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Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 618–624
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Photochemical stabilities of some dihydropyridine calcium-channel blockers in powdered pharmaceutical tablets

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Received 2 October 2007; received in revised form 19 January 2008; accepted 23 January 2008
Available online 7 February 2008

Abstract

Photostabilities of some dihydropyridine calcium-channel blockers in pulverized pharmaceutical tablets were studied. Powdered tablets including amlodipine, nifedipine, or nilvadipine were exposed to D65 daylight lamp radiation according to an ICH guideline (ICH Q1B). The photodegradation of pharmaceutical components and their degradation products were monitored by HPLC using a reversed phase column with UV detection; their peak components were identified using MS analysis. Photochemical reactions involved in the photodegradation of these pharmaceuticals include aromatization of the dihydropyridine moiety and conversion to nitroso group from the nitro group in benzene rings. Chemical stability studies of these drugs indicate that nifedipine is the most photosensitive. The rate constant of nifedipine is indicated as seven times higher than those of the other two drugs.

Keywords: Photostability; Dihydropyridine calcium-channel blocker; Amlodipine; Nifedipine; Nilvadipine; Photodegradation

1. Introduction

Most pharmaceuticals have been provided in various dosage forms such as tablets, capsules, and granules. Dosage forms are designed by manufacturers to maintain the quality of pharmaceuticals under the storage conditions and to enhance their gastrointestinal uptake and blood level control while increasing the half-life of drug components. Therefore, crushing the tablets and opening the capsules are not advisable in general, and of course, pulverization of these tablets is the out of the range of manufacturer's quality assurance. However, the tablets are sometimes pulverized to powders in clinical use to make them easy to ingest for elderly or disabled persons if they cannot be consumed in their whole form. The powder is then dissolved in a liquid or mixed with a food substance, which can be swallowed more easily. Pharmaceutical manufacturers provide the drug information concerned on the chemical stabilities of medicines in their provided form, but most provide no information related to chemical stabilities of drugs as crushed or opened.

Dihydropyridine (DHP) calcium-channel blockers were developed for injection or oral administration for use in the treatment of mild to moderate hypertension. To date, many derivatives of DHPs have been developed for clinical use [1]. The DHPs are known to be photolabile; most of their photochemical decomposition products have no pharmacological activity [2–6]. That fact implies that the pulverization of the tablets might induce photodegradation of DHP components depending on the room illumination. However, previous studies on the photodegradation of DHP drugs were conducted mainly using DHP alone in solid and solution states; some data were obtained after irradiation using a high-energy mercury lamp [7]. Therefore their kinetic evaluation is not directly applicable to the quality control of powdered tablets in clinical work. The International Conference on Harmonization (ICH), Expert Working Group (EWG) for photodegradation testing proposed guidelines for photostability experimentation using new drug substances and products. According to the ICH guidelines, two light sources are recommended. The guideline further suggest taking into account

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doi:10.1016/j.jpba.2008.01.042

the effect related to illumination of rooms in the studies of photodegradation of drugs, and to elimination of the influence of high-energy ultraviolet radiation [8]. Considering the increased use of DHP derivatives in medical treatment, not only in cardiology, but also in studies of powdered DHP drugs' photochemical stability are of considerable interest.

This study was intended to evaluate and compare the photostability of some DHPs in commercial tablets as their pulverized powders. We chose the three DHP drugs depicted in Fig. 1 for this study: nifedipine, [dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate]; nilvadipine, [5-isopropyl-3-methyl-2-cyano-1,4-dihydro-6-methyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate]; amlodipine besilate, [3-ethyl-5-methyl-2-[(2-aminoethoxy)methyl]-4-(*o*-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzenesulfonate]. The tablets were pulverized to powders and photodegradation was conducted by irradiation using a D65 lamp according to the ICH guidelines. Some kinetic parameters were obtained through quantitation of the degradation of DHPs and the generation of some decomposition products; they were compared to data from previous works.

2. Experimental

2.1. Materials

Nifedipine, nilvadipine and amlodipine besilate specimens were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Adalat® L (nifedipine 20 mg, Bayer Yakuhin, Osaka, Japan), Amlodin® (2.5 mg as amlodipine, Dainippon Sumitomo Pharma Co. Ltd., Osaka, Japan) and Nivadil® (nilvadipine 2 mg, Astellas Pharma Inc., Tokyo, Japan) were used for studies of photostability. Ethyl *p*-aminobenzoate (ABEE), formic acid, 28% ammonia, methanol and acetonitrile were purchased from Nacalai Tesque Inc. (Kyoto, Japan). In addition, PVDF-membrane filters were obtained from National Scientific (Rockwood, TN). Other solvents and specimens were of the highest commercially available grade.

2.2. Apparatus and experimental condition

Photostability tests were done using a D65 fluorescent light (FLR40S· D-EDL-D65/M; Toshiba Ltd., Tokyo, Japan) to simulate natural sunlight exposure. Irradiation was carried out in a dark room to prevent unintended light exposure. The HPLC apparatus consisted of a liquid chromatography pump (PU-850, JASCO Inc.), an injector (7125; Rheodyne LLC) with a 20 μl loop, a UV–vis spectrophotometric detector (870; JASCO Inc.) and a Windows PC-based data processor, SmartChrom (KYA Technologies Corp., Tokyo, Japan). Photodegradation products were separated using a column (4.6 mm i.d., 250 mm, Cosmosil ODS; Nacalai Tesque Inc., Kyoto, Japan); they were detected by an absorption at 234 nm. Analytical conditions were optimized referred to office data of manufacturers. Photoexposed samples of Adalat® L were eluted using aqueous 65% (v/v) methanol at a 0.45 ml/min flow rate. Samples of Amlodin® were eluted using 50 mM ammonium formate (pH 4.4)–acetonitrile (6:4, v/v) at 0.5 ml/min flow rate. Nivadil® were analyzed using 50 mM ammonium formate (pH 4.4)–acetonitrile (4:6, v/v) at 0.8 ml/min flow rate. Numbers of the peaks of photodegradation products were also confirmed using capillary electrophoresis in micellar electrokinetic chromatography mode (data not shown). The EI-MS spectra of peak components were obtained using a JEOL mass spectrometer. The mass spectra were recorded within *m/z* 100–900 at positive polarity.

2.3. Photostability testing

Commercial preparations of DHP tablets were pulverized in a mortar and filtered through a sieve (<105 μm). Powder quantities corresponding to those of tablets of the respective drugs were dispersed to 2 cm^2, re-closable gas-tight storage bags made of polyethylene. The samples were placed horizontally and exposed to D65 light to irradiate the sample surface with 11,111 lx light energy. Each sample bag was turned upside down every hour to expose the powder surface homogeneously. After the proposed period, one bag of the photoexposed sample was dissolved in 2 ml of solvent (methanol for Adalat® L, and acetonitrile for Amlodin® and Nivadil®) including ABEE as an internal standard (60 μmol for Adalat® L, 6 μmol for Amlodin® and 5 μmol for Nivadil®); then the solutions were filtered through a PVDF membrane (0.45 μm). Then the sample solution was injected to an HPLC column to elicit information related to the kinetics of photodegradation process. Disappearance of the drug and the generation of decomposition products were monitored over the period of 108 h because ICH guideline proposed 1,200,000 lx h as the total irradiation energy.

2.4. Characterization of photodecomposition products by EI-MS

Every peak component appearing in HPLC was collected in flasks and evaporated repeatedly with the addition of a few milliliters of methanol until the odor of eluent components used was lost. The residues were dissolved in a small amount of appropriate solvent and were subjected to EI-MS. The mass spectra (*m/z* and relative abundance in parentheses) of DHP drugs and their photodegradation compounds were recorded as follows.

Nifedipine (Adalat®, MW 346): 346 (M, 15%), 282 (M*, 100%), 269 (M–NO_2–OCH_3, 73%), 251 (M*–OCH_3, 82%), and 223 (M*–CO, 28%). Dehydro nifedipine (**I**, MW 344): 344 (M, 0.8%), 313 (M–OCH_3, 6%), 298 (M–NO_2, 100%), 267 (M–NO_2–OCH_3, 8%), 282 (M*, 27%), 251 (M*–OCH_3, 24%), and 223 (M*–CO, 8%): Dehydronitroso nifedipine (**II**, MW 328): 328 (M, 10%), 298 (M–NO, 7%), 297 (M–OCH_3, 4%), 267 (M–NO_2–OCH_3, 28%), 282 (M*, 100%), 251 (M*–OCH_3, 85%), and 223 (M*–CO, 28%).

Nilvadipine (Nivadil®, MW 385): 385 (M, 8%), 368 (M–OH, 9%), 342 (M–C_3H_7, 30%), 326 (M–C_3H_7O, 15%), 298 (M–C_3H_7O–CO, 17%), 263 (M–$C_6H_4NO_2$, 55%), and 221 (M–$C_6H_4NO_2$–C_3H_6, 100%). Dehydro nilvadipine (**III**, MW 383): 383 (M, 20%), 353 (M–OCH_3, 12%), 341 (M–C_3H_6, 40%), 324 (M–$COOCH_3$, 100%), 310 (M–OCH_3–C_3H_6, 32%), 282 (M–C_3H_6–$COOCH_3$, 28%), and 263 (M–C_3H_6–HNO_2,

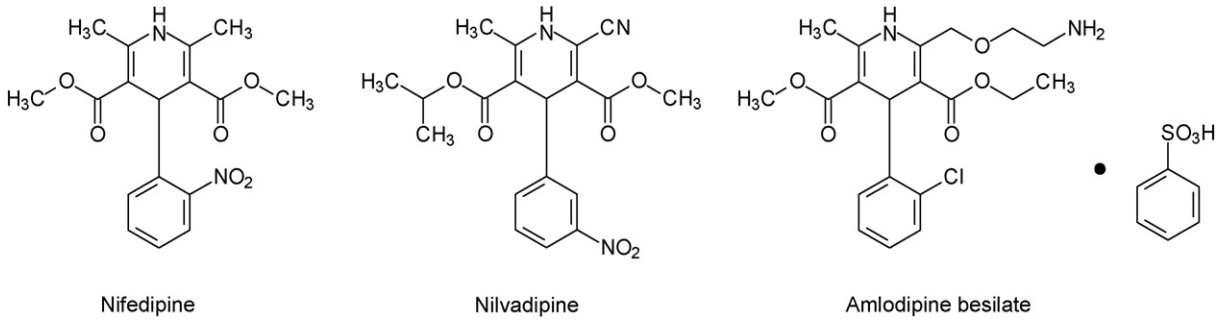

Fig. 1. Structures of the calcium-channel blockers used in this study.

19%). Dehydronitroso form (**IV**, MW 367): 367 (M, 3%), 325 (M–C_3H_6, 100%), 308 (M–C_3H_7O, 10%), and 294 (M–OCH_3–C_3H_6, 20%). Amlodipine (Amlodin®, MW 408.5), 408 (M, 3%), 364 (M–C_2H_6N, 4%), 297 (M–C_6H_4Cl, 100%), 254 (M–C_6H_4Cl–C_2H_5N, 24%). Dehydro amlodipine (**V**, MW 406.5): 406 (M, 3%), 347 (M–C_2H_5NO, 35%), 318 (M–C_2H_5NO–C_2H_5, 18%), 295 (M–C_6H_4Cl, 2%), 282 (M–C_6H_4Cl–CH_2, 2%).

In those data, M* indicates commonly found ions corresponding to 5,6-dihydro-2,4-dimethyl-5-oxo-benzo[*c*][2,7]naphthyridine-1-carboxylic acid methyl ester ($C_{16}H_{14}N_2$: 282).

2.5. Calibration and kinetic study

Working standard solutions of amlodipine, nifedipine, and nilvadipine specimens (0.1–10 mg/ml) were prepared in ethanol containing ABEE at a concentration of 5 mM; these solutions were analyzed using HPLC under the isocratic elution described in Section 2.2. A calibration graph for these specimens was then constructed by plotting the peak area of the drug versus the corresponding drug concentration, which indicates good linearity ($r^2 > 0.999$) for all of these DHP drugs.

The kinetics of photodegradation for all drugs were evaluated by plotting the peak areas of each main drug and photodecomposition products versus irradiation time. Linear relationships were observed for dissipation of all drugs according to the equation: $\ln A = \ln A_0 - kt$ (apparent first-order kinetics), where A is the remaining peak area, A_0 is the initial peak area of the drug (100%), k represents the slope, and t is the irradiation time (min).

3. Results and discussion

Photostability studies of powdered tablets of nifedipine, nilvadipine, and amlodipine pharmaceuticals were aimed at the evaluation of their photochemical properties. To this end, a D65 lamp was used as a solar simulator for photostability studies according to the option Q1B of ICH guidelines [8]. In addition, the photosusceptibilities of these three DHP drugs were compared to elicit chemical information on the handling and storage of these drugs. Finally, the most appropriate drug was determined for clinical use in cases where crushing DHP tablets is necessary.

3.1. Photochemical behavior of nifedipine pharmaceuticals

Photostability of nifedipine substance in pulverized tablets (Adalat® L) was verified by irradiation with a D65 lamp for 1–108 h at ambient temperature. After the powdered tablets were treated as described in Sections 2.2 and 2.3, the photoproducts were analyzed using HPLC with a UV detector and offline EI-MS. Fig. 2a shows selected HPLC profiles and peak assignments of the photoreaction samples of nifedipine drugs. Nifedipine seems unstable as a mixed solid, even with the existence of a large amount of additives; it forms two main photodegradation products (**I** and **II**), which are shown as peaks I and II in Fig. 2a, eluted at *ca.* 12 and 14 min, respectively. Results of EI-MS analysis indicated these two peaks as respectively assignable to dehydro (aromatization of DHP ring) and dehydration (dehydronitroso compound) products. Their structures are shown in Fig. 2b. Previous studies of the photostability of nifedipine as the solid state of the specimen [9] and its acetonitrile solution [10] indicated that nifedipine is converted photochemically to mainly these two products. In our examination, irrespective of the tablets, including those with large amount of vehicles, these two compounds were also generated to a great extent. The decreasing rate of a nifedipine peak on UV-detection HPLC was evaluated as a function of time, as described in Section 2.5, to obtain kinetic parameters of photodegradation process. We observed linearity in the relationship between the logarithm of peak area of nifedipine and the reaction time, which indicates that the reaction proceeds according to the apparent first-order kinetics. The kinetic analysis shown in Fig. 5 shows that the rate constant k thus obtained was 0.0813 h^{-1} and the half-life $t_{1/2}$ was 8.5 h. We evaluated the photodegradation of opened entire tablet of nifedipine as a reference. However, it showed no drug content variation or photoproduct formation, even after 108 h of irradiation (data not shown), meaning that the crushing of tablets must be important for the photodegradation of nifedipine. Teraoka et al. reported the rate constant of solid state nifedipine as 0.063 h^{-1} by D65 lamp irradiation [9], thereby indicating that crushing nifedipine tablets induces photodecomposition to that of nifedipine alone. Large amounts of additives have almost no effect on the photochemical decomposition of this drug. However, the plots of dissipation kinetics reflect biphasic character, and the plots comprised a fast initial step (0–10 h) and late second step (11 h–) phases. This phenomenon might indicate the shielding of some drugs from light irradiation in powdered

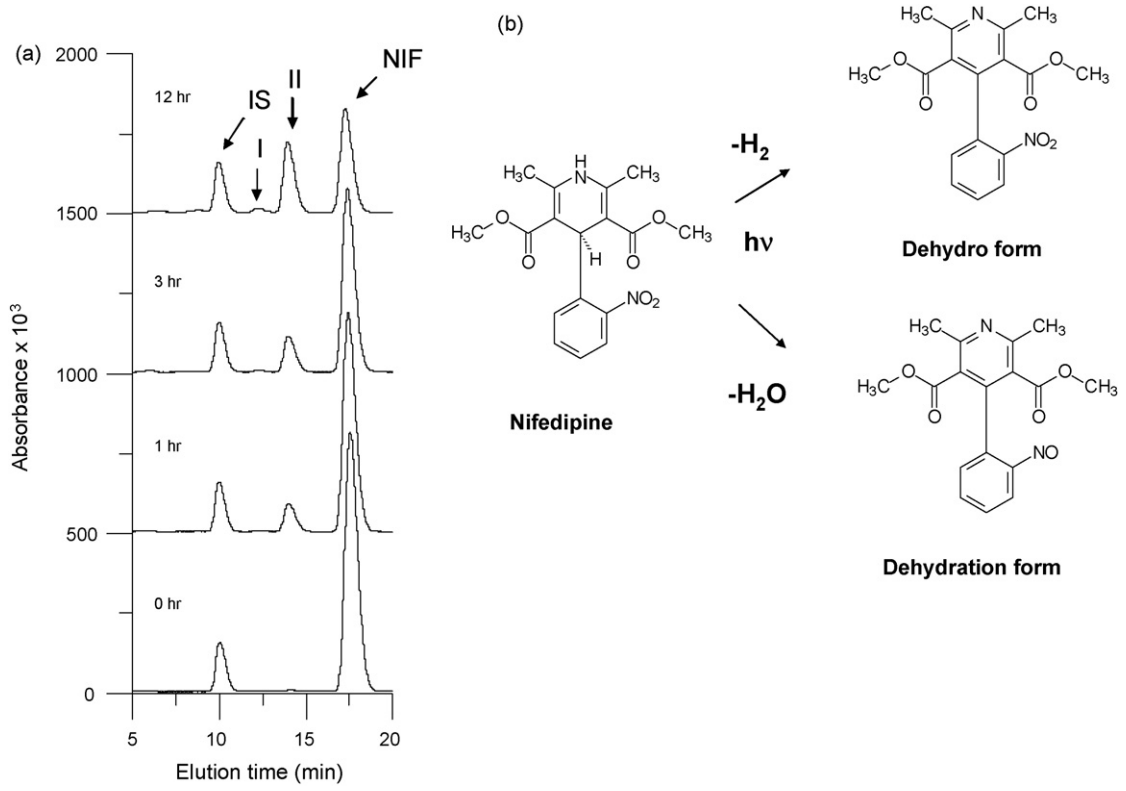

Fig. 2. Time course of the photodegradation of nifedipine (a), and the proposed reaction pathway (b). IS = ABEE; **I** = dehydro form; **II** = dehydronitroso form; NIF = nifedipine. Analytical conditions: column, cosmosil C18 (4.6 mm × 250 mm); eluent, H_2O–MeOH (35:65, v/v); flow rate, 0.45 ml/min; detection, absorbance at 234 nm.

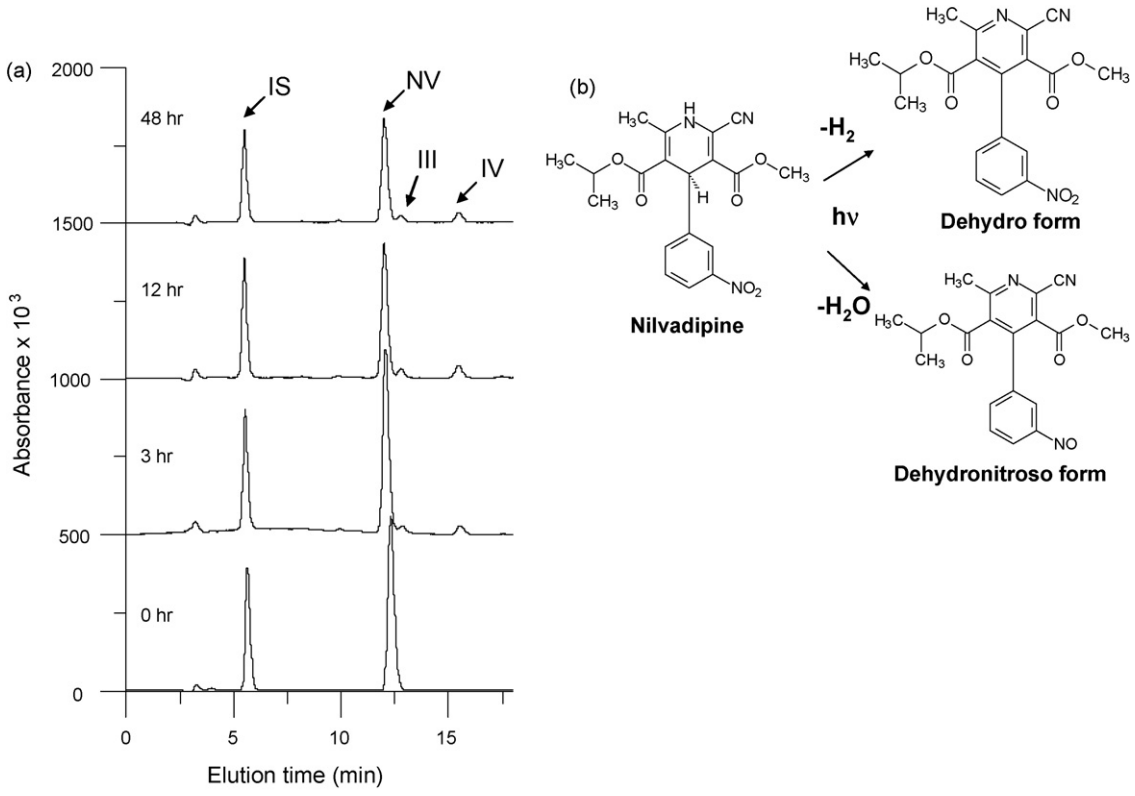

Fig. 3. Time course of the photodegradation of nilvadipine (a), and proposed reaction pathway (b). IS = ABEE; NV = nilvadipine; **III** = dehydro form; **IV** = dehydronitroso form. Analytical conditions: column, cosmosil C18 (4.6 mm × 250 mm); eluent, CH_3CN–NH_4^+/$HCOO^-$ (60:40, v/v) pH 4.4; flow rate, 0.8 ml/min; detection, absorbance at 234 nm.

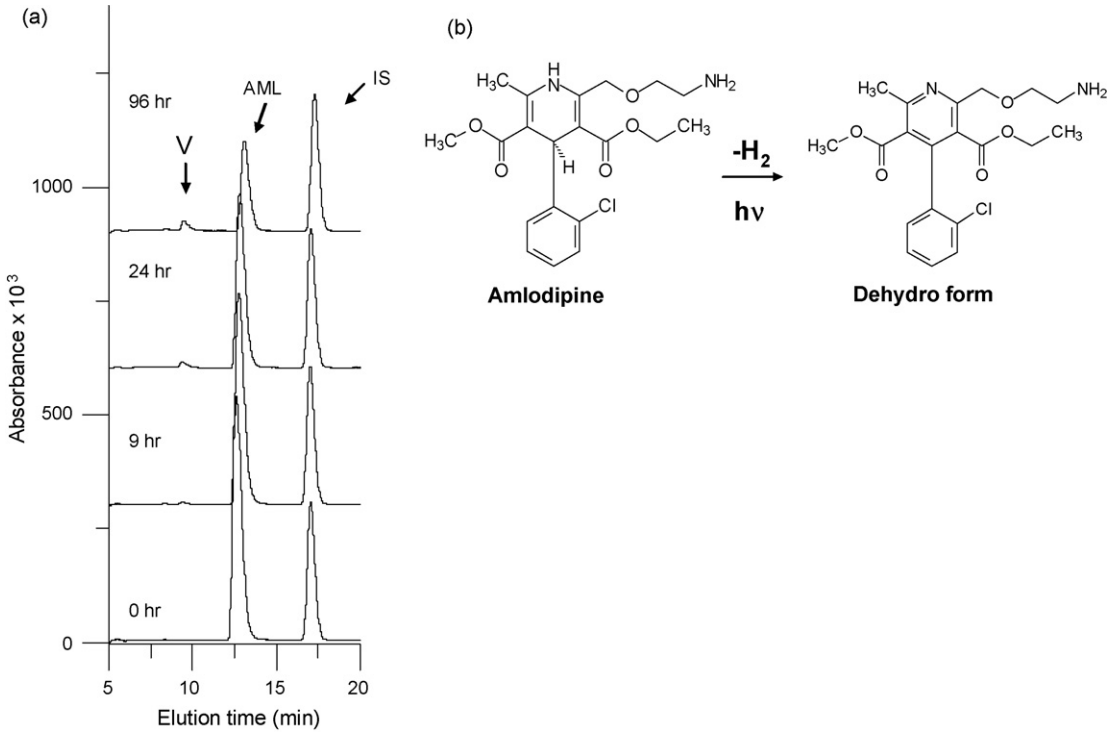

Fig. 4. Time course of the photodegradation of amlodipine (a), and photodegradation pathway (b). **V** = dehydro form; AML = amlodipine; IS = ABEE. Analytical conditions: column, cosmosil C18 (4.6 mm × 250 mm); eluent, CH_3CN–NH_4^+/$HCOO^-$ (40:60, v/v) pH 4.4; flow rate, 0.5 ml/min; detection, absorbance at 234 nm.

form, which might be surrounded by other additives or other drugs.

3.2. Photochemical behavior of nilvadipine pharmaceuticals

Photoexposure tests were also conducted using pulverized tablets of nilvadipine pharmaceuticals. Nilvadipine comprises a substituted dihydropyridine ring and a 3-nitrophenyl ring: its structure closely resembles that of nifedipine. Fig. 3a shows the selected chromatograms of photodegradation of the powders of nilvadipine tablets. Nilvadipine was eluted at 12.5 min; ABEE, as internal standard, was eluted at 5.5 min. In addition, many small peaks were observed at 7–12 min, although these minor peaks disappeared according to the increase of irradiation time. In contrast to nilvadipine, the peaks of major decomposition products indicate increased retention. These two major degradation products (**III** and **IV**) were eluted at 13 and 15 min. The MS studies show that these two peaks are, respectively, assignable to dehydration (aromatization of dihydropyridine ring and conversion of nitro group to nitroso groups) and dehydro (formation of pyridine ring) compounds. However, these small peaks disappeared according to the increase of irradiation time. Mielcarek et al. reported that the acetonitrile solution of nilvadipine specimen forms five decomposition products by irradiation of a high-pressure UV lamp with a 365 nm filter: M–H_2 (dehydro form), M–HCN (dehydro, denitrilo form), M–H_3CN (dehydro, denitrilo, intramolecular cyclized form) and two other unidentified compounds (*m/z* 356 and 316) [11]. Discrepancy of the products might be related to the difference of the wavelength and energy of light and the state of sample. Fig. 5 presents that kinetic evaluations of photodegradation of nilvadipine in powdered pharmaceuticals show a rate constant of the first order reaction $k_{obs} = 0.0119\,h^{-1}$ and the half-life $t_{1/2} = 58.2$ h.

3.3. Photochemical behavior of amlodipine pharmaceuticals

The same photoexposure tests were also applied to powders of amlodipine tablets. Fig. 4a shows the selected chromatograms

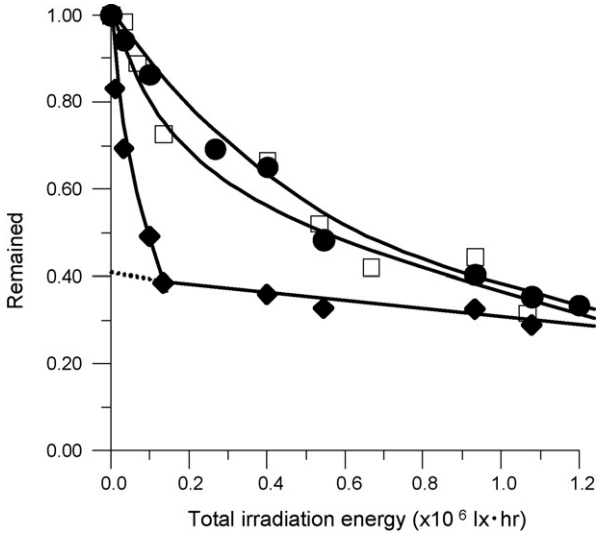

Fig. 5. Time course of the photodegradation of three DHP drugs in their pulverized form: (♦) nifedipine; (□) nilvadipine; (●) amlodipine besilate.

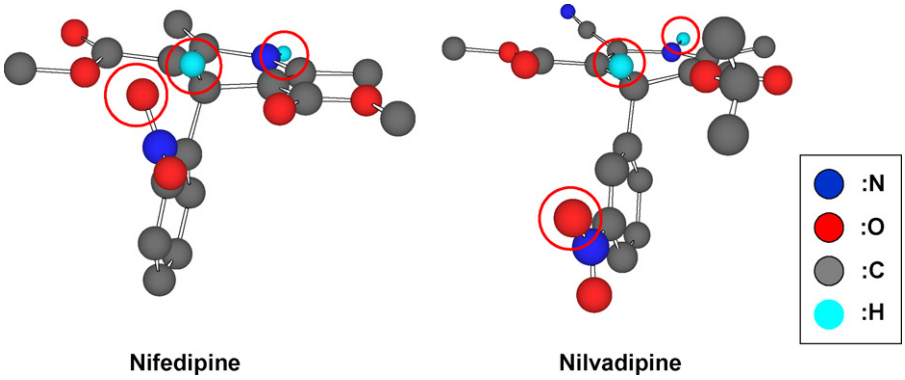

Fig. 6. Comparison of three-dimensional structures of nifedipine and nilvadipine. Circled atoms are oxygen atoms of nitro groups and hydrogen atoms on dihydropyridine rings.

of the photoreaction products of amlodipine drug. In contrast to the nifedipine, the amlodipine drug substance was found to be stable as a mixed solid with a large amount of additives. After irradiation for 24 h, more than 80% of amlodipine remained intact. A small peak corresponding to the dehydrocompound (peak V in Fig. 4) eluted at 9.5 min appeared only after 24-h irradiation. The kinetic study of the photodegradation process indicates the rate constant of amlodipine disappearance as $k = 0.0110\,h^{-1}$ and the half-life as $t_{1/2} = 60$ h, which is the same level of nilvadipine tablets. The photodegradation proceeded linearly over the 108 h period. Ragno et al. examined photostability of whole tablets of amlodipine pharmaceuticals with the distance of 30 cm under irradiation of daylight lamp (280–360 nm), and reported $0.0035\,h^{-1}$ as the photodegradation constants [12]. Because of difference of irradiation conditions, this result might not be directly comparable to ours, but the reaction of amlodipine degradation must be accelerated by pulverizing the tablet about three times as a rate constant. The reaction generates only one degradation product assignable to aromatization of dihydropyridine ring. The chlorophenyl ring indicated no conversion. Generation of dehydro derivative production reached its maximum at 60 h and then decreased gradually, indicating the decomposition of dehydrogenation products; nevertheless, we were unable to observe that phenomenon using UV detection HPLC.

3.4. Comparison of the photostabilities of three dihydropyridine derivatives

Fig. 5 shows that the rate constants of photodegradation of nifedipine, nilvadipine and amlodipine in pulverized pharmaceuticals were, respectively, 0.0813, 0.0119 and $0.0110\,h^{-1}$. When the illumination in working environment assumes 750 lx and 8 h as light irradiation time per day, 1 week irradiation gives 42,000 lx h, which corresponds to 3.8 h irradiation of D65 light. Therefore the expiry time ($t_{0.95}$) as 5% of clinical tolerated impurity for these drugs were estimated to be 1.2 days for nifedipine, 8.5 days for amlodipine, and 7.9 days for nilvadipine. Although the respective photosusceptibilities of nilvadipine and amlodipine are of the same level, nifedipine shows a greater than seven times higher degradation rate than either of other two compounds. The most characteristic feature of nifedipine is the presence of *o*-nitro groups in the phenyl ring, which strongly withdraws electrons from the 1,4-dihydropyridine ring, and which might enhance the aromatization of dihydropyridine ring. However, amlodipine has *o*-chlorophenyl groups which might also withdraw electrons with some extent and stimulate the dehydrogenation of dihydropyridine ring. Nilvadipine also has an *m*-nitrophenyl group. We simulate the stereo structure of nifedipine and nilvadipine based on the X-ray structural data to clarify this discrepancy [13,14]. Fig. 6 shows that nitro groups of nifedipine in phenyl ring comes close to hydrogen atom bound to the 4-position of the DHP ring. The electonegativity of the oxygen atom in nitro groups attracts hydrogen atoms of the dihydropyridine ring, which enhances these compounds' dehydration. In contrast, the nitro group of nilvadipine is positioned to the counter side of the dihydropyridine ring and inaccessible to the hydrogen atom of the dihydropyridine ring. But the electronegativity reduces electron densities of DHP ring and enhances DHP dehydrogenation. Consequently, we infer that the difference of the steric characteristics might enhance the photosusceptibity of nifedipine.

4. Conclusion

Dihydropyridine-type calcium-channel blockers are now widely used for management of patients with a various cardiovascular disorders; DHPs now number more than 30. For this study, we chose three DHP drugs and compared their photosusceptibilities in their pulverized forms: nifedipine (first generation), nilvadipine (second generation), and amlodipine (third generation). Results showed that these drugs were commonly converted to dehydro compounds or dehydronitroso compounds when including nitrophenyl group in their structures. Photodegradation of nifedipine was seven times faster than that of either of the other two drugs. Susceptibility of nifedipine may be attributable to the propinquity of the nitro group in phenyl ring to the hydrogen atom at the C-4 position of dihydropyridine ring. These results suggest that nifedipine should not be used or stored in pulverized form.

Acknowledgements

The authors are grateful to a grant from Higashi-Osaka City General Hospital (Fonds of Higashi-Osaka City). This work was also supported as a "High-Tech Research Center" Project for Private Universities: a matching fund subsidy from Ministry of Education, Culture, Sports, Science and Technology (MEXT), 2007-2011.

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