Pteridines
Vol. 11, 2000, pp. 94 - 99

Neopterin and kynurenine concentrations in aqueous humour of the anterior chamber of the eye and in serum of cataract patients with pseudoexfoliation

Guna Laganovska[1], Agris Martinsons[1], Bronislavs Pitrans[1], Bernhard Widner[2] and Dietmar Fuchs[2]

[1] Latvia Medical Academy, Riga, Latvia, [2] Institute of Medical Chemistry and Biochemistry, Leopold Franzens University, and Ludwig Boltzmann Institute of AIDS-Research, Innsbruck, Austria

Summary

In 40 cataract patients and in 51 patients without pseudoexfoliation (PES) we determined serum concentrations of neopterin, kynurenine, and selenium and concentrations of neopterin in aqueous humour from the anterior chamber of the eye. In addition, selenium content in lenses was determined. Significantly increased kynurenine and neopterin concentrations in serum and neopterin concentrations in aqueous humour were observed in mature cataract patients with PES compared to those without. These patients also presented with the lowest content of selenium in serum and lens, compared with cataract patients without PES. Increased concentrations of neopterin in serum and aqueous humour of the anterior chamber of eyes suggest an increased degree of oxidative stress in patients with PES. Thus, the results support the role of oxidative stress in the development of PES in cataract patients. The decreased content of selenium may elicit immune system activation via an increased oxidative stress as it is indicated by the increased formation of kynurenine and neopterin.

Key words: Neopterin, Kynurenine, Selenium, Cataract, Pseudoexfoliation

Introduction

Cataract, opacity of the lens of any type and origin, afflicts more than 50 million persons worldwide (1). It impedes penetration of the light rays into the eye and their focusing on the retina, in that way decreasing visual acuity. Research on the mechanisms of lens opacification during last years has revealed a number of individual cataractogenic stressors in man. They include formation of osmotic cataracts (diabetic, galactosemic and hypoglycemic cataracts), radiation cataracts (X-ray irradiation, near ultraviolet irradiation and microwave radiation) and conversion of soluble low molecular weight cytoplasmatic proteins to soluble high molecular weight aggregates, insoluble phases, and insoluble membrane-protein matrices in senile cataract (2).

The basic method of the cataract treatment is surgery: evacuation of the opaque lens and replacement of it by an artificial intraocular lens. During surgery the central part of the lens anterior capsule is removed, the lens nucleus is evacuated and the remaining lens mass is irrigated. The lens capsular bag, the posterior capsule and the peripheral part of the anterior capsule remains in the eye. The artificial lens is placed into the capsular bag. Complications during surgery, e.g. accidental ruptures of the capsular bag, occur more often in case of pseudoexfoliation.

The pseudoexfoliation syndrome (PES) clinically is characterized by the precipitation of specific material in the anterior parts of the eye (3). For a long period it was considered to be the lens capsule particles detached locally where the iris is moving on the anterior surface of the lens. In turn, this specific material was found in the posterior parts of the eye, extraocular and even in other organs, e.g. lien, aorta (3). From the data it was hypothesized that PES is a symptom of an underlying general disease. The question of the origin of this material and its connection with other ophthalmic and general diseases is still to be discussed (4). PES is often found in Scandinavian and Baltic countries (5), but is very rare in Africans and Americans. In PES amorphous flakes blocking the aqueous outlets inside the eye can lead to the development of increased intraocular pressure and glaucoma.

Some evidence has emerged that imbalances of tryptophan and other aminoacids, deficiences of

Correspondence to: Dr. Agris Martinsons, Kuldigas 23 - 4, Riga, LV 1007 Latvia,
fax: ++371 761 4168 3519, ++371 731 0034, e-mail: agrism@latnet.lv

calcium or selenium, or excessive intake of selenium may play some role in human senile cataract (6). Increased urinary excretion of tryptophan metabolites like kynurenine has been reported in patients affected by senile cataract, but no significant differences were found in the serum tryptophan levels between healthy subjects and cataract patients (6, 7). Oxidative stress has emerged as a common denominator for many changes in senile cataract, e.g. oxidative events with a decrease of free radical scavangers are involved in the onset of development of cataract in diabetes mellitus (8).

In vitro, large amounts of neopterin are produced by human monocytes/macrophages upon stimulation with interferon-gamma. In vivo, increased neopterin concentrations in human serum and urine indicate activation of cell-mediated (Th1-type) immune response, e.g., during virus infections, autoimmune diseases, allograft rejection and in certain types of malignancy (9). The amount of neopterin produced by activated monocytes/macrophages correlates with their capacity to release reactive oxygen species and more recently neopterin was found itself to be capable of enhancing toxic effects induced by reactive oxygen species (10). With this background, neopterin concentrations in body fluids can be regarded as an indirect estimate of the degree of oxidative stress emerging during cell-mediated immune response (11).

In this study we compared concentrations of neopterin, kynurenine and selenium in cataract patients, to further evaluate the pathogenetic role of immune activation and oxidative stress in the development of cataract with and without PES.

Patients and methods

We examined 40 patients with various types of cataract with PES and 51 patients without PES. Patients, aged 69 to 82, were divided into 3 groups depending on the stage of disease: 18 with subcapsular cataract, 19 with central cataract, and 14 with mature cataract. Patients with other ophthalmic diseases (e.g., glaucoma) were not included into this study. All patients have been operated at the P.Stradins Hospital, Department of Ophthalmology, Riga, Latvia, and informed consent was obtained from the patients to particpate in this study. The lens was obtained intraoperatively during the cataract surgery using a special forceps. In the part of the lens we examined the concentration of selenium (12). During the eye surgery we collected the aqueous humour from anterior chamber of the eye, and determined the concentration of neopterin by radioimmunoassay (13). In serum of all our patients the concentrations of selenium (12), neopterin (13), tryptophan and kynurenine (14) have been examined, and the kyn/trp ratio (= kynurenine/tryptophan x 100) was calculated as an estimate for IDO activity (14).

For statistical comparisons of groups, Student's t-test was used, p-values <0.05 were considered to indicate statistically significant differences. Correlations between parameters have been calculated using Spearman's rank correlation coefficients.

Results

The concentrations of all examined parameters were similar in all groups of our patients without PES, except the concentration of selenium in lenses of patients with central and mature cataract was lower compared with subcapsular cataract (Table 1). The concentrations of neopterin in aqueous humour of anterior chamber of the eye were on average <1 nmol/L which is approximately 10 – 15% of corresponding serum concentrations (Table 1). However, there was no difference of neopterin concentrations between the different groups of patients without PES.

Table 1. Serum neopterin, tryptophan, kynurenine, and selenium concentrations, and kynurenine/tryptophan (=kyn/trp) ratio *1000 in serum, and neopterin in aqueous humour and selenium in lenses in 3 groups of cataract patients without pseudoexfoliation (mean values ± S.D. are shown; #p<0.001 for all comparisons: A vs. B, A vs. C, B vs. C)

	Subcapsular cataract (n=18)	Central cataract (n=19)	Mature cataract (n=14)
Serum: Neopterin (nmol/L)	6.80 ± 0.65	6.52 ± 0.49	7.19 ± 0.57
Tryptophan (µmol/L)	75.35 ± 5.82	78.39 ± 5.88	72.65 ± 5.93
Kynurenine (µmol/L)	2.85 ± 0.67	2.32 ± 0.76	3.11 ± 0.85
Kyn/trp ratio (*1000)	47.73 ± 3.97	42.53 ± 4.21	51.63 ± 4.86
Selenium (mg/L)	0.091 ± 0.018	0.087 ± 0.012	0.081 ± 0.011
Lens: Selenium (mg/L)	0.523 ± 0.02#	0.389 ± 0.02#	0.289 ± 0.02#
Acqeous humour: Neopterin (nmol/L)	0.76 ± 0.17	0.83 ± 0.20	0.75 ± 0.21

In the case of subcapsular and central cataract with PES we found normal concentrations of tryptophan, kynurenine and neopterin in serum (Table 2). In the group of patients with mature cataract with PES we

observed an increase in serum concentrations of kynurenine and neopterin, as well as an increase of neopterin concentrations in the aqueous humour of the anterior chamber, compared with patients with subcapsular cataract. There was a tendency of tryptophan concentrations to decrease with progressed disease. However, the differences between groups did not reach statistical significance (Table 2). The concentration of selenium in serum was decreased, and in all groups of patients with PES we detected a very low content of selenium in lenses. There was a trend towards lower concentrations in patients with more advanced disease, but no significant difference between the groups was observed. Selenium was however statistically lower in patients with mature cataract with PES compared with the same form of cataract without PES (Table 1 vs. Table 2; $p<0.05$). Also the difference between selenium concentrations in serum in these groups was statistically significant ($p<0.05$). Neopterin content in aqueous humour of eye in mature cataract patients with PES was higher than in patients without PES ($p<0.05$).

Table 2. Serum neopterin, tryptophan, kynurenine, and selenium concentrations, and kynurenine/tryptophan (= kyn/trp) ratio in serum, neopterin in aqueous humour and selenium in lenses in 3 groups of cataract patients with pseudoexfoliation (mean values ± S.D. are shown; #$p<0.05$ when comparing A with C)

	Subcapsular cataract (n=18)	Central cataract (n=19)	Mature cataract (n=14)
Serum: Neopterin (nmol/L)	6.70 ± 0.53	6.82 ± 0.43	8.49 ± 0.59#
Tryptophan (µmol/L)	78.15 ± 6.12	76.37 ± 5.88	71.99 ± 6.23
Kynurenine (µmol/L)	2.72 ± 0.62	2.92 ± 0.73	4.79 ± 0.77#
Kyn/trp ratio (*1000)	46.77 ± 3.87	49.56 ± 4.01	59.65 ± 4.56#
Selenium (mg/L)	0.085 ± 0.015	0.063 ± 0.018	0.041 ± 0.012#
Lens: Selenium (mg/L)	0.277 ± 0.02	0.286 ± 0.02	0.203 ± 0.04
Acqeous humour: Neopterin (nmol/L)	0.8 ± 0.15	0.98 ± 0.23	1.33 ± 0.22#

There existed a positive correlation ($p<0.002$) between serum neopterin and the kyn/trp ratio (Fig.1), as well as serum kynurenine and neopterin ($p<0.01$) in all patients with PES. And there was a negative correlation of kynurenine with selenium in serum ($p<0.01$) in mature cataract patients with PES (Fig.2). A similar relationship seemed to exist for neopterin, but the correlation was not significant (Fig.2). The neopterin concentrations in aqueous humour of the anterior chamber of eye correlated with the neopterin concentration in serum ($p<0.05$; Fig.3), but we did not find a correlation between neopterin concentrations in aqueous humour and selenium in serum.

Fig.1: Correlation between serum neopterin and the kynurenine/tryptophan (kyn/trp) ratio * 1000 in cataract patients with pseudoexfoliation ($rs=0.59$; $p<0.002$)

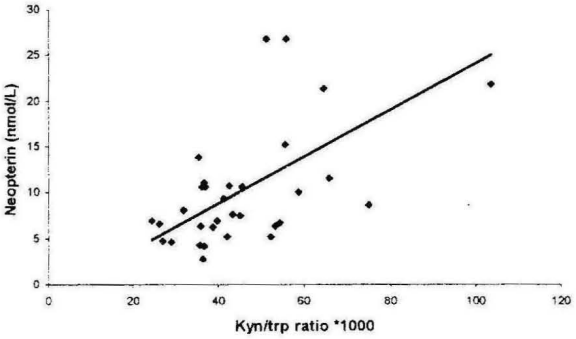
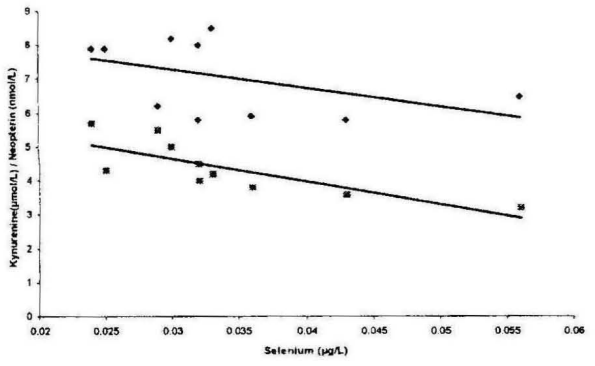


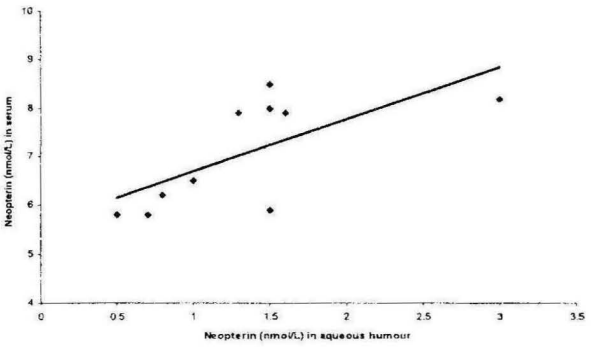
Fig.2: Correlation of serum kynurenine (squares) and neopterin (rhombs) concentrations with serum selenium content (neopterin: $rs=0.45$, not significant; kynurenine: $rs=0.78$, $p<0.01$)



Discussion

Our study demonstrates increased concentrations of neopterin and kynurenine in patients with mature cataract with PES. We detected increased concentrations of neopterin in aqueous humour of the

Fig.3: Correlation between neopterin in serum and in the aqueous humour of the eye in patients with mature cataract and pseudoexfoliation (rs=0.67; p<0.05)



anterior chamber of the eye in these patients. The concentration of neopterin in the aqueous humour wasrelated to the corresponding plasma levels. In the same group of patients we observed a decreased concentration of selenium in serum. In our patients with mature cataract without PES we detected a decreased content of selenium in lenses when compared with other groups, but there were no differences in concentrations of other metabolites.

Oxidative stress is one initiating factor for the development of cataract (8). A significant proportion of lenses and aqueous humour taken from cataract patients have elevated H_2O_2 levels, which can cause lens opacification (5). It is known that selenium as antioxidant works against the development of cataract. In Baltic countries there exists a biogeochemical deficit of selenium. The question about the role of selenium in the development of cataract is still open: there are some more positive studies, confirming the role of decreased concentration of selenium in the development of cataract (16), but not all authors agree with this opinion (17,18). The available data suggest that decreased selenium in aqueous humour and serum of patients with senile cataract may reflect defective antioxidative defence systems which may lead to the formation of cataract (16).

Indoleamine 2,3-dioxygenase (IDO) is the first enzyme of the extrahepatic tryptophan degradation pathway. IDO has been demonstrated to be able to scavenge O_2^- and therefore has the potential to act as a protective enzyme (19). IDO may work as an antioxidant also in the eye (20). Enzyme activity can be expressed as the quantity of kynurenine formed from the substrate tryptophan (kynurenine/tryptophan ratio; Ref. 21). The increase of serum kynurenine and kyn/trp ratio in the mature cataract patients with PES reflects this activity of IDO. The induction of active IDO formation is usually paralled by neopterin biosynthesis by activated monocytes/macrophages (21, 22). In these cells, the neopterin level correlates to the ability of these cells to produce oxygen radicals and therefore allows to estimate the expression of oxidative stress during cell-mediated immune activation (11). It has been reported that the activities of the pteridine synthesizing enzymes and tetrahydropteridine content were decreased in human senile cataract as compared with age-matched clear human lenses. The loss of tetrahydropteridine may result in lenticular proteins more susceptible to oxidation and contribute to high molecular weight protein formation in cataracts (23).

High serum levels of kynurenine and neopterin could lead to higher levels of these compounds in the aqueous humour and then in the lens; once inside the lens it can be transformed into other compounds contributing to the formation of the yellow-brown pigments, that are one of the major features of senile cataracts.

Primate lenses are unique in that they convert tryptophan into 3-hydroxykynurenine glucoside, probably via the intermediate kynurenine. 3-hydroxykynurenine glucoside is the major short-wave absorbing pigment present in human lenses and may play a role in protecting the eye from UV-induced photodamage (24). It has been also shown that low micromolar concentrations of 5-hydroxytryptophan, 3-hydroxykynurenine, or 3-hydroxyanthranilic acid, but not their corresponding non-hydroxylated metabolic precursors, scavanged peroxyl radicals with high efficiency (25). Experimental studies have indicated that elevated content of 3-hydroxykynurenine can accumulate in the lens (26), or via transamination it can lead to the formation of xanthurenic acid and its derivatives, which are responsible for the increase of lens fluorescence during cataract development (27). It is also possible that increased neopterin content in serum and aqueous humour of anterior chamber of eye reflects the activity of cell-mediated immunity against the specific material, which forms in the case of PES (e.g., melanin, amyloid P; ref. 28). Interestingly the neopterin concentrations in the acqueous humour of the eye were much lower than that obesreved in the serum of the same patients. This aspect would favour the view that the increase of neopterin concentrations with disease progression merely stems from an immune activation process going on in the blood and from there neopterin is leaking into the eye. As a consequence from the systemic immune activation process, the antioxidant pools including selenium are diminished. A similar situation was only recently described in the cerebrospinal fluid of patients with dementias, in whom higher neopterin concentrations have been found to correlate inversely with antioxidant α-tocopherol (29).

We suggest that in our cataract patients with PES the activation of IDO with increased formation of kynurenine could be considered as a defensive mechanism against massive oxidative stress due to decreased content of selenium or other antioxidative factors. The degree of oxidative stress can be estimated by the concentration of neopterin in serum and aqueous humour in patients with mature cataract with PES. Oxidative stress seems to play a certain role in the development of PES in cataract patients.

Acknowledgement

This work was supported by the Austrian Federal Ministry of Social Affairs and Generations and by the Austrian Funds "Zur Förderung der wissenschaftlichen Forschung", project 10776 Med.

References

1. Taylor A. Associations between nutrition andcataract. Nutr Rev 1989; 47: 225–234.
2. Chylack LT. Mechanisms of senile cataractformation. Ophthalmology 1984; 91: 596–602.
3. Kuchle M, Sclotzer-Schrehardt U, Nauman GO.Occurance of pseudoexfoliative material in parabulbar structures in pseudoexfoliation syndrome. Acta Ophthalmol; 1991; 69: 124–130.
4. Ringvold A, Blika S, Sandvik L. Pseudo-exfoliation and mortality. Acta Ophthalmol Scand 1997: 75: 255–256.
5. Alfaiate M, Leite E, Mira J. Prevalence and surgical complication of pseudoexfoliation syndrome in Portuguese patients with senile cataract. J Cataract Refractive Surg 1996; 22: 972–976.
6. Bunce GE, Kinoshita J, Horwitz J. Nutritionalfactors in cataract. Annu Rev Nutr 1990; 10: 233–254.
7. Allergri G, Angi MR, Costa C, Bettero A. Tryptophan and kynurenine in senile cataract. In: Progress in Tryptophan and Serotonin Research; Walter der Gruyter, Berlin – New York, 1984: 469–472.
8. Altomare E, Grattagliano I, Vendemaile G, Miceli – Ferrari T. Oxidative protein damage in human diabetic eye: evidence of retinal participation. Eur J Clin Invest 1997; 27: 141–147.
9. *Widner B, Murr C, Wirleitner B, Mayr C, Spöttl* N, Baier-Bitterlich G, Fuchs D. The importance of neopterin as a laboratory diagnostic marker of immune activation. Pteridines 1999; 10: 101-111.
10. Widner B, Baier-Bitterlich G, Wede I, Wirleitner B, Wachter H, Fuchs D. Neopterin: indicator of oxidative stress and part of the cytotoxic armature of activated macrophages in humans. Pteridines 1998; 9: 91-102.
11. Murr C, Fuith LC, Widner B, Wirleither B, Baier-Bitterlich G, Fuchs D. Increased neopterin concentrations in patients with cancer: indicator of oxidative stress? Anticancer Res 1999; 19: 1721–1728.
12. Husunuma R, Ogawa T, Kavanishi Y. Fluorometric determination of selenium in nanogram amounts in biological materials using 2,3-diaminonaphthaline. Anal Biochem 1982; 126: 242–245.
13. Fuchs D, Werner ER, Wachter H. Soluble products of immune activation: Neopterin. In: Manual of Clinical Laboratory Immunology (4th Edition) Rose RR, deMarcario EC, Fahey JL, Friedman H, Penn GM (eds), American Society of Microbiology, Washington, D.C., 1992, pp. 251–255.
14. Widner B, Werner ER, Schennach H, Wachter H, Fuchs D: Simultaneous measurement of serum tryptophan and kynurenine by HPLC. Clin Chem 1997; 43: 2424-2426.
15. Spector A. Oxidative stress-induced cataract: mechanism of action. FASEB J 1995; 9: 1173-1182.
16. Karakucuk S, Ertugrul Mirza G, Faruk Ekinciler O. Selenium concentrations in serum, lens and aqueous humour of patients with senile cataract. Acta Ophthalmol Scand 1995; 73: 329-332.
17. Knekt P, Heliovaara M, Rissanen A, Aromaa A, Aaran RK. Serum antioxidant vitamins and risk of cataract. Brit Med J 1992; 305: 1392–1394.
18. Rasi V, Costantini S, Moramarco A, Giordano R, Giustoisi R, Balacco Gabrieli C. Inorganic element concentrations in cataractous human lenses, Ann Ophthalmol 1992; 24: 459 – 464.
19. Daley-Yates PT, Powell AP, Smith LL. Pulmonary indoleamine 2,3 dioxygenase activity and its significance in the response of rats, mice, and rabbits to oxidative stress. Toxicol Appl Pharmacol 1988: 96: 22 – 232.
20. Malina HZ, Martin XD. Indoleamine 2,3-dioxygenase: antioxidant enzyme in the human eye, Graefes Arch Clin Exp Ophthalmol 1996; 234: 457 – 462.
21. Fuchs D, Möller AA, Reibnegger G, Stöckle E, Werner ER, Wachter H. Decreased serum tryptophan in patients with HIV-1 infection *correlates with increased serum neopterin and* with neurologic/psychiatric symptoms. J Acquir Immune Defic Syndr 1990;3:873-876.
22. Werner ER, Bitterlich G, Fuchs D, Hausen A, Reibnegger G, Szabo G, Dierich MP, Wachter H. Human macrophages degrade tryptophan upon induction by interferon-gamma. Life Sci 1987;41:273-280.

23. Rao GN, Cotlier E. The enzymatic activities of GTP cyclohydrolase, sepiapterin reductase, dihydropteridine reductase and dihydrofolate reductase; and tetrahydrobiopterin content in mammalian ocular tissues and in human senile cataracts. Comp Biochem Physiol 1985: 80: 61 – 66.
24. Wood AM, Truscott RJ. UV filters in human lenses: tryptophan catabolism. Exp Eye Res 1993; 56: 317 – 325.
25. Christen S, Peterhans E, Stocker R. Antioxidant activities of some tryptophan metabolites: possible implication for inflammatory diseases. Proc Natl Acad Sci USA 1990; 87: 2506 – 2510.
26. Chiarugi A, Rapizzi E, Moroni F. The kynurenine metabolic pathway in the eye: studies on 3-hydroxykynurenine, a putative cataractogenic compound. FEBS Lett 1999; 453:197-200.
27. Malina HZ, Martin XD. 3-hydroxykynurenine transamination leads to the formation of fluorescent substances in human lenses. Eur J Ophthalmol 1996; 6:250-256.
28. Li ZY, Streeten BN, Yobai N. Amyloid P protein in pseudoexfoliation fibrillopathy. Curr Eur Res 1984; 8: 217 – 227.
29. Sattler W, Leblhuber F, Walli J, Widner B, Fuchs D. Cerebrospinal fluid levels of a-tocopherol and neopterin in patients with dementia. Pteridines 1999; 10:220-224.