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Cyclodextrin complexation highly enhances efficacy of arylsulfonylureido benzenesulfonamide carbonic anhydrase inhibitors as a topical antiglaucoma agents

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ABSTRACT

Two new sulfonamides incorporating arylsulfonylureido moieties were complexed with gamma cyclodextrin (γ-CD), hydroxypropyl-gamma cyclodextrin (HPγ-CD), hydroxypropyl-beta cyclodextrin (HPβ-CD) and hydroxyethyl-beta cyclodextrin (HEβ-CD) in order to obtain drug formulations with effective topical intraocular pressure (IOP) lowering effects, in an animal model of glaucoma. The HPγ-CD was the best solubilizing agent for the two sulfonamides and its complexes were characterized in detail and administered to rabbits with eye hypertension of 45–50 mm Hg. The peak IOP lowering was observed after 1 h post-administration and was of 36–37 mm Hg. A low IOP pressure (of around—35 mm Hg) was then maintained for the next 24 h post-administration, which has not been observed before with any IOP lowering drug.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides endowed with a hydrophilic outer surface and a hydrophobic inner cavity, able to form inclusion complexes with a wide variety of guest molecules, positively affecting their physicochemical properties. In the pharmaceutical field, CD complexation has been mainly used to increase the solubility and dissolution properties, and, consequently, the bioavailability of poorly soluble drugs.[1–3] Moreover, CDs complexation can also be effective to enhance the chemical stability of drugs susceptible to degradation, to reduce gastric, dermal or ocular irritation effects, to increase the drug release rate from different kinds of delivery systems and/or to improve the permeation of the guest molecules through biological membranes.[4–6] It has been hypothesized that CDs act as permeation enhancers by keeping the hydrophobic drug molecules in solution and delivering them to the surface of the biological membrane, thus leading to a constant high concentration of dissolved drug at membrane surface.[7] Finally, CD complexation can solve problems of formulation of pharmaceutical liquid preparations, especially those designed for parenteral or ocular administration. All these benefits have been exploited in the development of ophthalmic preparations, where the use of CD complexes allowed for increasing the water solubility and, consequently, the corneal permeation rate and the therapeutic efficacy of a number of poorly soluble drugs.[8–11] In particular, some of us successfully employed the complexation with the highly-water soluble hydroxypropyl derivative of βCD for improving the solubility and bioavailability of various sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs) acting as topical antiglaucoma agents.[10]

Based on these considerations, in the present work we investigated the effectiveness of CD complexation in improving the water solubility and bioavailability of two new benzen-sulfonamides carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs), as compounds belonging to this family of pharmacological agents are widely used antiglaucoma drugs.[12,13] Indeed, compounds such as acetazolamide **AAZ**, methazolamide **MZA** and dichlorophenamide **DCP** were and are still clinically used systemic antiglaucoma agents, but as they act as pan-inhibitors of these enzymes (of which 15 isoforms are known in humans)[13] they show many side effects. A newer drug, dorzolamide **DRZ** has fewer side effects as it is more water soluble compared to the systemic inhibitors and may be administered as eye drops. The same is true for the structurally-related derivative brinzolamide **BRZ** (Fig. 1A). However their efficacy is rather limited and this is the reason why many new

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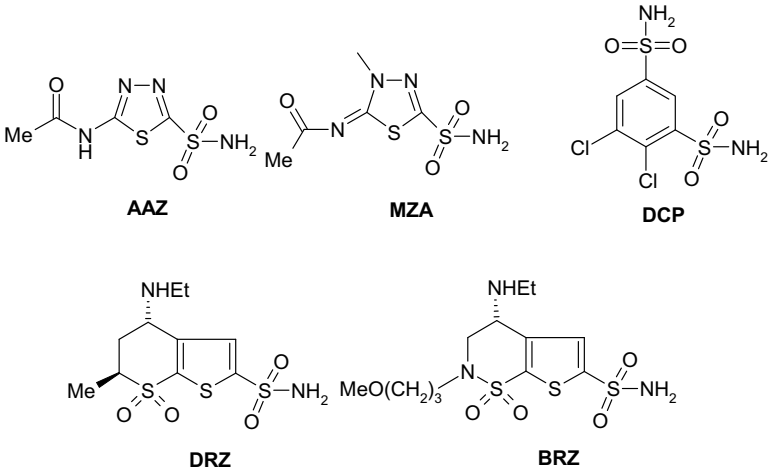

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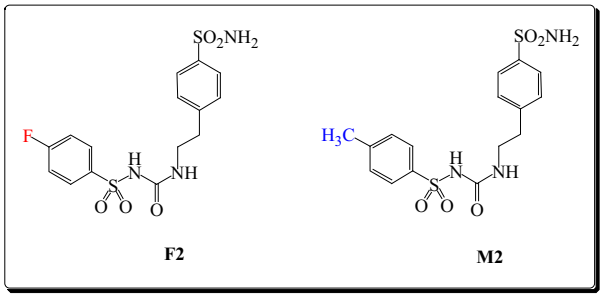

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Figure 1. (A) Clinically used sulfonamide CAIs. (B) Chemical structure of the benzene-sulfonamides **F2** and **M2**[13] used for complexation with CDs in this work.

approaches/compounds with CA inhibitory activity and enhanced efficacy are being investigayted.[12–14]

Recently we reported some new effective sulfonamide CAIs, among which the 4-methylpnehylsulfonylureido-derivative **M2** and the 4-fluorophenylsulfonylureido derivative **F2** possessing a 4-aminoethylbenzenesulfonamide scaffold (see Fig. 1), purposely synthesized by our research group as potential new antiglaucoma agents.[13] In fact, many studies are still in act in the search of even more efficacious topically acting CAIs, without the limits and side effects presented by the currently available drugs.[14] The different CDs used for this study were selected on the basis of their favorable toxicological profile, relatively high solubility, good complexation capabilities and reduced haemolytic effects.[8–11]

2. Results and discussion

2.1. Chemistry

Primary sulfonamides are well-known to act as potent CAIs due to their strong binding (as sulfonamidate anions) to the Zn(II) ion within the CA active site. In addition the organic scaffold of the sulfonamide also participates in many interactions with the enzyme active site which may lead to a stabilization or destabilization of the enzyme-inhibitor adduct.[12,14] In addition to this, the scaffold is important for assuring the right balance between hydro- and lipo-solubility of the drug, which is crucial for designing CAIs with effective anti-glaucoma activity. In fact sulfonamides are generally rather poorly soluble in water and this is one of their main limitations for use as anti-glaucoma, topically-acting drugs.[14] Recently, we reported[13] a class of sulfonamides possessing arylsulfonylureido tails in their molecules, which were low nanomolar inhibitors of isoforms CA II and XII (upregulated or overexpressed in glaucoma),[13] and showed effective in vivo intraocular pressure (IOP) lowering effects in an animal model of the disease. Among the compounds reported in the previous study there were pyridine-3-sulfonamide as well as 4-aminoethyl-benzenesulfonamide derivatives (incorporating the above mentioned arylsulfonylureido tails). As the pyridine-3-sulfonamides were more water soluble compared to the benzenesulfonamides, we investigated those compounds as antiglaucoma agents in the previous work.[13] However, the *p*–aminoethyl-benzenesulfonamide derivatives such as **F2** and **M2** showed very interesting enzyme inhibitory activity,[13] but were not investigated as IOP lowering agents due to their low water solubility. Thus, we decided to investigate their possible complexation by CDs and the possibility to use such complexes in anti-glaucoma studies in vivo. The two compounds differ only by the moiety present in the 4-position of the substituted-arylsulfonylureido fragment of the molecule, with fluorine being present in **F2** and methyl in **M2** (Fig. 1B).

2.2. Cyclodextrin solubilization studies

Preliminary studies were carried out to evaluate and compare the solubilizing efficacy of different CDs towards the two drugs. With this aim, the equilibrium saturation solubility at 25 °C in pH 7.4 buffered saline solution of the compounds **F2** and **M2**, as such and in the presence of a 20 mM concentration of each of the different CDs was determined (Table 1). The greater aqueous solubility of compound **F2** with respect to compound **M2** can be explained with its higher polarity, due to the presence of the