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Investigation of the photostability properties of memoquin, a quinone derivative for the treatment of Alzheimer's disease

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ABSTRACT

The photostability properties of memoquin, a multifunctional compound in preclinical development for the treatment of Alzheimer's disease (AD) were investigated in solutions exposed to radiations, using a xenon arc lamp to simulate the natural sunlight. Reversed phase liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (LC–UV/DAD–ESI-MS/MS) was applied to follow the photodegradation and disappearance of memoquin after irradiation. Under optimized chromatographic conditions, memoquin was separated with high resolution from the photoproducts formed in the photoexposed solutions. The results showed that memoquin is more stable at physiological and acid pHs, while it has a slow degradation pattern at more drastic conditions such as basic pH ($t_{1/2}$ = 389 min) and in methanolic solutions ($t_{1/2}$ = 465 min). In the irradiated solutions the appearance of photoproducts with lower retention times and molecular weight than memoquin was observed, thus indicating that some fragments were lost from its structure. The photodegradation products were characterized by LC–ESI-MS/MS and LC–UV/DAD analysis. The photoreactive centers were found on the amino groups of the side chains while the 1,4-benzoquinone functionality was maintained. Conversely, memoquin was found to be stable in the dark. These results suggest that, with appropriate handling and storage, memoquin's activity is not impaired.

1. Introduction

Memoquin (2,5-bis(diamine)-1,4-benzoquinone derivative [1]) is a multifunctional molecule structurally characterized by the presence of a 1,4-benzoquinone functionality in a polyamine skeleton, rationally designed to hit different biological targets involved in Alzheimer's disease (AD) neurodegeneration [2,3]. Memoquin affects several mechanisms relevant to AD: the formation of reactive oxygen species, the processing and aggregation of amyloid β (Aβ) peptides, and acetylcholinesterase activity. In animal models, it causes a remarkable decrease in the formation of AD's neurodegenerative hallmarks and a significant reversal of behavioral deficits. Based on this unique pharmacological profile, memoquin is a promising drug candidate for the treatment of AD. Memoquin is currently in preclinical development at the University of Bologna. At this stage, a more detailed characterization of the photochemical properties of memoquin is required. Information on kinetics, degradation pathways, and the identities of degradation products will be valuable for future formulation and metabolism studies.

In order to achieve a more detailed characterization of the photochemical properties of memoquin, specific photostability studies were performed. Light-induced decomposition can impair drug potency and induce phototoxic effects [4,5]. Quinones are known to be photoreactive compounds, acting as cofactors in photosynthetic reaction centers of photo system II and I or as electron carriers through cell membranes [6,7]. The presence of side chains may change a quinone's photochemistry, as is the case with phylloquinones [8] and other similar quinones [9–11]. In memoquin's structure there are two long and symmetric polyamine side chains that might increase quinone stability and determine a protective effect against photoinduced reactions.

Thus, the aim of the present study was to investigate the photochemical properties of memoquin in solutions exposed to UV radiations, using a xenon arc lamp (solar simulator). We evaluated the effect of the solvent (methanol) and pH (acetate buffer and phosphate buffer in the pH range of 4.0–9.5) on memoquin's photochemical properties and on the degradation kinetics. Memoquin and its photodegradation products were separated by a selective liquid chromatographic (LC) method, suitable for coupling with the mass detection system. The main photoproducts were characterized by LC-DAD and LC–MS/MS analysis, using an electrospray ionization source (ESI) and an ion trap analyzer.

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2. Experimental

2.1. Materials

Memoquin and the photoproduct **PP4** were synthesized as previously reported [12]. Sodium acetate, triethylamine, disodium hydrogen phosphate, phenol and acetic acid were from Sigma–Aldrich (Milan, Italy). Phenomenex Luna phenyl-hexyl stationary phase (150 mm × 3.0 mm I.D.) was from Phenomenex Italia (Bologna, Italy). Acetonitrile and all the other chemicals were of analytical reagent grade (Carlo Erba Reagenti and Sigma–Aldrich, Milan, Italy) and were used without further purification. Water used for the preparation of solutions and mobile phases was purified by a Milli-Rx apparatus (Millipore, Milford, MA, USA).

The buffer solutions were filtered through a 0.45 μm membrane filter and degassed before their use in HPLC.

2.2. Apparatus and experimental conditions

Tests on memoquin's photochemical stability were carried out at room temperature (25 ± 2 °C) using a xenon arc source to simulate natural sunlight exposure. Specifically, a 150 W xenon arc lamp (solar simulator, model 68805, Oriel Corporation, Stratford, CT, USA) was used, with a dichroic mirror (Oriel, model 81405) to block visible and IR radiation in order to minimize sample heating. An air-mass filter 1.5 (Oriel, model 81090) was used to simulate solar conditions and a UV-B-C blocking filter was employed to attenuate the UV-B component. A "beam-turning assembly", containing the dichroic mirror, directed the output beam downward. The UV dose (J cm^{-2}) from the xenon-arc lamp was measured using a Oriel Goldilux model 70127 radiometer fitted with external interchangeable probes for UV-A and UV-B (UV-A dose: 85 μW cm^{-2} and UV-B dose: 15 μW cm^{-2}).

Chromatographic analyses were performed at room temperature (25 ± 2 °C). All analyses were performed on a Phenomenex Luna phenyl-hexyl stationary phase (150 mm × 3.0 mm I.D.) under the following chromatographic conditions: mobile phase composed of 20 mM triethanolamine acetate buffer (pH 4.0)/acetonitrile (71/29, v/v), flow rate 0.4 ml/min.

Chromatographic analysis were carried out on an HPLC system consisting of a Jasco PU-1580 (Jasco, Cremella, Italy) solvent delivery system connected to a Jasco auto sampler model AS-2055 and a Jasco DAD-V-530 system.

LC–MS analyses were performed on a Jasco PU-1585 (Jasco, Cremella, Italy) equipped with a Reodyne Model 7125 injection valve (sample loop of 20 μl) connected to a LCQ DUO ion trap mass spectrometer (MS) with electrospray ionization (ESI) ion source, controlled by Xcalibur software 1.3 (Thermo Finnigan, San Jose, CA, USA). Nitrogen was used as sheath gas and helium (0.1 Pa) served as damping and collision gas. Data acquisition and analysis were conducted using Xcalibur software (version 1.0 SR1, Thermoquest, USA). The ESI system employed a 4.5 kV spray voltage (positive polarity), a capillary temperature of 200 °C, and a cone voltage of 14 V. A postcolumn T-splitter (split ratio: 1/3) was used to direct a low amount of the mobile phase into the mass spectrometer via the ESI interface.

The detection was performed with an ion trap mass spectrometer in positive polarity (full scan 100–650 m/z) and in single ion monitoring (SIM) mode on the generated cations at m/z = 633.4 (memoquin), m/z = 485.5 (photoproduct 2, **PP2**), m/z = 513.0 (photoproduct 3, **PP3**), m/z = 577.9 (photoproduct 4, **PP4**), and m/z = 605.0 (photoproduct 5, **PP5**).

MS/MS analyses were performed with an isolation width of 1 Th (m/z); the activation amplitude was around 35% of ejection RF amplitude, which corresponds to 1.85 V.

2.3. Photo stability kinetics studies

To evaluate the effect of different solvent and pH conditions on memoquin's photostability, studies were performed on the compound (0.025 mg ml^{-1}) dissolved in methanol, sodium acetate buffer (0.02 M; pH 4.0–7.0–8.3) and in the mixture composed of phosphate buffer (0.02 M, pH 9.5) and methanol (50/50, v/v). Aliquots (3 ml) of each solution were placed in quartz cells (path length: 1 cm) and closed with screw caps. Quartz cells were placed horizontally and exposed to UV-A radiation (Xe arc lamp) for increasing irradiation times (0–24 h, corresponding to increasing UV doses). Time course experiments were carried out by analyzing the photoexposed solutions by HPLC to follow the disappearance of memoquin. Chromatographic analyses were carried out as described in Section 2.2.

Main compound peak areas were integrated and the percentage of memoquin peak area at the corresponding irradiation time was plotted against time by using a non-linear regression fit (one phase exponential decay equation) and a logarithm linear regression fit. The computer program used to analyze these data was GraphPad Prism 4.0 (GraphPad Software Inc.). These analyses were also carried out on memoquin sample solutions stored in darkness, i.e. an aliquot of 3 ml in a 1 cm quartz cell wrapped in aluminium foil during the simultaneous radiation exposure.

2.4. Photoproducts characterization: LC-DAD and LC–ESI-MS/MS analysis

The photoexposed solutions, treated as described in Section 2.3, were subjected to LC–ESI-MS/MS analysis. The peak apex mass spectra were recorded within 100–650 m/z full scan (positive polarity), providing the total ion current (TIC) chromatograms and the pseudomolecular mass of the analytes. The MS/MS spectra of memoquin (retention time: 14.5 min) and its photoproducts were obtained on the molecular ion with 35 or 40% collision energy. With LC-DAD analyses, memoquin and related photoproducts were also characterized by chromatographic peak apex UV–vis spectra.

2.5. Method validation

Standard stock solutions of memoquin (c = 2.2 mg ml^{-1}) were prepared in methanol and diluted with the mobile phase to the required concentration (range: 2.2–45 μg ml^{-1}). The solutions obtained were analyzed by LC under the chromatographic conditions described in Section 2.2, using DAD detection set at 340 nm. Each solution was injected in duplicate. Calibration graph was then constructed by plotting memoquin peak area versus the corresponding concentration (μg ml^{-1}).

The repeatability of the chromatographic method was evaluated by injecting the same standard solution three times on the same day; the peak areas were integrated and the standard deviations calculated. Method sensitivity was evaluated by progressive dilution of memoquin standard solution by detecting the peak area height at 340 nm.

3. Results and discussion

The aim of the present work was the photostability study of memoquin, a multifunctional drug candidate for the treatment of AD [1–3]. This study was aimed at determining memoquin's photophysical and photochemical properties and structurally characterizing its main photoproducts. In fact, irradiation of benzoquinone [13,14] and other substituted 1,4-benzoquinones [15] in water is known to produce products such as benzene-1,2,4-triol, semiquinone and hydroxylated quinone [16–18]. Since memoquin is characterized by the presence of two symmetric polyamine