CHAPTER 9

Catalytic Strategies: The Case of the Crushed Mailbox

CASE HISTORY

A 65-year-old African American man returns to clinic for routine follow-up of mild systemic hypertension that has been controlled with diet. He offers no specific complaints at this visit but his wife states that she has noticed that he frequently "bumps into things" such as doorways. He recently ran over the mailbox when pulling into his driveway, failing to notice that he had gotten so close to it. He has no other symptoms and does not complain of problems with his vision.

His physical examination was remarkable for a mild elevation of blood pressure (142/90 mmHg) and no focal neurological deficits. Detailed ophthalmological examination revealed no eye tenderness, no scleral or conjunctival redness, and normally reactive pupils. Slit lamp examination of the eye revealed a normal anterior chamber and no evidence for angle closure. Examination of the fundus of the eye demonstrated an increase in the size of the optic cup relative to the optic disk with no other abnormalities. His intraocular pressures were 32 mmHg OD (right eye) and 28 mmHg OS (left eye) [Upper limits of normal: 20 mmHg]. Visual field testing revealed bilateral visual field loss.

What part of the visual field appears to be affected in this patient?

What is a slit-lamp examination and what does it reveal?

How is the fundus of the eye visualized?

How is intraocular pressure measured?

DIAGNOSIS AND TREATMENT

The physician made the diagnosis of glaucoma and the patient was treated with a beta adrenergic blocker (Timolol maleate 0.25%) and an alpha adrenergic agonist (apraclonidine 0.5%) administered topically in both eyes. He returned in one month with intraocular pressures of 29 mmHg OD and 26 mmHg OS and treatment with a topical carbonic anhydrase inhibitor (dorzolamide 2%) was initiated. The patient was seen two months later and intraocular pressures were down to within the normal range of 18mmHg OD and 17mmHG OS. Reexamination of his eyes and visual field testing indicated no further impairment of vision. The patient was advised to continue the course of treatment and to return for reexamination on a semiannual basis. His wife purchased a new mailbox and moved it well away from the driveway to avoid damage to it in the future.

What appears to be the goal of treatment?

Was the initial drug treatment effective?

Did the patient's vision improve?

DISCUSSION

Symptoms and Etiology

Primary open-angle glaucoma (POAG) accounts for 60–70% of all glaucomas. Its causes are largely unknown, but it is characterized by poor drainage of the aqueous humor from the anterior chamber of the eye (see Figure 9.1), resulting in increased intraocular pressure (IOP). Typically, it has no symptoms besides the gradual loss of peripheral vision, which often goes

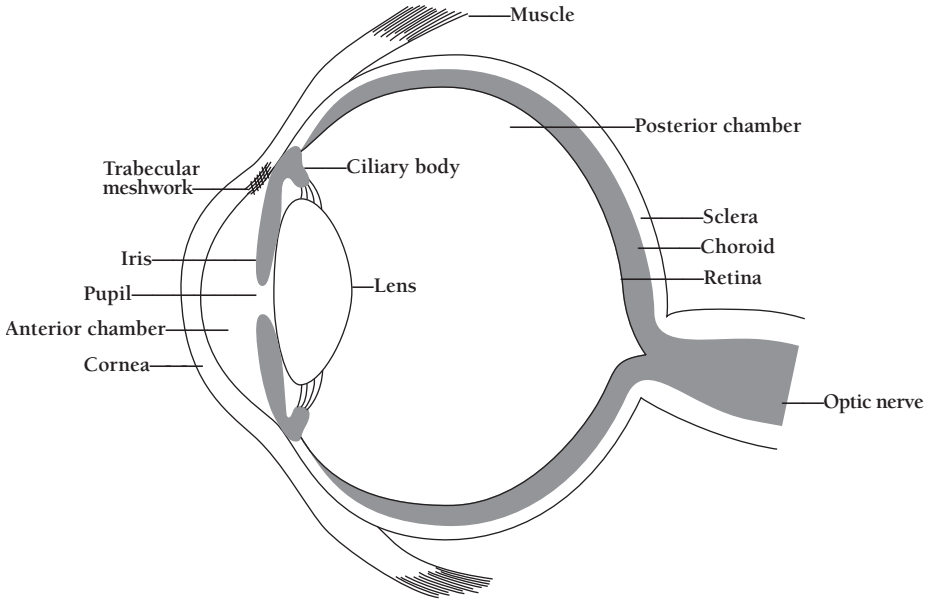

FIGURE 9.1 Anatomy of the eye.

unnoticed until significant damage has occurred. Unfortunately, the development of symptoms signals irreversible damage, and contemporary therapy is aimed at halting further progression of disease and preservation of the remaining vision.

Diagnosing Glaucoma

Three tests are commonly used to diagnose glaucoma. Examination of the optic nerve is done using an opthalmoscope, which allows the physician to view the fundus through the patient's pupil. Enlargement, or notching of the optic cup are tell-tale signs of damage to the optic nerve, and glaucoma. Additionally, a tonometer is used to measure the pressure within the eye, and a visual field test will reveal the characteristic shrinking of the breadth of peripheral vision.

Management of Glaucoma

Although laser treatment and surgery are options in the treatment of POAG, drug treatment is usually first-line therapy for newly diagnosed patients. A number of types of drugs are used, among them beta-blockers, alpha-agonists, and carbonic anhydrase inhibitors. All of these agents act to decrease the IOP by reducing the flow of fluid into the anterior chamber and/or increasing the drainage out of it, and the choice of drug is based on effectiveness and severity of side effects for each individual patient. Often, a combination of drugs is used, as for the patient described here. When the combination of timolol maleate and apraclonidine proved ineffective for this individual, the carbonic anhydrase inhibitor, dorzolamide, was prescribed, this time with success.

Treatment of Glaucoma by Inhibition of Carbonic Anhydrase

Carbonic anhydrase (CA) is a zinc-containing enzyme that catalyzes the hydration of carbon dioxide to yield a bicarbonate ion and a proton (see section 9.2 of *Biochemistry,* 5e). Inhibition of the enzyme leads to decreased production of bicarbonate, a key step in aqueous humor production. Bicarbonate formed in the ciliary body associates with sodium ions and is secreted into the posterior chamber of the eye (see Figure 9.1). Due to osmosis, a passive flow of water ensues and continues on into the anterior chamber to form the aqueous humor. Thus, CA inhibitors reduce IOP by decreasing fluid flow into the eye.

Biochemical Basis for Dorzolamide's Action

Dorzolamide, introduced in 1995, was the first topical carbonic anhydrase inhibitor to appear on the market. The difficulty in developing topical drugs was that clinical effectiveness necessitated inhibition of CAII, the most active human isoform, by virtually 100%. This requires both a high degree of inhibitory activity and efficient penetration of the eye. Dorzolamide, with its high affinity for the enzyme (Kd = 0.37 nM) and efficient ocular penetration, was shown to reduce IOP to comparable extents as the previously used systemic CA inhibitors acetazolamide and methazolamide.

Dorzolamide is chemically classified as a sulfonamide (see Figure 9.2) and acts by binding to the active site of CA and preventing the binding of substrates, water, and carbon dioxide. It thus acts as a competitive inhibitor. The nitrogen atom of dorzolamide binds the zinc atom and displaces the hydroxide group normally bound in this position

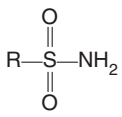

FIGURE 9.2 Structure of a sulfonamide.

(compare Figures 9.3a and 9.3b). The nitrogen also donates a hydrogen bond to the hydroxyl side chain of threonine-199, which in turn donates a hydrogen bond to the side chain of glutamate-106. In addition, one of the oxygen molecules of the sulfonamide group makes a hydrogen bond with the peptide backbone at threonine-199.

The similarities between binding of dorzolamide and the binding of the enzyme's substrates are clear. In addition, dorzolamide makes contacts with the walls of the cone-shaped active site via its bicyclic ring structure (− R).

Dorzolamide is a Transition State Analog

A key concept in catalysis is that enzymes decrease the activation barrier of a reaction by lowering the free energy associated with the transition state (see Section 8.3 of *Biochemistry,* 5e). The transition state is the most unfavorable, or highest energy state the reaction passes through, and enzymes act by lowering its energy, thereby increasing the rate of reaction. Thus, enzyme structures have evolved to specifically bind the transition state of the reactions they catalyze. Dorzolamide is an effective inhibitor of carbonic anhydrase because it mimics the transition state of the reaction, and hence binds tightly to the enzyme.

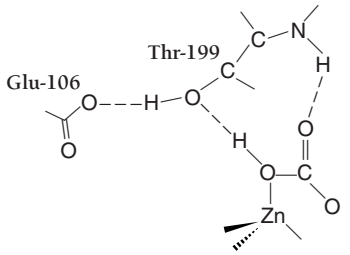

FIGURE 9.3a Schematic drawing of substrate binding to the active site of carbonic anhydrase.

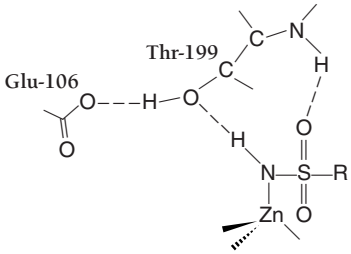

FIGURE 9.3b Schematic drawing of a sulfonamide binding to the active site of carbonic anhydrase.

QUESTIONS

1. Altitude sickness is caused by the reduced partial pressure of oxygen at high elevations, which results in hypoxemia, a lowered oxygen level in the blood. This stimulates hyperventilation, which can lead to alkalosis (increased alkalinity, or pH, of bodily fluids) and the symptoms of mountain sickness, which include fatigue, nausea, headache and a rapid and forceful heartbeat. The carbonic anhydrase inhibitor, acetazolamide, taken orally as of the first day of ascent, can be given as a prophylactic treatment to mountain climbers. How do you think the drug might work in this context?
2. If you were to design a new carbonic anhydrase inhibitor drug for the treatment of glaucoma, what physicochemical properties of the drug might you need to consider?
3. Sulfonamides are also used as antimicrobials (the "sulfa drugs") but those used to treat glaucoma do not have antibacterial activity. By comparing the structures of the following antimicrobial sulfonamides with the carbonic anhydrase inhibitors used to treat glaucoma (see Figure 9.4a) can you identify the structural component important for antibacterial activity?

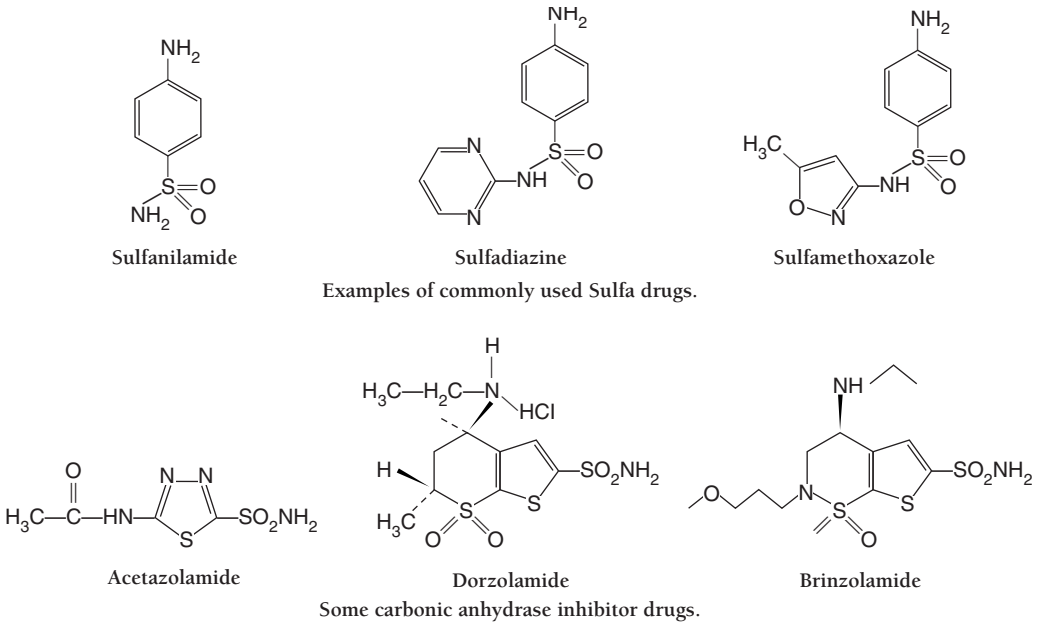

FIGURE 9.4a

4. Prior to the development of dorzolamide, the only available carbonic anhydrase inhibitors were taken orally and were associated with a number of side-effects, including polyuria, fatigue, and gastrointestinal disturbances. Can you explain the physiological basis for these side-effects?
5. About 25% of patients experience a bitter or metal-like taste upon administration of dorzolamide eye drops. Can you explain why this might occur? [Hint: Lacrimal fluid from the eye drains into the oropharynx.]

6. A patient who wears soft contact lenses would like to know if she can take dorzolamide to treat the glaucoma from which she suffers. Dorzolamide itself is not contraindicated in those with soft contact lenses, however, can you think of any other factors that should be considered before advising her?
7. A less common form of glaucoma called acute closed-angle glaucoma occurs when, for a variety of reasons, the root of the iris presses against the trabecular meshwork, from which fluid drains from the eye. With the blockage of the outflow channel, the IOP can rapidly rise to dangerously high levels, causing painful swelling of the eye, nausea, and dizziness. Acute closed-angle glaucoma can lead to blindness in as little as a day or two unless treated immediately. Do you think medication can be used to treat this form of glaucoma?
8. Latanoprost, a prostaglandin analog, was approved by the FDA in 1996 for the topical treatment of glaucoma. It functions by stimulating uveoscleral fluid outflow (flow of fluid through the front of the eye). Given their modes of action, would you expect latanoprost to have an IOP lowering effect on top of that produced by dorzolamide?
9. The use of systemic carbonic anhydrase inhibitors in post-menopausal women has been associated with inhibition of osteoporosis. Can you speculate as to how CAI might inhibit bone loss?

FURTHER READING

1. Infeld, D. A. and O'Shea, J. G. Glaucoma: Diagnosis and Treatment. *Postgraduate Medical Journal* (1998) 74:709–715.
2. Lindskog, S. Structure and Mechanism of Carbonic Anhydrase. *Pharmacol. Ther.* (1997) 74(1):1–20.
3. Herkel, U. and Pfeiffer, N. Update on Topical Carbonic Anhydrase Inhibitors. *Current Opinion in Opthalmology* (2001) 12(2):88–93.

For further information, see the following web sites:

National Eye Institute: www.nei.nih.gov/

American Academy of Opthalmology: www.eyenet.org

The Glaucoma Foundation: www.glaucoma-foundation.org/info

The Glaucoma Research Foundation: www.glaucoma.org

National Library of Medicine-Medlineplus: www.nlm.nih.gov/medlineplus/glaucoma.html