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Desmethyldiazepam: A Specific Radioimmunoassay

Keyphrases □ Desmethyldiazepam—radioimmunoassay □ Radioimmunoassay—desmethyldiazepam □ Diazepam, desmethyl metabolite—radioimmunoassay □ Benzodiazepines—desmethyldiazepam, radioimmunoassay

To the Editor:

Desmethyldiazepam, a 1,4-benzodiazepine, has particular clinical significance because it is a pharmacologically active metabolite of diazepam and several other benzodiazepines (1). Thus, blood levels of desmethyldiazepam and the parent compounds are important parameters in the pharmacokinetic understanding of anxiolytic drug therapy and in the biopharmaceutical evaluation of different drug formulations.

Although many analytical procedures, primarily employing electron-capture GLC and high-performance liquid chromatography, have been reported for the quantitation of desmethyldiazepam in blood or plasma, none achieves the high sample throughput attainable with radioimmunoassay in which the compound may be measured directly in microsamples. Radioimmunoassay is particularly advantageous in bioequivalency studies, where numerous samples may be generated, and in pharmacokinetic studies in the neonate, where a limited sample volume is available for analysis. We now report the development of a specific radioimmunoassay for desmethyldiazepam which satisfies the requirements for both high throughput and small samples.

The hapten 7-chloro-1,3-dihydro-5-(4-hydrazinocarbonylmethoxyphenyl)-2*H*-1,4-benzodiazepin-2-one was converted to its reactive acyl azide with nitrous acid and was coupled covalently to bovine serum albumin as described previously (2). The immunogen consisted of 15 moles of hapten covalently coupled to 1 mole of albumin. Rabbits were immunized intradermally, and the antiserum with the highest titer (1:7500 dilution) of antibodies to desmethyldiazepam was used for all studies.

The radioligand 9-^{3}H-desmethyldiazepam was prepared by catalytic exchange of 9-iododesmethyldiazepam with tritium gas. The reaction was carried out in tetrahydrofuran containing 0.5% triethylamine with a 10% palladium-on-charcoal catalyst. After removal of the labile tritium by repeated freeze drying from methanol, the reaction product was chromatographed on a silica gel column packed in ethyl acetate. The appropriate fractions were combined to yield 9-^{3}H-desmethyldiazepam with a specific activity of 26.5 Ci/mmole.

The radioimmunoassay was identical to that described recently for diazepam (3). A logit-log calibration curve for desmethyldiazepam was linear from 30 to 2000 pg/tube; thus a working sensitivity limit of 3 ng/ml was achieved using a 10-μl plasma sample. This sensitivity is comparable to that achieved by electron-capture GLC with a 1-ml sample. The intra- and interassay coefficients of variation did not exceed 6 and 11%, respectively.

The specificity of the antiserum was evaluated by cross-reactivity studies with those drugs and/or their metabolites of which desmethyldiazepam is a known metabolite. Diazepam, chlordiazepoxide, desmethylchlordiazepoxide, demoxepam, prazepam, and medazepam all cross-reacted less than 1% relative to desmethyldiazepam (100% cross-reactivity). Clorazepate exhibited an apparent cross-reactivity of >50%, which undoubtedly was due in part to its decomposition to desmethyldiazepam during the assay incubation period. Tricyclic antidepressants, commonly prescribed in conjunction with benzodiazepines, did not cross-react with the antiserum.

Further evidence for the specificity of the radioimmunoassay was obtained by comparison with an established electron-capture GLC method for the determination of desmethyldiazepam (4). The joint determinations of desmethyldiazepam in 30 plasma samples from subjects who had received various doses of clorazepate or diazepam were subjected to linear regression analysis by a method (5) using a 95% confidence ellipse. Over a nearly 300-fold desmethyldiazepam concentration range (24–6400 ng/ml), the correlation coefficient, regression line slope, and y-intercept were 0.998, 1.07, and −5.8, respectively. Thus, the radioimmunoassay measures desmethyldiazepam as precisely and specifically as the electron-capture GLC method.

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