
<td>1.9±0.5</td>
<td>3.2±0.4</td>
</tr>
</tbody>
</table>

The experimental groups are: untreated diabetic rats (D); diabetic rats treated with stobadine (0.05% w/w stobadine dipalmitate; D+STB); diabetic rats treated with vitamin E (0.1% w/w D,L-alpha-tocopheryl acetate; D+E); diabetic rats treated with t-butyl hydroxytoluene (0.4% w/w t-butyl hydroxytoluene; D+BHT); and diabetic rats treated with a mixture of stobadine (0.05% w/w stobadine dipalmitate) and vitamin E (0.1% w/w D,L-alpha-tocopheryl acetate; D+STB+E). Values shown are mean±SEM (n=8).

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Figure 2. Cataract progression in STZ-diabetic rats treated with the combination of STB and vitamin E based on biweekly scoring of lens opacity. Cataract formation was scored as described in Figure 1. The experimental group was diabetic rats treated with the mixture of stobadine (0.05% w/w stobadine dipalmitate) and vitamin E (0.1% w/w D.L-alpha-tocopheryl acetate; D+STB+E).

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fortified with stobadine dipalmitate (0.05% w/w), were determined to be 5.6±0.8 ng/ml.

**DISCUSSION**

In diabetic animals, the antioxidants tested (when administered individually) apparently delayed the onset of advanced stages of cataract (stage III and IV). Considering values of the average cataract scores, stobadine or BHT treatment was found to be more effective than that of vitamin E for which a significant effect was seen only in week 12. The anticataract action of BHT was described by other authors in galactosemic and STZ-diabetic rats [10,29-31,43]. Several clinical studies pointed to a diminution of incidence of cataract after an adequate supply of antioxidant vitamins in food [14-16,32-34]. Equivocal effects of the natural antioxidant vitamin E have been described in its relation to sugar cataract development in rodents: a significant prevention of cataractogenesis [9,11,35], or an improvement of lens biochemical indices, without affecting the visual cataract score [36,37].

At the end of our experiment (week 18), biochemical analyses of eye lens proteins showed significant diminution of sulfhydryl groups and elevation of carbonyl groups in diabetic animals in comparison to healthy controls. These results point to the pro-oxidant role of hyperglycemia and are in agreement with findings of other authors (discussed in the following paragraphs). In their study on human cataractous lenses, Boscia et al. [8] identified a quantitative threshold of protein oxidation above which clinically significant cataracts develop.

A progressive decrease of protein sulfhydryls was observed during the development of diabetic and senile cataracts [8,44-46]. Sulfhydryl oxidation is thought to be one of the main pathological events leading, through disulfide cross-linking and molecular aggregation, to protein precipitation and lens opacification [47-52].

The presence of increased levels of free carbonyls in proteins of the cataractous diabetic eye lens, as described also by others [6,8,53,54], can be interpreted as a result of oxidative insult initiated by hyperglycemia, since the DNPH assay [42,55] was found to be selective enough to discriminate between protein bound carbonyls produced by metal catalyzed oxidations and those formed in the early glycation steps [56,57].

Dietary supplementation with any of the antioxidants studied in our experiments did not influence the levels of the biomarkers (>CO, sulfhydryl) significantly, in spite of the fact that a markedly decreased incidence of cataract was observed in diabetic groups treated with STB or BHT at week 18 when compared with untreated diabetic animals. The results indicate that oxidative modifications of eye lens proteins demonstrated by a decrease of sulfhydryl and increase of >CO groups may not be directly associated with the development of diabetic cataract. Cataractogenesis in diabetes seems to be a rather complex process, presumably including additional pathways, not connected with direct free radical oxidative modification of lens crystallins (e.g., mitochondrial damage). Damage of mitochondria of superficial cortex fiber cells at the lens equatorial region.
tor where the first globular degeneration is seen in glucose cataract [58], followed by calcium release and activation of calcium sensitive calpain proteases [59-61], was suggested as a likely cause of lens opacification in diabetes. Mitochondrial oxidative damage of lens epithelial cells leading to severe depletion of intracellular ATP was reported to be one of the initiating events contributing to the formation of cataract. Beneficial effects of estrogen therapy against cataract in postmenopausal women was explained by stabilization of the mitochondrial membrane due to the antioxidant action of the hormone, independently of estrogen receptors [62,63]. Anticataract actions of the antioxidants studied in our experiment may be hypothetically explained by a plausible protection of lens mitochondria. At least for stobadine, other authors reported its ability to preserve fine mitochondrial structure in cerebral capillaries and neurons under conditions of global cerebral ischemia-reperfusion injury in dogs [64]. Stobadine was found to ameliorate the mitochondrial energy production reduced in the diabetic kidney [65]. Stobadine was also reported to prevent calcium accumulation in diabetic tissues [22].

Another conceivable explanation may be consistent with an increased lipid peroxidation rate in diabetes mellitus [5,6,66-68]. Dyslipidemia, including hypertriglyceridemia, is considered a significant and independent risk factor for diabetic complications [68,69]. Decomposition of lipid peroxides initiates chain reactions that produce reactive carbonyl compounds. One of the byproducts of lipid peroxidation is the toxic compound malondialdehyde (MDA), whose involvement in cataractogenesis has been suggested, mainly due to its cross-linking ability [70-74]. Lens MDA may be the result of lipid peroxidation of the lens cell membranes or may represent the consequence of its migration from the readily peroxidizable retina or from the central body compartment [5,6,75-77]. According to the results presented here and the previous findings of Perkiner et al. [22], stobadine supplementation of diabetic animals significantly attenuated plasma levels of MDA, an index of systemic oxidative damage. In our previous papers we reported on the ability of stobadine to reduce oxidative damage of the diabetic heart and kidney tissue as measured by conjugated dienes [19,21] or MDA [78]. Stobadine also caused a significant correction of hypercholesterolemia and hypertriglyceridemia in diabetic rats [19,22], consistent with beneficial effects of stobadine on dyslipidemia in a short term clinical trial in humans [17]. The ability of stobadine to attenuate lipoxidation reactions in diabetes, and thus the production of toxic aldehydes, may account, at least partly, for its observed anticataract action.

In vivo, it is very likely that an antioxidant would not exert its effect by itself, but would act in combination with other antioxidants present (e.g., antioxidant vitamins). Such interactions may affect the final antioxidant capacity. In their pulse radiolytic study on stobadine, Steenken et al. [79] demonstrated the ability of a water soluble analog of alpha-tocopherol, Trolox, to recycle stobadine from its one electron oxidation product on giving a corresponding Trolox radical. Kagan et al. [80] reported that the antioxidant potency of stobadine in vivo might thus be increased by its interaction with other antioxidants having more negative redox potentials (e.g., vitamin E). Indeed, Rackova et al. [81] showed that when Trolox and stobadine were present simultaneously in liposomal incubations, Trolox spared stobadine markedly in a dose dependent manner. This sparing effect of Trolox was inferred by its direct interaction with stobadiny radicals leading to stobadine regeneration.

By analogy, we anticipated an enhanced antioxidant and anticataract action of stobadine when administered to diabetic rats in a mixture with vitamin E. However, contrary to our expectations, the combined treatment STB+E increased the progression of the advanced stages of cataract. Moreover, biochemical analyses showed further decrease of lens protein free silyhydrys in diabetic rats treated with the mixture STB+E in comparison with the untreated diabetic group, indicating a prooxidant effect of the STB+E cocktail. The reason for the observed detrimental effect of the antioxidant mixture STB+E is not yet known. The experimental data presented here were obtained by using synthetic D,L-alpha-tocopherol which may behave differently from its natural D-isomer. Nevertheless, the result is reminiscent of the studies by Clarke et al. [82] on RCS strain rats. They found that individually taken vitamin E, C, or A were effective in reducing cataract risk, while the combination of the three supplements potentiated cataract formation. Other authors described beneficial effects of the combination STB+E. Similar or even enhanced protection in comparison with treatments by individual antioxidants was observed in rodent diabetic kidney and brain [78], diabetic heart and liver [22], or peripheral nerves (unpublished). We can only hypothesize that in diabetic animals treated with the mixture STB+E, the resulting total antioxidant concentration in the lens may have exceeded the level above which the reduction potential of the antioxidant mixture to recycle transition metals in their lower valence may prevail (e.g., Fe^{3+} to Fe^{2+}), thus driving the production of detrimental hydroxyl radicals via the Fenton reaction with endogenous hydrogen peroxide. A similar mechanism has been suggested for pro-oxidant action at elevated concentrations of vitamin E [83,84].

In conclusion, the results are in accordance with the postulated pro-oxidant role of chronic hyperglycemia. However, the direct oxidative free radical damage of eye lens proteins does not seem to be the key mechanism effective in the development of diabetic cataract. Sugar cataractogenesis appears to be a multifactorial process in which apart from the reported non-oxidative pathways and free radical directed protein oxidative modifications other mechanisms may be involved, including consequences of the overt oxidative stress in diabetes (e.g., protein modifying potential of toxic aldehydes generated as byproducts of carbohydrate auto-oxidation and lipid peroxidation). The ability of stobadine to attenuate lipoxidation reactions in diabetes may account, at least partly, for its observed anticataract action.

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