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ORIGINAL ARTICLE

Indigotine, azorubine, and cochineal red as photoprotectors of manidipine

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Abstract

Background: The calcium antagonists from the group of 1,4-dihydropyridine (DHP) derivatives are photolabile, and the products of their photochemical decomposition have no pharmacological activity. Like the other DHP derivatives, after exposure to light, manidipine undergoes decomposition leading to a weakening of the pharmacological activity. *Method*: The protective influence of selected dyes on manidipine has been tested. The photodegradation of manidipine was performed according to the first version of the ICH Guideline for photostability testing. The solutions were illuminated by a high-pressure lamp HBO-200, and the methods used for evaluation of manidipine photodegradation were HPLC, HPLC–MS, and UV–vis spectrophotometry. Quantitative evaluation of photodegradation was made on the basis of the quantum yields of the processes with the use of Reinecke salt as a chemical actinometer. *Results*: Results have shown that all the dyes studied, indigotine, azorubine, and cochineal red, slow down the process of manidipine photodegradation, and the most effective is azorubine. The role of the dyes has been determined on the basis of the Stern–Volmer dependence. *Conclusion*: The dyes studied, indigotine, azorubine, and cochineal red, can be potential photoprotectors of manidipine as they quench the excited states of the compound.

Key words: *Calcium channel blockers; dihydropyridine derivatives; dyes; manidipine; photoprotection*

Introduction

Dihydropyridine (DHP) calcium channel blockers show high efficiency in the cardiovascular treatment, particularly in the treatment to decrease hypertension[1,2]. They control blood pressure and are used in angina pectoris and Raynaud's or ischemic heart disease therapy[3–6]. Calcium antagonists have beneficial effect on diastolic function and atherosclerosis[7]. Besides the cardiological applications, calcium channel blockers are recommended in the treatment of other diseases like asthma or migraine[8,9]. According to the classification based on pharmacokinetic and pharmacodynamic properties, taking into account the chemical structure, tissue selectivity, or duration of activity, DHPs are divided into the first (e.g., nifedipine), the second (e.g., nisoldipine), and the third generations (lacidipine)[10].

DHP derivative 2-[(4-diphenylmethyl)-1-piperazinyl]ethyl methyl(±)-1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate [manidipine (MND)] has been selected as a potent and long-acting antihypertensive drug from a series of analogues with piperazinylalkyl ester side chains. MND is a second-generation calcium antagonist with peculiar and favorable characteristics like the ability in increasing hematic renal flow and giving rise to a remarkable sodium excretion without modifying glomerular filtrate[11]. MND, as a representative of the latest generation of calcium antagonists, is an agent for good hypotensive effectiveness and advantageous metabolic profile. It is particularly recommended in therapy of mild-to-moderate hypertension, in treatment of hypertension in elderly, and in patients with impaired renal function. But the most important feature of MND, causing its popularity in everyday practice, is

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lower frequency of adverse events when compared to other calcium antagonists applied so far[12].

The chemical structure of MND is characterized by the presence of a side chain containing a –$COOCH_2CH_2$–piperazin–$CH(Ph)_2$ group that was introduced to improve the lipophilic properties and the activity duration of the drug. From a physicochemical point of view, MND is slightly soluble in water, but it is more soluble in some widely used solvents as well as ethanol and methanol, or mixture of water–organic solvents[13]. Due to its chemical features, MND is sensitive to light, a behavior common to all members of DHP class of compounds.

Nowadays, photostability of drugs is an emerging topic in the pharmaceutical research as the number of drugs that are revealed to be light sensitive is notably increasing. Accordingly, many new technology-based pharmaceutical systems are presently proposed in order to enhance the stability of such compounds[14–16]. Therefore, photosensitive drugs can be protected from light during preparation or administration (i.e., topical application) by means of photo-absorbent additives directly included into the formulation[17–19]. Recently, microspheres and microcapsules have attracted the attention as inclusion systems for the potential protection of photosensitive drugs[20].

According to some authors, also dyes used for coloration of different drug formulations have photoprotecting effect[21]. The aim of this study was to test the photoprotective influence of selected dyes on MND.

Experimental

Materials and instruments

MND ($C_{35}H_{38}N_4O_6$·2HCl; mol.wt. = 683.65 g/mol) was supplied by Industria Chemica et Farmaceutica S.P.A (Milano, Italy).

The dyes were purchased from Hoffmann (Steszew, Poland).

Azorubine: disodium 4-hydroxy-2-[(*E*)-(4-sulfonato-1-naphthyl) diazenyl] naphthalene-1-sulfonate.

Cochineal red: 1-(1-naphthylazo)-2-hydroxynaphthalene-4′,6,8-trisulfonate.

Indigotine: disodium 3,3′-dioxo-2,2′-bi-indolylidene-5,5′-disulfonate.

Methanol was obtained from J.T. Backer (Deventer, Holland); the samples were tested on a spectrophotometer UV-160 A Shimadzu (PC 160 Plus software, Tokyo, Japan).

Liquid chromatography-mass spectrometry (LC-MS) was performed on Waters model 2690 high-performance liquid chromatography (HPLC) instrument equipped with an electrospray ionization (ESI) interface (Waters, Milford, MA, USA).

Methodology of photochemical degradation study

Photodegradation of MND solutions (concentration 6.18×10^{-5} mol/L) was performed in a light chamber equipped with a mercuric burner HBO-200 according to the ICH Guideline for photostability testing. The wavelength of the maximum radiation absorption $\lambda = 365$ nm was obtained as a result of Wood filter selection. The process was performed in the cylindrical quartz cell ($V = 2.8$ mL**)** at a constant temperature of 25°C. All solutions of MND were exposed to the radiation of intensity of 561.86×10^6 lux h.

Quantum yield of photodegradation process

The quantum yield (Table 1) of MND photodecomposition was determined with the use of Reinecke salt (SR) used as a chemical actinometer. The SR salt solution was irradiated with $\lambda = 365$ nm for 60 seconds. Knowing I_{SR}, the number of quanta absorbed by the sample was calculated as $I_{abs} = I_{SR}(1–10^{-A})$. The quantum yield of photodegradation for a certain percent of MND conversion was calculated from $\varphi = \Delta c \cdot N_A / I_{abs} \cdot t$ according to the procedure described by Mielcarek et al.[22]

Photoprotective role of dyes

Methanol solutions of MND (~1×10^{-5} mol L^{-1}), with indigotine, azorubine, and cochineal red in concentrations given in Table 1, were irradiated in the conditions described in Section '*Methodology of photochemical degradation study*'. After a certain time of irradiation, the UV–vis spectra were taken in the range 200–700 nm and the absorbency was measured at λ = 360 m. Furthermore, photodegradation of MND was analyzed by the HPLC–ESI–MS. The chromatographic separation was carried out

Table 1. Quantum yields of MND with dyes used in the photodegradation process.

MND concentration [10^5 (mol/L)]	Concentration [10^6 (mol/L)]			Quantum yield (φ)·(10^3)		
	Indigotine	Azorubine	Cochineal red	Indigotine	Azorubine	Cochineal red
6.47	1.32	2.33	1.54	6.04	5.69	4.66
	2.64	3.10	3.09	5.52	5.53	4.60
	4.62	3.88	4.63	5.09	5.50	4.47
	5.94	5.43	6.18	4.81	5.37	3.38
	7.26	6.98	7.52	4.70	4.25	4.30

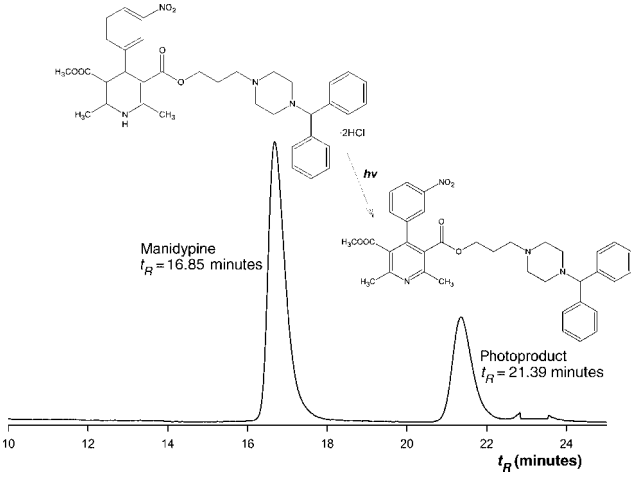

Figure 1. HPLC chromatogram of a manidipine solution irradiated in the presence of indigotine; the photodegradation time—60 seconds.

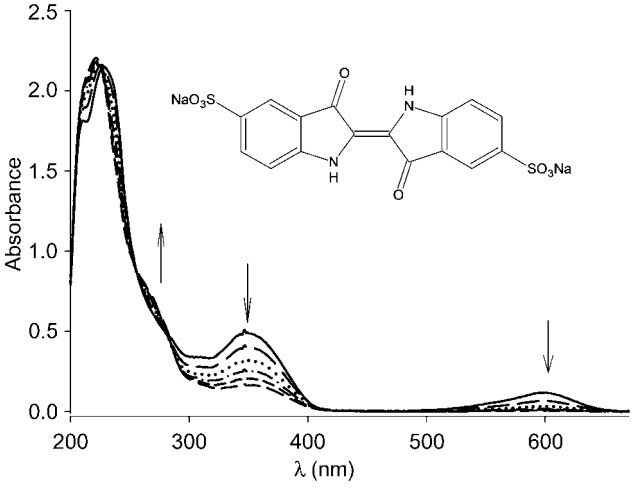

Figure 2. Changes in the electron absorption spectrum of manidipine in the presence of indigotine; the photodegradation time – 300 seconds.

in the isocratic mode on a column C-18 Nova-Pak in the conditions described by Mielcarek et al.[20] Elution was isocratic with the flow rate adjusted at 0.5 mL/min. The mobile phase consisted of ACN–H_2O–10% HCOOH (50:45:5, v/v/v/v). Mass spectra were recorded in the range of 100–1000 m/z. ESI was sufficient to obtain molecular ions. The chromatogram and electron absorption spectra of the MND solution irradiated in the presence of indigotine are shown in Figures 1 and 2, respectively. The actual quantum yields of MND photodegradation in the presence of the dyes are given in Table 1.

Results and discussion

DHP derivatives are highly sensitive to light[23]. Upon irradiation, the molecules undergo irreversible chemical changes leading to the disappearance of pharmacological effect. As follows from Figure 1, the irradiation of MND in the presence of indigotine was reflected in the spectrum by a hipsochromic shift of the absorption band. This effect was assigned to the $\pi \rightarrow \pi^*$ type electron transitions in the coupled diene system of the unsaturated DHP ring. The appearance of the new absorption band in the range 255–315 nm (λ_{max} = 275 nm) is a consequence of aromatization of the DHP ring. The HPLC–MS measurements permitted separation and identification of the main products of the photochemical decomposition of MND taking place in the presence of the dyes. MND ($[M + zH]^+ = 611$) irradiated in the presence of the dyes was found to decompose with the generation of a single stable photoproduct ($[M + zH]^+ = 609$) being nitrophenylpyridine derivative (photoproduct) (Figure 2).

At the next stage, the process of MND photodegradation was quantitatively evaluated by the determination of the quantum yields of the substrate decay. The number of quanta emitted was determined with the use of Reinecke salt as a chemical actinometer. The number of the quanta absorbed by the actinometer (I_{SR}) was 1.134×10^{17}. The measurements permitted determination of the apparent quantum yields, which were extrapolated to zero time of irradiation; the extrapolated real quantum yields are presented in Table 1.

The photoprotective effect of the selected dyes (azorubine, indigotine, and cochineal red) was then tested. This stage of the study was preceded by the evaluation of the photostability of the dyes applied. Azorubine and cochineal red were found stable in the assumed model of irradiation, whereas indigotine underwent decomposition revealed by a gradual disappearance of the long wavelength absorption band with a maximum at λ_{max} = 599 nm. The slow decomposition of indigotine is a result of a low quantum yield of photodegradation from the singlet state and a small quantum yield of the intersystem crossing (φ_{ISC}) singlet–triplet in the molecule of this dye. Therefore, it can be assumed that the probability of population of the triplet state in indigotine as a result of the triplet–triplet sensitization is greater than that as a result of the singlet–triplet intersystem crossing (intramolecular process).

A comparison of the quantum yields collected in Table 1 has shown that the presence of all dyes inhibits the process of photochemical decomposition of MND. The strongest photoprotective effect was observed to have cochineal red; in the presence of this dye, the photolysis of MND was twice slower. As mentioned above, in the irradiation conditions, applied indigotine underwent photodegradation ($\varphi = 1.97 \times 10^{-5}$); however, in the presence of MND, its photochemical degradation was much faster with the yield of $\varphi = 4.5 \times 10^{-4}$. MND was found to act as a sensitizer toward indigotine

and its presence accelerated the dye decomposition by three to four times. It can be supposed that the energy of excitation is directly transferred from the triplet state of MND to the triplet state of indigotine.

As the rate of NMD photodegradation in the presence and in the absence of the dyes was different, an attempt was made to explain their role in this process. The dyes can act as sensitizers, inner filters, or quenchers. An effective way of checking the mechanisms of photochemical reaction mechanism is to investigate the phenomenon of their quenching. The knowledge of the quenching nature permits indirect determination of the kinetics and mechanisms of certain photophysical processes, preceding or competing with the photochemical reactions, and determination of the lifetime of the excited state under quenching.

To find out the role of dyes in the process of MND photodegradation, first their effectiveness as the inner filter was tested. The lifetime of the excited state under quenching was calculated using the Stern–Volmer equation according to the procedure presented by Mielcarek et al.[20]

On the basis of the below equation, the intensities of the absorbed radiation by MND in the presence of dyes were determined:

$$I_{\text{abs}}^{\text{MND+DYE}} = I_0(1-10^{-(\varepsilon_{\text{MND}}\cdot C_{\text{MND}} + \varepsilon_{\text{DYE}}\cdot C_{\text{DYE}})\cdot l}), \quad (1)$$

where $I_{\text{abs}}^{\text{MND}}$ is the intensity of irradiation absorbed by MND, $I_{\text{abs}}^{\text{MND(DYE)}}$ is the intensity of irradiation absorbed by MND in the presence of the dye, ε_{MND} is the molar absorption coefficient of MND (6655 $\text{mol}^{-1}\cdot\text{L/cm}$; λ = 350 nm), ε_{DYE} is the dye molar absorption coefficient (Table 2), and l is the optical path length.

The Stern–Volmer equation presents the dependence of the dye concentration on the ratio of $\Delta A^{\text{MND}}/\Delta A^{\text{MND + DYE}}$, found after a certain time of irradiation [22].

$$\frac{\Delta A^{\text{MND}}}{\Delta A^{\text{MND+DYE}}}(1+K_{\text{SV}}[\text{DYE}]), \quad (2)$$

where $\Delta A^{\text{MND}}/\Delta A^{\text{MND + DYE}}$, the values of absorbance of MND taking into account the inner filter effect of dyes; ΔA, the difference in MND absorbency without (ΔA^{MND}) and in the presence of the dye ($\Delta A^{\text{MND + DYE}}$);

Table 2. Values of the dyes' molar absorption coefficient.

	$\varepsilon_{\text{DYE}}\cdot 10^3$ ($\text{mol}^{-1}\cdot\text{L/cm}$)		
λ (nm)	Indigotine	Azorubine	Cochineal red
350	6.182	5.218	5.349
353	6.044	4.926	4.992
363	2.155	4.365	3.750

Table 3. Lifetime of manidipine with indigotine, azorubine, and cochineal red.

Lifetime of manidipine in the absence and in the presence of dyes (seconds)			
Manidipine	Indigotine	Azorubine	Cochineal red
4.29×10^{-5}	1.61×10^{-6}	6.30×10^{-6}	2.64×10^{-6}

K_{SV}, Stern–Volmer constant; and [DYE], the concentration of the dye [mol/L].

The parameters describing the linear dependence described by Equation (2) for particular dyes were

$$\text{indigotine} \quad y = 19320 + 1.1386 \quad r = 0.958,$$

$$\text{azorubine} \quad y = 75{,}600 + 1.2984 \quad r = 0.949,$$

$$\text{cochineal red} \quad y = 31{,}680 + 1.1832 \quad r = 0.968.$$

The values obtained were used for the determination of the lifetimes of the excited states:

$$K_{\text{SV}} = k_{\text{q}}\cdot\tau_0.$$

It was assumed that the energy transfer process is diffusion controlled and the quenching rate constant (k_{q}) is equal to the diffusion constant (D):

k_{q}—the quenching rate constant for methanol is 1.2×10^{10} [L/mol/s]

τ_0—the lifetime of the excited state [s].

The calculated lifetimes of the excited states (t_0) are given in Table 3. The values obtained are of the order of 10^{-6} seconds, which suggest that the photodegradation of MND takes place from the triplet state.

Conclusion

The dyes tested in the studied systems have been found to play the role of quenchers of photochemical reactions.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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