Free Radical Scavenging and Antioxidant Activity of Allopurinol and Oxypurinol in Experimental Lens-Induced Uveitis

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Purpose. In addition to the inhibition of xanthine oxidase, allopurinol is known to act, dependent on the dose, as a free radical scavenger, an antioxidant, and a "scavenger" of hypochlorous acid. This activity was investigated using a model of lens-induced uveitis.

Methods. Lipid peroxides (LPO) were determined in aqueous humor and in retinal tissue. Reduced and oxidized glutathione (GSH and GSSG) of the aqueous humor and myeloperoxidase (MPO) activity in the iris–ciliary body complex were analyzed. Allopurinol and oxypurinol concentrations were determined by high-performance liquid chromatography in aqueous humor and retinal tissue of both control eyes and eyes with uveitis. These measurements were performed 6 hours after intravenous application of allopurinol.

Results. In lens-induced uveitis, LPO are significantly elevated, GSH is reduced, and GSSG and MPO are increased. A xanthine oxidase inhibition dose (<10 mg/kg body weight) of allopurinol showed no effects on oxidative tissue damage in the model used in this study. Higher doses, however, were able to reduce the oxidative damage. Allopurinol (20 mg/kg body weight) had slight effects on GSH and GSSG. All parameters improved using a dose of 50 mg/kg body weight; a dose of 100 mg/kg body weight only showed additional improvement in GSH and GSSG. There was no further change in the other parameters. Allopurinol and oxypurinol concentrations in aqueous humor and retinal tissue showed a dose dependency reaching scavenger concentrations after application of 50 mg/kg body weight of allopurinol.

Conclusions. These results suggest that the xanthine oxidase mechanism plays a minor role in the oxidative tissue damage due to lens-induced uveitis. Free radicals and oxidants are generated by activated leukocytes; therefore, the effect of higher doses of allopurinol is due to its free radical scavenging and antioxidative activity. Invest Ophthalmol Vis Sci. 1994;35:3897–3904.

Allopurinol is widely used in clinical medicine for the treatment of hyperuricemia. A reduction in uric acid is achieved by the inhibition of the enzyme xanthine oxidase. In ischemic diseases, xanthine oxidase uses oxygen as a reduction equivalent, leading to the formation of superoxideanion radicals. Therefore, xanthine oxidase is thought to contribute to oxidative tissue damage after ischemia and reperfusion. Using allopurinol as an inhibitor of xanthine oxidase, successful experimental attempts have been made in reducing ischemia-induced oxidative tissue damage.[1] Furthermore, allopurinol itself and its metabolite oxypurinol can act as scavengers of both the hydroxyl radical and hypochlorous acid. These scavenging effects are dose dependent.[2,3]

Oxygen-free radicals are thought to play a major role in the pathogenesis of immunologically induced inflammations, such as certain manifestations of uveitis.[4] Using a model of immunologically induced uveitis (lens-induced uveitis), the aim of this study was to examine whether allopurinol has a dose-dependent

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effect on oxidative stress, inflammation, and lipid peroxidation.

MATERIALS AND METHODS

Animal Experiments

The treatment of animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experiments were performed with male Wistar rats (n = 46).

The animals receiving allopurinol therapy were randomly divided into six groups (n = 5 animals each). The first group (base) was used for evaluation of the basic tissue levels. The animals were anesthetized, and the tissue samples were taken as described below.

Experimental uveitis was induced according to the method described by Rao et al[5] (groups 2 to 6). The animals were sensitized every 2 weeks over a 2-month period with four subcutaneous injections of 10 mg bovine lens protein in complete Freund's adjuvant (Sigma, Deisenhofen, Germany). One week after the last injection the lens of one eye was ruptured using the tip of a 30-gauge needle. At the time of lens rupture, the therapy groups were given different doses of allopurinol (10, 20, 50, and 100 mg/kg body weight; Henning, Berlin, Germany) intravenously. One group served as the control and received saline instead of allopurinol.

In separate experimental series, the concentration of both allopurinol and oxypurinol in aqueous humor and retinal tissue was determined in healthy eyes (left eye, a) and eyes with lens-induced uveitis (right eye, b) 6 hours after intravenous administration of different doses of allopurinol (group 7: 10 mg/kg body weight, n = 4; group 8: 20 mg/kg body weight, n = 4; group 9: 50 mg/kg body weight, n = 4; group 10: 100 mg/kg body weight, n = 4).

Tissue Sampling

Twenty-four hours after lens rupture, the animals were anesthetized, paracentesis was performed, and aqueous humor was taken using a microcapillary. The aqueous humor samples of each group were pooled, yielding 55 to 70 μl per group. Iris, ciliary body, and neurosensory retina were surgically removed using microforceps and an operating microscope. Biochemical analysis was then performed. The same procedure was performed with the first group (base values), except for lens rupture. All animals were killed afterward by exsanguination.

Biochemical Analysis

Lipid Peroxide Levels. Determination of lipid peroxide (LPO) levels in the aqueous humor and retinal tissue samples, expressed as "malondialdehyde-like substances" (MDA), was accomplished by high-performance liquid chromatography (HPLC), according to the method of Esterbauer et al.[6] Determination of lipid peroxides with the thiobarbituric acid (TBA) assay, expressed as TBA-reactive substances (TBARS), was performed according to Ohkawa et al,[7] as modified by Augustin and Lutz[8] for retinal samples, and according to Yagi[9] for aqueous humor samples. The results of the two methods (MDA and TBARS) were compared, and a correlation was calculated.

Malondialdehyde-Like Substances. High-performance liquid chromatography was carried out using an instrument by Bio-Rad (Munich, Germany).

Retinal Samples. The retinal sample was suspended in acetonitrile, homogenized using an Ultra Turrax blender (Janke & Kunkel, Staufen, Germany) and centrifuged at 3000g for 5 minutes. Twenty microliters of the clear supernatant was injected into the HPLC apparatus.

Aqueous Humor Samples. Before injection into the HPLC apparatus, 20 μl of the pooled aqueous humor was diluted with an equal volume of acetonitrile, mixed on a vortex mixer, and centrifuged at 3000g for 5 minutes; 20 μl of the clear supernatant was injected into the HPLC apparatus. The following columns for direct determination of MDA were used: 1, 250 × 4.5 mm S5 NH_2 spherisorb (aminophase); 2, precolumn 50 × 4.6 mm (aminophase) (Promochem, Wesel, Germany). The upper pressure limit of the high-pressure pump was set to 5000 psi. An injection valve with a 20-μl loop and a 50-μl syringe were used. The UV detector was set to 270 nm. Before injecting the sample, approximately 50 ml of eluent (acetonitrile–0.03-M Tris, pH 7.4 [1:9 vol/vol]) was used for equilibration of the column. The flow rate was 1 ml/min. A stock solution of MDA (10 mM) for calibration was prepared from malonaldehydebisdiethylacetal (Merck, Darmstadt, Germany), with 10 ml of 1% sulfuric acid to 50 μl of acetal, and maintained at room temperature. For HPLC calibration, 3 ml of Tris buffer was added to 12.5 μl of stock solution and injected into the HPLC apparatus.

Values were calculated automatically with the aid of a computer and correlated with the protein content of the retinal sample, expressed as water-soluble protein per milliliter. MDA values of the retinal samples are expressed as means of MDA/mg water-soluble protein (±SEM). MDA values of the aqueous humor are expressed as means per milliliter.

Thiobarbituric Acid Reactive Substances. *Retinal Samples.* Tissue samples were homogenized in 0.9 ml 1.15% KCl for 30 seconds with an Ultra Turrax blender and centrifuged at 3000g for 5 minutes. The assay mixture consisted of 0.1 ml of the supernatant, 2 ml of 0.9% NaCl, 0.2 ml of sodium dodecylsulfate,

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and 3 ml of TBA, containing equal parts of 0.8% aqueous TBA and acetic acid. The mixture was heated for 75 minutes at 95°C and then cooled with tap water. The resulting stain was transferred from the reaction site to a stable organic layer by adding 5 ml of n-butanol (Uvasol; Merck), with vigorous shaking of the mixture. After centrifugation at 3000 rpm for 15 minutes, the supernatants were fluorimetrically evaluated at 515-nm excitation and at 553-nm emission. Varying amounts of 1-, 2-, and 3-nmol tetramethoxypropane served as external standards and were assayed using the method described above. TBARS values are reported as means of TBARS/mg water-soluble protein (±SEM).

Aqueous Humor Samples. Ten microliters of aqueous humor was added to 4 ml of distilled water and 1 ml of the TBA reagent (a mixture of equal volumes of 0.67% TBA aqueous solution and glacial acetic acid). The reaction mixture was heated for 60 minutes at 95°C. After cooling with tap water, 5 ml of n-butanol (Uvasol) was added, and the mixture was shaken vigorously. After centrifugation (3000 rpm, 15 minutes), the organic layer was fluorimetrically evaluated at 515-nm excitation and 553-nm emission. Varying amounts of 0.1-, 0.2-, and 0.3-nmol tetramethoxypropane served as external standards and were assayed using the method described above. TBARS values are reported as means of TBARS/ml.

Myeloperoxidase. Myeloperoxidase (MPO) activity in the iris and ciliary body was determined using the method of Bradley et al.[10] To free MPO from primary granules of neutrophilic leukocytes, specimens were homogenized for 5 seconds in 3 ml of hexadecyl-trimethylammonium bromide (HTBA) solution (0.5 HTBA in 50-mM phosphate buffer, pH 6.0) with an Ultra Turrax blender. The homogenate was sonicated for 10 seconds, freeze thawed three times, and centrifuged at 40,000*g* for 15 minutes at 4°C, resulting in the formation of a stable pellet. The supernatant was assayed for MPO activity by spectrophotometry, using a mixture of 0.1 ml of supernatant and 2.9 ml of 50-mM phosphate buffer, pH 6.0, containing 0.167 mg/ml of o-Dianisidine hydrochloride and 0.0005% H_2O_2. The change in light absorbance at 460 nm was measured with a spectrophotometer. Myeloperoxidase values are expressed as U/mg water-soluble protein (±SEM).

Glutathione. Determination of reduced and oxidized glutathione (GSH and GSSG, respectively) was performed according to the method described by Bergmeyer.[11] The chemicals were purchased from Fluka (methylglyoxal; Neu-Ulm, Germany) and Boehringer (glyoxalase I, NADPH, glutathione reductase; Mannheim, Germany).

To the samples was added 100 μl $HClO_4$, and the mixture was sonicated for 10 seconds and centrifuged (Eppendorf-centrifuge; Eppendorf, Hamburg, Germany) at 13,000 rpm for 30 minutes (4°C). pH was adjusted to 6.5 to 7.0 using K_3PO_4. Thereafter, the samples were centrifuged (Eppendorf-centrifuge) for 15 minutes (13,000 rpm, 4°C). The supernatant was diluted to a total of 550 μl.

GSH Measurement.

Principle:

$$\text{GSH} + \text{methylglyoxal} \xrightarrow{\text{glyoxylase I}} \text{S-lactoyl GSH} \quad (1)$$

The sample was assayed by spectrophotometry at 240 nm using a mixture of distilled water (0.15 ml), glyoxylase I (0.01 ml, 12.5 U/ml), methylglyoxal (0.01 ml, 0.015 M), and diluted supernatant of the sample (50 μl drawn from the total of 550 μl). The extinction coefficient for S-lactoyl GSH (240 nm) was 3.37/cm per μmol.

GSSG Measurement.

Principle:

$$\text{GSSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{glutathione reductase}} 2\,\text{GSH} + \text{NADP} \quad (2)$$

The sample was assayed by spectrophotometry at 340 nm using a mixture of NADPH (0.01 ml, 2.5 mM), glutathione reductase (0.01 ml, 120 U/ml) and diluted supernatant of the sample (200 μl drawn from the total of 550 μl). The extinction coefficient for NADPH (340 nm) was 6.3/cm per μmol.

Determination of Allopurinol and Oxypurinol

Determination of allopurinol and oxypurinol was performed by HPLC using an instrument by Bio-Rad. The tissue was sampled from each animal separately as described above.

Retinal Samples. The retinal sample was suspended in 0.6-M perchloric acid, homogenized using an Ultra Turrax blender, and centrifuged at 3000*g* for 5 minutes. Twenty microliters of the clear supernatant was injected into the HPLC apparatus.

Aqueous Humor Samples. Before injection into the HPLC apparatus, the amount of aqueous humor sampled was determined and diluted up to a total of 25 μl, using 0.6-M perchloric acid, mixed on a vortex mixer, and centrifuged at 3000*g* for 5 minutes; 20 μl of the clear supernatant was injected into the HPLC apparatus.

The following columns were used: 1, spherisorb 50 × 4.6 mmt S5 ODS2, 80A, octadecyl, 20 cm; 2, precolumn spherisorb 50 × 4.6 mm S5 ODS2, 80A, octadecyl, 5 cm (Promochem). The upper pressure limit of the high pressure pump was set to 2600 psi. An injection valve with a 20-μl loop and a 50-μl syringe

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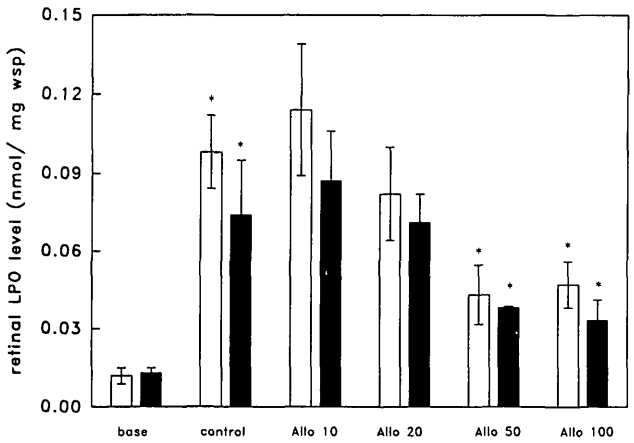

FIGURE 1. Lipid peroxide levels in retinal tissue as base values (base), untreated lens-induced uveitis (control), and after different doses of allopurinol (Allo 10, 20, 50, and 100 mg/kg body weight). Untreated uveitis controls are statistically compared to base levels. All treatment values are statistically compared to untreated uveitis controls. Filled bars = MDA; open bars = TBARS. $*P < 0.05$.

were used. The UV detector was set to 235 nm. Before injecting the sample, approximately 50 ml of eluent (KH_2 phosphate buffer [30 mmol; pH = 4.5] plus 0.5% methanol) was used for equilibration of the column. The flow rate was 1 ml/min.

Allopurinol and oxypurinol were identified by retention times of known standards of allopurinol (Henning) and oxypurinol (Sigma).

The values of the retinal samples are expressed as means of allopurinol and of oxypurinol per gram of tissue (±SEM). Allopurinol and oxypurinol values of the aqueous humor are expressed as means ± SEM per liter.

Protein Analysis

Protein analysis was done according to the method of Schacterle and Pollack.[12]

Statistics

Statistical analysis of MDA and TBARS values in retinal tissue samples, MPO values, and allopurinol and oxypurinol values was performed using Student's *t*-test.

The correlation of MDA, TBARS, GSH, GSSG, and the GSH–GSSG ratio versus the allopurinol dosages was calculated using SPSS-PC (SPSS, Chicago, IL) software to test for dose dependency (Pearson's coefficient of correlation). Normal distribution was assumed.

RESULTS

The lens capsule was found to be disrupted in all eyes studied.

Retina

Retinal lipid peroxides values increased significantly ($P < 0.05$) in untreated uveitis controls, compared to base values. A significant ($P < 0.05$) decrease compared to untreated uveitis controls was achieved with a dose of 50 mg/kg body weight of allopurinol; a dose of 100 mg/kg body weight resulted in no further change (Fig. 1). The coefficient of correlation between the two methods of determining LPO (i.e., MDA and TBARS values) was 0.85. The dose dependency of the allopurinol treatment is reflected in a correlation coefficient of $r = -0.83$ ($P < 0.05$) for allopurinol dosage versus TBARS, and $r = -0.89$ ($P < 0.05$) for allopurinol dosage versus MDA in retinal tissue.

Aqueous Humor

In aqueous humor of untreated uveitis controls, lipid peroxides measured as MDA and TBARS were markedly increased compared to base values. The correlation between the two methods was >0.85. Allopurinol showed a dose-dependent effect on MDA and TBARS. The dose of 20 mg/kg body weight of allopurinol showed only a slight reduction of MDA and TBARS compared to untreated uveitis controls, whereas 50 mg/kg body weight produced a more pronounced reduction. A dose of 100 mg/kg body weight showed no further changes (Table 1). The coefficient of correlation for TBARS and MDA values versus the dose of allopurinol was $r = -0.86$ and $r = -0.92$, respectively ($P < 0.05$ for both values).

Reduced glutathione in aqueous humor of untreated uveitis controls decreased as compared to base values. This resulted in a consecutive increase in GSSG. A minor increase in GSH and decrease in GSSG compared to untreated uveitis controls was achieved with a dose of 20 mg/kg body weight of allopurinol. A dose of allopurinol of 50 mg/kg body weight and 100 mg/kg body weight, respectively, resulted in a further recovery of the redox system GSH–GSSG (Ta-

TABLE 1. Aqueous Humor Results

Group	*GSH*	*GSSG*	*MDA*	*TBARS*
Base	0.286	0.055	0.07	0.05
Control	0.058	0.130	3.20	3.90
Allo 10	0.061	0.142	3.11	4.08
Allo 20	0.091	0.121	2.95	3.13
Allo 50	0.192	0.107	1.59	1.76
Allo 100	0.228	0.081	1.42	1.89

Reduced and oxidized glutathione (GSH, GSSG) (μmol/ml) and lipid peroxide levels as malondialdehyde-like substances (MDA) and thiobarbituric acid reactive substances (TBARS) (nmol/ml) in aqueous humor as base values (Base), untreated lens-induced uveitis (Control) and after different doses of allopurinol (Allo 10, 20, 50, and 100 mg/kg body weight).

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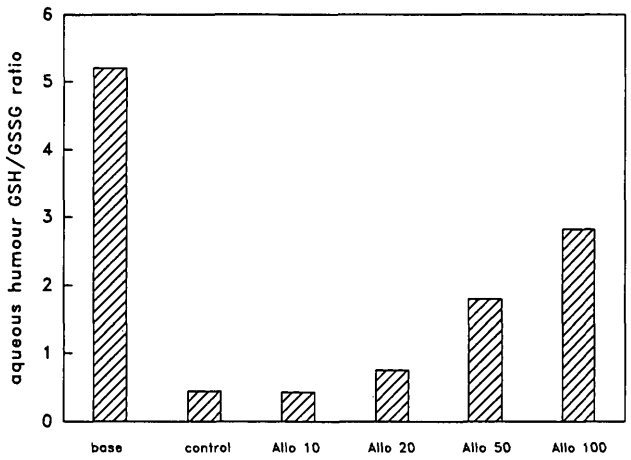

FIGURE 2. GSH–GSSG ratio in aqueous humor calculated from the values of Table 1.

ble 1). Using the GSH–GSSG ratio, the effects become more evident (Fig. 2). The coefficient of correlation between GSH, GSSG, and the GSH–GSSG ratio versus the dose of allopurinol was $r = 0.95$, -0.96, and 0.99, respectively ($P < 0.01$ for all values).

Iris and Ciliary Body

Iris and ciliary body showed a significant increase in myeloperoxidase activity ($P < 0.01$) compared to base values. When compared to untreated uveitis controls, doses of 10 and 20 mg/kg body weight of allopurinol were found to have no effect on this parameter compared to untreated uveitis controls. A significant ($P < 0.01$) reduction in MPO was achieved using 50 mg/kg body weight of allopurinol, but increasing the dose to 100 mg/kg body weight resulted in no further change (Fig. 3).

Allopurinol and Oxypurinol Measurements

The concentration of both allopurinol and oxypurinol in aqueous humor and retinal tissue 6 hours after intravenous application of allopurinol was dose dependent for each step ($P < 0.05$) (Table 2).

Scavenger concentrations of allopurinol in aqueous humor were reached after application of 50 mg/kg body weight and greater (Table 2). The concentrations of allopurinol and oxypurinol in eyes with uveitis were generally elevated compared to those in healthy eyes. Figure 4 shows an HPLC registration of allopurinol and oxypurinol in aqueous humor.

DISCUSSION

Determination of Lipid Peroxides

Because of its low specificity, the determination of LPO by the thiobarbituric acid method is not highly regarded. However, the advantage of this method is its high sensitivity in detecting peroxides. Therefore, additional measurement of LPO by HPLC as MDA was proposed as a method of higher specificity. The correlation between the results obtained by the two methods should exceed 0.8[13] to exclude false positive readings of the TBA method caused by certain flavines and peroxides of other origin. This was proven in both aqueous humor and retinal samples.

Oxidative Ocular Tissue Damage Due to Lens-Induced Uveitis

Lens-induced uveitis represents a severe Arthus reaction with pronounced tissue inflammation. This model is ideal for studying the pathophysiology of immune complex-mediated uveitis. In our study, oxidative stress (GSH–GSSG), inflammation (MPO), and oxidative tissue damage (LPO) of the affected ocular structures were evaluated systematically.

As shown in previous studies using free radical scavengers,[5,14,15] lens-induced uveitis leads to ocular oxidative tissue damage. The initial accumulation of inflammatory components ("phakitis") is followed by retinitis and choroiditis. The retinal tissue is especially susceptible to oxidative damage because of its great amount of polyunsaturated fatty acids. Therefore, permanent functional impairment due to severe uveitis could be caused by oxidative damage. Our study confirms previous results indicating retinal lipid peroxidation in uveitis.[15–17]

The presence of lipid peroxides in aqueous humor can be caused by peroxidation of tissues of the anterior segment of the eye secondary to an inflammatory response, with increased myeloperoxidase activities resulting in OCl^- production. An additional source of peroxides in aqueous humor may be the

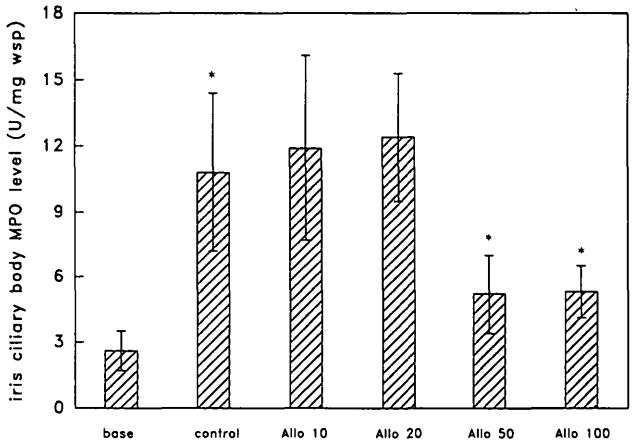

FIGURE 3. Myeloperoxidase activity in iris and ciliary body as base values (base), untreated lens-induced uveitis (control), and after different doses of allopurinol (Allo 10, 20, 50, and 100 mg/kg body weight). Untreated uveitis controls are statistically compared to basic levels. All treatment values are statistically compared to untreated uveitis controls. *$P < 0.05$.

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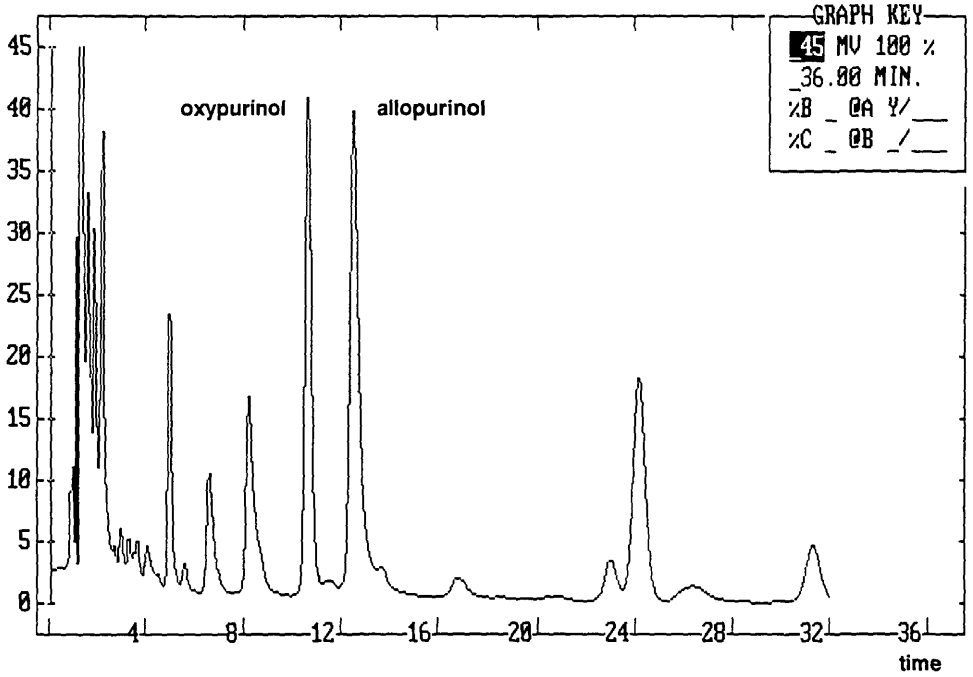

FIGURE 4. HPLC registration curve of allopurinol and oxypurinol in aqueous humor.

retinal tissue. MDA was also identified among the products of oxidative decomposition of amino acids, complex carbohydrates and pentoses, and hexoses, and as a byproduct of prostaglandin biosynthesis. However, peroxidation products of fatty acids are thought to be the major source of MDA.[18] Thus, our data may not allow any definite conclusions concerning the origin of LPO in aqueous humor. It is, however, conceivable that the peroxides measured in aqueous humor originate partly from the retinal tissue, because the combination of both methods to determine lipid peroxides ensures that MDA is measured (because MDA is mostly a product of peroxidation of fatty acids) and because the retinal tissue is known to be rich in polyunsaturated fatty acids.

Mechanisms Leading to Oxidative Tissue Damage

Inflammation. Oxidative tissue damage in lens-induced uveitis is caused by several types of free radicals that are mainly produced through a superoxide anion radical. Despite the presence of xanthine oxidase in ocular tissues,[19] the respiratory burst of neutrophils is the major source of superoxide anion radicals, initiating the oxidative tissue damage. The immigration and activation of neutrophils, as shown by our data, are thought to be a sign of an inadequate host defense.[4,15]

The arachidonic acid pathway of activated phagocytes is an additional pathway for the production of oxidative metabolites such as singlet O_2, hydroxyl radicals, and hydro(pero)xy acids, leading to the peroxidation of unsaturated fatty acids.[4]

Propagation of Peroxidation. A further source for oxygen-free radicals in this model is the retina itself. The polyunsaturated fatty acids of the retina become oxidized, resulting in fatty acid radicals capable of the further oxidation and chemoattraction of neutrophils, making this system self propagating.[17,20]

Allopurinol and Oxypurinol Concentration in Ocular Tissues

In this study, we have proved for the first time that allopurinol and its major metabolite oxypurinol reach the ocular tissues. After intravenous application of 10 mg/kg body weight and 20 mg/kg body weight, the concentration of allopurinol in aqueous humor was similar to the concentrations obtained by Zimmermann et al[21] in extracellular fluid. These authors demonstrated that such low levels of allopurinol lead to an 80% inhibition of xanthine oxidase. Thus, it is conceivable that xanthine oxidase is not the major source of free radicals in experimental model.

Conversely, Moorhouse and coworkers[3] demonstrated that, after application of 50 mg/kg body weight of allopurinol, tissue concentrations capable of scavenging radicals are achieved. Similar scavenging concentrations in aqueous humor were attained in our experiments (both in control animals and in animals with uveitis) after administration of the same amount of allopurinol.

Considering the half-life of the allopurinol molecule of 2 to 3 hours, concentrations of up to 1 mmol should have been achieved in aqueous humor 3 hours after application. Those concentrations of allopurinol

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TABLE 2. Allopurinol and Oxypurinol Concentrations

	Aqueous Humor (μmol/l)				*Retina (nmol/g)*			
Dose (Group)	*ALLO Controls (a)*	*ALLO LIU (b)*	*OXY Controls (a)*	*OXY LIU (b)*	*ALLO Controls (a)*	*ALLO LIU (b)*	*OXY Controls (a)*	*OXY LIU (b)*
Allo 10 (7)	44.8 ± 8.7	32.0 ± 6.3	3.2 ± 0.9	4.0 ± 0.5	60.6 ± 7.6	80.8 ± 9.3	4.6 ± 0.7	6.6 ± 1.2
Allo 20 (8)	55.0 ± 8.7	75.4 ± 9.4	8.9 ± 1.4	11.6 ± 0.8	81.9 ± 11.3	123.8 ± 12.4	17.3 ± 3.1	16.7 ± 3.7
Allo 50 (9)	160.1 ± 17.6	285.5 ± 25.1	21.8 ± 2.2	27.1 ± 4.5	177.1 ± 12.7	198.7 ± 17.8	25.4 ± 4.7	34.1 ± 4.9
Allo 100 (10)	442.2 ± 21.8	503.6 ± 39.4	47.5 ± 4.2	71.6 ± 3.7	412.0 ± 36.7	392.2 ± 29.3	44.7 ± 3.3	61.6 ± 6.5

Concentrations of allopurinol (ALLO) and oxypurinol (OXY) in aqueous humor (μmol/l) and retinal tissue (nmol/g tissue) 6 hours after intravenous application of different doses of allopurinol (Allo 10, 20, 50, and 100 mg/kg bw) in control eyes (Controls) and in eyes suffering from lens-induced uveitis (LIU). Uveitis was induced at the time of allopurinol injection. The dose dependency is significant for each step ($P < 0.05$).

are known to exhibit electron transfer activities.[22] This may be an additional explanation for the strong scavenger activities of allopurinol because, according to these authors, allopurinol decreases the leakage of electrons and the formation of incompletely reduced oxygen species.[22]

The elevated concentrations of allopurinol and oxypurinol in the tissues of eyes with uveitis are explained by the results of Rao and coworkers,[5] who postulated a breakdown in the blood-ocular barriers after lens disruption.

Effects of Allopurinol

Oxidative tissue damage in this experimental model is caused by different species of radicals and oxidants. Therefore, therapy using scavengers acting only on the 4-electron chain reduction of oxygen to water, such as superoxide dismutase and catalase–peroxidases, does not seem to be suitable as antioxidant therapy. Using doses higher than the xanthine oxidase inhibition dose of <10 mg/kg body weight,[21] powerful antioxidant activity regarding hydroxyl radical and OCl^- was shown for allopurinol and its derivatives.[2,3]

In our study, allopurinol exhibited this dose-dependent effect on the parameters of oxidative tissue damage and inflammatory activity in the anterior segment and retinal tissue. This dose dependency has been demonstrated in other experimental models as well.[23] The chemical structure of allopurinol is similar to that of purines, which are also known to be hydroxyl radical scavengers.[24] For example, uric acid is thought to be an important antioxidant of the human body.[2]

During investigations of allopurinol as a radical scavenger, Moorhouse and coworkers[3] have demonstrated that allopurinol's major metabolite, oxypurinol, which is produced by catalysis of xanthine oxidase, has even more powerful radical scavenging activity. Moreover, reaction kinetics studies[3] showed that antioxidative action of allopurinol regarding hypochlorous acid is thermodynamically impossible. Therefore, scavenging of hypochlorous acid is caused by the oxypurinol, which was shown to reach the ocular tissues.

It is known from several studies that allopurinol also has immunologic effects[25] and may alter the function of lymphocytes.[26] However, although cell-mediated immunologic alteration appears to be responsible for the majority of experimental uveitis models, Marak et al[27] demonstrated that lens-induced uveitis is not a cell-mediated disease. Despite the immunologic effects of allopurinol, it should be noted that both free radicals and granulocytes with MPO activity are involved in the action of the immune system. Thus, an additional explanation for the immunologic effects of allopurinol in this experimental model may be that allopurinol acts as an electron transfer agent or as a free radical scavenger, whereas hypochlorus acid generated from MPO is scavenged by its metabolite oxypurinol.

Because of the broad therapeutic range and the low incidence of unwanted side effects, the scavenging and antioxidative activities of allopurinol and oxypurinol should be further investigated in the therapy of inflammatory ophthalmic diseases.

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Key Words

uveitis, radicals, oxidation, scavenger, allopurinol

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