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Pharmacokinetic properties of MH84, a γ-secretase modulator with PPARγ agonistic activity

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ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia. Since no causative treatment is available, new therapeutic options are utmost needed. Several pirinixic acid derivatives, including MH84 (2-((4,6-bis(4-(trifluoromethyl)phenethoxy)pyrimidin-2-yl)thio)hexanoic acid), have shown promising *in vitro* results as γ-secretase modulators as well as PPARγ activators as potential pharmacological compounds against AD.

Using a newly developed and validated sensitive LC–MS (APCI-qTOF mass analyzer) method, the pharmacokinetic and long-term accumulating properties as well as the blood–brain-barrier permeability of MH84 were evaluated in a preclinical animal study. MH84 was administered to mice by oral gavage with a dose of 12 mg/kg. Nine time points from 0.5 to 48 h with 6 animals per point were investigated. Additionally 6 animals were fed daily, for 21 days with an identical dose to determine possible long-term accumulation in plasma and brain tissue.

The sample preparation was performed by a liquid-liquid extraction on Extrelut® columns whereas the LC separation was operated on a MulthoHigh 100 RP 18-5 μ column (125 × 4 mm) using an isocratic mobile phase of formic acid (0.1% (v/v))–methanol mixture (11:89 (v/v)) at a flow rate of 1 ml/min. The validation confirmed the new LC–MS method to be precise, accurate and reliable. After oral application, C_{max} and T_{max} of unmetabolized MH84 was determined to be 10.90 μg/ml and 3 h in plasma. In brain tissue a constant level of 300 to maximum 320.64 ng/g was found after 1.5–6 h. Daily gavage for 21 days did not lead to a long-term drug accumulation in the brain. The efficacy of the obtained MH84 levels needs to be investigated in further preclinical pharmacodynamic animal studies.

1. Introduction

Over the last three decades enormous efforts were made to understand the neurobiological mechanisms of Alzheimer's disease (AD). Besides the typical well characterized pathological hallmarks of extracellular amyloid-β deposits and intracellular tau tangles, many other changes were found in the brain of Alzheimer's patients. These changes comprise inflammatory responses, insulin deficiency and activated microglia leading to synaptic dysfunction and neural loss [1,2]. The identification of the interplay between these alterations is a main current topic of research.

The investigated compound MH84 is a small molecule, which shows a dual activity concerning two important neurobiological targets of Alzheimer's disease: γ-secretase and PPARγ (peroxisome proliferator-activated receptor gamma). MH84 was chosen based on the most desirable *in vitro* activity (γ-secretase: IC_{50}(Aβ42) = 6.0 μM; EC_{50}(Aβ38) = 1.8 μM and PPARγ: EC_{50} = 11.0 μM, max. activation: 112%) of a small in-house synthesized SAR library [3].

Along with β-secretase, γ-secretase is responsible for the amyloidogenic cleavage of amyloid-β precursor protein (APP) producing Aβ 37–43 peptides and cell signaling peptides in normal and pathological pathways [4,5]. It is an aspartyl protease complex with presenilin in the catalytic center. In AD the cleavage process is altered leading to an excessive formation of Aβ42. This

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peptide has a high tendency to form oligomers and fibrils [6], which are toxic in neuronal cell cultures [7]. Due to interferences with the physiological Notch signaling pathway, the pharmacological use of γ-secretase inhibitors is limited [8]. Therefore γ-secretase modulators that shift the APP cleavage from Aβ42 to shorter more soluble and less toxic Aβ38, which do not alter the Notch signaling pathway, might be more desirable [9].

PPARγ, a nuclear receptor regulating lipid and carbohydrate metabolism represents another promising target for AD therapy [10]. Agonists of PPARγ directly influence the APP cleavage by suppressing the transcription of APP processing enzyme BACE1 (β-secretase) thus leading to decreased Aβ levels [11]. Besides the alteration of Aβ production, the clearance of preexisting excessive Aβ by microglia and IDE (insulin-degrading enzyme) is affected by PPARγ. In AD microglia are chronically activated leading to a continuous microglial-driven inflammation [12]. The chronic inflammation has a negative impact on microglial clearance functions and leads to cytotoxic effects. It was reported that the stimulation by PPARγ agonists (DSP-8658 and pioglitazone) enhances the degradation of Aβ by microglia [12] and inhibits proinflammatory gene expression [13,14]. Additionally, inflammation is proposed to be the link to the formation of neurofibrillary tangles by changing the substrate specificity of kinases/phosphatases leading to tau phosphorylation [15].

Currently, there is a controversial discussion, if elevated serum cholesterol levels increase the incidence of AD [16]. Obviously, several genes regulating the cholesterol homeostasis are linked to AD. The best described are ApoE (Apolipoprotein E) and ABCA1 (ATP-binding cassette A1). The ApoE4 allele is proven to be the principal genetic risk factor for the sporadic late-onset variant of the disease [17]. The activation of PPARγ and Liver X receptors (LXRs) can increase brain ApoE and ABCA1 levels. This improves Aβ degradation and leads to reduced Aβ levels and plaque formation [18,19].

The risk of developing AD in patients with type 2 diabetes is twice as high in healthy patients [20]. Brain insulin receptor signaling is significantly decreased in AD according to insulin resistance [21]. IDE, which is reduced in the brain of late-onset AD patients, degrades extracellular Aβ [22]. PPARγ agonists increase IDE expression and its proteolytic activity resulting in decreased extracellular Aβ levels [23].

Thus, MH84 with its action on both γ-secretase and PPARγ, represents a promising pharmacological compound for further preclinical and clinical investigations. We determined MH84 in plasma and brain tissue and thereby obtained the pharmacokinetic profiles using a newly developed and validated sensitive LC–MS method.

2. Experimental

2.1. Materials

MH84 and its internal standard (MH41) were obtained by in-house synthesis. The purity was proven by NMR and MS spectra as well as elementary analysis. The Extrelut® liquid–liquid separation columns were purchased by VWR international. PALL GHP Acrodisc syringe filters (45 μm GHP membrane) were obtained from Analytics shop.com (Altmann Analytik). Methanol (LC–MS), formic acid (0.1%; LC–MS), tert-butylmethylether (HPLC) and TRIS ultra quality (≥99.9%) for buffer preparation were purchased by Carl Roth Germany. Water used for the buffer preparation and water-methanol (50:50, v/v) mixture was obtained by additional distilling of water obtained by a purifying system (ELGA, Purelab Ultra®).

2.2. Solution and sample preparation

2.2.1. Preparation of stock solutions

A freshly weight amount of MH84 (500–1000 μg) was dissolved in 1 ml DMSO and further diluted with DMSO to obtain DMSO stock solution (250 μg/ml). This solution was further diluted with methanol–water mixture (50:50, v/v) for spiking solutions. The maximum content of DMSO at the spiked calibration point is 0.1% (at a concentration of 250 ng/ml in mouse plasma). The internal standard solution (c = 1 μg/ml) was obtained by the same procedure.

2.2.2. Preparation of calibration curves and QC solutions from plasma

MH84 from solutions in methanol–water (50:50, v/v) was spiked to 30 μl of rat plasma to concentrations between 20 and 250 ng/ml to get a matrix-assisted calibration curve. Therefore, an amount of 370 μl water, 100 μl of internal standard solution (1 μg/ml) and 400 μl of acetone for protein precipitation were added (total volume 1 ml). The QC samples were obtained in the same manner to get low QC (20 ng/ml), mid QC (100 ng/ml) and high QC (200 ng/ml) solutions. The equivalence of rat and mouse plasma (samples from the application study) was proved in a cross-validation measurement. The plasma from the application study was obtained by centrifugation at only 900 × g for 10 min to prevent centrifugation deficiency (induced by high molecular weight of MH84) observed during method development. Samples from the application study, which exceeded the calibration range were diluted with rat plasma to quantify the amount by recalculation. For pharmacokinetic values obtained after 24 and 48 h, a calibration curve in the upper concentration range (up to 2 ng/ml) was used to quantify small concentrations of MH84.

2.2.3. Preparation of calibration curves and QC solutions from brain tissue

A homogenate of pig brain (188.9 g) in TRIS buffer (5 mM, pH = 7.4) was obtained preparing 10% (w/w) brain to TRIS buffer in an ultra-turrax homogenating step (3 min, 10.000 rpm). Aliquots of this homogenate were frozen at −20 °C and used for calibration curves and QC samples. The homogenates from samples of the application studies were obtained by ultra-turrax procedure (20 s, 8000 rpm) in 10% (w/w) mouse brain to TRIS buffer. The homogenation time and rate gave comparable results due to different amounts of brain used for the homogenate. The equivalence of pig brain and mouse brain homogenate was proven in a cross-validation measurement. For the preparation of calibration curves, QC samples and samples from the application study, 400 μl brain tissue homogenate was used. Two different calibration curves had to be utilized to meet validation criteria of precision and accuracy. Therefore MH84 was spiked from solution in methanol–water (50:50, v/v) in a concentration range 2–20 ng/ml (low concentration calibration curve) and 20–150 ng/ml (high concentration calibration curve). Additionally, 100 μl internal standard solution (c = 1 μg/ml in methanol–water (50:50, v/v)) and 400 μl of acetone for protein precipitation were added (total volume 1 ml). The QC samples were prepared by the same procedure to get low QC (5 ng/ml), mid QC (20 ng/ml) and high QC (100 ng/ml) due to the expected concentration range of the application study.

2.2.4. Sample preparation

For every calibration point, QC sample and sample from the application study, 1 ml of matrix-solvent mixture containing MH84 and the internal standard was obtained (see Sections 2.2.2 and 2.2.3). The mixtures including brain homogenate were centrifuged for 10 min at 900 × g. Afterwards 850 μl were taken from the supernatant (brain homogenate) or matrix–solvent mixture (plasma)