THE DETERMINATION OF THE PENETRATION OF THE β-BLOCKER (-)-BUNOLOL INTO THE HUMAN EYE

D.G. Watson[1], C.N.J. McGhee[2] and J.M. Midgley[2]
1. The Biomedical GC-MS Unit, Department of Pharmacy, University of Strathclyde
2. The Tennent Institute of Ophthalmology, Western Infirmary, Glasgow G12.

In earlier studies we have reported data for the penetration of anti-inflammatory steroids into the human eye (Watson 1988, Midgley 1988, McGhee 1989). ß-blocker drops are used to treat glaucoma and are generally applied twice a day. No data exists on the amouts of the ß-blocker penetrating into the aqueous humour of the human eye where it is presumed to act via binding to receptor sites on the ciliary body with consequent reduction in the rate of aqueous humour production. We have devised a highly sensitive and specific procedure using gas chromatography-negative ion chemical ionisation mass spectrometry (GC-NICIMS) to determine subnanogram amounts of the ß-blockers used in ophthalmological practice. The procedure involves formation of the pentafluorobezamide derivative of the ß-blocker in aqueous alkaline solution followed by extraction into organic solvent and trimethylsilylation of remaining free hydroxyl groups. The mass spectra of the ß-blockers yield a high abundance of significant ions which facilitate analysis by selected ion monitoring. We have applied our method to a study of the penetration of L-bunolol into the human eye. The ß-blocker preparation (50μL, 0.5% L-Bunolol. Hydrochloride, Betagan, Allergan UK Ltd.) was applied to the eyes of volunteers at various time intervals before elective cataract surgery. Samples (50-100μL) of aqueous humour obtained per-operatively were then subjected to derivatisation as described above and are then analysed by GC-NICIMS. The mean concentration of L-bunolol and its dihydrometabolite in samples of human aqueous humour at the average time of sampling are shown in:

Table 1

Concentration (ng/ml of aqueous humour SEM)		Sample	Mean(Min)
L-Bunolol	Dihydro-L-bunolol	Number	Time SD
197.3 ± 65.7	251.4 ± 53.4	9	30 ± 8.8

The dihydrometabolite of L-bunolol is rapidly and stereospecifically formed giving only one peak in the chromatogram. This is in contrast to a chemically synthesised sample which yields two peaks due to the two possible diastereoisomers resulting from reduction of the keto group in the molecule.

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