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Reversed-Phase High-Performance Liquid Chromatographic Determination of Gliclazide in Human Plasma

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A high-performance liquid chromatography (HPLC) procedure was developed for the determination of plasma gliclazide, a new oral hypoglycemic drug.

Gliclazide, tolbutamide (used as an internal standard) and plasma components in plasma were successfully separated as sharp peaks by reversed-phase HPLC on a Jasco SC-01 column with methyl alcohol containing 0.2% acetic acid (3: 2, v/v) as the mobile phase (retention time; gliclazide: 16 min, IS: 10 min, plasma components: 5.5 and 7.0 min).

The relationship between the plasma levels of gliclazide and the peak height ratio (gliclazide/IS) was linear (correlation coefficient, $r=1.00$) in the range of 0.3 to 10 μg/ml plasma. The detection limit and the reproducibility (CV) of the present method were 0.3 μg/ml and 4.0% ($n=8$), respectively.

The decay curve of plasma gliclazide in a diabetic patient given 80 mg of the drug orally was determined by the present method and GLC (ECD method). The two results agreed well.

Reversed-phase HPLC was shown to be suitable for the routine clinical assay of gliclazide in plasma

Keywords——gliclazide; tolbutamide; hypoglycemic drug; high-performance liquid chromatography; reversed-phase high-performance liquid chromatography; decay curve of plasma gliclazide

Introduction

Gliclazide, N-(4-methylphenylsulfonyl)-N′-(3-azabicyclo[3,3,0]octyl)-urea, is one of a series of sulfonylureas recently evaluated as potential oral hypoglycemic drugs. Studies on the dynamic profiles of the drug in plasma were required simultaneously with determination of the pharmacologic responses to glucose and insulin levels in plasma after oral administration of gliclazide in normal and diabetic subjects.

Conventional methods for the determination of sulfonylureas in plasma have based on colorimetry,[2] gas-liquid chromatography (GLC)[3] or high-performance liquid chromatography (HPLC).[4] The former two methods, however, are troublesome, and in particular, colorimetry is less sensitive and specific than HPLC.

In the present work, we developed a specific, rapid and accurate HPLC method for the determination of gliclazide levels in plasma.

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Experimental

Apparatus and Conditions——A liquid chromatograph (Jasco Tri Rotar) was operated in two different modes; in the normal phase mode, Lichrosorb SI-60 (Merck, ϕ 4.0×250 mm) was used as a stationary phase with a mobile phase of mixture of dichloromethane and methyl alcohol (3: 2, v/v), while in the reversed-phase mode, SC-01 (Jasco, ϕ 4.6×125 mm) was used with a mixture of methyl alcohol and 0.2% acetic acid (3: 2, v/v). Several other packings and mobile phases were used for tests of the HPLC conditions. The effluents were monitored with a variable wavelength spectrometric detector (Jasco Uvidec 100-II) at the absorption maximum of the drug (227 nm). The column temperature was ambient (25—27°) and a 10 μl sample was injected with a microsyringe (Microliter, Hamilton Co., U.S.A.). The internal standard (IS) was tolbutamide.

Reagents——Gliclazide was purchased from Dainihon Seiyaku Co., Ltd., Japan. Tolbutamide was a product of Hoechst Japan Co., Ltd., Japan. Methyl alcohol, dichloromethane, chloroform and acetic acid were products (special grade for liquid chromatography) of Wako Pure Chemical Industries Ltd., Japan. All other chemicals were of analytical reagent grade. All aqueous solutions containing reagents were passed through a filter (Millipore type CS, 0.22 μm, Millipore Co., U.S.A.) before use.

Extraction Procedure——A mixture of 2.5 ml of human plasma and 1 ml of 0.25 M acetate buffer (pH 3.95) was shaken vigorously for 15 min with 4 ml of chloroform containing 0.5 μg of IS (tolbutamide). The mixture was centrifuged (2900 rpm, 15 min), then 3 ml of the chloroform layer was withdrawn, mixed with 2 ml of 1 N NaOH solution, and shaken for 10 min. After centrifugation of the mixture for 5 min, 1.5 ml of the aqueous layer was added to a mixture of 0.5 ml of 3 N HCl solution and 2.5 ml each of 0.25 M acetate buffer (pH 3.95) and chloroform, followed by shaking for 10 min. After centrifugation for 5 min, 2 ml of the chloroform layer was evaporated to dryness at 50° under an N_2 stream. The residue was dissolved in 50 μl of methyl alcohol, and 10 μl of aliquot was injected into the HPLC column. The internal standard (tolbutamide) was dissolved in the chloroform used for extraction at a concentration of 0.5 μg/4 ml. The extractability of gliclazide from plasma using the present procedure was over 95%. The methyl alcohol solution containing the extracted drug was stored at 2—4°, and assayed within 3 days.

Results and Discussion

The peaks of gliclazide and plasma components were observed at retention times of 2.2 and 2—3 min in the HPLC chromatogram on the normal phase (Lichrosorb SI-60). Thus, normal phase HPLC did not seem suitable for the determination of plasma gliclazide under these conditions.

Subsequently, several reversed-phase HPLC were examined using the following packings: Lichrosorb RP-2 (Merck), Nucleosil C_{18} (Nagel), Jasco SC-01 (Jasco), Lichrosorb RP-8 (Merck) and Lichrosorb RP-18 (Merck). The retention times of gliclazide became shorter in this order, and the separation of peaks due to the drug and ordinary plasma components was satisfactory with the SC-01 column.

Several mobile phases were tested in reversed-phase (Jasco SC-01) HPLC. A mixture of methyl alcohol and 0.2% acetic acid (3: 2, v/v) produced the best HPLC chromatogram, as shown in Fig. 1.

The retention times of gliclazide and IS were 16 and 10 min, respectively, and those of the main plasma components were 5.5 and 7.0 min. The relationship between the gliclazide level and the peak height ratio (gliclazide/IS) was linear (correlation coefficient, r=1.00) in the range of 0.3 to 10 μg/ml plasma.

Figure 2 shows typical decay curves of plasma gliclazide in a diabetic patient given 80 mg of the drug (2 tablets containing 40 mg of gliclazide) orally. The decay curve obtained by the present reversed-phase HPLC was compared with that by GLC (ECD method).[5] Both curves agreed well. The plasma level of gliclazide rapidly reached a maximum (8.1 μg/ml plasma) at about 2 hr after administration of the drug; it subsequently declined nearly to the pre-administration level within 48 hr.

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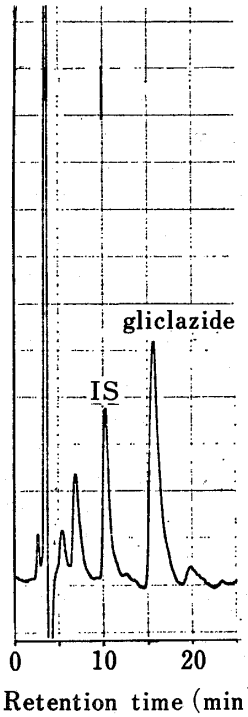

Fig. 1. HPLC Separation of Gliclazide and IS from Plasma Components

Column: SC-01 (Jasco, ϕ 4.6×125 mm).
Mobile phase: methyl alcohol–0.2% acetic acid (3: 2, v/v).
Flow rate: 0.4 ml/min.
IS: tolbutamide.

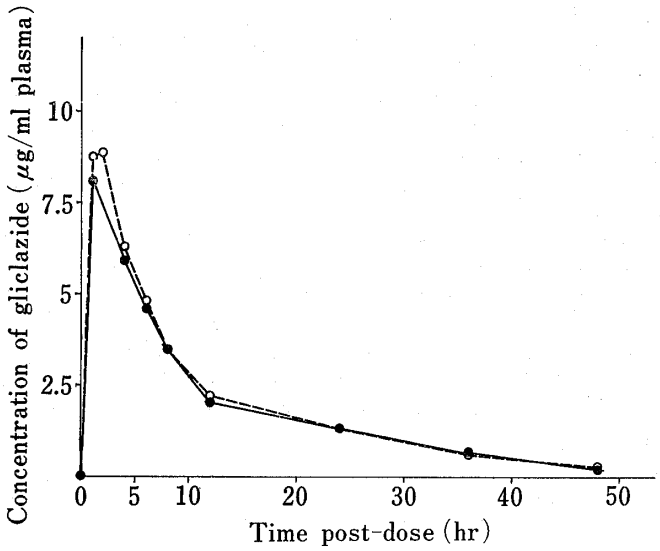

Fig. 2. Decay Curves of Plasma Gliclazide Levels after Oral Administration of 80 mg to a Diabetic Patient

Each point represents the mean value determined in duplicate. The same sample was determined by reversed-phase HPLC (●) and by gas-liquid chromatography (ECD method) (○).

The effective oral dose of gliclazide, which is a more potent hypoglycemic than other sufonylureas, was not more than 1/4 of that of tolbutamide. However, the detection limits of the conventional methods for assay of plasma sulfonylureas by colorimetry and GLC (pyrolysis method) are 10[6] and 5 μg/ml,[6] respectively, so that these methods are not satisfactory for the determination of plasma gliclazide. On the other hand, the detection limit of GLC (ECD method) is 0.1 μg/ml of gliclazide,[5] which would seem to be useful for clinical examination of plasma gliclazide, but this method usually requires tedious procedures such as acylation of the extracted sample, and gives lower reproducibility.

In conclusion, the present method for the determination of plasma gliclazide by reversed-phase HPLC is comparable to the GLC (ECD method) in sensitivity (0.3 μg/ml), specificity and rapidity (shorter retention time). The present method seems preferable for routine clinical examination because of its simplicity and high reproducibility (CV: 4.0%, n=8).

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