Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 837 (2006) 87–91
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of 5-chloro-7-iodo-8-quinolinol (clioquinol) in plasma and tissues of hamsters by high-performance liquid chromatography and electrochemical detection

G.P. Bondiolotti [a], C. Pollera [b], R. Pirola [a], S.R. Bareggi [a,*]

[a] *Department of Pharmacology Chemotherapy and Medical Toxicology, School of Medicine, University of Milan, Italy*
[b] *Department of Animal Pathology, Hygiene and Public Health, Section of Microbiology and Immunology, School of Veterinary Medicine, University of Milan, Italy*

Received 19 December 2005; accepted 5 April 2006

Abstract

This paper describes a method of determining clioquinol levels in hamster plasma and tissue by means of HPLC and electrochemical detection. Clioquinol was separated on a Nucleosil C18 300 mm × 3.9 mm i.d. 7 μm column at 1 ml/min using a phosphate/citrate buffer 0.1 M (400 ml) with 600 ml of a methanol:acetonitrile (1:1, v/v) mobile phase. The retention times of clioquinol and the IS were, respectively, 11.6 and 8.1 min; the quantitation limit (CV > 8%) was 5 ng/ml in plasma and 10 ng/ml in tissues. The intra- and inter-assay accuracies of the method were more than 95%, with coefficients of variation between 3.0 and 7.7%, and plasma and tissue recovery rates of 72–77%. There was a linear response to clioquinol 5–2000 ng/ml in plasma, and 10–1000 ng/g in tissues. The method is highly sensitive and selective, makes it possible to study the pharmacokinetics of plasma clioquinol after oral administration and the distribution of clioquinol in tissues, and could be used to monitor plasma clioquinol levels in humans.

Keywords: Clioquinol; Hamsters; HPLC; Electrochemical detection

1. Introduction

Clioquinol (5-chloro-7-iodo-8-quinolinol) acts as a zinc and copper chelator. Metal chelation is a potential therapeutic strategy for Alzheimer's disease because the interaction of zinc and copper is involved in the deposition and stabilisation of amyloid plaques, and chelating agents can dissolve the amyloid deposits by preventing metal-A-beta interactions [1–3]. As Alzheimer's disease and prion disease are CNS degenerative disorders characterised by amyloid deposits, it is conceivable that some drugs may be active in preventing both.

Transmissible spongiform encephalopathies (TSE) form a group of progressive, fatal neurodegenerative diseases affecting the central nervous system of humans (kuru, Creutzfeldt-Jacob disease) and animals (scrapie, bovine spongiform encephalopathy) [4–6]. It is believed [7] that the causative agents are proteinaceous infectious particles ("prions") completely devoid of any nucleic acids that represent the altered counterpart of a cell protein, and are resistant to proteolytic digestion, high temperatures, denaturating agents and the disinfectants usually used for sterilisation. The pathological protein (PrP^{sc}) is the protease-resistant isoform of a GPI-anchored cell transmembrane molecule (PrP^{c}) that is mainly expressed in CNS neurons, but also in many other cell types. As it is the main component of amyloid deposits, and the cause of neurodegenerative CNS lesions, PrP^{sc} is the primary target for therapeutic strategies [8,9].

The hamster model is particularly suitable for TSE studies because the period required for the development of experimental scrapie is shorter than in mouse; when hamsters are intracerebrally infected by the 263 K prion strain, the incubation period lasts 2 months and death occurs after about 1 month [10,11]. Preliminary results indicate that clioquinol may improve

* Corresponding author at: Department of Pharmacology, Via Vanvitelli 32, 20129 Milano, Italy. Tel.: +39 0250316946; fax: +39 0250316949.
E-mail address: silvio.bareggi@unimi.it (S.R. Bareggi).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.04.014

cognitive symptoms and prolong the survival of infected animals [12].

After oral administration in rodents (mice and rats, but not hamsters), clioquinol is extensively metabolised to glucuronate and sulfate metabolites [13–18], but these animal and human studies made use of relatively insensitive and non-specific HPLC methods with UV detection, and thus required complex extraction procedures in order to determine tissue clioquinol levels. An even more complex GC method with electron-capture detection after acetylation has been developed by Jack and Riess [19], which also used solvent extraction with a sensitivity of 50 ng/ml. Finally, a highly sensitive GC–MS method has been developed that uses benzene extraction and the conversion of clioquinol into pentafluorobenzyl ether [20].

As studying the pharmacokinetics of clioquinol and its tissue distribution may be relevant to understanding its targets and its mechanism of inhibiting prion infection, we have developed a simple, sensitive and specific method of determining clioquinol levels in plasma and tissues by means of HPLC and electrochemical detection.

2. Experimental methods

2.1. Chemicals and reagents

The 5-chloro-7-iodo-8-hydroxyquinoline, 5,7-dichloro-8-hydroxyquinoline, phenolphthalein glucuronic acid and β-glucuronidase (25,000 units/0.4 ml) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and the analytical reagents from Merck (Darmstadt). The aqueous solutions were in reagent-grade water obtained using a Milli-Q System (Millipore, Bedford, MA, USA). Stock solutions (0.5 mg/ml in methanol) were prepared every month and stored at 4 °C. In these conditions solutions have found stable for more than 1 month. Standard working solutions were prepared every day immediately before use in distilled water.

2.2. Biological samples

The plasma, and the brain, spleen and liver tissues were obtained from Syrian hamsters treated orally with clioquinol 50 mg/kg. Sixty minutes after administration, the animals were anesthetised with diethyl ether, their blood was drawn by heart puncture and centrifuged, and the plasma was stored at −25 °C until analysis. After the animals were sacrificed, the tissues were removed, washed with physiological solution, wiped with clean paper, weighed, and frozen at −25 °C.

At the beginning of the study, three series of standard plasma and tissue samples from drug-free animals were prepared as described below, and spiked with low, medium and high concentrations of clioquinol (10, 100 and 2000 ng/ml in plasma; 20, 100 and 500 ng/ml in tissues: quality control samples), and frozen at −25 °C. One sample of each known concentration was analysed at each run of the unknown samples. The samples were analysed within 2 weeks.

2.3. Sample preparation

2.3.1. Plasma

A volume of 0.2 ml of plasma was deproteinised with an equal volume of 0.6N perchloric acid containing sodium disulfite 1 mg/ml, disodium ethylendiaminotetracetate (EDTA) 0.5 mg/ml and 40 ng of 5,7-dichloro-8-hydroxyquinoline as internal standard (IS); methanol 0.2 ml was added and mixed by vortexing. The antioxidant disulfite was added to protect clioquinol from possible degradation due to the presence of perchloric acid; EDTA was added to prevent the formation of complexes that may reduce recovery and be induced by clioquinol (a potent chelator of many ions); and methanol was added to improve the solubility of clioquinol in the extraction medium. The lowest possible methanol concentration was used in order to prevent the extraction of other interfering compounds.

The proteins were precipitated by centrifugation at 13,000 × g × 15 min at 4 °C; 50 μl of the clear acid supernatant were suitable for use in the chromatographic system.

Standard plasma samples were prepared by spiking 0.2 ml of drug-free plasma with known amounts of clioquinol (5–2000 ng/ml) and 40 ng of IS. The samples were analysed as described above.

2.3.2. Tissue

Brain, spleen and liver (about 450 mg of brain, 150 mg of spleen, 200 mg of liver) were homogenised in an Ultra-Turrax apparatus with 1 ml of 0.6N perchloric acid and 120 ng of internal standard; methanol 0.5 ml was added and the mixture was re-homogenised. After centrifugation as above, 50 μl of the supernatant were injected into the chromatographic system.

Standard tissue samples obtained from drug-free animals were prepared by spiking before homogenisation 1 ml of 0.6N perchloric acid with known amounts of clioquinol (10–1000 ng/ml) and 120 ng of IS. The samples were analysed as described above.

Calibration curves were calculated by analysing the linear regression of the ratios of the peak clioquinol and IS area against the clioquinol concentrations in the standard samples, and the curves were used to calculate the concentrations of clioquinol in the unknown samples.

2.4. Clioquinol glucuronate and sulfate

Clioquinol glucuronate was determined in plasma according to the method of Chen et al. [21] with modifications: after enzymatic hydrolysis: 400 μl of distilled water, 100 μl of 1 M acetate buffer, pH 5, and β-glucuronidase at a final concentration of 200 units/ml were added to a sample of 500 μl of plasma; phenolphthalein glucuronide was used as a standard to check the efficiency of the deconjugation reaction. The samples were incubated at 37 °C for 2 h, and the unconjugated clioquinol and IS were extracted and determined as previously described. To check the deconjugation reaction, the samples were reinjected under different chromatographic conditions (see below) in order to obtain adequate separation and unconjugated phenolphthalein