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## Bioanalysis and pharmacokinetics of Oxyphenonium Bromide

Greving, Jan Egbert

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## SUMMARY

Oxyphenonium bromide (OxBr, Antrenyl<sup>R</sup>) is the  $\alpha$ -cyclohexyl- $\alpha$ -phenyl glycolic acid ester of diethyl-(2-hydroxy-ethyl)-methyl ammonium bromide, a drug developed in the years 1945 - 1950, which possesses a strong anticholinergic effect. The compound is applied in the treatment of spasms in the gastrointestinal tract, bile ducts and urogenital system, ulcus pepticum and as a component of preanesthetic medication. Because of its anticholinergic effect OxBr is also applied for the relaxation of smooth muscle in the bronchial tree in the treatment of patients with Chronic Aspecific Respiratory Affections (CARA; more or less equivalent terms are: CNSLD, COLD, "Asthma", "Bronchitis").

In general OxBr is administered in a dose of 5 - 15 mg up to 4 times a day for oral use, 1 - 2 mg up to 4 times a day for intramuscular (IM) or subcutaneous use and 0.5 - 2 mg time for intravenous (IV) use.

OxBr was chosen as a model compound in order to study the effects of a "purely" anticholinergic drug in the bronchus obstruction in CARA, because OxBr exerts little or no other activities, such as an antihistaminic effect, as present in a large number of other anticholinergics, among which thiazinamium methylsulphate.

OxBr is a quaternary ammonium compound and cannot easily pass lipid membranes because of its ionic character in aqueous medium. Its pharmacokinetics and particularly its bioavailability after oral administration has not yet been investigated in man, probably due to the lack of a sensitive and selective assay method for OxBr in biological fluids such as plasma and urine.

The aim of the study, described in this thesis, was the development of a method of determination of OxBr in plasma and urine in order to investigate the pharmacokinetics in patients after single dose administration of OxBr by IV, IM and oral routes.

This Thesis is divided into 3 Parts:

In Part I the introduction, divided in 2 Chapters is given.

In Chapter 1 the characteristics of OxBr, such as its physico-chemical and pharmacological properties are described.

In Chapter 2 a survey of the strategies and developments in the bioanalysis of quaternary ammonium compounds, particularly aggravated upon quantitative chromatographic analysis systems is presented.

Part II encloses the bioanalysis of OxBr and is divided in 4 Chapters.

In Chapter 3 is described the ion-pair extraction of OxBr and benactyzine methyl iodide (BenMI), a compound with a strong structural agreement. The general principles of ion-pair partition are given as well as the possible side reactions such as dissociation of the ion-pair in the organic phase. The ion-pair extraction of oxyphenonium ( $Ox^+$ ) and methylbenactyzine ( $BenM^+$ ) were studied from aqueous medium into 1,2-dichloroethane as organic phase using iodide, perchlorate and picrate as counter ions. Besides ion-pair partition, dissociation in the organic phase was also observed, which enhanced the extraction yield in the organic phase in the lower concentration range. For the isolation of  $Ox^+$  from biological fluids an ion-pair extraction with perchlorate as a counter ion was chosen.

In Chapter 4 the hydrolysis rate of OxBr in neutral and alkaline media is described. It appeared, that OxBr is relatively stable in neutral medium. In alkaline medium it rapidly hydrolyses into  $\alpha$ -cyclohexyl- $\alpha$ -phenyl glycolic acid and diethyl-methyl-ethanol ammonium. This hydrolysis enables splitting OxBr for at least 99% at a pH  $\approx$  12 at a temperature of 40°C within 30 minutes.

In Chapter 5 the derivatization is described of  $\alpha$ -cyclohexyl- $\alpha$ -phenyl glycolic acid and analogous carboxylic acids with pentafluorobenzyl bromide for a quantitative determination of these acids in the picomole range by means of gas chromatography-electron capture detection. The derivatization reaction was carried out after ion-pair extraction of the acids with tetrapentylammonium as counter ion into dichloromethane as organic phase, while after taking to dryness, the reaction was performed in dichloromethane or acetone with a low concentration of pentafluorobenzyl bromide. The minimum detectable amount of the pentafluorobenzyl derivatives of the acids amounted about 100 femtogram, which is about 500 attomol ( $5 \times 10^{-16}$  mol).

In Chapter 6 the total procedure of determination of OxBr in plasma

and urine to which BenMI is added as the internal standard is described. After isolation by means of ion-pair extraction with perchlorate as counter-ion and subsequent re-extraction a hydrolysis is performed in alkaline medium. The resulting carboxylic acids are derivatized with pentafluorobenzyl bromide and determined by gas chromatography-electron capture detection. Much attention has been paid to avoiding interferences of other carboxylic acids, which may disturb particularly fatty acids from the biological sample or present as contaminants in glassware and chemicals. OxBr could be determined down to 0.5 ng/ml using 2 ml plasma and down to 10 ng/ml using 0.1 ml urine. The method is sufficiently sensitive for the determination of OxBr in plasma and urine after single administration of therapeutic doses of OxBr.

Part III goes into the subject of the pharmacokinetics of OxBr in man and is divided into 4 Chapters.

In Chapter 7 an introduction is given of the pharmacokinetics as far as of interest for adequately analysis of the plasma concentration-time curves and urinary excretion data observed after IV, IM and oral administration. In this Chapter attention is also paid to factors, which might influence the bioavailability of a drug. Moreover, it describes the method used for curve fitting by means of non-linear regression analysis computer programs, such as NONLIN and the influence of data weighting. As a method for data weighting the log-fit approach is proposed in such cases, in which the precision of the data points is unknown and first-order kinetics are assumed. This log-fit weighting assumes a constant variance after logarithmic transformation of the data and has been applied for the analysis of all the plasma concentration-time curves.

In the following Chapters the pharmacokinetics are described after single dose administration of OxBr in CARA-patients (volunteers) under standardized conditions, in which the plasma concentration, lung function such as  $FEV_{1.0}$  and VC, and side effects such as heart frequency during 7 hours were measured and urine excretion during 24 hours was traced.

In Chapter 8 the pharmacokinetics after IV administration of 2 mg OxBr are described. An open three-compartment model is proposed. The harmonic mean  $t_{1/2}$  elimination was found to be 120 min. The volume of the central compartment  $V_C$  was 2.4 h, the volume of distribution  $V_B$  was 60 l.

and the body plasma clearance  $V_Q$  was 340 ml/min.

In Chapter 9 the pharmacokinetics are described after IM administration of 2 mg OxBr. An open two-compartment model with a first-order absorption and a lag time is proposed. The lag time was found to be 1.2 min the  $t_{1/2}$  absorption was 5 min., the  $t_{1/2}$  elimination was 130 min., the  $V_C$  was 14 l. and the  $V_B$  was 65 l. The mean bioavailability  $F$  calculated from areas under the curves was found to be 79%. The maximum plasma concentration was reached after 15 min and was found to be 80 ng/ml.

In Chapter 10 the pharmacokinetics are described after oral administration of 10 mg OxBr in the form of 2 tablets Antrenyl<sup>R</sup>. A relatively rapid absorption was found, followed by a flat slowly descending plasma concentration curve. An open one-compartment model is proposed. The  $t_{1/2}$  absorption was found to be 23 min., the  $t_{1/2}$  elimination was 190 min., the  $V_B$  was 93 l. The longer  $t_{1/2}$  elimination may be due to a prolonged slow (re)absorption and distribution from the gastrointestinal and hepatic systems. The bioavailability  $F$  was found to be 6%. The maximum plasma concentration of about 5 ng/ml was reached after about 80 min. The mean plasma concentrations in the terminal phase after oral administration of 10 mg OxBr was found to be somewhat higher than found after IV or IM injection of 2 mg OxBr, despite the low bioavailability.

All kinetic values mentioned were calculated from the means of estimates in a group of 7 patients. For the individual data and curves the reader is referred to the Chapters concerned.

The excretion of OxBr in urine after IV, IM and oral administration is also described in Chapters 8, 9 and 10, respectively. The urine was sampled as much as possible every hour during the first 7 hours, and then pooled from 7 to 24 hours. The evaluation of the excretion rate as a function of time appeared to be not well possible because complete emptying of the urinary bladders was difficult particularly after IV and IM administration. This may be due to the anticholinergic effect of OxBr on the smooth muscle of the bladder.

The average renal clearances measured over 24 hours were found to be 190, 180 and 140 ml/min after IV, IM and oral administration, respectively. Assuming, that  $Ox^+$  is partly bound to plasma proteins, the occurrence of tubular secretion can be taken into account beside glomerular filtration. Indications for a saturation of this active secretion process were not found. The coefficients of correlation of the average renal

clearances after different routes of administration in the group of seven patients were low. Moreover, the coefficients of correlation of the bioavailability based on urinary excretion data and areas under the curves was also low so that calculating the bioavailability from urinary excretion data cannot be recommended. The mean fraction of OxBr excreted unchanged in urine during 24 hours were found to be 58, 59 and 47% after IV, IM and oral administration, respectively.