CHAPTER 1

GENERAL INTRODUCTION
1.1 FLURBIPROFEN - AN OVERVIEW

Introduction
Flurbiprofen, CAS registry number (Substance name) 5104-49-4, a member of the phenylalkanoic acids (1), a white (or almost white) crystalline powder, melting point 114-117°C, practically insoluble in water, but readily soluble in most organic solvents, also known as a hydatropic acid analog (2), was already subjected for evaluation of its platelet aggregation inhibiting action in 1973 (3). Chemically it is known as: 2-fluoro-α-methyl-[1,1'-biphenyl]-4-acetic acid; 2-fluoro-α-methyl-4-biphenyl-acetic acid; 2-(2-fluoro-4-biphenyl)propionic acid; 3-fluoro-4-phenylhydatropic acid (4). In 1993 its potent anti-platelet activity was evaluated in a double-blind, placebo-controlled, multi-centre study for efficacy on preventing reinfarction and reocclusion after successful thrombolysis or angioplasty in acute myocardial infarction (5).

Flurbiprofen

Flurbiprofen is described in the recent editions of the United States (USP), European (EP), British Pharmacopoeia (BP) and Japanese Pharmacopoeia (JP). In the USP both flurbiprofen and its sodium salt are described in the racemic form. In the EP the racemic form is also described; however in the EP and BP monograph of flurbiprofen the existence of an enantiomer is alluded to ("and enantiomer"). The Japanese Pharmacopoeia (JPXIII) gives no hint of the chiral nature of the flurbiprofen molecule. A solution of flurbiprofen in methanol giving no optical rotation is the only description given thereof.

A monograph for flurbiprofen eyedrops is mentioned in the USP as "Flurbiprofen sodium ophthalmic solution" and in the BP as "Flurbiprofen eyedrops". They contain not less than 90.0% and not more than 110.0% of the prescribed or stated amount. The sodium salt of flurbiprofen in the USP and BP is available in the dihydrate form.

In the EP, five impurities for flurbiprofen are mentioned. Interestingly 4 are chiral (one chiral centre) and one diastereomeric in nature (two chiral centers).
Pharmacodynamics

The major pharmacological properties have already been reviewed by Adam et al. in 1975 (1). Several discriminating techniques were applied to determine the lowest effective oral dose (mg/kg) as anti-inflammatory, analgesic and antipyretic drug. In the anti-inflammatory tests three animal species were used: guinea pig, rat and mouse. In the guinea pig the UV-erythema test was employed in which the reference compound (acetylsalicylic acid, 80 mg/kg) was found to correspond to 0.25 mg/kg of flurbiprofen. In the mouse model the capillary permeability of the peritoneum was evaluated by use of a dye (Pontamine sky blue). Acetylsalicylic acid at 120 mg/kg was equivalent to 0.47 mg/kg of flurbiprofen. In the rat three methods were employed: the carageenan edema test, and two adjuvant arthritis models for the developing state and the established state. Reference compounds were, respectively, acetylsalicylic acid (81 mg/kg) in the first and indomethacin (1 mg/kg) and phenylbutazone (10 mg/kg) in the two latter models. The corresponding lowest effective dose for flurbiprofen was, 0.11 mg/kg, and 0.33 mg/kg in the two latter models.

With the carageenan edema test a subgroup of rats was also tested who were bilaterally adrenalectomised to rule out any adrenocortical interference. Several conclusions were drawn from this study. Flurbiprofen was devoid of adrenocortical-stimulating properties and was one of the most potent agents of this type reported yet; at least 10 times more potent than ibuprofen. It was postulated that the mode of action in the mouse and rat was not identical to that of acetylsalicylic acid.

In US patent 3,755,427 (August 28th 1973) (6) it was stated that flurbiprofen was between 75 to over 100 times as potent as acetylsalicylic acid.

Pharmacokinetics

Pharmacokinetic properties have been assessed in different species (7). In man (8), when assessed by HPLC of the racemic molecule, a two-compartment open model appeared the most appropriate for flurbiprofen. Drug absorption efficiency was found independent of the oral dose. The intact drug resides mainly in the
peripheral and central compartments, disappearing with a terminal half life of approximately 5.5 hours. More than 99% of flurbiprofen is bound to serum proteins. The serum flurbiprofen concentrations in clinical use however show an occupancy of less than 10% of the primary binding sites. The binding site differs from that of drugs like oral anticoagulants and sulphonamides. Drug interactions will therefore not automatically occur with simultaneous use.

Oxidation and conjugation are the main pathways of metabolism. More than 95% of an oral dose is excreted via the kidney within 24 hours. Forty to 47% of a daily oral dose is excreted as 2-[2-fluoro-4'-hydroxy-4-biphenylyl]propionic acid; 5% as 2-[2-fluoro-3',4'-hydroxy-4-biphenylyl]propionic acid; 20-30% as 2-[2-fluoro-3'-hydroxy-4'-methoxy-4-biphenylyl]propionic acid and 20-25% as the parent molecule flurbiprofen. Between 65 - 85% of flurbiprofen and its metabolites are present as glucuronide and sulfate conjugates.

Stereoselective HPLC of human plasma has also been performed (9). After oral administration of 25 mg of the R(-) enantiomer of flurbiprofen no indication was found that inversion to the S(+) enantiomer occurred. This was confirmed in healthy volunteers taking either 50 mg R(-) flurbiprofen or S(+) flurbiprofen (10). Several studies on the pharmacokinetics of flurbiprofen in the rat have been performed all showing that in this species a minimal amount of inversion could take place (approx. 5%), the inversion halftime being approximately half an hour (11,12,13,14).

Stereoselective studies have been performed following the disposition of flurbiprofen in normal volunteers after a single 50 mg racemic dose (15), in healthy female subjects following oral administration of the single enantiomers of flurbiprofen, 50 mg S(+)-flurbiprofen or R(-)-flurbiprofen or 100 mg R(-)-flurbiprofen or placebo, in a 4-way crossover design with placebo (16); in patients with end-stage renal disease undergoing continuous ambulatory peritoneal dialysis (CAPD) after administration of a single 100 mg racemic dose (17), and stereoselective disposition of racemic flurbiprofen in single and multiple dosing in uraemic patients (18). On the basis of pharmacokinetics, adjustment of flurbiprofen dosing in uraemic patients is not necessary. In CAPD patients circulating plasma levels of flurbiprofen proved 40-50% lower than in normal subjects implying that analgesia could be less than expected in this selected group of patients. Accumulation of the 2-[2-fluoro-4'-hydroxy-4-biphenylyl]propionic acid metabolite, which has minimal anti-inflammatory activity, does occur in this group of patients but the clinical significance is not established. In patients with liver disease with ascites and in renal failure patients with a creatinine clearance of less than 10 ml.min-1, significant higher free fractions of R(-) and S(+) flurbiprofen were detected in conjunction with lower albumin concentrations (19). An overview of the clinical pharmacokinetics of flurbiprofen and its enantiomers is presented in (20).
A different model was introduced for the investigation of the pharmacokinetics of flurbiprofen enantiomers and the simultaneous inhibition of prostanoid production (21). This study was performed in healthy volunteers in whom, after receiving orally either 75 mg R(-), S(+) flurbiprofen or no medication in a randomised 3-way cross-over design, flurbiprofen pharmacokinetics were analysed by HPLC and prostanoid production was monitored by enzyme immuno assay and chemiluminescence assay. Here also no clinically relevant inversion of R(-) to S(+) flurbiprofen was seen. However, the study showed unexplained discrepancies in several stages of the pharmacokinetic and pharmacodynamic parameters of the flurbiprofen enantiomers.

Chiral inversion of R(-) flurbiprofen to S(+) flurbiprofen has been studied in vitro to investigate the mechanism behind this phenomenon which is not shared by all 2-arylpropionic acids (22). With crude rat liver homogenates it was demonstrated that an acyl-CoA synthase enzyme in conjunction with ATP and Mg2+ is obligatory. The first step comprises the metabolic formation of a CoA thioester of R(-) ibuprofen. It has been made plausible that this step takes place in adipose tissue since after administration of R(-) flurbiprofen significant amounts of both enantiomers are found in adipose tissue. By way of a non-stereoselective racemase (epimerase) this product is converted to its S(+) ibuprofen CoA thioester. Through action of a hydrolase, S(+) ibuprofen is released from its CoA thioester form. This unidirectional enantioselective chiral inversion in man has not been reported for flurbiprofen, carprofen and ketoprofen (23,24,25). Research on the enzymatic inversion at the chiral carbon atom had been done earlier by use of deuterated ibuprofen. In that study it was noted that the R(-) isomer is the only substrate for the epimerisation reaction (26). A summary of the metabolic chiral inversion of 2-arylpropionic acid derivatives, the variations between species and the complexity that can arise due to formed chiral metabolites has been presented in (27).

Biliary excretion has been examined in normal and bile-duct cannulated rats for the enantiomers of flurbiprofen after intravenous dosing of 10 mg/kg of each enantiomer (28). It was found that the fraction of enterohepatic circulation was greater for R(-) flurbiprofen than for its antipode. Although the S(+) flurbiprofen enantiomer was excreted to a greater extent in bile, reabsorption from the intestine was insignificant. One reason for this phenomenon may be the presumed stereoselective hydrolysis of the flurbiprofen conjugates with preference for the R(-) enantiomer. However in later similar experiments (29) enterohepatic cycling of both flurbiprofen enantiomers could be demonstrated.

Glucuronidation in rat and human liver microsomes proceeds faster for the R(-) enantiomer than for its S(+) antipode. Glucuronidation is facilitated by the enzyme complex UDP-glucuronosyltransferase of which there exist several isoforms (30). However not the identity of the isoenzyme but the stereoselective interaction of the enantiomer influences the reaction velocity.
In a review (31) on the binding of flurbiprofen to albumin in human plasma it was reported that at low therapeutic concentrations the $S(+)\overline{\text{enantiomer}}$ has a higher protein binding than its $R(-)\overline{\text{antipode}}$. At high drug concentrations there is no measurable difference, however. In an ultrafiltration study done with normal volunteers the free fraction of $R(-)\overline{\text{flurbiprofen}}$ was higher than its $S(+)\overline{\text{antipode}}$ at low drug levels but similar for both enantiomers at higher drug levels. Patients with renal impairment and patients exhibiting hypoalbuminaemia have higher free fractions of flurbiprofen enantiomers than normal volunteers. Plasma protein binding of an enantiomer is not influenced by its own concentration or the presence of its antipode under clinical therapeutic conditions (32).

In a model study using isolated perfused rabbit lungs it was demonstrated that flurbiprofen does not undergo pulmonary metabolism to any extent (33).

As mentioned above (8) the main routes of biotransformation of flurbiprofen are through oxidation and conjugation. Oxidation has been investigated more specifically (34) for the enantiomers of flurbiprofen utilizing human liver microsomes. The most prominent oxidative metabolism route is by cytochrome P450. It was established that cytochrome P450 2C9 and its allelic variant R144C catalysed the oxidative reaction. Interestingly, there was no stereoselective preference of one enantiomer over the other.

Safety for intestinal permeability changes when using the racemate or the separate enantiomers of flurbiprofen was studied in rats for which species it was established that only a minimal inversion of the $R(-)\overline{\text{enantiomer}}$ takes place. Intestinal permeability was measured by urinary excretion of $^{51}\text{Cr-EDTA}$ (35). It was established that at both dosages used ($1 \text{ mg/kg}$ and $3 \text{ mg/kg}$ for the racemic drug and half for the enantiomers) permeability was significantly different from control. $R(-)\overline{\text{flurbiprofen}}$ was safest in both dosage ranges. $S(+)\overline{\text{flurbiprofen}}$ inflicted similar damage as the racemic form.

In (36) it was shown that in rats $R(-)\overline{\text{flurbiprofen}}$ gave the same increase of intestinal permeability, but the difference was that the impact on mucosal prostanoid production was smaller and not accompanied by ulcerative changes in the small intestine.

Although it would seem attractive to develop therapeutic $R(-)\overline{\text{enantiomers}}$ of 2-arylproionic acids due to its supposedly lower toxicological profile it must be borne in mind that the presumed pharmacological action required for reducing inflammation is inhibition of prostaglandin synthesis. This property resides primarily, in the case of flurbiprofen, in the $S(+)\overline{\text{enantiomer}}$ for which a difference of 30 to 100 times compared to the $R(-)\overline{\text{enantiomer}}$ was established depending on the model used. Only with full metabolic inversion of a $R(-)\overline{\text{enantiomer}}$ to a $S(+)\overline{\text{enantiomer}}$ would such a therapeutic drug be a possibility. For flurbiprofen this is not the case in humans (37,38,39).
In a comparative study (40) done in rabbits, the inhibitory effect on rise in intraocular pressure and increase in aqueous humor protein after topical application of arachidonic acid (5% in peanut oil) by 14 nonsteroidal anti-inflammatory inhibitors was measured. For 50% inhibition of the intraocular pressure response, flurbiprofen ranked second best with an effective concentration of approximately 0.06%. Indomethacin (suspension in water, not further specified) ranked 4th with an approximate concentration of 0.2%.

In a short review (41) the importance of the involvement of prostaglandins to certain eye conditions is discussed. The rise in intraocular pressure and the breakdown of the blood-aqueous barrier were related to these compounds. A search for the best drug in inhibiting prostaglandin mediated diseases was called for before testing them in the human eye.

The comparative in vivo inhibitory effects of flurbiprofen, indomethacin and acetylsalicylic acid, all as sodium salt solutions, have been tested in the rabbit anterior uvea and conjunctiva after topical (0.5% solutions) and intraperitoneal administration (42). In both methods of administration acetylsalicylic acid almost completely abolished prostaglandin synthesis. Flurbiprofen given intraperitoneally was more potent than indomethacin which inhibited prostaglandin synthesis only partially, even at twice the dose of acetylsalicylic acid and flurbiprofen. Topical administration revealed that acetylsalicylic acid performed well even at a dose as low as 0.01% but indomethacin and flurbiprofen performed poor.

Use of flurbiprofen (0.01% and 0.1%) was evaluated in comparison to 1% prednisolone as an inhibitor of corneal neovascularization in New Zealand albino rabbits (43). Flurbiprofen 0.1% and prednisolon 1% were equally effective in inhibiting vessel growth.

As an alternative possibility for the use of topical administration of corticosteroids a nonsteroidal anti-inflammatory drug was considered (44). Flurbiprofen was tested in a double-blind fashion to see if intraocular pressure would change and if its use could block corticosteroid induced ocular hypertension. In a selected group of patients, with known intraocular sensitivity towards corticosteroids, flurbiprofen eye-drops (0.03%) did not alter intraocular pressure following six weeks of treatment. Also pretreatment by flurbiprofen did not block corticosteroid-induced ocular hypertension.

Flurbiprofen was also investigated for human use in the prevention of intraocular inflammation (45). In a randomised double-blind parallel group study, placebo or flurbiprofen (100 mg thrice daily) was given orally for 8 days starting 24 hours before routine cataract extraction. Flurbiprofen was only favoured over placebo for the resolution of corneal inflammation at day 6. Interestingly, flurbiprofen concentrations in the aqueous were detected up to 4 hours after the last dose with a con-
centration of 0.57 mg/L (2.3x10^{-6}M). The authors conclude that flurbiprofen may be of value in the treatment of uveitis and other kinds of intraocular inflammation. In a study (46) involving female New Zealand rabbits, eyedrop disposition was studied after application of ^14C labeled flurbiprofen in a concentration of 0.03%. No metabolism was detected for flurbiprofen in the eye. The total amount present in ocular tissues (cornea, aqueous humor, iris, ciliary body, choroid and retina) in normal rabbit eyes 30 minutes after application of 50 microliter of a 0.03% solution, was 4.25%. At 6 hours this was 1.59%. Following ocular application 77.51±8.79% was found in a 24-urine collection period. Unchanged flurbiprofen accounted for 25.3±3.6%.

Ocular availability was studied in female albino rabbits (47) receiving 50 microliter of 0.3% or 0.15% solution of flurbiprofen. The ocular bioavailability of the 0.3% solution was 10% and for the 0.15% solution 7%. The elimination half-life in the aqueous humor was 93 minutes which approximates the turnover rate of aqueous humor in the rabbit and indicates that drainage is the main route of elimination.

Bioavailability was determined after a single dose and after multiple doses of labeled flurbiprofen in rabbit eyes using topical application (48). Multiple dosing of a flurbiprofen solution of 0.03%, every half hour three doses, gave levels in the eye high enough to prevent prostaglandin synthesis. It compared favourably to the use of a single drop of 0.1% solution possibly because of less irritation of the eye and thus less stimulation of tear flow. Peak tissue concentrations were reached between 30 minutes and 60 minutes and were 2 to 6 times higher in all tissues than seen after one drop of 0.1% solution.

To determine the intraocular concentration of flurbiprofen sodium in the human aqueous humor of patients undergoing cataract surgery, samples were taken after receiving flurbiprofen sodium at selected times prior to surgery (49). Only single drop instillation was done. Samples of aqueous humor were analysed by HPLC. Flurbiprofen concentrations were detectable in the aqueous between 30 minutes to 7.25 hours after topical application.

To determine if and how much drug can penetrate to the posterior segment of the eye, a study was done in white New Zealand albino rabbits, by single drop method of dosing, using paracetamol 1% in saline and bendazac lysine 0.5% in saline or other solvent (50). When comparing the data with literature data it appeared that paracetamol behaved similar to flurbiprofen as regards penetration into the aqueous humor, having a very poor entry into the vitreous and attaining higher concentrations in the retina than paracetamol. In all three eye compartments bendazac lysine permeated poorly. The data suggest an alternative entry route to the posterior segments of the eye. It appears that the lens acts as a barrier for the entry from the aqueous.
In a study done by Carabaza et al. inhibition of prostaglandin synthesis was investigated using the enantiomers of three NSAIDs (ketoprofen, flurbiprofen and ketorolac), including stereoselective inhibition of inducible COX-2 (51). It became apparent that inhibition by the three enantiomer pairs is comparable for COX-1 and COX-2. With both cyclooxygenase isoenzymes inhibition resides almost exclusively in the S(+) isomer.

One of the most frequent problems encountered during cataract surgery is involuntary pupillary constriction. In the past, several pharmacological interventions have been tried as a remedy, but without success. Albino rabbits have been used to study the effect of topical administration of indomethacin (1% aqueous solution with no further specification of buffer and pH used) and flurbiprofen (0.03% aqueous solution of sodium flurbiprofen) on this unwanted condition (52). In this set-up also local anaesthetics, capsaicin, sympathomimetic agents and an anticholinergic were involved all according to a specified protocol. Flurbiprofen demonstrated a significant inhibitory effect on miosis while topical indomethacin failed. However no single agent or combination of agents blocked the miotic response completely.

Although nonsteroidal anti-inflammatory drugs are pharmacologically effective inhibitors of cyclooxygenase activity and prostaglandin synthesis (53), cyclooxygenase-independent anti-inflammatory actions of NSAIDs are also known (54). Since it was reported that sodium salicylate and acetylsalicylic acid inhibit the action of the transcription factor nuclear factor kappa B (NF-κB), the enantiomers of flurbiprofen were tested in a zymosan-induced paw inflammation model. Although R(-) flurbiprofen does not inhibit cyclooxygenase to a significant extent, it is more potent than S(+) flurbiprofen and almost as effective as dexamethasone in this inflammatory model. Inhibition of NF-κB by R(-) flurbiprofen resulted in a reduced expression of COX-2 and tumor necrosis factor α (TNF-α).

Nitric oxide formed by the inducible NO synthase (iNOS) has been implicated as a mediator of pain and tissue injury in various inflammatory and autoimmune diseases. In an in vitro model involving RAW 264.7 macrophages, it could be demonstrated that iNOS mRNA expression is equipotently suppressed by the enantiomers of flurbiprofen. S(+) flurbiprofen and R(-) flurbiprofen did not inhibit LPS induced COX-2 mRNA expression but did inhibit LPS-induced prostaglandin E₂ formation enantioselectively, with the S(+) antipode being 46 times more active than the R(-) flurbiprofen (IC₅₀ 0.0061µM and 0.28 µM respectively). Collectively, these findings would suggest that the pharmacological (i.e. anti-inflammatory) activity of the flurbiprofen enantiomers is not only related to inhibition of cyclooxygenase enzyme activities but also to inhibition of transcription factor activation like NF-κB and AP-1, resulting in diminished formation of pro-inflammatory factors like iNOS and TNF-α (55,56).
REFERENCES


1.2 Cataract and Cataractogenesis

Cataract
Alterations in lens transparency increase with age. It seems possible that the lens stays transparent until the age of 120 years. In the fifth decade of life however approximately 65% of people will have some form of lens opacity. This can vary from small spots to complete opacification. The patient will not immediately notice the development of cataractogenesis as this process does not proceed with an overt inflammatory process nor is any pain experienced. Symptoms accompanying such a process are difficulty in reading, in recognizing faces, watching television, seeing in bright light and during driving (1,2). A simple test is available to assess visual function, the Snellen chart, but reliability warrants consideration (3).

On a global scale cataract is the commonest cause of visual disability and by far the single largest cause of blindness (4). Traditional eye medicines in rural Africa inflict corneal ulcers and cause blindness in children in a quarter of cases (5). The best known medication to cause cataracts are corticosteroids (6) with evidence that phenothiazines, amiodarone, chloroquine and possibly acetylsalicylic acid also might be associated with increased risk (7). There are still limitations in the identification of the causes of cataracts, not only in developing countries but also in industrialised countries (8). Research is ongoing to gain a better understanding of the genetics of human cataract. It can be envisaged that knowledge of congenital cataract will provide more insight into the putative role of genes in age-related cataract (9).

There is still no effective, pharmacological, remedy for established cataracts although a theoretical and experimental basis is building up to address age-onset cataractogenesis by anti-cataract agents (10).

Treatment is purely surgical with an established success rate >90%. Two basic techniques are in use for management: extracapsular cataract surgery and intra-capsular cataract surgery.

The surgical removal of an opacified lens was first reported in 1745 (April 8th, Marseille) performed by Jacques Daviel (1693-1762). In 1752 two lectures were presented by him at the Académie Royale de Chirurgie in Paris where an account was given of 206 lens extractions. Of these 182 were successful: 88%. Almost two centuries later Sir Harold Ridley performed the first successful lens implantation (London, November 29th 1949).

Innovation is still improving cataract surgery, especially by the technological advancement of extracapsular extraction and posterior-chamber intraocular lens implantation (11). These substantial improvements should become available to an increasing group of patients on the waiting list (12,13,14). Outpatient cataract sur-
gery seems very well possible without loss of quality (15). Bilateral cataract extraction can be safely done within 48 hours (16).

Caractogenesis
Age related, or senile cataract is the most common form and inflicts blindness worldwide. There are two types of age-related cataract, nuclear and cortical. One of the possibilities that has been investigated for nuclear cataractogenesis is through hydroxyl radical-attack of lens proteins which causes cross-linking and protein aggregation, ultimately resulting in opacity of the lens (17). In another model it is proposed that cataract is essentially a conformational disease in which non-enzymatic modification of amino groups e.g. by sugars and steroids destabilize the lens proteins and causes conformational changes. The interaction between the amino acid of a lens protein and a sugar, well known as the Maillard reaction, will not only give rise to a colored reaction product but will also cause the protein to cross-link, aggregate and eventually to become insoluble which in turn will opacify the lens (18). In another sugar-related process it was investigated whether the polyol pathway was involved in the process of cataractogenesis. By the enzyme aldose reductase glucose can be converted to sorbitol. However, when a limited amount of antioxidants is available a significant amount of hydrogen peroxide can be formed. This will give rise to the production of hydroxyl radicals and will lead to the initial stage of a "sugar" cataract (19). Another reported mechanism on the formation of cataract was the kynurenine metabolic pathway. The tryptophan metabolite, 3-hydroxykynurenine is present at elevated concentrations in the lens and is able to absorb UV radiation. However, an excessive amount in the lens has been reportedly associated with cataract formation. In the rabbit eye enzymes, leading to the formation of this metabolite, are present in the iris/ciliary body. The formed metabolite is taken up by the lens for formation of UV-filtering products. If however an excess of 3-hydroxykynurenine is present in the lens, free radical formation may occur, which will ultimately lead to tissue injury like lens opacification (20,21). Oxidative damage of lens proteins seems to be a major factor in cataract formation. A threshold of protein oxidation has been identified at which opacification will take place (22). Radiation-induced cataractogenesis will commence above 1 Gy as has been observed in survivors of the Hiroshima and Nagasaki atomic bombs (23).

The lens is not a purely passive optical element but maintains an internal circulation for lens transparency. Sodium-potassium pumps have been identified in the lens as well as a major intrinsic protein belonging to the aquaporin family of water channels. Glucose is transported from the aqueous humor to the lens for energy support. It has been postulated that dysfunction of any of these links in this chain of events may ultimately lead to cataract formation (24).
REFERENCES

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1.3 CYSTOID MACULAR EDEMA

In 1942 a report was published on a toxic ocular reaction. The characteristic finding was that the primary aqueous (aqueous humor obtained on a first paracentesis) did coagulate in contrast to the usual finding that coagulation only takes place in secondary aqueous (aqueous humor obtained on a second paracentesis). The nature of this phenomenon in the - as it was termed - plasmoidtoxic aqueous- of the primary aqueous, was investigated in some detail. Although no exact culprit could be defined it became clear that the increased permeability of the ciliary body was a major contributing factor in the toxic ocular reaction and that the fibrinogen system played an essential role in the coagulation process of the plasmoidtoxic aqueous (1).

In 1953 a paper was published in which a complication was described following common intracapsular cataract extraction. One of the features of this complication was the development of postoperative macular changes, and of ultimate reduction of vision as a result of vitreous opacities or macular degeneration. Of the 1,068 cataract extractions 894 were intracapsular extractions. Of these 483 occurred intact; the remaining 222 showed complications varying from marked prolapse of the vitreous into the anterior chamber without rupture to late rupture of the anterior hyaloid with or without adhesions. The percentage of patients encountered with poor vision as a result of vitreous opacities or macular degeneration was found to be 2%. This was similar to the postoperative detachment of the retina after cataract surgery, as found in a total of reviewed 1,200 cases (2).

In 1966 a new study was presented showing the advantage of the use of intravenous sodium fluoresceinate to detect the lesion. It was demonstrated that resolution of fluorescein leakage into the retina and optic nerve generally parallels the clinical resolution of edema of the macula and optic disc. The earlier reports of an incidence of 2% of cystoid macular edema was questioned and it was expected to be higher as experience was gained with this new staining technique (3).

A review on the complication of cystoid macular edema in 1976 showed that this complication following cataract surgery was the most common and troublesome (4). The progress in surgical techniques was impressive enough to diminish the majority of complications other than cystoid maculopathy. The incidence of clinically significant cystoid macular edema remained 2 - 6%. In an attempt to grasp the etiological factors the author (A.R. Irvine) put forward the possibility of "vasoactive factors from inflammatory cells in the vitreous to penetrate the retina preferentially at the macula and disc". Of the possibilities to produce aphakic cystoid macular edema, inflammation and increased permeability were major steps in the reaction sequence. Medication seemed straight forward as to use steroids. However oral therapy proved of transient value just like periocular steroid injections and had unfavourable side effects. Topical steroid therapy was found to be ineffective. New perspectives
appeared following the elucidation of the role of prostaglandins in inflammatory vascular permeability changes. However the controlled study mentioned in this review using indomethacin (orally 25 mg tid for 3 weeks) failed to demonstrate any beneficial effect (4).

In 1985 a hypothesis was put forward for aphakic cystoid macular edema. Based on the results of a randomised double blind trial that showed a reduction in incidence of 50% for aphakic cystoid macular edema by use of an ultraviolet radiation-absorbing chromophore in a posterior chamber intraocular lens, it was postulated that postoperative exposure to near-ultraviolet radiation generates free radicals. These radicals would facilitate the synthesis of inflammatory mediators like prostaglandins. Prostaglandins are involved in the breakdown of blood-ocular barriers. It follows that a combination of factors like UV-A radiation and the synthesis of prostaglandins is a possibility worth testing as a contributing factor toward cystoid macular edema and therefore amenable to medical treatment (5).

In an update of the pharmacological therapy it was mentioned that topical nonsteroidal anti-inflammatory agents were still not commercially available. However, topical indomethacin was mentioned as the one agent effective in the prophylaxis of angiographic aphakic cystoid macular edema. Other nonsteroidal anti-inflammatory agents and corticosteroids are mentioned but no evidence was presented other than anecdotal, not detailed enough or in number too small to evaluate statistically (6).

Further studies on cystoid macular edema revealed that any disturbance of the vitreous can lead to this syndrome. In particular three possibilities are mentioned by which intraocular lenses can give rise to chronic cystoid macular edema (7). These are iris chaffing in combination with an uveitis-glaucome-hyphema syndrome after posterior chamber intraocular lens implantation, movement of the intraocular lens with intermittent corneal touchings and the corneo-retinal inflammatory syndrome compromising both the cornea and the retina. When one of these three situations occur intraocular lens removal is required to prevent permanent macular damage (7).

Cystoid macular edema has also been described in the French literature as Irvine-Gass syndrome. An extensive treatise is presented in (8).

In the German literature a report was published on the safety and efficacy of a 1% indomethacin suspension for the prevention of cystoid macular edema (also known as Irvine-Gass-Norton syndrome or Irvine syndrome). The incidence of cystoid macular edema was 1.34%. Side effects of the eyedrops, as observed in 10% of the cases, were mainly conjunctival in origin (9). Another pharmacological approach for failing visual acuity, local application of steroids and an injection of tolazoline (α-adrenergic antagonist, having some cholinergic, H₂-histaminergic, and 5HT₁ receptor antagonistic properties as well) in Tenon’s capsule, is proposed (10).

In the meantime the FDA has approved several topical NSAIDs for clinical use in ophthalmology (11). The approvals are restricted to specific indications, however;
flurbiprofen sodium and suprofen for the prophylaxis of surgical miosis, ketorolac for the relief of itching due to allergic conjunctivitis and diclofenac for the treatment of postcataract inflammation. For intraoperative miosis no conclusive evidence has been presented that an NSAID is effective. For the prevention of postcataract surgical inflammation the NSAIDs are at least as effective and perhaps more effective than corticosteroids in preventing disruption of the blood-aqueous barrier. For cystoid macular edema the evidence is that topical NSAIDs are better than topical corticosteroids.

In a Canadian report the incidence of aphakic/pseudophakic cystoid macular edema in 90 studies from 1979 to 1991 is presented using three different techniques (12). For intracapsular and extracapsular cataract extraction and the phacoemulsification technique it varied between 2 - 10%, 0 - 7.6% and 0.6 - 6.0%, respectively. However when using fluorescein angiography the incidence varied between 40 - 60%, 2.7 - 11.3% and 2.1 - 6.0%, respectively. Interestingly, aphakic cystoid macular edema occurs more frequently with intracapsular than extracapsular cataract extraction, and even less with placement of an intraocular lens in an intact capsular bag. It seems that the capsular bag encompasses properties other than just for support. The lens barrier protects, it seems, against access of inflammatory agents into the vitreous. Permanent visual impairment due to cystoid macular edema will vary between 0.5% and 2%. Treatment of clinically or angiographically proven cystoid macular edema with indomethacin decreased the incidence of cystoid macular edema; however there was no difference in visual outcome between active and placebo treated groups. No long-term effectiveness was shown yet with treatment by a NSAID. In the same report carbonic anhydrase inhibitors (e.g. acetazolamide) are mentioned as possibly effective drugs in cystoid macular edema caused by changes in the external blood-retinal barrier (retinitis pigmentosa). However, a recent report on gastric mucosa samples obtained by biopsy showed that NSAIDs (acetylsalicylic acid, indomethacin, naproxen, diclofenac and piroxicam) can activate the carbonic anhydrase isoenzymes I, II and IV (13).

An extensive review was published in 1998 (14) in which the view is held that the incidence, pathogenesis and treatment of cystoid macular edema following cataract surgery are still poorly understood. Incidence of cystoid macular edema is greatest following an intracapsular cataract extraction with implantation of an iris clip lens in an older population with systemic vascular disease. Clinical characteristics of cystoid macular edema are a nonuniform distribution of the retinal intravascular fluid within the macula leading to accumulation of transudate and ultimately to a symptomatic or asymptomatic decrease in visual acuity. Preferential leakage from perifoveal capillaries in eyes with cystoid macular edema cannot be explained yet and possibly reflects a result of an unknown capillary vitreous interaction. Inflammation, however, is the mainstay in the development of cystoid macular edema. Presumably,
breakdown of the blood aqueous barrier is associated with the development of cystoid macular edema. This was established in rabbits in which the topical activity of NSAIDs in stabilizing the blood aqueous barrier following paracentesis was studied by monitoring the integrity of the barrier using anterior ocular fluorophotometry before and after paracentesis. The integrity was followed by changes in fluorescein concentrations measured after intravenous administration of fluorescein sodium in the anterior chamber of the eye. All NSAIDs studied (flurbiprofen 0.03%, 0.1% diclofenac, 0.5% ketorolac and 1% suprofen) stabilized the blood aqueous barrier after paracentesis.

In a Swiss overview it was concluded that NSAIDs with potent anti-inflammatory properties allow good control of ocular inflammation, effective maintenance of mydriasis during surgery and delay the onset of cystoid macular edema (15). A recent review on topical NSAIDs for ophthalmic use concluded that the benefit-risk ratio is still favorable when they are applied in an appropriate and judicious manner (16).

REFERENCES

1.4 PROSTANOIDS

In 1929 it was documented that feeding of rats not only should include essential elements like amino acids, vitamins and minerals but also small amounts of unsaturated fat. Analysis uncovered the essential fatty acid linoleic acid, an eighteen carbon atom chain with two double bonds.

Screening of many types of polyunsaturated fatty acids showed arachidonic acid (20 carbon atoms and 4 double bonds) to be the most active fatty acid to prevent manifestations of nutritional deficiency.

In 1930 a factor was discovered in human semen that contracted the uterus; it also lowered blood pressure. Von Euler demonstrated this to be a fatty acid and introduced in 1935 the name prostaglandin. In 1960 Bergström elucidated the structure of some prostaglandins, one of them being prostaglandin E₂. In 1964 van Dorp carried out experiments in the Unilever Research laboratories (Vlaardingen) and demonstrated by use of labeled arachidonic acid that it was converted into prostaglandin E₂ by the medium of homogenized sheep seminal vessels. This finding was published in 1964 jointly with Bergström in the same journal. In 1970 Vane published his discovery that the biosynthesis of prostaglandins was inhibited by aspirin-like drugs. Four years later Samuelsson discovered the bioconversion by platelets of arachidonic acid into thromboxane A₂. Two years later prostacyclin was discovered by Vane.

The first comprehensive reviews were published in 1974 summarizing results from the already expanding field of prostaglandin research (1,2). All efforts were now geared to ‘visualize’ the prostaglandin endoperoxide synthase enzyme (Cyclooxygenase, COX). Known features were that cyclooxygenase is a polypeptide, homodimeric in nature (approximately 70 kDa) and in monotopic arrangement in the cell membrane. It carried two distinct functional enzyme activities, catalyzing both the bisoxygenation of arachidonic acid to its hydroperoxy arachidonate metabolite prostaglandin G₂ and consecutively catalyzing the peroxidative reduction of prostaglandin G₂ to its endoperoxide H₂ (3). The peroxidase activity of the enzyme complex is not affected by NSAIDs.

A major advance in the field of eicosanoid research was the discovery of a second inducible cyclooxygenase isoenzyme, COX-2 (4,5). The two isoforms, COX-1 and COX-2, were believed to explain the therapeutic but also the adverse effects of the frequently used NSAIDs. By hypothesizing that the COX-1 enzyme was the constitutive enzyme, designed to be available for physiological functions, the COX-2 enzyme was thought primarily to act in pathophysiological processes (COX dogma). An important aim would be to develop COX-2 specific NSAIDs that would aid in fighting inflammatory processes and not displaying unwanted side-effects related to inhibition of the COX-1 enzyme (6,7).
Evidence was delivered that localization of the COX-1 enzyme primarily was in the smooth endoplasmic reticulum and the COX-2 enzyme in the nuclear envelope. Cell membrane receptors for prostanoids have been localized on several tissues including the eye (8,9).

In studying the dynamics of inhibition by flurbiprofen and indomethacin of the human prostaglandin H synthases it became evident that these compounds influenced at least five processes, including the rate of catalytic activation, the rate of substrate turnover, the rate of autoinactivation of the enzyme complex and the association and dissociation rates of the inhibitor with the complex. Overall, indomethacin and flurbiprofen behaved similarly towards the human prostaglandin endoperoxide H synthase-1 and -2 enzymes, although the individual kinetic parameters differed (10,11). In an earlier study it was concluded that the inhibitor-enzyme complex is more stable for the flurbiprofen-prostaglandin H synthase-1 than for flurbiprofen-prostaglandin H synthase-2 complex (12).

Each isoenzyme, prostaglandin endoperoxide H synthase-1 and prostaglandin endoperoxide H synthase-2, is encoded by a different gene. When activated and with adequate arachidonic acid and oxygen present, a single prostaglandin endoperoxide H synthase (COX) molecule can produce $10^3$ molecules of prostaglandin G$_2$, a hydroperoxide, which is catalytically reduced to its alcoholic form (PGH$_2$) by peroxidase (13). The COX-1 and COX-2 enzymes are homodimers. Each dimer consists of three independent folding units: a membrane-binding domain, an enzymatic/catalytic domain and an epidermal growth factor-like domain. The enzymatic/catalytic domain consists of two separate, closely spaced, but interdependent areas encompassing 80% of the protein. The cyclooxygenase site is situated at the apex of a long hydrophobic channel in the prostaglandin endoperoxide H synthase molecule. A marked difference between COX-2 and COX-1 is the larger channel and the approximately 20% larger binding site in the former. This difference has been exploited to examine the possibility of designing selective COX-2 inhibitors (14). Although it has been suggested that the COX-1 enzyme complex is mainly active for maintenance purposes and the COX-2 enzyme, by nature of its rapid induction capabilities, for the contribution to prostaglandin related inflammation, pain and fever, implying that selective COX-2 inhibitors would benefit the patient, some doubt has arisen concerning this elegant theory (15,16). In a more recent study using a COX-1 selective inhibitor as well as COX-2 selective and non-selective inhibitors in normal and monoarthritic rats and mice with paw inflammation, it was concluded that inhibition of both COX-isoenzymes was needed for effective analgesia in inflammation (17). In studies involving the use of cyclooxygenase knockout mice it became apparent that deficiency of COX-2 had more pronounced effects on the physiological maintenance of the body than deficiency of COX-1 (18). To initiate the cyclooxygenase reaction, activation of the peroxidase active site is
necessary (19). When activated, an aqueous insoluble, nonchiral, arachidonic acid molecule, liberated by phospholipase A2 from the membrane phospholipids, will be 'sucked' into the hydrophobic channel where it will be converted to the prostaglandin G2 endoperoxide. After bioconversion of arachidonic acid into the prostaglandin G2 endoperoxide it will be transported to a reservoir type enclave formed by the dimeric cyclooxygenase enzym complex. From here transport of prostaglandin G2 to the peroxidase catalytic site is possible, where it will be transformed into prostaglandin H2 endoperoxide.

Closer examination of the interaction of the NSAID flurbiprofen, a representative of the 2-phenylpropionic acid class, with the enzyme channel, reveals that the amino acid tyrosine 355, situated near the entrance of the channel, creates a local narrowing which in turn provides a handsome explanation why the S(+) flurbiprofen enantiomer of this molecule will have a better fit than its counterpart R(-) flurbiprofen (20,21). The carboxylate group of flurbiprofen as well as of indomethacin will complex with the guanidinium group of arginine 120 just like the carboxylate group of arachidonic acid. Preparation of neutral NSAIDs by transforming the carboxylate group into an ester or amide function may enhance COX-2 selectivity (22).

The cyclooxygenase enzymes produce prostanoids from the polyunsaturated fatty acid, arachidonic acid. These prostaglandins, D2, E2, F2α, I2 and thromboxane A2, act via their respective receptors to elicit various physiological reactions (23).

A number of receptors have been identified (24). Prostaglandins are involved, together with histamine and bradykinine, in the local increase in vascular permeability and edema in which PGE2 and PGI2 are prominently involved. It has become evident that they elicit these vascular changes as well as inflammatory pain via EP- and IP-type receptors, respectively.

In a study involving knock-out mice with a EP1 receptor deficient status, the pain-sensitivity responses were tested in two acute prostaglandin-dependent models (25). The animals' reaction was reduced by approximately 50%, the same amount of reduction that could be achieved by pharmacological interventions in wild-type mice.

In determining the inhibitory effects of the flurbiprofen enantiomers on the COX-1 and COX-2 isoenzymes, it is of advantage to take the biological surroundings of the NSAID in the human body, e.g. causing protein binding, into consideration. Use of human whole blood, as an ex vivo method for quantification of the inhibitory effects of an NSAID on the synthesis of prostanoids has become an established procedure (26,27). As an unequivocal example of functional COX-1 the Ca2+-ionophore stimulated platelet is used, measuring the metabolite of thromboxane A2. The lipopolysaccharide stimulated monocyte can be taken as an activity indicator of functional COX-2. Any interference by platelets is excluded by acetylation of the COX-1 enzyme by prior administration of acetylsalicylic acid.
Prostaglandin levels have been measured using radioimmunoassays in 41 human eyes of patients undergoing vitrectomy (28). In 'quiet eyes' undergoing routine cataract extraction physiological prostaglandin levels of around 100 pg/ml were reported. In patients with a diagnosis of cataract and cystoid macular edema mean levels of PGI2 varied between 49 and 360 pg/ml.

Induction of COX-2 mRNA can take place in the rabbit eye within three hours following glaucoma filtration surgery (29). Paracentesis however fails to induce COX-2 mRNA, possibly because of the minimal disturbance by this procedure.

Prostaglandin synthase activity exists both in vascular and avascular structures of the eye, being most abundant in the iris-ciliary body.

Although the iris-ciliary body of the rabbit eye has a high functional capacity to synthesize prostanoids following paracentesis, only a transient ocular inflammatory response follows which resolves within 3 - 4 hours (30).

Eicosanoid measurements in the aqueous obtained during paracentesis of the rabbit eye showed a strong and rapid rise in PGE2 levels in the aqueous humor with peak values at 20 minutes, followed by recovery to baseline within 48 hours (31). No prostacyclin (measured as the stable metabolite 6-keto-PGF1α) was detected in the aqueous humor at the start but was markedly present at ten and twenty minutes after the initial trauma. Prostaglandin synthesis was followed shortly by an increase in aqueous humor protein, with peak levels achieved within 30 minutes after paracentesis. Both PGE2 and protein levels declined gradually to near baseline levels 48 hours after trauma.

Experimental evidence has shown that the inducible COX-2 mRNA is present in the first hours after injury and is possibly assisting in wound healing (32,33). COX-1 knockout mice do not show signs of spontaneous gastrointestinal ulceration as would have been expected when the generation of prostaglandins by the COX-1 enzyme is involved in maintenance and integrity keeping purposes (34). Classical NSAIDs still show efficacy when the COX-2 enzyme is no longer present, as shown by Western blotting (35). Administering COX-2 inhibitors then does not seem indicated. Interestingly, a COX-2 enzyme complex showed up again near resolution of inflammation and produced anti-inflammatory prostaglandins, PGD2, PGF2α and a member of the PGJ2 family (36). This is in contrast with the view hinting that cyclooxygenase-2 inhibitors might be the new approach to therapy in ocular inflammation (37).

The case against the use of COX-2 inhibitors seems more compelling now that it has become clear that in the field of rheumatology a five fold higher risk of cardiac complications can occur (38,39,40,41). In a well designed study using knockout
mice deficient of a prostacyclin receptor or a thromboxane receptor, it was shown that efficient cross talk exists between prostacyclin- and thromboxane-dependent signaling pathways (42). Inhibition of one signaling pathway might induce the other. This could account for unforeseen complications with the use of cyclooxygenase-2 inhibitors (43). Also the presence of mutations in cytochrome P450 isoenzymes that metabolize arachidonic acid could play a role in diseases involving clotting and inflammatory disorders (44). Potential drug alternatives, however, are being developed (45).

Flurbiprofen is an NSAID with preferential cyclooxygenase-1 inhibiting capacity like indomethacin. Its use as a NSAID eyedrop for combatting inflammatory responses following cataract surgery therefore seems appropriate.

REFERENCES