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INVESTIGATIONS OF IBUPROFEN AND PARACETAMOL BINDING TO LENS PROTEINS TO EXPLORE THEIR PROTECTIVE ROLE AGAINST CATARACT

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Abstract—There is evidence that ibuprofen and paracetamol can act as anti-cataract drugs. [^{14}C]-Ibuprofen labelled at the methyl group of the propanoic acid moiety was synthesized. The labelled ibuprofen was found to bind non-covalently to α-crystallin but not to β- and γ-crystallin of the bovine lens. Labelled paracetamol binds to total lens soluble proteins. Both drugs penetrate into the lens cortex and nucleus within 24 hr. Affinity chromatographic studies suggest that the lipophilic isobutyl group of ibuprofen hinders binding to the lens proteins. Hence, in the light of weak binding of ibuprofen and paracetamol and strong binding of the ibuprofen analogue used in the affinity chromatography, it is suggested, in this paper, that the protection against cataract by these analgesics is possibly due to their metabolites interacting with the lens proteins.

Cataract, a major cause of blindness, is partly the result of structural modifications of the lens proteins, which culminates in the opacity of the hitherto transparent lens [1]. The opacification results in the deterioration of vision, or in extreme cases, in blindness.

Patients suffering from persistent diarrhoea, renal failure and diabetes mellitus are at greater risk of developing cataract than healthy individuals [2–5]. In severe diarrhoea and kidney failure patients, the blood level of urea is elevated. Urea, in turn, is in chemical equilibrium with cyanate, a reactive agent capable of carbamylating the amino and thiol groups of proteins [6]. The carbamylation irreversibly modifies the conformation of the lens proteins [7], giving rise to disulphide bond formation. Cyanate incubated with crystallins causes almost all the proteins to form large aggregates held together by disulfide bonds [8]. These aggregates would scatter light and decrease lens transparency.

One proposed mechanism for the formation of cataract in diabetes involves non-enzymic reaction of glucose with proteins to give a Schiff base which is quickly converted into the more stable Amadori product [9, 10]. Intermolecular cross-links are formed by the reaction of carbonyl groups on such Amadori products. Glycation may induce conformational changes, leading to increased disulphide bond formation. Glucose-6-phosphate, the concentration of which is elevated in diabetic lens, caused conformational changes to both α- and γ-crystallin by reacting non-enzymically with these proteins [9, 10]. Other sugars, such as galactose [11], glucosamine [12], a metabolite of glucose, and fructose [13] also cause changes in the structure of lens proteins by reacting with their amino groups [14].

Consumption of ibuprofen and paracetamol as well as aspirin were associated, in epidemiological studies, with a reduced risk of developing cataract [15, 16]. Aspirin may achieve this effect by acetylation of the lens crystallins [17]. Additionally, aspirin and ibuprofen inhibit glycation [12] and carbamylation of these proteins [17, 18]. Ibuprofen reduces cyanate and galactose binding, in a dose-dependent manner and it was suggested that ibuprofen probably competes for cyanate and galactose binding sites. The binding of glucose-6-phosphate is not inhibited [18]. Ibuprofen also protects against opacities produced by cyanate-induced phase separation and prevents formation of cataract in incubated lenses [18]. Both ibuprofen and paracetamol protect diabetic rats against cataractogenesis [19].

In efforts to possibly uncover the underlying biochemical basis of the anti-cataract effect of ibuprofen and paracetamol, the present study was undertaken to explore the penetration of these drugs into the lens and their binding to lens proteins.

MATERIALS AND METHODS

Materials. The reagents used were of analytical grade. The boiling range of hexane and petroleum ether was 60–80°. [^{14}C]Methyl iodide was purchased from Amersham International (Amersham, U.K.). Silica gel (70–230 mesh, 60 Å) and silica plates of thickness 0.25 mm were from the Aldrich Chemical Co. (Gillingham, U.K.). Sepharose 4B was obtained from Pharmacia (Uppsala, Sweden). Scintran Cocktail T and Fractogel TSK HW-55 (S) were obtained from B.D.H. Chemicals (Poole, U.K.). [^{3}H-G]Paracetamol was obtained from Du Pont (Washington, DC, U.S.A.) as a solution in ethanol. Bovine eyes were obtained fresh and used immediately. Minimum essential medium (MEM†) was made up according to Ref. 20.

4-Isobutylacetophenone. Isobutylbenzene (100 g, 0.745 mol), $AlCl_3$ (200 g, 1.5 mol) and CS_2 (200 mL) protected from moisture, were mixed before acetyl

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† Abbreviations: MEM, minimal essential medium; TCA, trichloroacetic acid.

chloride (58.5 g, 0.745 mol) was added dropwise over a period of 30 min. The reaction mixture was stirred at room temperature for 30 min and the contents of the flask were poured onto ice (700 g). The organic layer was separated and the aqueous phase extracted with ether (200 mL). The CS_2 phase and the ether extract were combined, washed with 10% aqueous HCl (40 mL), 10% Na_2CO_3 (40 mL) and water (50 mL) and dried with Na_2SO_4. Removal of the solvents *in vacuo* gave a brownish red liquid which was then distilled under vacuum, affording a colourless liquid which was then used without further purification, although a portion (0.5 g) was purified by chromatography on silica gel (30 × 2.3 cm) in 10% ethyl acetate in hexane; yield: 42%; TLC R_f value: 0.50 (10% ethyl acetate in hexane). ^1H NMR (CD_3COCD_3, 200 MHz) spectrum of the chromatographed product had δ 7.88 (*d*, 2 × ArH, J = 7.33 Hz), 7.28 (*d*, 2 × ArH, J = 7.33 Hz), 2.52 (*s*, CH_3–CO; *d*, 2 × 1′-H, $J_{1',2'}$ = 7.23 Hz), 1.98 (*m*, 2′-H, $J_{2',1'} = J_{2',3'}$ = 7.23 Hz), 0.88 (*d*, 6 × 3′-H, $J_{3',2'}$ = 7.23 Hz), consistent with the structure of 4-isobutylacetophenone.

4-Isobutylphenylacetic acid (ibufenac). 4-Isobutylphenylacetic acid was prepared from 4-isobutylacetophenone by the Kindler modification of the Willgerodt reaction, following the methodology of Nicholson and Adams [21]. Most of the coloured impurities were removed on a silical gel column (60 × 4.5 cm) eluted with 25% ethyl acetate in hexane. Recrystallization from petroleum ether gave white needles. The overall yield, based on isobutylbenzene, was 28.5%. One further recrystallization from the same solvent afforded shiny white needles; m.p.: 86–88° (literature [21]: 85.5–87.5°); TLC R_f value: 0.54 (ethyl acetate–hexane, 7:10). The ^1H NMR ($CDCl_3$, 200 MHz) spectrum of the product showed δ 7.23 (*d*, 2 × ArH, J = 7.86 Hz), 7.13 (*d*, 2 × ArH, J = 7.86 Hz), 3.64 (*s*, 2 × 2-H), 2.49 (*d*, 2 × 1′-H, $J_{1',2'}$ = 6.82 Hz), 1.89 (*m*, 2′-H, $J_{2',1'} = J_{2',3'}$ = 6.82 Hz), 0.93 (*d*, 6 × 3′-H, $J_{3',2'}$ = 6.82 Hz), in accordance with the structure of the title compound.

Synthesis of α-methyl-^{14}C-*labelled ibuprofen.* (Adapted from the method used by Pfeffer and Silbert [22] to prepare α-substituted long-chain aliphatic acids.)

Lithium diisopropylamide mono(tetrahydrofuran) (2.20 mmol) in cyclohexane (1.4 mL) was placed in a dry ice-cooled 5-mL flask maintained at 0° under nitrogen throughout the reaction. 4-Isobutylphenylacetic acid (200 mg, 1.04 mmol) in anhydrous tetrahydrofuran (3 mL) was added with stirring. After 10 min, hexamethylphosphoramide (0.45 mL) was added and the solution was stirred for 15 min. [^{14}C]Methyl iodide (1 mCi, 64 μL, 1.03 mmol) was then added. The reaction mixture was stirred under nitrogen at 0° for 1 hr, after which time a further volume of "cold" methyl iodide (50 μL, 0.80 mmol) was added and the solution stirred for another hour.

The solution was acidified with 10% aqueous HCl and extracted with ethyl acetate (2 × 30 mL). The organic layer was separated, washed with the aqueous acid (3 × 10 mL) and water (20 mL), dried with Na_2SO_4 and the solvent removed *in vacuo*. The residue was applied to a silica gel column (16 × 1.5 cm) and eluted with 25% ethyl acetate in hexane. Fractions (each of 2 mL) were collected and monitored by TLC. The fractions containing the product were pooled and evaporated to dryness, affording a white solid (148 mg, 44.4 μCi; 61.8 μCi/mmol); chemical yield = 69% and radioactivity yield = 4.4%. Recrystallization from a small volume of petroleum ether yielded a white powder (120 mg); m.p.: 75–77° (literature [21] 75–77.5°); TLC R_f value: 0.53 (ethyl acetate–hexane, 7:10).

The ^1H NMR ($CDCl_3$, 200 MHz) spectrum of the product showed δ 11.5–9.9 (broad, deuterium-exchangeable, –COOH), 7.27 (*d*, 2 × ArH_a, $J_{a,b}$ = 8.21 Hz), 7.14 (*d*, 2 × ArH_b, $J_{b,a}$ = 8.21 Hz), 3.75 (*q*, 2-H, $J_{2,3}$ = 7.18 Hz), 2.49 (*d*, 2 × 1′-H, $J_{1',2'}$ = 7.07 Hz), 1.88 (*m*, 2′-H, $J_{2',1'} = J_{2',3'}$ = 7.07 Hz), 1.53 (*d*, 3 × 3-H, $J_{3,2}$ = 7.18 Hz), 0.93 (*d*, 6 × 3′-H, $J_{3',2'}$ = 7.07 Hz), consistent with the structure of ibuprofen.

Extraction of soluble proteins from lens. Four fresh bovine lenses were homogenized with water (40 mL). The homogenate was centrifuged at 11,500 *g* and 4° for 20 min. The supernatant was freeze-dried and stored at −20°.

Equilibrium dialysis study of ibuprofen and paracetamol binding to lens proteins. Stock solutions of radio-labelled ibuprofen (9.2 mg, 45 μmol, 2.4 μCi) in 0.1 M phosphate buffer (pH 6.9) and 0.05% in sodium azide (140 mL) and of total lens soluble proteins (0.6 g, 30 μmol) in the phosphate buffer (10 mL) were prepared. Seven aliquots of the stock ibuprofen solution (20 mL each) were added to seven vials, each containing enough "cold" ibuprofen to give final concentrations of 0.34, 3, 6, 12, 18, 24 and 30 mM. Seven dialysis sacs containing the protein solution (1 mL each) were placed separately in each of the ibuprofen solutions, and were incubated at 37° for 25 hr. The progress of dialysis in the experiment, where a solution of 3 mM ibuprofen was used, was monitored by measuring the radioactivity of quadruplicate samples each of 0.1 mL from the external buffer at intervals of 0, 1, 3, 7 and 24 hr. The activities of the inside and outside of all the sacs were also determined at the end of the experiment by scintillation counting in Scintran Cocktail T (4.5 mL) to which water (10% v/v) had been added.

Similar experiments were carried out with paracetamol (0.96, 3, 6, 12, 24, 48 and 90 mM) incubated for 24 hr.

Fractionation of lens proteins incubated with ibuprofen and paracetamol. Total lens soluble proteins (30 mg, 1.5 μmol) were added to a solution of ibuprofen (1.57 mg, 7.6 μmol, 0.47 μCi) or paracetamol (0.7 mg, 4.7 μmol, 0.06 μCi) in the phosphate buffer (1.5 mL). The solution was incubated at 37° for 25 hr before chromatography on a TSK HW-55 (S) Fractogel column (85 × 1.5 cm) collecting 1.5-mL fractions until all the proteins had eluted when 5-mL fractions were collected. In the case of the ibuprofen experiment, having collected a total of 400 mL of the eluent, the eluting solution was changed to 5% aqueous NH_3. All fractions were monitored for radioactivity by scintillation counting.

Preparation of crystallins. Total lens soluble proteins (200 mg) were applied to a Fractogel column

(135 × 2.5 cm). The column was eluted with the phosphate buffer at 20 mL/min. Fractions of 2-mL volumes were collected and their absorbances at 280 nm were measured. The α-, β- and γ-crystallin fractions were appropriately pooled and dialysed against distilled water over 24 hr with three changes. The dialysed solutions were then freeze-dried and used in the next experiment.

Equilibrium dialysis examination of ibuprofen binding to separated crystallins. The crystallins prepared as described above were dissolved separately in the phosphate buffer (1.5 mL) and dialysed against a solution of ibuprofen (0.83 mg, 4 μmol, 0.2 μCi) in the phosphate buffer (20 mL). The amount of protein in each solution was estimated by the method of Lowry *et al.* [23] after 25 hr of incubation, following centrifugation to remove any precipitated proteins. α-, β- and γ-Crystallins still in solution were estimated to be 74, 50 and 27 mg, respectively. Radioactivity was measured in quadruplicate samples of 0.1 mL each from inside and outside the sacs.

Penetration of ibuprofen and paracetamol into the whole lens. Four bovine lenses were removed from the eyeballs, washed, gently blotted and placed in MEM solution (10 mL). Ibuprofen (0.82 mg, 4 μmol, 0.19 μCi) or paracetamol (0.6 mg, 4 μmol, 0.15 μCi) was added and the lenses were incubated at 37° with rocking. The activity of the medium was measured at intervals for up to 24 hr, at which time the lenses were carefully blotted with a filter paper and separated immediately into capsule, cortex and nucleus which were weighed separately. The latter two were homogenized individually in phosphate buffer (5 mL). Quadruplicate samples of 0.1 mL each were taken for radioactivity measurements. The activity of the unhomogenized capsules was also measured.

The cortex and nucleus homogenates were centrifuged at 11,500 *g* for 30 min. The supernatant was removed and the insoluble material washed twice by resuspension in the buffer (5 mL each time), followed by centrifugation. The combined washings were added to the main supernatant. The radioactivity of the combined soluble fractions and of the insoluble materials was measured.

Four aliquots (0.1 mL) of the soluble fractions were removed, and to each was added 10% aqueous TCA (1 mL). The resulting suspension was allowed to stand at 4° overnight. The precipitated proteins were filtered off using glass fibre filter paper and washed with 5% aqueous TCA (5 × 5 mL). The radioactivity of the filter paper containing the isolated proteins and of the filtrate was determined by liquid scintillation counting.

Covalent binding of ibuprofen and paracetamol to lens soluble proteins. Lens soluble protein (100 mg), was dissolved in the phosphate buffer (10 mL) containing ibuprofen (10.5 mg, 51 μmol, 3.1 μCi) or paracetamol (7.6 mg, 50 μmol, 4.5 μCi). The resulting solution was incubated at 37° for 5 weeks and aliquots (4 × 0.1 mL) of the solution were removed at intervals of 0, 1, 2, 4, 8, 16, 25 and 35 days and treated with 10% aqueous TCA as described above. Radioactivity in both the protein precipitates and the filtrate was determined by scintillation counting.

Preparation of affinity column. 2-(4-Aminophenyl)propanoic acid was prepared by aqueous $NH_3/FeSO_4$ reduction of the corresponding nitro-compound, following the method of Jacobs and Heidelberger [24] for the preparation of 4-aminophenylacetic acid. The product was recrystallized from chloroform, giving white needles; yield = 45.4%, m.p.: 137.5–139°, TLC R_f value: 0.59 (ethyl acetate–petroleum ether, 3:1). ^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of the product had δ 6.68 (*d*, 2 × ArH, J = 8.71 Hz), 6.21 (*d*, 2 × ArH, J = 8.71 Hz), 5.40 (broad, deuterium-exchangeable, 2 × –NH), 3.15 (*q*, 2-H, J = 6.82 Hz) and 0.97 (*d*, 3 × 3-H, J = 6.82 Hz), indicating the structure of 2-(4-aminophenyl)propanoic acid.

Sepharose 4B (50 mL) was activated with CNBr [25], and coupled to 6-amino-*n*-hexanoic acid [26]. The coupled agarose was suspended in a solution of 2-(4-aminophenyl)propanoic acid (330 mg, 2 mmol) in water (80 mL). The pH of the suspension was brought to 4.62 with 1 M HCl. The coupling reagent, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.1 g, 5.74 mmol) dissolved in water (6.5 mL), was added dropwise to the suspension over 5 min with gentle shaking at room temperature. Shaking continued for 24 hr. The gel was filtered off, washed with a large volume of the 0.1 M phosphate buffer (pH 6.9), resuspended in the phosphate buffer and packed into a glass tube.

Affinity chromatography of lens proteins on a column coupled with an ibuprofen analogue. A solution of total lens proteins (50 mg) dissolved in the phosphate buffer (2 mL) was applied to the affinity column (13 × 1.5 cm). The column was washed successively with the 0.1 M phosphate buffer (50 mL), 0.5 M phosphate buffer (pH 6.9) containing 1 M sodium chloride (50 mL), and urea of molarity 2, 4, 6 and 10 M (100 mL each) in the 0.1 M phosphate buffer. The protein contents of the collected 5-mL fractions were estimated by measuring their absorbance at 280 nm.

RESULTS

The mean values of four samples were used for calculations. Standard deviations are represented by SD. Student's *t*-test was used for comparison of groups.

Synthesis

The melting points, ^{1}H NMR spectra and thin-layer chromatographic behaviours of the ibuprofen prepared in this work and of an authentic sample were identical. The chemical routes for the synthesis of the radio-labelled ibuprofen and the preparation and coupling of the affinity material to the agarose are depicted in Figs 1 and 2, respectively.

Equilibrium dialysis

Binding of ibuprofen and paracetamol to lens proteins was explored by equilibrium dialysis. Dialysis of the solution of total lens soluble proteins against ibuprofen or paracetamol reached equilibrium after approximately 7 and 2 hr, respectively. The concentration of the drugs inside the

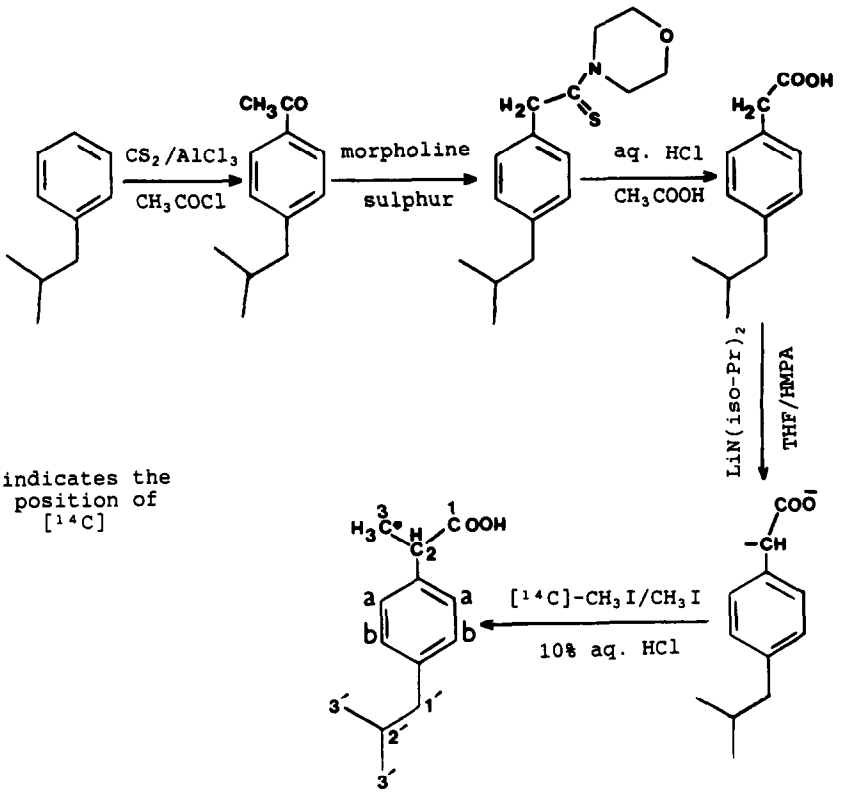


Fig. 1. Synthesis of ^{14}C-labelled ibuprofen. THF = tetrahydrofuran; HMPA = hexamethylphosphoramide.

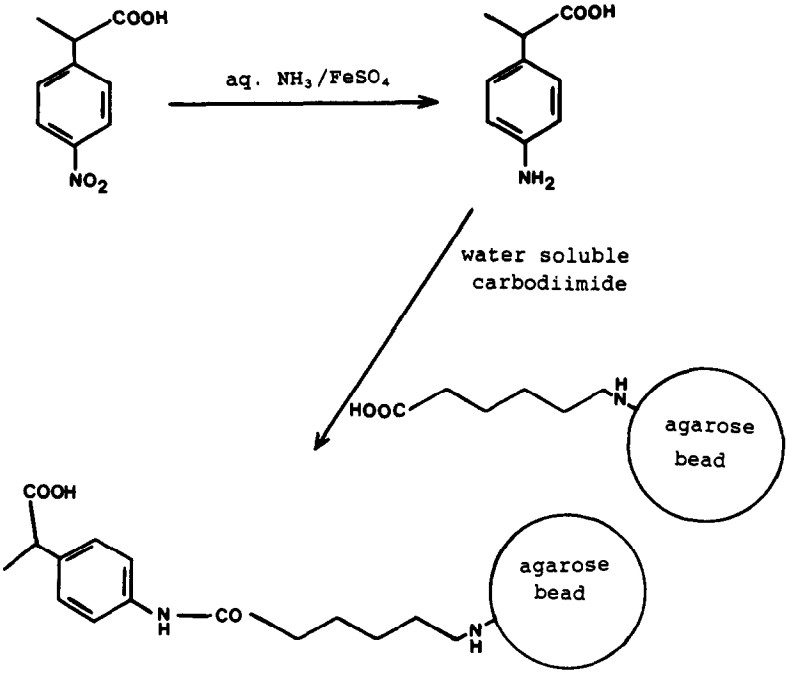


Fig. 2. Preparation of 2-(4-aminophenyl)propanoic acid and its subsequent coupling to Sepharose 4B through the linker.

Table 1. Equilibrium dialysis of lens proteins against paracetamol and ibuprofen

[drug]/[P] used	[I]/[P] bound	[Pa]/[P] bound
0.114	0.049 ± 0.006*	—
0.32	—	0.018 ± 0.007*
1	0.192 ± 0.0125*	0.072 ± 0.029*
2	0.595 ± 0.037*	0.150 ± 0.024†
4	1.28 ± 0.06*	0.322 ± 0.083†
6	2.37 ± 0.11*	—
8	5.67 ± 0.09*	0.684 ± 0.097*
10	7.87 ± 0.22*	—
16	—	1.375 ± 0.170*
30	—	2.60 ± 0.22*

[I]/[P] = moles ibuprofen bound per mole protein subunit (M_r = 20,000). [Pa]/[P] = moles paracetamol bound per mole protein subunit. [drug]/[P] = ratio of drug and protein subunits concentrations used.

Values are means ± SD.

* $P < 0.001$; † $P < 0.015$ (Student's t-test between the drug concentrations inside and outside the sacs).

dialysis sacs for increasing amounts of the drugs were higher than those outside, depending on the drug–protein concentration ratios used. The amounts of bound drugs increase with the drug–protein concentration ratios used (Table 1).

In experiments with the separated proteins, α-crystallin bound ibuprofen in a ratio of 1 ibuprofen to 25 α-crystallin subunits of 20,000 molecular weight (Student's t-test comparison between the ibuprofen concentrations inside and outside the sac, $P < 0.001$), but there was no binding to the other crystallins.

Covalent binding of ibuprofen and paracetamol to lens soluble proteins

Proteins precipitated with 10% aqueous TCA in the ibuprofen and paracetamol experiments up to 5 weeks of incubation showed no radioactivity. All the activity was found in the filtrate, indicating the lack of covalent interaction.

Penetration of drugs into whole lenses

Following incubation of lenses with ibuprofen and paracetamol, the radioactivities of whole capsule/epithelial fraction and samples from cortex and nucleus homogenates were measured and the values clearly showed that these drugs permeate into the lens and that their concentrations were higher in cortex than in nucleus (Table 2). Separation of cortex and nucleus into soluble and insoluble fractions by centrifugation revealed ibuprofen and paracetamol to be almost exclusively (more than 98%) in the soluble fractions. The small amounts of activity exhibited by capsules and the insoluble proteins may be due to their contamination rather than due to any binding.

The TCA-precipitated proteins from the soluble fractions of both cortex and nucleus of both ibuprofen and paracetamol incubations were shown to be virtually devoid of activity and the filtrate contained almost all the activity (more than 98%), indicating that little, if any, drug was covalently bound to the lens proteins in these experiments with whole lenses.

Table 2. Concentration of ibuprofen and paracetamol in different regions of the lens following 24 hr of incubation

Lens constituents	Concentration (μmol/g wet weight) Ibuprofen	Paracetamol
Capsule/epithelium	0.025 ± 0.014	0.021 ± 0.006
Total cortex	0.42 ± 0.03*	0.26 ± 0.03†
Total nucleus	0.20 ± 0.01*	0.14 ± 0.04†

Values are means ± SD.

* $P = 0.001$; † $P = 0.002$ (Student's t-test between ibuprofen concentrations in cortex and nucleus).

Fractionation of lens soluble proteins incubated with ibuprofen and paracetamol

Size-exclusion chromatography (Fig. 3) of the lens soluble proteins incubated with labelled ibuprofen and paracetamol revealed no radioactivity in the protein fractions, the last of which, γ-crystallin, was eluted before 120 mL while paracetamol left the column after 140 mL, well clear of the proteins. Ibuprofen remained bound to the column and was only eluted after 5% aqueous NH_3 was applied to the column.

Affinity chromatography

The lens proteins were tightly bound to the affinity column containing 2-(4-aminophenyl)propanoic acid attached to the linker through an amide linkage. Severe dissociating conditions up to 10 M urea, had to be employed to release the proteins from the column (Fig. 4).

DISCUSSION

A protective action of ibuprofen and paracetamol against cataract has been indicated by epidemiological studies [15, 16], *in vitro* experiments and by studies of diabetic rats [19]. The binding studies here were an attempt to explore this activity. In the equilibrium dialysis experiments the concentrations of ibuprofen and paracetamol inside the dialysis sac exceeded those outside the sac. As the free drug can traverse the semi-permeable membrane in both directions, the excess drug inside the sac is a measure of the amount of drug bound to the proteins in the dialysis sac (Table 1). Only α-crystallin bound ibuprofen in the equilibrium dialysis. The binding of ibuprofen and paracetamol to the proteins is not covalent as indicated by TCA precipitation of proteins, and indeed it is very weak as revealed by the observation that the protein-containing fractions from the size-exclusion column showed no radioactivity. The radioactive drugs were eluted later than the proteins (Fig. 3). Presumably such weak binding does not account for the therapeutic effect of the drugs, since, in order to protect the proteins a drug must be able to reside on the protein molecule tightly enough to compete with cataractogenic agents for binding sites.

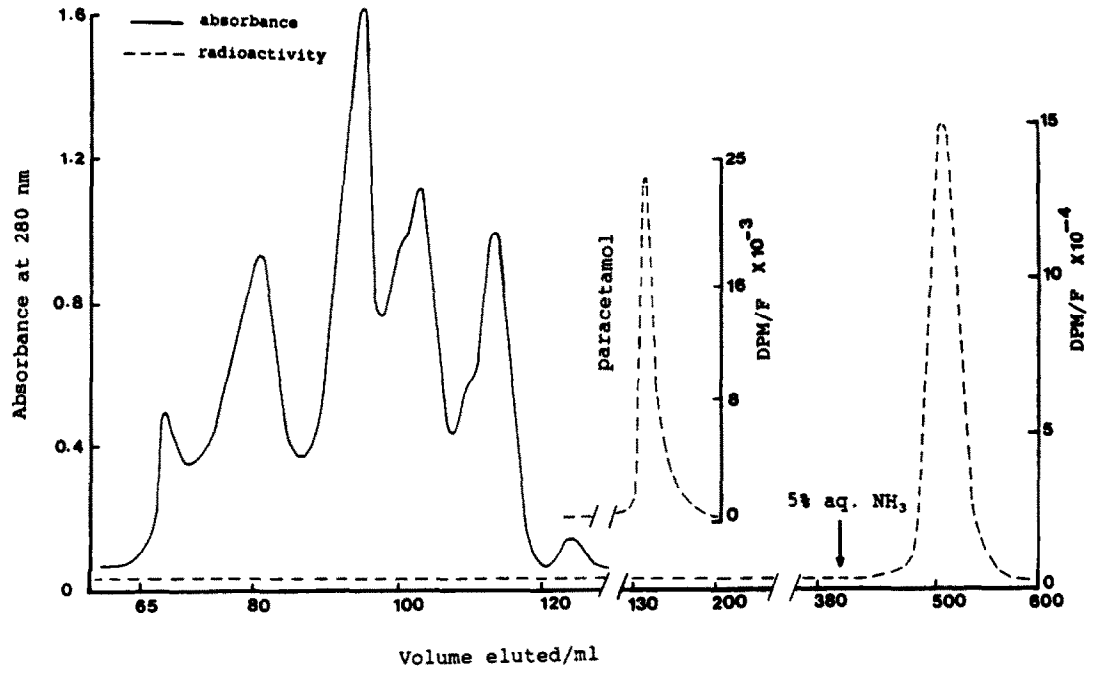


Fig. 3. Gel chromatography of bovine lens soluble proteins incubated with [^{14}C]ibuprofen and with [^{3}H]paracetamol separately. The inset shows the paracetamol peak from a separate run on the same column. DPM/F = disintegrations per minute per fraction.

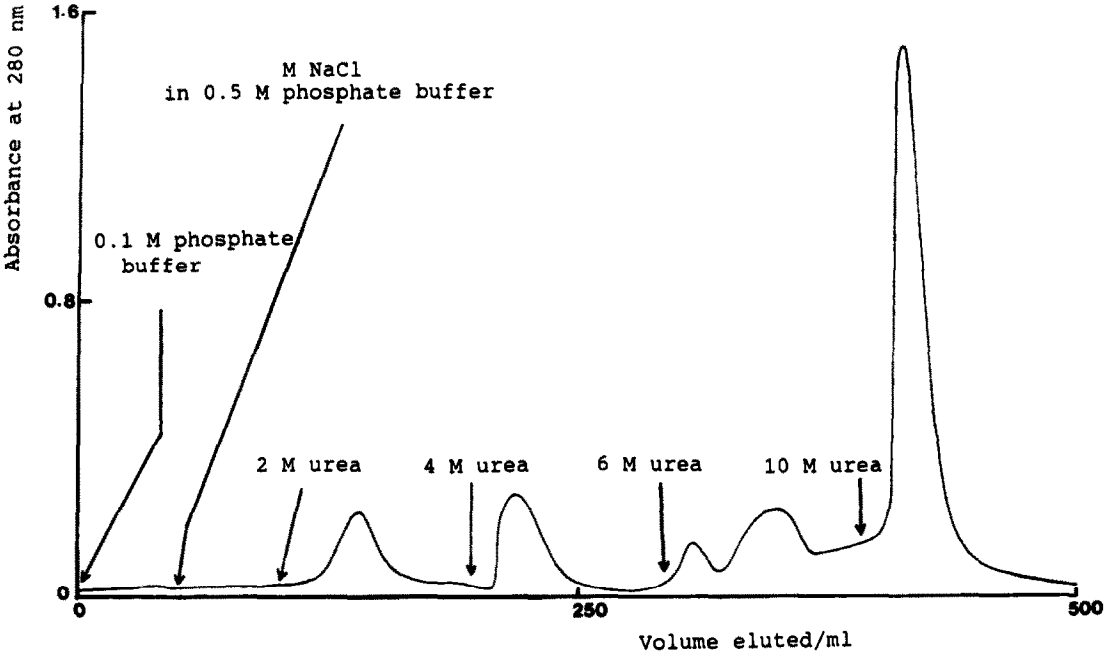
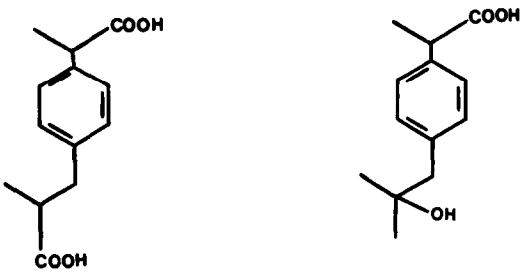


Fig. 4. Affinity chromatography of lens proteins on Sepharose 4B coupled to an ibuprofen analogue.

The protective effect of aspirin on lens proteins has been explained by acetylation [17]. Ibuprofen and paracetamol permeate into the cortex and nucleus (Table 2) showing that if they reach the aqueous humour after oral dosing they could reach the parts of the lens susceptible to cataract. The insoluble proteins of cortex and nucleus bind neither ibuprofen nor paracetamol, so presumably these drugs do not



(I) (II)

Fig. 5. Major metabolites of ibuprofen.

bind to the plasma membranes which are present in this fraction and therefore do not protect lens by action at the membrane.

The equilibrium dialysis and size-exclusion chromatography indicated weak binding of paracetamol and ibuprofen to lens proteins but the affinity chromatography seemed to indicate strong binding (Fig. 4). This is presumably because the affinity column was made with a homologue of ibuprofen. This was 2-(4-aminophenyl)propanoic acid which has a carboxamide group in place of the isobutyl group of ibuprofen (Fig. 2). This suggests that the binding site for the ibuprofen analogue is hydrophilic in nature and that the isobutyl chain is too lipophilic to allow substantial interactions. Therefore, it is possible that cataract prevention is not due to ibuprofen *per se* but due to a metabolite perhaps with enhanced hydrophilicity. Two known metabolites of ibuprofen (Fig. 5) [27] are more hydrophilic than the parent compound. These metabolites each have an additional hydrogen bonding species and might be formed in the lens or might reach the lens after formation elsewhere in the body. Similarly, paracetamol may need metabolic activation and/or conjugation in order to protect against cataract. A case in point for the therapeutic importance of metabolism is bendazac which appears not to be effective against the binding of galactose and glucose-6-phosphate to proteins, whereas 5-hydroxybendazac, a metabolite of bendazac, does ensure such protection [28].

Nevertheless, the possibility of ibuprofen and paracetamol achieving their cataract-prevention effects by some other mechanism(s) than direct interaction with the proteins still exists. Other possibilities that have been suggested include inhibition of prostaglandin synthesis, decreasing blood glucose level, increasing blood flow to the eye [29,*]. The possibility that the drugs act by membrane interactions seems unlikely as they do not bind to the membrane-containing fraction.

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* E. Linnér, personal communication.

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