Bioanalytical assay of clozapine and its N-oxide metabolite and the determination of their blood levels in the dog

J. MEIER (introduced by B. BERDE)

Division of Pharmaceutical Research and Development, Sandoz Ltd., Basle, Switzerland

Clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo-[b,e][1,4]diazepine) is a neuroleptic drug, the special feature of which is its low propensity for causing extrapyramidal effects (Ayd, 1974). The N-oxide of clozapine has been shown to be the principal metabolite in the dog and in man (Gauch & Michaelis, 1971). Since N-oxidation of tertiary amines and subsequent reduction back to the corresponding base have been established as common metabolic routes (Bickel, 1969), this study was aimed at clarifying the oxidation and reduction processes which occur following administration of clozapine, by simultaneously measuring the pharmacokinetics of clozapine and its N-oxide metabolite in the blood of dogs.

None of the known bioanalytical methods based on gas-liquid chromatography, thin-layer plate chromatography, polarography or radio-immunoassay, was found to be sufficiently sensitive to detect and differentiate between clozapine and its N-oxide metabolite. In this study, a method using high-pressure liquid chromatography (HPLC) was developed for this purpose and then applied in some preliminary experiments in dogs.

The drug and its N-oxide metabolite were extracted from samples of blood and urine with chloroform. The condensed extract was applied with a sample loop to a column (250 x 3 mm) which was slurry-packed with the stationary phase Merckosorb SI 60 (10 μm). A gradient of chloroform/methanol/water served as the mobile phase. Using a Hewlett Packard instrument with u.v. detection, clozapine and its N-oxide could be determined to a minimum concentration of 10 ng/ml of blood or urine (5% significance level).

The blood levels of clozapine and clozapine-N-oxide were measured with this HPLC method in 2 beagles over periods of 48 h following administration. Each dog received on separate weeks 300 mg clozapine orally, 300 mg clozapine-N-oxide orally and either 50 mg clozapine or 50 mg clozapine-N-oxide i.v. Both clozapine and clozapine-N-oxide were rapidly absorbed after oral administration. Oxidation of clozapine and reduction of clozapine-N-oxide occurred in the blood very soon after either oral or intravenous administration. The reduction process was faster than the oxidation process; the maximum level of clozapine was observed 10 min after intravenous administration of the N-oxide, whereas the maximum level of the N-oxide was not measured until 1 h after intravenous administration of clozapine. The clozapine/clozapine-N-oxide ratio oscillated during the first 6 h after administration. No common continuous pharmacokinetic model could describe the observed data due to the inter- and intra-subject variations in the levels of the two compounds.

It is concluded that both clozapine and its N-oxide should be determined in future pharmacokinetic studies in the dog and in man since the two compounds are so readily converted to each other *in vivo*. The HPLC method described would be of value in this type of study.

References

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