Antioxidant activity of plasma from subjects with and without senile cataract


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Abstract. The relationship between overall plasma antioxidant activity and senile cataract was examined in 148 subjects aged 37–90 years. Antioxidant activity of plasma from 52 control and 96 patients with senile cataract was determined by a method which measures the capacity of plasma to inhibit auto-oxidation of bovine brain homogenate. In contrast to previous studies which demonstrated a relationship between decreased levels of antioxidant components in blood and the occurrence of lens opacities, the present study does not show any significant difference between subjects with and without senile cataract.

Introduction

Numerous reports indicate that oxidative stress is involved in cataractogenesis. Many epidemiological studies [1–4], although controversial [5], have shown that sunlight exposure, which generates reactive oxygen, could represent a major risk factor for senile cataract. Experimental data have also established that oxygen–derived free radicals induce lens opacification in animals [6–9], and that antioxidant therapy can delay the onset of cataract in animal models [10–12]. Moreover, many analytical investigations have demonstrated that cataractous lenses contain products of oxidative processes [13–15] and are deficient in antioxidant defences [16–20]. More recently, it has been proposed that such a deficiency could also be found at the plasma level and that subjects with high contents of at least two of the three antioxidants: tocopherol, ascorbate or carotenoids, have a reduced risk of cataract as opposed to subjects with low levels of one or more of these compounds [21]. These very attractive data led us to carry out the present study: we determined the total antioxidant capacity of plasma from control and cataractous subjects by a method used successfully in other free radical–related diseases [22, 23].
Materials and methods

Subject selection. Ninety-six patients aged from 45 to 90 years (70.5 ± 8.8), undergoing surgery for all types of cataract, were examined in this study. Fifty-two subjects aged from 37 to 80 (58.5 ± 11.9) without any loss of lens transparency were recruited as controls. Patients as well as controls were recruited in four distinct hospitals. All subjects were given a detailed ophthalmic examination including visual acuity, IOP determination and observation of lenses. We excluded subjects for the following reasons:
1. cataracts of non-senile origin (traumatic, congenital, endocrinal);
2. other diseases (malignant hypertension, diabetes, rheumatoid arthritis, severe myopia, retinopathy);
3. drug treatments (anti-inflammatory drugs, barbiturates, tocopherol, vitamin C, vasoprotective and hepatoprotective drugs containing potential free radical scavengers).
Control subjects without any lens opacity or vacuoles when observed with a slit lamp were recruited in the same ophthalmology departments, on the same exclusion criteria as far as disease and treatment were concerned.

Plasma antioxidant activity. Venous blood samples were collected on heparin and immediately centrifuged for plasma preparation. Plasma samples were frozen and stored at -20 °C until use. The samples were analysed between 1 and 3 months after collection. In these conditions, the antioxidant activity of the plasma samples was observed, by us and by others [24], to be stable for several months. Inhibition of brain homogenate auto-oxidation by plasma was measured according to the method described by Stocks et al. [24]. Briefly, bovine brains were obtained from recently slaughtered animals and immediately packed in ice. While removing the blood vessels and meninges from the surface, the brains were washed with ice-cold saline and then homogenized with phosphate buffer 4-fold their weight. The homogenate was centrifuged at 1,000 g for 15 minutes. The supernatant was stored at -80 °C. Before use, the supernatant was diluted with phosphate buffer three times its volume. The homogenate was then incubated and shaken at 37 °C for 1 hour in the presence of various quantities of plasma. At the end of incubation 0.5 ml trichloroacetic acid (28%) was added to 1 ml homogenate. After vortexing, the sample was centrifuged and 1 ml supernatant collected. Thiobarbituric acid (TBA 0.37%, 0.25 ml) was added to the supernatant, and the mixture was heated at 100 °C for 15 minutes. When heated under acidic conditions, TBA forms adducts with products of lipid peroxidation. Sample absorbance was measured at 532 nm. The antioxidant index was expressed as the volume (in μl) of plasma per ml homogenate which totally inhibited autoxidation. This value was the x intercept of the straight line, derived by linear regression from experimental values. The Student's t test was used for statistical analysis of the data.