Reflections on flurbiprofen eyedrops
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SUMMARY
SUMMARY AND OUTLINE OF THIS THESIS

Surgery of the eye lens has become the treatment of choice for senile cataract. As from the first description of this therapy, there have been reports on a serious complication in the macula of the retina (macula retinae) affecting the vision of the patient. This complication, first described in 1953, has become known as cystoid macular oedema. The incidence varies between 2 and 50%, but can incidentally go up to 70%. Publications on eye research mainly of Japanese origin have described the use of topically applied indomethacin to prevent cystoid macular oedema after lens extraction. In response to these reports, in the clinics of Arnhem a question about the feasibility of preparing indomethacin eyedrops was put forward in 1980 by one the ophthalmologists to the hospital pharmacist. This question has triggered the research described in this thesis.

Following these reports on the prevention of cystoid macular edema after lens extraction, several formulations of indomethacin eyedrops have been described in the Dutch and international literature. Indomethacin acts by inhibition of the synthesis of prostaglandins, mediators involved in the inflammatory reaction, that is at the basis of this complication. Indomethacin was introduced into the field of ophthalmology in different types of formulations, including solutions in sesame oil, aqueous suspensions and aqueous solutions. Concentrations in oily solutions varied from 0.1 to 1%, in suspensions from 0.5% to 1% and in aqueous solutions from 0.35 to 1%.

Unfortunately, the sesame oil based solutions turned out to be less suitable, since they caused blurring of vision as a result of difference in refractive index. The aqueous solutions on the other hand, being either a suspension or a solution, were irritating to the eye (burning sensation). In order to alleviate this undesirable side effect a reduction in concentration was suggested from 1% to 0.2 or 0.1% indomethacin. In 1981 it was shown that four different indomethacin suspension eyedrops, all 0.5% in concentration, gave different prostaglandin synthesis inhibiting activities, which was attributed to differences in physicochemical properties. It was concluded that, apart from the subjective complaints of irritation of the eye, the use of eyedrops as a suspension gives rise to non-reproducible pharmacokinetic and pharmacodynamic behaviour.

As a result of the afore-mentioned question, a phosphate buffered (pH 7.4) aqueous solution of indomethacin was prepared by means of the organic base meglumine, in a concentration of 0.1%. These eyedrops were introduced in clinical practice in 1981 and produced no burning sensation when applied to the patient's eye. In 1984 Indoptol®, an aqueous suspension eyedrop of 1% indomethacin, was introduced in the Dutch market and in 1986 Indocid® of comparable composition was introduced in France. In 1987 a new presentation of indomethacin followed in
France as Indocollyre® 0.1%, which was introduced in The Netherlands in 1994. This formulation contains indomethacin as a lyophilized (freeze-dried) product, which is brought into solution by addition of a sterile borate buffer. Ongoing own research on our in-house developed eyedrops with different bases, L-lysine, D-lysine, L-arginine, D-arginine and tromethamol (not published), did not provide suitable pharmaceutical alternatives.

In the meantime our first introduced (1981) solution of indomethacin 0.1%, without the need of extra pharmaceutical excipients, remained the mainstay of the eye clinic. This solution was tested in a pharmacological setting in the rabbit eye using a paracentesis model of removing aqueous humor and measuring the influx of protein and fluorescein into the secondary aqueous humor which reflects the breakdown of the blood-aqueous barrier. The results showed that in a concentration of indomethacin as low as 0.05%, already 90 - 100% of the pharmacological activity is obtained, as demonstrated by the inhibition of fluorescein and protein influx. Following these results our indomethacin 0.1% formulation was incorporated in the Dutch National Formulary (FNA) in 1986. Impracticalities with indomethacin in aqueous solution - no sterilisation possibility and only a relatively short shelf-life when in solution - prompted us to explore the possibility of formulating eyedrops based on a different NSAID.

In 1990, topically applied S(+)-ibuprofen was reported to be effective in a rabbit model of interleukin-1 or paracentesis induced uveitis at relatively elevated concentrations (0.9% and 0.8% respectively). Also with S(+)-naproxen, marketed by Syntex as an enantiomeric pure NSAID, the anti-inflammatory effect of eyedrops (0.5%) was demonstrated experimentally. In our search for a pharmaceutically more acceptable solution of an NSAID - the introduction to the Dutch market of a diclofenac ophthalmic 0.1% solution (Naclof®) being imminent - we turned to the USP in which a flurbiprofen sodium ophthalmic solution is mentioned. We embarked on a study to manufacture flurbiprofen eyedrops by the protocol of June 1992. A letter of consent, with restricted financial aid, for the project (9206SO.008) was issued January 8th 1993 by the SWOR (Stichting ter bevordering van Wetenschappelijk Onderzoek in ziekenhuis Rijnstate).

The aim of this thesis was to investigate and to evaluate the pharmaceutical application of flurbiprofen in eyedrops as well as the pharmacology of this nonsteroidal anti-inflammatory drug. Flurbiprofen is a chiral molecule implying that the racemate is presumably not the preferred pharmacological form to prepare such eyedrops. Therefore it was deemed necessary to characterize the contribution of each enantiomer. At the start of the investigations, the pharmacological action of flurbiprofen was attributed to the inhibition of the cyclooxygenase enzyme (COX), which is known to be responsible for prostaglandin synthesis. In 1991 it became apparent
that not one but two isoenzymes are capable of synthesising prostaglandins (COX-1 and COX-2). Our investigations became more challenging as it was postulated that this second isoenzyme would be the more relevant target for nonsteroidal anti-inflammatory drugs as its activity would be related more closely to inflammation. It was decided to study both the racemic form and the individual enantiomers of flurbiprofen for prostaglandin synthesis inhibiting activity. S(+) flurbiprofen showed a marked selectivity for inhibition of COX-1 compared to COX-2, both in an extra-ocular matrix (human whole blood) and in human iris/ciliary body preparations. Since the susceptibility of iris COX-1 for S(+)flurbiprofen was 70-fold higher than for blood COX-1 the hypothesis was raised that a splice variant of COX-1 in the human iris could be responsible for this observation.

In chapter 2 the rationale for the chosen buffer in the S(+) flurbiprofen eyedrop preparation is described. Although the eye, especially the nasal corner, the eyelids and skin surrounding the eye are sensitive to external stimuli, physiological reactions due to deviations outside the normal values for osmolarity or pH are not always seen. In a state of ill-health or during regular use of ophthalmic preparations, this situation may be more outspoken, however. The active component in the eyedrop can provoke, when not properly dissolved, a prickling or burning sensation leading to lacrimal discharge, occasional haemorrhage or endangering blinking reflexes during surgery. Lacrimal discharge will cause an unwanted dilution and drainage of medicine. Individual sensitivity may vary and physiological values of tear fluid can fluctuate, dependant on the health condition of the individual eye. Eye irritation must be discerned from an allergy, which requires the choice of a different pharmacological agent.

Non-irritating eyedrops should in principle comply with: (1) sterility, (2) isotonicity and (3) pH value. Sterility is of paramount importance when the ophthalmic solution is applied to the injured eye. Isotonicity with respect to the tear fluid will reduce irritation and adverse reactions of the eye. The pH value of the formulation is of utmost importance both for stability during storage and for keeping the formulation within physiological limits. During storage hydroxyl ions, released from the glass containers, can raise the pH which may endanger the stability of the active principle. On the other hand, the lacrimal fluid has only limited buffering capacity. Therefore the choice for a buffer applied to the ophthalmic solution of flurbiprofen is governed by the following issues: (1) flurbiprofen is unstable at high pH, (2) the solubility of flurbiprofen in aqueous solution is problematic at pH values below 7, (3) the natural pH of the tear fluid is 7.4 and (4) the tolerability for the patient is in the pH range of pH 6.6 - 7.8.

Based on practical experience with the indomethacin eyedrops a choice was made for the phosphate buffer (pH 7.4) in the preparation of the S(+) flurbiprofen eyedrops. A citrate buffer of pH 6.45, as is employed in Ocuflur® (racemic flur-
biprofen sodium), was not considered favourable as it has an extreme in its buffering capacity at pH 6.5. The same choice was made later on, in a United States patent 4,996,209, describing the preparation of a single enantiomer in a phosphate buffer. Although additional components were also present in that composition, the eyedrops described in this thesis are free of other components.

In chapter 3 the stability of the formulation is addressed. The active S(+) enantiomer of flurbiprofen was formulated into a stereoselective, ballast free ophthalmic solution in a concentration of 0.015%. Analysis by capillary zone electrophoresis shows shelflife stability of up to four years at room temperature of this enantiomer. The inhibitory effect of S(+) flurbiprofen on the synthesis of prostaglandins, as measured in a homogenate of bovine iris/ciliary body, remained unaffected during a shelflife period of three years after manufacture.

Chapter 4 describes the pharmacological activity of different flurbiprofen preparations in isolated bovine iris/ciliary body homogenates. Concentration-response curves have been determined for S(+) flurbiprofen, R(-) flurbiprofen as well as the racemate, to inhibit PGE\textsubscript{2} production mediated by COX-1. A significant difference between the enantiomers was established. S(+) flurbiprofen proved one hundred times more potent than R(-) flurbiprofen. It was concluded that S(+) flurbiprofen is the active component that should be incorporated in the ophthalmic solution.

Using the human whole blood assay as described by Patrignani et al. (1994), in chapter 5 differences were detected between S(+) flurbiprofen, R(-) flurbiprofen and racemic flurbiprofen for inhibition of COX-1 and COX-2. COX-1 activity was monitored by measuring TxB\textsubscript{2} (the stable metabolite of TxA\textsubscript{2}) production from the platelets, whereas COX-2 activity was determined using PGE\textsubscript{2} production in monocytes, following induction of this enzyme by LPS. The stereoselectivity of S(+) flurbiprofen compared to R(-) flurbiprofen, expressed as the reciprocal of the ratio of the concentrations giving 50% inhibition (IC\textsubscript{50}), amounted to 340 for COX-1 and 56 for COX-2. The selectivity of racemic flurbiprofen for COX-1 versus COX-2, was 16-fold.

In chapter 6 the interaction of S(+) flurbiprofen with COX-1 and COX-2 in the human iris was studied. After LPS-treatment for 24h, substantial amounts of COX-2 immunoreactivity could be visualized for the first time in human iris/ciliary body preparations. Remarkably, S(+) flurbiprofen showed a 3,600-fold higher potency for inhibiting COX-1 compared to COX-2. Furthermore, the susceptibility of human iris COX-1 for inhibition by S(+) flurbiprofen was 70-fold higher than of COX-1 in human blood.

In chapter 7 the distribution of a flurbiprofen analogue in the human eye has been visualised. Technetium labelled diflunisal, sharing pharmacological and chemical resemblance with flurbiprofen as an NSAID being fluorinated and possessing a biphenyl ring, was used in an attempt to visualize COX-activity in the internal structures of the eye. The scintigraphic results obtained with this labelled drug were compared with instillation of the same volume and activity of pertechnetate. An
amount of 3% of instilled technetium labelled diflunisal could be localized in the eye. Activity could be visualized in the area of the iris indeed. Diflunisal was used because the labelling efficiency of S(+) flurbiprofen proved not appropriate (Chapter 8). R(-) flurbiprofen would chemically be a good labelling agent, but not pharmaco-logically, for obvious reasons (Chapters 5 and 6).

In the final Chapter the occurrence of alternative splicing of COX-1 in RNA in the human iris was explored, as a possible explanation of the remarkably high affinity of S(+) flurbiprofen reported in Chapter 6. Indeed an alternatively spliced mRNA COX-1 splice variant (SV) could be detected in the human iris tissue from 3 patients. However, the same splice variant was also found in blood cells derived from four individuals. The amount of COX-1SV present in the human iris was not significantly different from the amount present in blood cells, implying that the occurrence of the COX-1 splice variant in the iris can not explain the observed difference in IC$_{50}$ by S(+) flurbiprofen between human iris (0.8 nM) and human blood (56 nM) for COX-1 inhibition.