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Studies on curcumin and curcuminoids. XI. Stabilization of photolabile drugs in serum samples by addition of curcumin

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Summary

Curcumin dissolved in polyethyleneglycol PEG 400 was added to serum samples spiked with several photolabile drugs. The samples were exposed to light and analyzed for the content of undecomposed drug by means of HPLC. Curcumin had a slight stabilizing effect on furosemide and clonazepam in serum. The photostability of nifedipine in serum, however, was clearly improved, with a 6-fold increase in half-life by addition of curcumin. The photochemical degradation of nifedipine followed first-order kinetics independent of addition of curcumin. Addition of curcumin to serum samples in clinical laboratories may improve the results of nifedipine determination.

Introduction

Relationships between serum levels and therapeutic and toxic effects have been clearly established for several drugs. Therefore, serum level monitoring is of great importance as a guide for the appropriate adjustment and maintenance of dosage regimens necessary to maintain optimal serum levels for each prescribed drug. However, high analytical quality is essential if serum levels are to be of value in the clinical management of patients. The analytical methods must be adequate with respect to specificity, sensitivity, accuracy, and the time to perform a given drug assay.

Many drugs are known to be photolabile in the solid state or in solution. Great effort is taken to protect pharmaceutical preparations of such drugs from photolytic degradation during the storage period. However, little work has been done to evaluate the stability of photolabile drugs in serum samples. Unless the sampling conditions and analytical conditions are well standardized, the observed drug concentration in serum can be strongly influenced by the light sources in the laboratory giving a day-to-day variation. Recently, there have been a few reports describing the photochemical degradation of clonazepam and nifedipine in serum. Clonazepam is strongly affected by artificial UV-light and sunlight (Wad, 1986). The half-life of clonazepam in serum is one hour or less depending upon the conditions. The half-life of nifedipine in serum under the influence of daylight is reported to be between 44 min and 2.7 h (Bach, 1983; Hamann and McAllister, 1983; Kleinbloesem et al., 1984).

The analytical procedure used in serum level

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monitoring of drugs often requires one or more extraction steps with organic solvents. In addition to the storage conditions of the serum samples, the photochemical stability of the drug in organic solvents therefore strongly influence the results obtained. As an example, the half-life of nifedipine in dichlormethane–pentane–methanol (3 : 7 : 1) is reported to be only 15 min (Kleinbloesem, 1985). As a consequence of such findings, it is recommended that photolabile drugs in serum are protected from light by wrapping the tubes in aluminum foil or by using dark tubes, and by performing the analysis in yellow or red light. Petters et al. (1984) succeeded in protecting 7-aminoclonazepam from chemical decomposition in organic solvents by adding a chemically similar substance to the plasma before the extraction procedure. It should be possible to stabilize drugs photochemically in a similar way by adding a "protector" to the serum samples. This would be of great importance for serum level monitoring of several photolabile drugs.

Curcumin is the main coloured compound isolated from the rhizomes of the plant *Curcuma longa* L. (Zingiberaceae). Curcumin is a crystalline compound with a bright orange-yellow colour. It is frequently used as a colouring agent in food products, cosmetics and textiles. Unfortunately, the stability of curcumin is pH-dependent (Tønnesen and Karlsen, 1985). The colour also shows severe fading under the influence of light (Tønnesen et al., 1986) However, curcumin is shown to have a photostabilizing effect on some drugs (nitrofurazone, fluphenazin and nifedipine) in different formulations (Thoma, 1982). Curcumin is further shown to be useful as a formulation aid to protect light sensitive drugs in gelatin capsules (Tønnesen and Karlsen, 1987). Curcumin was therefore chosen as a possible protector for photolabile drugs in serum samples. For routine analysis, curcumin should be available in solution which easily can be added to the serum samples. Curcumin is not sufficiently solubile in water at pH below 8, and the stability of curcumin above this pH is low. To make a stable and convenient formulation of curcumin, the substance was dissolved in polyethylene glycol 400 (PEG 400). A small volume of this solution was added to the serum samples together with the drug of choice. The samples were irradiated, and after extraction with organic solvents the drug content was measured by means of HPLC. In this work the stabilizing effect of curcumin on nifedipine, clonazepam, furosemide and chloramphenicol in serum samples was investigated.

Materials and Methods

Materials

Pure curcumin is not commercially available and was synthesized after the method of Pabon (1964) and tested according to Tønnesen and Karlsen (1983). Nifedipine was provided by C.F.M. Milan, Italy. Clonazepam was a gift from The National Center for Epilepsy, Norway. Furosemide was obtained by mixing furosemide injection solution (Lasix 10 mg/ml, Hoechst) with 1 M hydrochloric acid. Furosemide precipitated immediately, and was separated from the solution by centrifugation. Chloramphenicol was provided by Norsk Medisinaldepot, Norway. Control serum was obtained from The National Center for Epilepsy, Norway.

Preparation of samples

Control serum samples (0.4 ml) were spiked with 0.1 ml PEG 400 containing the drug of choice (1 mg/ml) or curcumin (0.45 mg/ml) in combination with the drug of choice (1 mg/ml). After exposure to light, the samples were extracted with organic solvents. The drug content was quantified by means of HPLC. All the analytical steps were carried out in sodium light, without the influence of daylight. The extraction procedures were as follows.

Nifedipine. (a) Serum samples were transferred to a 15-ml centrifuge tube containing 0.5 ml of 1 M sodium hydroxide. The mixture was extracted with 5.0 ml dichlormethane–pentane (3 : 7) on a vortex whirl-mixer for 20 s and centrifuged for 5 min at 2300 *g*. 4.0 ml of the organic layer were transferred to a 15-ml centrifuge tube and evaporated to dryness under vacuum. The residue was dissolved in 5.0 ml methanol before injection. (b) 5.0 ml of methanol were added to the serum sample. The mixture was centrifuged for 5 min at

2300 *g*. The methanol layer was injected directly.

Chloramphenicol. 5.0 ml of ethyl acetate were added to the serum sample. After mixing, 3.5 ml of the organic layer were transferred to a 15-ml centrifuge tube and evaporated to dryness under vacuum. The residue was dissolved in 6.0 ml methanol before injection.

Furosemide. 9.0 ml acetonitrile were added to the serum sample. After mixing, the sample was centrifuged for 5 min at 2300 *g*. The acetonitrile-layer was injected directly.

Clonazepam. 5.0 ml of acetonitrile were added to the serum sample. The further procedure was as described for furosemide.

HPLC analysis

For HPLC analysis of the serum samples a Spectra-Physics SP 8700 liquid chromatograph was used. The injector system was a Shimadzu Sil-6A auto injector. The analysis was carried out at ambient temperature. The detector was a LDC Spectro-Monitor III. The stationary phase was Bondapak C-18 (Waters), particle size 10 μm, pre-packed in a 300 mm $\times$ 3.9 mm i.d. column with a guard column, stationary phase Supelcosil C-18 (Supelco), particle size 5 μm, pre-packed in a 20 mm $\times$ 4.6 mm i.d. column. The following conditions were used for quantification of the drugs.

Nifedipine: mobile phase 0.01 M disodium hydrogen phosphate buffer (pH = 6.1)/methanol (45 : 55); detection wavelength 254 nm; flow rate 1.3 ml/min (Pietta et al., 1981).

Chloramphenicol: mobile phase methanol/0.05% phosphoric acid (4 : 6); detection wavelength 280 nm; flow rate 1.3 ml/min (Oseekey et al., 1980).

Furosemide: mobile phase acetonitrile/0.05% phosphoric acid (3 : 7); detection wavelength 280 nm; flow rate 1.3 ml/min (Nation et al., 1979).

Clonazepam: mobile phase acetonitrile/water (38 : 62); detection wavelength 320 nm; flow rate 1.3 ml/min (modification of the method described by Wad, 1986).

Irradiation

The samples were exposed to an immersion lamp with emission 240–600 nm, 120 W (Hereaus immersion lamp system). The samples were placed 5 cm from the light source. Some samples were also exposed to normal laboratory light on a clear day, but were not exposed to direct sunlight.

Spectrophotometer

The transmission spectra of serum and curcumin samples were obtained by use of a Shimadzu spectrophotometer, model UV-260.

Results and Discussion

Curcumin is easily soluble in polyethylenglycol PEG 400 in the concentration range 0.2–0.6 mg/ml. The stability of stock solutions of curcumin in PEG 400 diluted in water to give a total concentration of curcumin in the range 0.02–0.12 mg/ml was evaluated. Concentrations below 0.10 mg/ml showed no sign of precipitation of curcumin after one week storage. A decrease in curcumin content of 22% was observed after 1.5 h exposure to the immersion lamp system when the sample was prepared from 2 ml stock solution (4.5 mg curcumin/10 ml PEG) in 10 ml water.

As a standard "protective" additive for the further studies on serum samples, a stock solution of 4.5 mg curcumin in 10 ml PEG 400 was chosen and added to serum in the ratio 2 : 10. Transmission spectra of blank serum, serum spiked with curcumin additive and curcumin additive in water are shown in Figs. 1–3. Blank serum diluted in water (1 : 10) has a UV cut-off at 294 nm (Fig. 1). Many photolabile drugs are sensitive to irradiation in the wavelength area 300–450 nm, and the serum will give no protection to such compounds. Curcumin additive in water (2 : 10) gives protection towards irradiation in the wavelength area 340–470 nm (Fig. 2). This area is extended towards somewhat longer wavelengths in serum (Fig. 3).

Due to the high pH of standard serum (8–8.5), curcumin will dissociate and the colour changes towards red.

Blank serum and serum containing curcumin additive were spiked with chloramphenicol, furosemide, clonazepam and nifedipine (0.2 mg/ml). The samples were irradiated with the Hereaus immersion lamp system. After given time intervals, samples were analyzed with respect to unde-

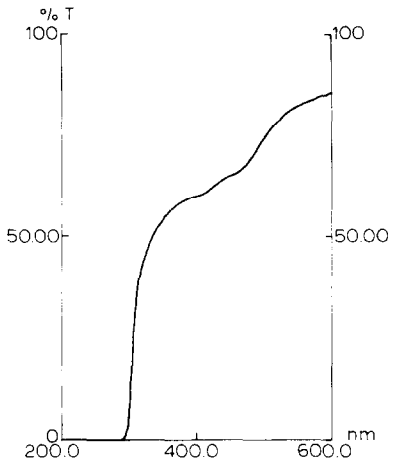

Fig. 1. Transmission spectrum of blank serum diluted in water (1 : 10).

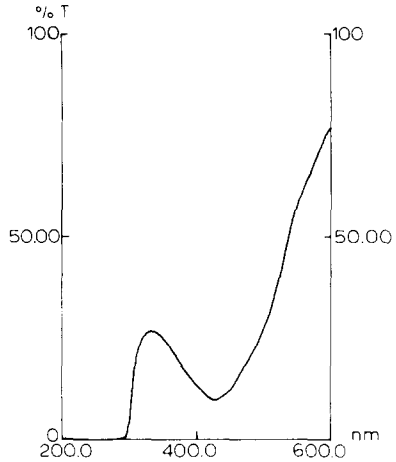

Fig. 3. Transmission spectrum of curcumin additive in serum (2 : 10) diluted in water (1 : 10).

composed drug. The addition of curcumin to the samples had no stabilizing effect on chloramphenicol as shown in Table 1. Curcumin additive showed a slightly stabilizing effect on furosemide and clonazepam (Table 2, Fig. 4, Table 3). For nifedipine, however, the stabilizing effect of curcumin was very obvious. After 5 min exposure to light, less than 1% nifedipine was present in the reference samples. In samples containing curcumin 78% nifedipine remained after 5 min irradiation under the same conditions. The half-life

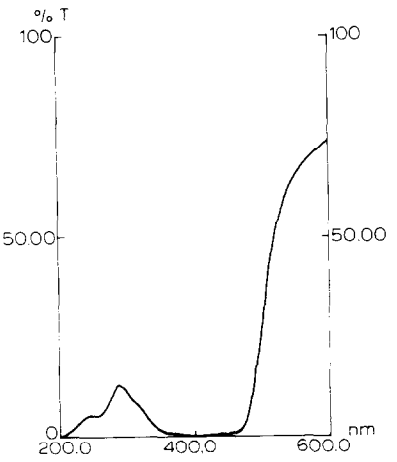

Fig. 2. Transmission spectrum of curcumin additive diluted in water (1 : 10).

of nifedipine was found to be 8.9 min. For this reason, only nifedipine was further studied in this work. Two methods were tried out for extraction of nifedipine from serum. Method (a) is based on extraction with apolar organic solvents (dichlormethane–pentane 3 : 7), which prevent co-extraction of curcumin. Organic solvents like acetonitrile, ethyl acetate and methanol which are commonly used for extraction of serum samples are all good solvents for curcumin. For drugs on which curcumin has a stabilizing effect in organic solvents, co-extraction of curcumin from serum will be an advantage. The stability of nifedipine in methanol with and without curcumin additive was investigated. The half-life of nifedipine in methanol was increased with a factor of 1.7 in presence of curcumin after exposure to daylight.

TABLE 1

% degradation of chloramphenicol in serum with and without curcumin additive

Irradiation time (min)	% degraded	
	without curcumin	with curcumin
15	33%	34%
30	31%	30%
60	46%	39%

TABLE 2

% degradation of furosemide in serum with and without curcumin additive

Irradiation time (min)	% degraded	
	without curcumin	with curcumin
30	24%	23%
75	45%	34%
120	58%	37%

TABLE 3

% degradation of clonazepam in serum with and without curcumin additive

Irradiation time (min)	% degraded	
	without curcumin	with curcumin
30	18%	10%
90	28%	31%
180	59%	46%

The following experiments were therefore performed with methanol-extraction of the samples (method (b)). Curcumin did not interfere with the HPLC-analysis of nifedipine.

Under certain conditions, curcumin is found to act as a sensitizer when exposed to light (Tønnesen et al., 1986). The HPLC-chromatograms of nifedipine in blank serum and in serum containing curcumin additive showed no different degradation patterns (irradiated, non-irradiated samples), which indicates that curcumin has no influence on the reaction mechanisms involved. This is further confirmed by calculating the reaction order for the photochemical process. Photochemical degradation of nifedipine in serum follows first-order kinetics, independent of the presence of curcumin.

Samples of nifedipine in serum were exposed to normal laboratory light on a clear day in May. The results are given in Figs. 5 and 6. In the presence of curcumin additive, the half-life of nifedipine in serum samples are increased from 50.2 min to 5.0 h. The half-life observed in serum samples without curcumin is in good agreement with what is reported in the literature (Kleinbloesem et al., 1984; Hamann and McAllister, 1983; Bach, 1983).

In conclusion, the photostability of nifedipine in serum can be clearly improved with a 6-fold increase in half-life by addition of curcumin dissolved in PEG 400 to the samples. By use of methanol for extraction, curcumin will have a stabilizing effect through the complete analytical procedure.

Curcumin has no stabilizing effect on chloramphenicol, but a slight effect on clonazepam

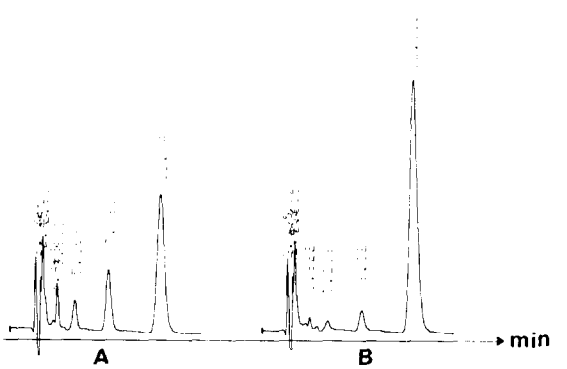

Fig. 4. HPLC-chromatogram of furosemide in serum exposed to light for 2 h. A: sample without curcumin. (The peak with retention time 12.1 minutes is furosemide.) B: sample with curcumin additive.

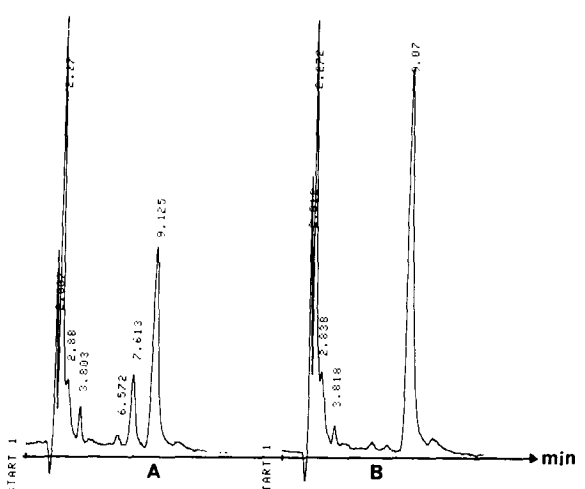

Fig. 5. HPLC-chromatogram of nifedipine in serum exposed to light for 1 h. A: sample without curcumin. (The peak with retention time 9.1 min is nifedipine.) B: sample with curcumin.

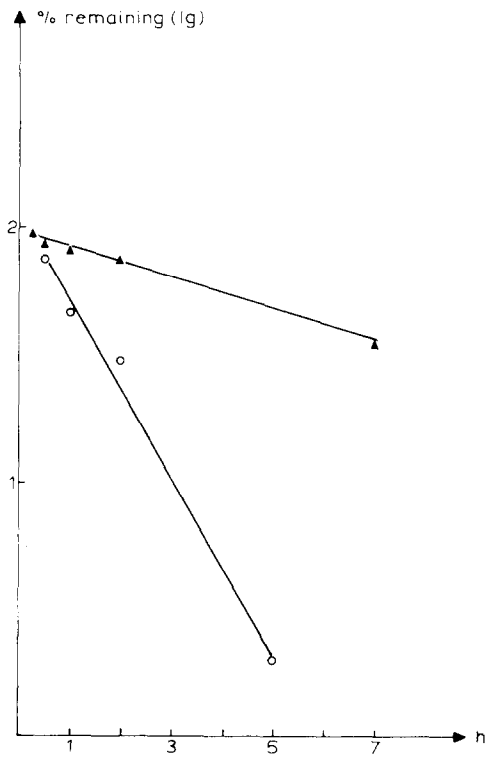

Fig. 6. 1 g (%) nifedipine remaining in serum after exposure to daylight. ○—○—○—○, samples without curcumin additive. ($y = -0.006x + 2.07$ Regr. $= -0.9950$.) ▲—▲—▲—▲, samples with curcumin additive. ($y = -0.001x + 1.99$; Regr. $= -0.9974$.)

and furosemide. These observations can possibly be explained from the absorption spectra of the compounds. The absorption spectrum of chloramphenicol in PEG/methanol (0.2 : 10) shows a maximum at 273 nm. The absorbance above 300 nm is low, which is the "protective" area of curcumin additive.

The absorption spectrum of furosemide in PEG/mobile phase (0.2 : 10) shows a peak in the area 300–380 nm with a maximum at 339 nm. The absorption spectrum of clonazepam in PEG/mobile phase (0.2 : 10) shows a peak in the area 290–380 nm with a maximum at 309 nm. The observed effect of curcumin on these compounds might be ascribed to a pure "filter" effect in the area above 340 nm. The absorption spectrum of nifedipine in PEG/methanol (0.2 : 10) shows a peak in the area 300–420 nm with a maximum at 334 nm. In the literature nifedipine in the solid state is reported to be sensitive to light over the range of wavelengths between 340 and 560 nm, especially 420 nm (Sugimoto et al., 1981). In the organic solvent, nifedipine is reported to be most sensitive to light in the wavelength range between 370 and 700 nm (Thoma and Klimek, 1985). Curcumin in PEG/methanol (0.2 : 10) shows an absorption maximum at 422 nm. It is therefore likely that the stabilizing effect of curcumin on nifedipine can be ascribed to a pure "filter" effect in solution.

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