Effect of Acetazolamide (Diamox®) on tear secretion

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Abstract. The side effects of acetazolamide (Diamox®) on lacrimation were measured in rats by means of the cotton-thread tear test. After a daily oral 1-mg dose (administered for five days), comparable to the dose used for adult humans on a drug-to-bodyweight basis, tear production remained unaffected but the lacrimal peroxidase secretion decreased by 60% of the baseline level. After withdrawal of acetazolamide the peroxidase secretion returned to the baseline level.

Introduction

Decreased lacrimation as a side effect of drug therapy has been reported for diuretics in man (Tripathi, 1975) and in rats (Thörig et al., 1984a). The widespread use of acetazolamide as an anti-glaucomatous drug suggests a need for evaluation of the effect of this drug on lacrimation. The fine-cotton-thread method according to Kurihashi, Yanagihara and Honda (1977) was used, as modified for rats (Thörig et al., 1983). In this study we investigated in rats the effect of daily oral administration of acetazolamide, equivalent to the dose used for adult humans on a drug-to-bodyweight basis (therapeutic dose), on both the tear production and the secretion of the major lacrimal enzyme, peroxidase (Thörig et al., 1984b).

Materials and methods

Cotton-thread tear test

Cotton-thread tear tests were performed on both eyes of sedated rats as described previously by Thörig et al. (1983). For oral administration acetazolamide (Diamox®) was dissolved in distilled water.

Peroxidase assay

Tear peroxidase was extracted from the stained moistened part of the cotton thread with phosphate-buffered saline pH 7.4 to obtain a 1%-tear-solution
Peroxidase (E.C.1.11.1.7.) was determined according to Herzog and Fahimi (1973, 1976), using a 5 mM-solution of 3,3'-diaminobenzidine (DAB) in 0.1 M phosphate-citrate buffer at pH 6.0. Enzyme activity is calculated as units per volume absorbed tearfluid.

**Experimental design**

A group of 5 male Wistar rats weighing 150–220 g, raised in the Central Institute for the Breeding of Laboratory Animals, Zeist, The Netherlands, was used. Tear production and tear peroxidase levels were measured daily in both eyes of the rats with the cotton-thread tear test, in three periods of five consecutive days. In the first period (control 1) data were obtained without acetazolamide administration. In the second period tear test values were obtained two hours after daily acetazolamide administration. Acetazolamide was administered orally as 1-ml-solution (1 mg/ml) via a plastic gastric tube. Data from this period were compared to the baseline tear test values obtained in the first period and evaluated for statistically significant differences. Afterwards a new period without acetazolamide administration (control 2) followed. Between each period intervals of two days without experimentation were introduced. The tear production and its peroxidase content were expressed separately as area under the curve (AUC) for each period and calculated respectively as µl x days and peroxidase units x days.

**Statistical analysis**

Paired Student's t-test was used to test the significance of differences between the AUC’s for each period of five consecutive days.

**Results and discussion**

Acetazolamide does not affect tear production, but reduces the lacrimal peroxidase secretion by approximately 60% (Figures 1 and 2). Peroxidase in tears may be considered as a marker enzyme of the lacrimal gland in rats (Van Haeringen et al., 1979; Thörig et al., 1984b), analogous to lysozyme in human tears (Van Haeringen, 1981). For this reason peroxidase is a useful enzyme for comparison of the lacrimal side effects of drugs. In the case of diuretics we compared three types differing in their degree of action on the nephron, which can be expressed as the percentage of extra sodium after maximal dose administration: furosemide 15%, acetazolamide 5–15% and amiloride 5% (Seely and Dirks, 1977).

The lacrimal side-effects of furosemide and amiloride have been determined recently in an identical way (Thörig et al., 1984a). Furosemide, given to rats on a drug-to-weight basis, reduced the mean baseline tear secretion by 30%, and decreased the peroxidase secretion dose-dependently. Amiloride, on the other hand, did not affect either tear production or lacrimal peroxidase secretion (Figures 1 and 2).