CHROMBIO. 5294

Note

Photodegradation of nifedipine relative to nitrendipine evaluated by liquid and gas chromatography

BARRY K. LOGAN

Department of Pathology, University of Tennessee, Memphis, TN 38163 (U.S.A.)

and

KENNERLY S. PATRICK*

*Department of Medicinal Chemistry, University of Tennessee, Memphis, TN 38163 (U.S.A.) and *Department of Pharmaceutical Sciences, College of Pharmacy, Medical University of South Carolina, Charleston, SC 29425-2303 (U.S.A.)*

Nifedipine is a dihydropyridine calcium channel blocker commonly used in the treatment of cardiovascular diseases. Therapeutic drug monitoring studies have been of limited value due to the low toxicity [1] and relatively broad range of therapeutic plasma concentrations (25–100 ng/ml) [2]. However, the pharmaceutical formulation of nifedipine can markedly affect bioavailability [3], underscoring the importance of bioequivalence testing. To this end, a sensitive and specific gas chromatographic–mass spectrometric (GC–MS) method for plasma nifedipine quantitation has recently been developed which incorporates nitrendipine, a structurally related dihydropyridine drug (Fig. 1), as an internal standard [4].

Ideally, chromatographic analysis of plasma nifedipine should incorporate an internal standard possessing physical and chemical properties similar to the drug [5] to most effectively control for variability in extraction recovery and any possible oxidative [6] and hydrolytic losses occurring during the preparation of the samples. High-performance liquid chromatographic (HPLC)

Fig. 1. Structures of nifedipine and nitrendipine. Nifedipine differs structurally from the internal standard nitrendipine in being an *ortho*-nitro isomer and ester homologue.

Fig. 2. Photodegradation pathways for nifedipine. Daylight frequencies dehydrate the drug, producing dehydronitrosonifedipine (upper right), while UV light oxidizes the drug to dehydronifedipine (lower right).

methods for nifedipine plasma determinations have utilized as internal standards spironolactone [7], 4-dimethylaminobenzaldehyde [8], 11-ketoprogesterone [9], ethinyl estradiol [10], chlorpropamide [11], nitrendipine [6,12,13] or none [14], while literature GC methods for plasma nifedipine have standardized with diazepam [15–18], methylnitrazepam [19], trifluperidol [20], nitrendipine [4,21], the diethyl ester homologue of dehydronifedipine [22], deuterated nifedipine [23] or none [24]. Of these several internal standards, only nitrendipine is both commercially available and structurally analogous to the analyte.

Nifedipine plasma sample collection, storage and preparation for analysis require handling in gold fluorescent light to prevent photodegradation [7]. Daylight frequencies (400–600 nm) very rapidly dehydrate nifedipine to the nitrosopyridine species [20] (Fig. 2), giving the parent drug a half-life in an organic solvent or plasma of only 15 or 44 min, respectively [6]. Further, UV light (254 nm) oxidizes nifedipine to dehydronifedipine [20], which is also a metabolite [25]. The present chromatographic study was conducted to deter-

mine if the photosensitive internal standard nitrendipine [26] photolyzes at a rate parallel to that of nifedipine, whereby this standard would control for the possible photolytic loss of nifedipine during sample preparation. The results of this study also have implications regarding lighting conditions for nitrendipine analyses.

EXPERIMENTAL

Chemicals

Nifedipine was purchased from Sigma (St. Louis, MO, U.S.A.). Nitrendipine was extracted with acetone from Baypress® 20-mg tablets (Bayer, Zurich, Switzerland) and crystallized. Dehydronifedipine, dehydronitrosonifedipine, dehydronifedipine monoester and dehydronifedipine lactone [5,7-dihydro-2-methyl-4-(2-nitrophenyl)-5-oxofuro[3,4-*b*]pyridine-3-carboxylic acid methyl ester] were from Bayer (Leverkusen, F.R.G.). Acetonitrile was obtained from Burdick and Jackson (Muskegon, MI, U.S.A.). Deionized water was doubly filtered and buffer components were of analytical grade.

Instrumentation and chromatography

The HPLC system utilized a gradient pump (Varian 5000 Series, Sugarland, TX, U.S.A.) and an injector (Rheodyne Model 7125, Berkeley, CA, U.S.A.) with a 10-μl loop. The UV–VIS detection was performed with a diode-array detector (Hewlett Packard Model 1040A, Palo Alto, CA, U.S.A.) and data system (Hewlett Packard 300 Series). The eluent was monitored at 200, 272, 276, 280 and 314 nm, and full UV–VIS spectra were acquired from 190 to 400 nm on all peaks over 5 mA.U. Liquid chromatography was performed on a 100 mm × 4.6 mm cyano cartridge (CS-MP Spheri-5, Brownlee Labs., Santa Clara, CA, U.S.A.). Acetonitrile in 0.05 M KH_2PO_4–H_3PO_4 (pH 3) buffer was pumped as a 10–40% gradient over 10 min, with a 5-min re-equilibration time. The flow-rate was 1.5 ml/min.

The photodegradation product of nifedipine was identified by comparison of the GC retention time and mass spectrum to that of the reference standard and literature spectra [20] using a Hewlett Packard Model 5790 gas chromatograph equipped with a 15 m × 0.25 mm, 0.25 μm film thickness, 5% phenylmethylsilicone fused-silica column (DB-5, J & W. Scientific, Folsom, CA, U.S.A.). A temperature program from 200 to 295°C at 10°C/min was used. Detection was either by total ion monitoring MS (Hewlett Packard Model 5970) or flame ionization.

Stability testing

Acetonitrile solutions (70 μM) of each dihydropyridine drug were placed in 75 mm × 12 mm borosilicate glass test tubes and stored in the dark at 25°C or exposed to light in two settings at 25°C: (1) in a laboratory lit only with flu-

orescent lighting; (2) in a laboratory lit with fluorescent lighting and window daylight. The laboratory measured 7 m × 8 m, lit by 24 fluorescent lights (GE, cool white f40 W) 120 cm long. These fixtures were 1.8 m above the test samples. For window daylight exposure, the samples were placed directly under a glass window on a fall day, starting at 11:00 a.m. These specifics, as related to light intensity, are reported to critically influence the rate of nifedipine photodegradation [27]. Aliquots of the irradiated solutions (10 μl) were taken at times 0, 40, 60, 80, 100, 120, 180, 280 and 310 min and liquid-chromatographed as described. Samples after 310 min were taken for GC analysis.

The concentrations of the dihydropyridine drugs were determined by chromatographic peak areas. The half-lives of nifedipine in both lighting conditions were determined by fitting the data to a first-order decay [28].

Nitrendipine in acetonitrile (70 μM) was placed in a quartz cuvette and exposed to direct sunlight from 10:00 a.m. until 5:00 p.m. at approximately 18°C, then analyzed by HPLC and GC.

RESULTS AND DISCUSSION

Chromatography

The present study was conducted to determine if the reportedly photosensitive internal standard nitrendipine [26] controls for the possible spurious photodegradative loss of the analyte nifedipine during preparation of plasma samples for analysis. A cyano column in reversed-phase mode served to separate nifedipine from the daylight degradation product dehydronitrosonifedipine [20] and nitrendipine. It also proved efficient in separating reference standards of the dehydronifedipine monoester metabolite [12,25] and the metabolic artifact nifedipine lactone [21,22] (Fig. 3).

This cyano phase failed to separate the UV photodegradation product dehydronifedipine [20] from dehydronitrosonifedipine. However, these products were separable using GC on a 5% phenylmethylsilicone column (Fig. 4). The degradation product generated from nifedipine under the two described laboratory lighting conditions was confirmed to be only dehydronitrosonifedipine by the HPLC–UV spectrum [λ_{max} 222, 283, 312 (shoulder) nm] [20] and by the GC retention time and mass spectrum [m/z 269 (100%), 328 (M$^+$)].

Rates of photodegration

Acetonitrile solutions were used as the irradiation medium because this solvent was used to extract nifedipine in a recent method for plasma analysis [4]. The rate of photodegradation of nifedipine has been reported to be highly dependent upon the light intensity [27]. In the present study, no degradation was evident for either nifedipine or nitrendipine when stored as acetonitrile solutions in the dark for six days. Under the fluorescent lighting conditions, nifedipine degraded according to first-order kinetics with a half-life of 110 min
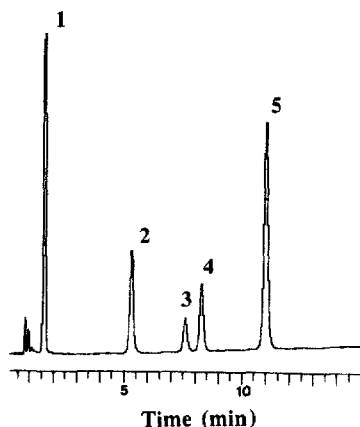
Fig. 3. Liquid chromatographic separation of dehydronifedipine monoester (1), dehydronifedipine lactone (2), unresolved dehydronifedipine and dehydronitrosonifedipine (3), nifedipine (4) and nitrendipine (5) eluted under high pressure from a cyano phase column using acetonitrile in 0.05 M KH$_2$PO$_4$-H$_3$PO$_4$ (pH 3) buffer as a 10-40% gradient over 10 min and detected by UV-VIS absorbance.
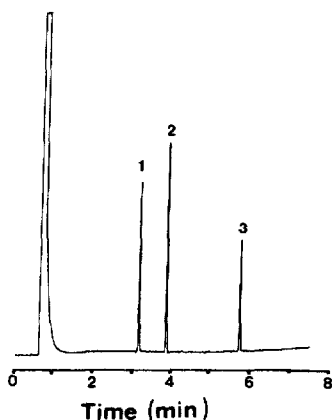

Fig. 4. Gas chromatographic separation of dehydronitrosonifedipine (1), dehydronifedipine (2) and nifedipine (3) on a 5% phenylmethylsilicone capillary column using flame ionization detection (see Experimental).

(Fig. 5). Combining the fluorescent irradiation with daylight through a window pane reduced the photolytic half-life of nifedipine to 13 min.

Nitrendipine was stable under both laboratory lighting conditions. This analogue of nifedipine exhibited no apparent degradation during the 310 min of fluorescent light or combined fluorescent and window light.

A solution of nitrendipine in a quartz cuvette exposed to direct sunlight for 6 h did generate detectable quantities of two degradation products. One prod-
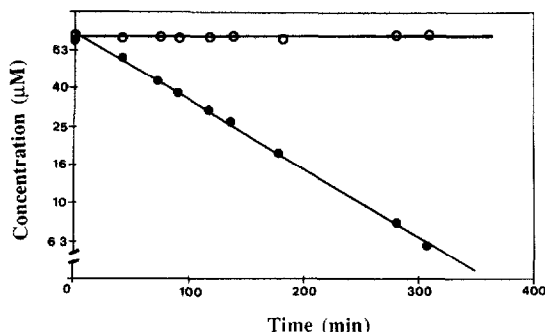
Fig. 5. Concentrations over time of irradiated nifedipine (●) and nitrendipine (○). Acetonitrile solutions of the drugs were contained in borosilicate tubes and exposed to fluorescent laboratory lighting (see Experimental).
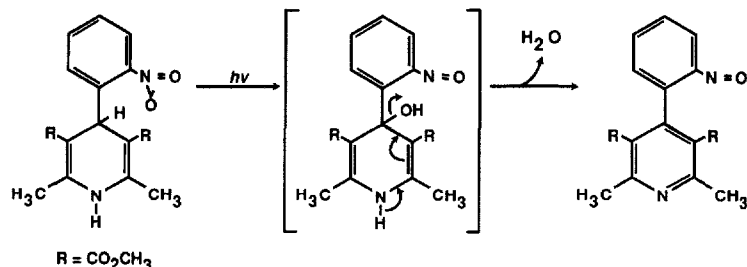

R = CO$_2$CH$_3$

Fig. 6. Postulated reaction mechanism for daylight (400–600 nm) induced degradation of nifedipine. This dehydration may proceed through an intramolecular disproportionation facilitated by the proximity of the nitro group to the benzylic hydrogen.

uct was identified as dehydronitrendipine (also a metabolite [29]) based on MS [m/z 313 (100%), 358 (M$^+$)] [30] and UV [λ_{max} 272 nm (−NO$_2$)] data. The second degradation product lacked the 300–350 nm chromophore characteristic of aromatic nitroso compounds and was not identified. This apparent reluctance of nitrendipine to form a nitroso photodegradation product may explain the observed laboratory light stability of this derivative.

The ability of the *ortho*-nitro group of nifedipine to participate in a favorable six-membered intramolecular disproportionation mechanism, followed by aromatization through dehydration (Fig. 6), is postulated to account for the much greater photosensitivity of this derivative relative to its *meta*-nitro homologue, nitrendipine. The intermediate formation of the proposed benzyl alcohol has precedents in related intramolecular redox reactions of *ortho*-substituted nitrobenzenes [31,32]. The difference in stability between these drugs does not appear to be related to the ester homology since the *meta*-nitro isomer of nifedipine is more photochemically stable than nifedipine [33].

In conclusion, the use of nitrendipine as an internal standard does not control for the photodegradative loss of nifedipine, reinforcing the need for special laboratory lighting conditions [7] to insure drug stability when handling nifedipine samples.

ACKNOWLEDGEMENT

REFERENCES

1 P.H. Stone, E.M. Antman, J.E. Muller and E. Braunwald, Ann. Intern. Med., 93 (1980) 886.
2 P.D. Henry, Am. J. Cardiol., 46 (1980) 1047.
3 I. Sugimoto, A. Kuchiki, H. Nakagawa, K. Tohgo, S. Knodo, I. Iwane and K. Takahashı, Drug Dev. Ind. Pharm., 6 (1980) 137
4 K.S. Patrick, E.J. Jarvi, A.B. Straughn and M.C. Meyer, J. Chromatogr., 495 (1989) 123.
5 K.H. Dudley, Natl. Bur. Stand. Publ., 519 (1979) 381.
6 C.H. Kleinbloesem, J. van Harten, P. van Brummelen and D.D. Breimer, J. Chromatogr., 308 (1984) 209.
7 J.J. Schaeck, S.T. Wu, L.Z. Benet and E.T. Lin, Pharm. Res., 3 (1986) 6S.
8 P. Pietta, A. Rava and P. Bondi, J. Chromatogr., 210 (1980) 516.
9 P.R. Bach, Clin. Chem., 29 (1983) 1344.
10 B.J. Curley, R.G. Buice and P. Sidhu, Ther. Drug Monit., 7 (1985) 321.
11 Anonymous, Supelco Reporter, 6 (1987) 6.
12 K.D. Raemsch and J. Sommer, Hypertension, 5 (Suppl. II) (1983) 18.
13 W. Snedden, P.G. Fernandez and C. Nath, Can. J. Physiol. Pharmacol., 64 (1986) 290.
14 V. Nitsche, H. Schutz and A. Eichinger, J. Chromatogr., 420 (1987) 207.
15 J. Dokladalova, J.A. Tykal, S.J. Coco, P.E. Durkee, G.T. Quercia and J.J. Korst, J. Chromatogr., 231 (1982) 451.
16 T.S. Foster, S.R. Hamann, V.R. Richards, P.J. Bryant, D.A. Graves and R.G. McAllister, J. Clin. Pharmacol., 23 (1983) 161.
17 S.R. Hamann and R.G. McAllister, Clin. Chem., 29 (1983) 158.
18 P. Jakobsen, O. Lederballe Pedersen and E. Mikkelsen, J. Chromatogr., 162 (1979) 81.
19 R. Tanner, A. Romagnoli and W.G. Kramer, J. Anal. Toxicol., 10 (1986) 250.
20 R. Testa, E. Dolfini, C. Reschiotto, C. Secchi and P.A. Biondi, Farmaco Ed. Prat., 34 (1979) 463.
21 B.J. Schmid, H.E. Perry and J.R. Idle, J. Chromatogr., 425 (1988) 10.
22 S. Kondo, A. Kuchiki, K. Yamamoto, K. Akimoto, K Takahashi, N. Awata and I. Sugimoto, Chem. Pharm. Bull., 28 (1980) 1.
23 S. Higuchi and Y. Shiobara, Biomed. Mass Spectrom., 5 (1978) 200.
24 F. Kozjek, S. Primozic, A. Mrhar, R. Karba, K.D. Raemsch and A. Janezic, Biopharm. Drug Dispos., 8 (1987) 23.
25 J. Kann, G.J. Krol, K.D. Raemsch, D.E. Burkholder and M.J. Levitt, J. Cardiovasc. Pharmacol., 6 (1984) S968.
26 Anonymous, Baypress® Brand of Nitredipine, Package Insert, Bayer, Leverkusen, 1985.
27 I.A. Majeed, W.J. Murray, D.W. Newton, S. Othman and W.A. Al-Turk, J. Pharm. Pharmacol., 39 (1987) 1044.
28 R. Schall, Chemical Kinetics of Homogeneous Systems, D. Reidel, Boston, MA, 1971.
29 P.A. Soons and D.D. Breimer, J. Chromatogr., 428 (1988) 362.
30 H. Meyer, D. Scherling and W. Karl, Arzneim.-Forsch., 33 (1983) 1528.
31 A. Patchornik, B. Amit and R.B. Woodward, J. Am. Chem. Soc., 92 (1970) 6333.
32 U. Zehavi, B. Amit and A. Patchornik, J. Org. Chem., 37 (1972) 2281.
33 K. Akimoto, K. Kurosaka, H. Nakagawa and I. Sugimoto, Chem. Pharm. Bull. (Tokyo), 36 (1988) 1483.