Contents lists available at SciVerse ScienceDirect



Free Radical Biology and Medicine



journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Comprehensive pharmacokinetic studies and oral bioavailability of two Mn porphyrin-based SOD mimics, MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺



Tin Weitner^a, Ivan Kos^{a,1}, Huaxin Sheng^{b,c}, Artak Tovmasyan^a, Julio S. Reboucas^{a,2}, Ping Fan^d, David S. Warner^{b,c}, Zeljko Vujaskovic^{a,3}, Ines Batinic-Haberle^a, Ivan Spasojevic^{d,*}

^a Department of Radiation Oncology, Duke University Medical Center, Durham, NC 27710, USA

^b Department of Anesthesiology, Duke University Medical Center, Durham, NC 27710, USA

^c Multidisciplinary Neuroprotection Laboratories, Duke University Medical Center, Durham, NC 27710, USA

^d Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA

ARTICLE INFO

Article history: Received 26 August 2012 Received in revised form 20 December 2012 Accepted 3 January 2013 Available online 15 January 2013

Keywords: Pharmacokinetics Oral availability Mn porphyrins MnTE-2-PyP⁵⁺ MnTnHex-2-PyP⁵⁺ SOD mimic LC-MS/MS Mouse plasma Mouse organs Free radicals

ABSTRACT

The cationic, ortho Mn(III) N-alkylpyridylporphyrins (alkyl=ethyl, E, and n-hexyl, nHex) MnTE-2-PyP⁵⁺ (AEOL10113, FBC-007) and MnTnHex-2-PyP⁵⁺ have proven efficacious in numerous in vivo animal models of diseases having oxidative stress in common. The remarkable therapeutic efficacy observed is due to their: (1) ability to catalytically remove O_2^{-} and $ONOO^{-}$ and other reactive species; (2) ability to modulate redox-based signaling pathways; (3) accumulation within critical cellular compartments, i.e., mitochondria; and (4) ability to cross the blood-brain barrier. The similar redox activities of both compounds are related to the similar electronic and electrostatic environments around the metal active sites, whereas their different bioavailabilities are presumably influenced by the differences in lipophilicity, bulkiness, and shape. Both porphyrins are water soluble, but MnTnHex-2-PyP⁵⁺ is approximately 4 orders of magnitude more lipophilic than MnTE-2-PyP⁵⁺, which should positively affect its ability to pass through biological membranes, making it more efficacious in vivo at lower doses. To gain insight into the in vivo tissue distribution of Mn porphyrins and its impact upon their therapeutic efficacy and mechanistic aspects of action, as well as to provide data that would ensure proper dosing regimens, we conducted comprehensive pharmacokinetic (PK) studies for 24 h after single-dose drug administration. The porphyrins were administered intravenously (iv), intraperitoneally (ip), and via oral gavage at the following doses: 10 mg/ kg MnTE-2-PyP⁵⁺ and 0.5 or 2 mg/kg MnTnHex-2-PyP⁵⁺. Drug levels in plasma and various organs (liver, kidney, spleen, heart, lung, brain) were determined and PK parameters calculated (C_{max} , $C_{24 h}$, t_{max} , and AUC). Regardless of high water solubility and pentacationic charge of these Mn porphyrins, they are orally available. The oral availability (based on plasma AUC_{oral}/AUC_{iv}) is 23% for MnTE-2-PyP⁵⁺ and 21% for MnTnHex-2-PvP⁵⁺. Despite the fivefold lower dose administered, the AUC values for liver, heart, and spleen are higher for MnTnHex-2-PyP⁵⁺ than for MnTE-2-PyP⁵⁺ (and comparable for other organs), clearly demonstrating the better tissue penetration and tissue retention of the more lipophilic MnTnHex-2-PyP⁵⁺. © 2013 Elsevier Inc. All rights reserved.

Introduction

MnTE-2-PyP⁵⁺ (AEOL10113, FBC-007), MnTnHex-2-PyP⁵⁺, and MnTDE-2-ImP⁵⁺ (AEOL10150) have been the most frequently

studied cationic metalloporphyrins in animal models of diseases that have oxidative stress in common [1–5]. The structures, aqueous physicochemical properties, and lipophilicity of MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺ are summarized in Table 1,

Abbreviations: SOD, superoxide dismutase; MnP, Mn metalloporphyrin; MnTM-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-methylpyridinium-2-yl)porphyrin; MnTE-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-n-hexylpyridinium-2-yl)porphyrin; AEOL10113, FBC-007; MnTnHex-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-n-hexylpyridinium-2-yl)porphyrin; MnTB-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-n-hexylpyridinium-2-yl)porphyrin; MnTBAP³⁻, Mn(III) *meso*-tetrakis(4-benzoic acid)porphyrin; MnTDE-2-ImP⁵⁺, AEOL10150 Mn(III) *meso*-tetrakis(*N*,*N*'-diethylimidazolium-2-yl)porphyrin; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MeCN, acetonitrile, PK, pharmacokinetics; ip, intraperitoneal; iv, intravenous; sc, subcutaneous; NF-kB, nuclear factor kB; 0^o/₂ –, superoxide; ONOO⁻, peroxynitrite; CO^o/₃ –, carbonate anion radical; ClO⁻, hypochlorite; BBB, blood–brain barrier; AUC, area under the curve up to the last measurement at 24 h

^{*} Corresponding author.

E-mail address: ivan.spasojevic@duke.edu (I. Spasojevic).

¹ Current address: Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovacica 1, 10 000 Zagreb, Croatia.

² Current address: Departamento de Química, CCEN, Universidade Federal da Paraíba, João Pessoa, PB 58051-970, Brazil.

³ Current address: Division of Translational Radiation Sciences, Department of Radiation Oncology, University of Maryland Baltimore, Baltimore, MD 21201.

^{0891-5849/}\$ - see front matter © 2013 Elsevier Inc. All rights reserved. http://dx.doi.org/10.1016/j.freeradbiomed.2013.01.006

Table 1

Comparison of MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺ with regard to their redox properties, reactivity toward superoxide and peroxynitrite, and lipophilicity.



Redox property is expressed as the metal-centered reduction potential, $E_{1/2}$, for the Mn^{III}P/Mn^{II}P redox couple, reactivity toward superoxide as log $k_{cat}(O_2^{\bullet-})$ and peroxynitrite as log $k_{red}(ONOO^-)$, and lipophilicity in terms of distribution between *n*-octanol and water, log P_{OW} . Data were taken from [1–3,7–9].

whereas their biologic and therapeutic effects are detailed elsewhere [1–4]. They were developed based on the structureactivity relationship between the metal-centered reduction potential, $E_{1/2}$, for the Mn^{III}P/Mn^{II}P redox couple and the rate constant, k_{cat} , for the catalysis of O₂⁻ dismutation [1–3,6]. The $k_{cat}(O_2^{-})$ parallels the rate constant for ONOO⁻ reduction, k_{red} [1–3,7–9]. Such relationship is due to the similar thermodynamic and kinetic factors governing the reaction of electron-deficient cationic MnPs with electron-donating anionic species, such as O₂⁻ and ONOO⁻ [1–3,10]. The same is valid for their reactivity toward CO₃⁻, CIO⁻, and deprotonated, anionic cysteine sites of signaling proteins [2,3,9,11,12]. These MnPs are able to oxidatively modify the cysteine of p50 and p65 of NF- κ B transcription factor, thereby preventing its activation [1,12,13].

The more lipophilic analogs with longer alkyl chains of up to 8 carbon atoms were synthesized with the goal of enhancing the bioavailability of MnPs while retaining the *ortho*-positioned pentacationic charge and in turn favorable redox properties [14,15]. MnTE-2-PyP⁵⁺ (2-carbon-atom ethyl chains) and MnTnHex-2-PyP⁵⁺ (6-carbon-atom *n*-hexyl chains) differ by \sim 4 orders of magnitude in lipophilicity (Table 1), which affects their mitochondrial distribution, transport across the BBB, and in turn their therapeutic effects [1,2,4,16–18]. Bioeffects differ less in magnitude (few to 120 folds) than the difference in lipophilicity would imply [16,19].

Pharmacokinetic studies provide invaluable information for researchers to understand the organ and cellular/subcellular accumulation and clearance of a drug and its mechanism of action. A limited rat PK study of MnTnHex-2-PyP⁵⁺ was performed recently [19], in a 90 min middle cerebral artery occlusion stroke model, in which rats were injected subcutaneously with 0.225 mg/kg twice daily for a week, starting at either 5 min or 6 h after reperfusion. The neurologic function was significantly improved and the total infarct size decreased with both early and delayed treatment. Naïve male Wistar rats were simultaneously given 75 μ g/kg iv and 2.25 μ g/kg sc of MnTnHex-2-PyP⁵⁺ every 24 h for 7 days. LC-MS/MS analysis of plasma and brain levels showed that the level of MnP in the brain steadily increases and at day 7 approaches the plasma level (25 nM in the brain vs 50 nM in plasma) [19]. This data set is the first to correlate directly the in vivo levels of MnP with its efficacy.

The common view is that hydrophilic drugs are not sufficiently orally available. The preliminary plasma PK set of data showed, surprisingly, that a highly hydrophilic MnTE-2-PyP⁵⁺ is orally available [20]. To increase our knowledge about the impact of charge and lipophilicity on Mn porphyrin bioavailability, we report herein a comprehensive pharmacokinetic study of two pentacationic metal complexes of very different lipophilicities

(Table 1), MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺, administered via intravenous (iv), intraperitoneal (ip), and oral gavage (oral) routes.

Materials and methods

Porphyrins and other chemicals

MnTM-2-PyP⁵⁺ (λ_{max} 453.4 nm, log ε =5.11), MnTE-2-PyP⁵⁺ (λ_{max} 454.0 nm, log ε =5.14), MnTnHex-2-PyP⁵⁺ (λ_{max} 454.5 nm, log ε =5.21), and MnTnHep-2-PyP⁵⁺ (λ_{max} 454.0 nm, log ε =5.19) were synthesized and characterized as previously described [14,21]. Other chemicals used were acetonitrile (MeCN) from Fisher Scientific, methanol (anhydrous, absolute) from Mallinck-rodt, glacial acetic acid from EM Science, heptafluorobutyric acid (HFBA) from Aldrich, and phosphate-buffered saline (50 mM sodium phosphate, 0.9% NaCl, pH 7.4) from Gibco.

Mice

The Duke University Medical Center Animal Facility has a program continuously accredited by AAALAC International. All experiments using animals were performed according to the approved protocol for humane care and use of animals. Tenweek-old C57BL/6 J female mice weighing 17-25 g were used. The concentration of aqueous solutions (for oral gavage) or saline solutions (for ip and iv injections) was adjusted so that the mice were injected with volumes of \sim 0.2 ml per dose. Mice did not have access to food 12 h or to water 3 h before oral gavage. In a dose-dependence study the mice were given orally 5 to 80 mg/kg MnTE-2-PyP⁵⁺ and 0.1 to 40 mg/kg MnTnHex-2-PyP⁵⁺ (see Supplementary Fig. S5). Mice were observed for 24 h and then euthanized and blood and organ samples were taken. In a timedependence study, mice were given a single dose of 10 mg/kg MnTE-2-PyP⁵⁺ or 0.5 or 2 mg/kg MnTnHex-2-PyP⁵⁺, via oral gavage or ip or iv, and were euthanized at various time points (5 min to 24 h). For the ip and iv studies 3 mice per time point sufficed. For the oral PK study there was a large variability at earlier time points. To increase accuracy, as many as 10 mice were used per time point up to 2 h after dosing, whereas for later time points 3 mice were sufficient. To collect tissues, mice were anesthetized with isoflurane, arterial blood was sampled, and the vasculature was briefly rinsed with 50 ml saline by transcardial perfusion before excision of liver, kidney, spleen, lung, heart, and brain. Plasma and hematocytes were separated immediately. Samples were kept at -80 °C.

Dosing of MnPs

In various efficacy studies performed thus far, MnPs have been administered as saline or phosphate-buffered saline solutions at various doses and via various routes: subcutaneous (sc), ip, iv, intramuscular, oral gavage, skin treatment, sc and iv osmotic pumps, and inhalation. At 3 mg/kg/day given ip for 4 days, MnTE-2-PyP⁵⁺ was able to fully reverse morphine tolerance in a mouse model [5]. In a rat lung pulmonary radioprotection study this drug nearly fully reversed lung fibrosis. It was given sc at 6 mg/ kg/day for 2 weeks, starting at various time points after radiation, ranging from 2 h to 8 weeks [22,23]. MnTE-2-PyP⁵⁺ radiosensitized tumors when given ip at 6 mg/kg/day for 3 days [24]. At 15 mg/kg/day for 3 weeks (given in two daily increments), MnTE-2-PyP⁵⁺ exerted an anticancer effect in a 4T1 mouse mammary model [25]. In a rat diabetes model, 10 mg/kg given ip every second day from the onset of disease until completion of the study prevented adaptive transfer of autoimmune diabetes by a

Download English Version:

https://daneshyari.com/en/article/8271605

Download Persian Version:

https://daneshyari.com/article/8271605

Daneshyari.com