University of Groningen

Childhood-onset movement disorders

Lambrechts, Roald Alexander

DOI:
10.33612/diss.101316004

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

[Link to publication in University of Groningen/UMCG research database](#)

Chapter 3

Synthesis and characterization of 4-thiobutyl-triphenylphosphonium-pantetheine, a pantetheine derivative

R.A. Lambrechts[1], B. Srinivasan[1], E.M. Geertsema[2], G.J. Poelarends[2], O.C.M. Sibon[1], M. de Villiers[1,2]

[1] Department of Cell Biology, University Medical Center Groningen, Groningen, The Netherlands.
[2] Department of Pharmaceutical Biology, Groningen Research Institute of Pharmacy, Groningen, The Netherlands.

ABSTRACT

Pantetheine, a low molecular weight thiol, has been found to ameliorate symptoms in various disease models but specifically in Pantothenate Kinase-Associated Neurodegeneration (PKAN). Pantetheine is usually administered in its disulfide form (i.e. pantethine) since pantethine is commercially available and is reduced to pantetheine in biological systems. The applicability and efficacy of pantethine (therefore also pantetheine) as a clinical therapeutic however is hampered since both forms can be degraded by pantetheinases present in the body. Here, we report the synthesis of a masked form of pantetheine, namely 4-thiobutyl triphenylphosphonium-pantetheine (TBTP-pantetheine), following our hypothesis that this pantetheine-derivative might be more stable in the presence of pantetheinases than pantetheine itself. Higher stability would enhance transport into the cytoplasm where TBTP-pantetheine is metabolized into pantetheine which can subsequently execute its medicinal action. We find that TBTP-pantetheine is stable in aqueous solution, however it was found to be less stable in 10% fetal calf serum (which contains pantetheinases) compared to pantethine, the commercially availabile disulfide of pantetheine. We show that TBTP-pantetheine has improved lipophilicity, but equal passive membrane permeability/diffusion, as compared with pantethine.
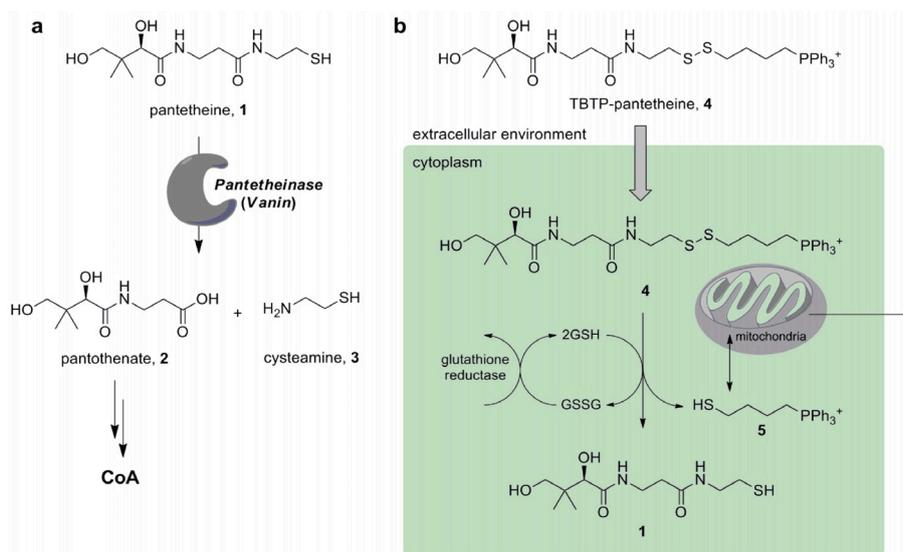
Keywords: TBTP-pantetheine; pantetheine; pantetheinase; Coenzyme A; PKAN; serum stability.

INTRODUCTION

Pantethine, the disulfide of pantetheine (scheme 1a, 1), has been investigated in the context of biological processes involved in several diseases, including hypercholesterolaemia [1], cataract disease [2] and cerebral malaria [3]. In addition, preclinical research has shown beneficial effects of pantethine on a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced mouse model for Parkinson's disease [4,5] and pantethine also prevented neuronal degeneration in animal models of Parkinson's disease [6,7]. In general, pantethine is investigated as possible treatment since it is commercially available, however the beneficial effects are due to the reduced form (i.e. pantetheine 1), which is formed *in vivo*. Recently, pantethine has been investigated as a potential rescue agent for Pantothenate Kinase-Associated Neurodegeneration (PKAN), a neurodegenerative disease caused by mutations in the human *Pank2* gene (one of the four human pantothenate kinase genes (*PANK1-4* known) [8,9]. The PANK2 protein is mainly localized in the brain [8,9]. It is believed that mutations in the human *PANK2* gene renders the enzyme pantothenate kinase, which is responsible for the pivotal, rate limiting step in the biosynthesis of coenzyme A (CoA) during which pantothenate (2, Vit B5, precursor for CoA) is phosphorylated to 4'-phosphopantothenate, inactive and therefore impairs the pathway to produce CoA which is an essential cofactor in all organisms. Pantethine has shown potential as rescue agent in a Drosophila model for PKAN where it restored CoA levels, rescued brain degeneration, mitochondrial dysfunction and locomotor disabilities [8].

Although pantethine has been shown to be effective in Drosophila PKAN models, a possible clinical implementation may be limited by the fact that pantethine is rapidly hydrolysed when administered orally [10]. Vanin proteins (also known as pantetheinases) present in humans contain pantetheinase activity and have the ability to degrade pantetheine (1), its natural substrate, to form pantothenate (2) and the antioxidant cysteamine (3) (scheme 1a). Since these enzymes are present in the gastrointestinal mucosa and human serum [11], they limit the use of pantethine (which has also been shown to be degraded by pantetheinases [12]) as a possible rescue agent for PKAN. Vanin proteins have also been shown to be an important implication in the use of pantothenamides as antimalarials, since this specific class of pantothenate derivatives has the same core structure as 1 and pantetheinases can hydrolyze pantothenamides with a wide range of structural modifications on the cysteamine moiety [13-16]. Inhibitors for pantetheinases have been described [17] and combining pantothenate derivatives (i.e. pantethine or pantothenamides) with such inhibitors has been proposed as a strategy to overcome the obstacle formed by pantetheinases [18]. Alternatively, small modifications in the core structure of pantothenamides led to increased stability towards pantetheinase activity and increased antiplasmodial activity towards the malaria parasite *Plasmodium falciparum* [16].

Scheme 1. Metabolism of pantetheine (1) and TBTP-pantetheine (4).

a) Natural pantetheine recycling through degradation of pantetheine (1) by pantetheinases to produce pantothenate (2) and cysteamine (3). As a result, 2 can be taken up by the cell and be converted to coenzyme A. b) Proposed mechanism of action of TBTP-pantetheine (4). The TBTP-moiety of 4 will enable transport of 4 into the cytoplasm where it can be reduced by glutathione reductase to release pantetheine (1) as rescue agent. The TBTP cation (5) will dispense to the mitochondria where it will slowly be released from the cell.

We set out to prepare the novel pantetheine derivative, 4-thiobutyl triphenylphosphonium-pantetheine (TBTP-pantetheine, 4, scheme 1b) in order to protect pantetheine against degradation by pantetheinases, so it can function as a better rescue agent for PKAN. Since the core pantetheine structure of the molecule must be intact in order to be converted to CoA, we envisioned to couple pantetheine (1) and 4-thiobutyltriphenylphosphonium (TBTP, 5), a lipophilic cation, to construct the pantetheine-derivative 4 with supposedly improved pharmacokinetic properties as compared to pantetheine [19,20]. Pantetheinases seem to be promiscuous towards the cysteamine moiety of pantetheine derivatives, and therefore we assumed that some degradation of 4 would still take place. Despite the latter, the TBTP-moiety might reduce the affinity of pantetheinases for 4 compared to that of 1 and also improve the cellular uptake, as was previously shown for other TBTP-linked compounds [21]. This might lead to shorter exposure of 4, as compared to 1, in the compartments where pantetheinases are abundant. Moreover, the TBTP-moiety of 4 may facilitate blood brain barrier crossing, as permeability depends highly on a compound's lipophilicity. Once TBTP-pantetheine (4) enters the cell it should be reduced by the glutathione pool present in the cytoplasm to release pantetheine (1) as rescue agent of the impaired CoA biosynthesis pathway present in PKAN patients. The TBTP cation 5 will dispense to the mitochondria where it will slowly be released from the cell [20]. Although TBTP-linked molecules have been found to accumulate in internal organs and the central nervous system, these compounds are generally well tolerated and have previously been used in clinical trials [21-23]. This study describes a simple synthesis of TBTP-pantetheine (4) in three chemical

steps and shows that 4 is stable over a prolonged period in aqueous solution at various temperatures. We also compared the stability of TBTP-pantetheine (4) to pantethine (disulphide of 1) in the presence of fetal calf serum, and investigated the membrane permeability of both these compounds.

MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich Chemical Co. and solvents were purchased from Biosolve or Sigma-Aldrich Chemical Co. Fetal calf serum (FCS) was obtained from Greiner Bio-one and Dulbeccos Phosphate Buffered Saline (PBS) was from Invitrogen. Centrifugal devices for protein removal were from PALL. Gentest pre-coated PAMPA plates were from BD Biosciences. UV 96-well flat-bottomed plates were from Greiner Bio-one.

UV absorbance was determined using a SPECTROstar Omega plate reader from BMG Labtech. NMR analyses were performed on a Varian Inova 200 and 300 machine at the NMR Center, University of Groningen. Chemical shifts (δ) are reported in parts per million (ppm). Mass Spectrometry was performed using Orbitrap high resolution mass spectrometry (HRMS) by the Mass Spectrometry core facility, University of Groningen.

High performance liquid chromatography (HPLC) analysis was performed on a Shimadzu LC-10AC system using a SCL-10A system controller, SIL-10AC automatic sample injector, and LC-10AT solvent delivery system. The peaks of interest were detected at 205 nm and/or 267 nm with a Shimadzu SPD-M10A photodiode array detectorafter injection of 25 µl of each sample. Separation was achieved by using a Synergi 4u Hydro-RP 80A (150 × 4.60 mm, 4 µm) C18 column from Phenomenex at 30°C while maintaining a flow rate of 1.0 ml/min. HPLC method: The column was equilibrated in solution A (20 mM KH_2PO_4, 0.1% hexanesulfonic acid, pH 3) and the sample was injected. After 5 min elution with 100% A, the following linear gradients were used: a linear gradient increasing solution B (acetonitrile) to 30% (5-18 min), a linear gradient increasing solution B to 50% (18-21 min), isocratic elution at 50 % solution B (21-23 min), a linear gradient decreasing solution B to 0% (23-24 min) and finally isocratic elution in 100% solution A (23-30 min).

Synthesis of 4-bromobutyl thiolacetate (7)

The synthesis of 7 was carried out according to a previously published method [19]. Briefly, thiolacetic acid (5.63 g, 73.1 mmol) was added to 4-bromo-1-butene 5 (5 g, 37.0 mmol) under nitrogen. After the addition of α,α′-azoisobutyronitrile (AIBN, 3.36 mg) the reaction was stirred for 60 min at 40°C. Dichloromethane was added to the reaction mixture and the solution was washed with water (3 × 8.5 ml). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo* after which purification by flash column chromatography (2:1 to 4:1 EtOAc: hexanes) afforded product 7 (2.14 g, 27%) as a yellow oil. TLC (EtOAc: hexanes, 2:1): R_f = 0.73; ^{1}H NMR (CDCl$_3$, 200MHz, 25°C): δ = 1.66–1.80 (m, 2H), 1.86–2.00 (m, 2H), 2.33 (s, 3H), 2.90 (t, *J* = 7 Hz, 2H), 3.41 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (CDCl$_3$, 75.5 MHz, 25°C): δ = 28.4, 28.4, 30.8, 31.8,

33.1, 195.9; HRMS: m/z [M]+ calcd for $C_6H_{12}OBrS$: 210.97879; found: 210.97879. ^{1}H NMR data is consistent with literature [19].

Synthesis of S-acetyl-thiobutyltriphenylphosphonium Bromide (8)

The synthesis of 8 was carried out according to a previously published method [19]. Briefly, 7 (2.14 g, 10.1 mmol) was dissolved in dry toluene (6 ml) under nitrogen followed by the addition of triphenylphosphine (2.66 g, 10.1 mmol). The reaction mixture was refluxed for 2 hours under nitrogen after which the heat was turned down and the reaction mixture left in the heating mantle to cool down slowly overnight. An upper colourless toluene layer and lower yellow layer formed. The mixture was stored at -20°C for a few hours until a white precipitate started to form. The upper toluene layer was removed with a pipet followed by washing of the bottom layer three times with pentane followed by three times with diethyl ether. Finally, 8 was collected as a white precipitate which was dried *in vacuo* (3.01 g, 63%). ^{1}H NMR (CDCl$_3$, 200MHz, 25°C): δ = 1.57–1.79 (m, 2H), 1.91–2.05 (q, *J* = 7.1 Hz, 2H), 2.19 (s, 3H), 2.91 (t, *J* = 6.8 Hz, 2H), 3.79–3.93 (m, 2H), 7.63–7.90 (m, 15H); ^{13}C NMR (CDCl$_3$, 75.5 MHz, 25°C): δ = 28.1, 28.1, 29.9, 30.2, 30.7, 117.5, 119.2, 129.9, 130.2, 130.5, 130.5, 130.8, 130.8, 133.8, 133.8, 133.8, 134.0, 134.0, 134.0, 134.1, 134.3, 135.1, 135.2, 196.3; HRMS: m/z [M]+ calcd for $C_{24}H_{26}OPS$: 393.1426; found: 393.14255. ^{1}H NMR data is consistent with literature [19].

Synthesis of S-thiobutyltriphenylphosphoniumpantetheine (TBTP-pantetheine, 4)

S-acetyl-thiobutyltriphenylphosphonium bromide (8, 4.50 g, 9.50 mmol) was dissolved in ethanol (13.6 ml) and 1 M NaOH (10.5 ml), followed by stirring for 30 min at room temperature. To this reaction mixture was added a solution of D-pantethine (2.63 g, 4.75 mmol) in ethanol (20.5 ml). The reaction mixture was stirred for 2 hours at room temperature, followed by removal of the ethanol *in vacuo*. The residue was dissolved in water for lyophilization overnight. The final product was purified by flash column chromatography (10-20% MeOH/DCM) to yield 4 as a white powder (2.14 g, 32%). ^{1}H NMR (CDCl$_3$, 200MHz, 25°C): δ = 0.88 (s, 3H), 0.93 (s, 3H), 1.66–1.85 (m, 2H), 1.92–2.12 (m, 2H), 2.48 (t, *J* = 5.8 Hz, 2H), 2.62–2.96 (m, 4H), 3.33–3.54 (m, 6H), 3.62–3.76 (m, 4H), 4.03 (s, 1H), 7.49 (br s, 1H), 7.66–7.87 (m, 15H), 8.05 (br s, 1H); ^{13}C NMR (CDCl$_3$, 75.5 MHz, 25°C): δ = 20.9, 22.1, 23.1, 24.5, 30.2, 30.2, 36.0, 36.8, 38.6, 38.8, 39.4, 42.8, 70.7, 117.4, 119.1, 130.7, 130.7, 130.7, 130.9, 130.9, 130.9, 133.7, 133.7, 133.7, 133.8, 133.9, 133.9, 133.9, 134.1, 135.4, 135.5, 172.4, 174.1; HRMS: *m/z* [M]+ calcd for $C_{33}H_{44}O_4N_2PS_2$: 627.2465; found: 627.24548.

Stability of TBTP-pantetheine (4) in water

A solution of TBTP-pantetheine (4, 1 mM) was prepared in water and kept at different temperatures (-20°C, 4°C, 25°C, and 37°C) for 14 days. Samples were injected on HPLC (method already described) on day 1, day 7 and day 14 in order to determine the concentration of 4 in solution, based on a standard curve of known concentrations prepared for TBTP-pantetheine (4). The experiment was performed in triplicate and the indicated errors are standard deviations.

Stability of TBTP-pantetheine (4) in Fetal Calf Serum

Solutions of TBTP-pantetheine (4, 1 mM) or D-pantethine (1 mM) were prepared in 10% FCS (pre-incubated at 37°C) in PBS in a final volume of 150 µl. Corresponding control samples either contained no FCS, no pantethine or no TBTP-pantetheine (4). The samples were incubated at 37°C for 60 min and degradation action of the enzyme stopped at specific timepoints (5, 10, 20, 45 and 60 min) by flash freezing in liquid nitrogen followed by centrifugation for 45 minutes at 14 000 rpm in 3 K centrifugal filters at 4°C. The experiment was performed in triplicate and each sample was analyzed by the already described HPLC method. The amount of pantothenate formed was calculated by correlation to a standard curve of known pantothenate (2) concentrations. The amount of pantothenate (2) formed upon degradation of pantethine, was divided by 2 in order to compensate for the 2:1 pantetheine (1) ratio between pantethine and TBTP-pantetheine (4). The errors bars indicate standard deviations.
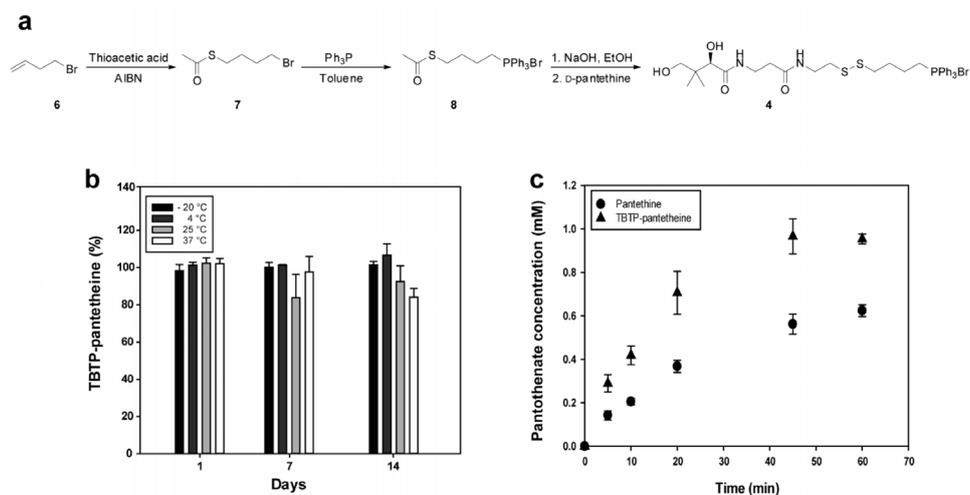
Membrane Permeability of TBTP-pantetheine (4)

To determine the membrane permeability potential of 4, a previously reported *in vitro* model was used [24]. Experiments were carried out according to the manufacturer's instructions. The donor well was filled with 300 µl of a solution of the test compound (either D-pantethine or 4) in PBS and the acceptor wells contained 200 µl PBS. Two different concentrations were tested: a low concentration of 800 µM and high concentration of 2 mM, with every condition tested in quadruplicate. The Parallel Artificial Membrane Permeability Assay (PAMPA) plate system was assembled and incubated for 5 hours at room temperature without agitation. After incubation, 100 µl of each donor and acceptor well solution was transferred to a UV 96-well plate where the absorbance was measured between 220 and 400 nm using a SPECTROstar Omega plate reader. The concentration of compound present in each solution was determined by comparison with standard curves determined for each compound. Caffeine and amiloride were measured as positive and negative control for membrane permeation, respectively [5]. Permeability and mass retention was determined.

RESULTS AND DISCUSSION

Synthesis

We set out to prepare TBTP-pantetheine (4) following our hypothesis that this pantetheine-derivative exhibits enhanced bioavailability and pharmacokinetic properties as compared to pantetheine itself. TBTP (5) was synthesized according to known literature procedures by firstly preparing the acetylated form of TBTP (8, Fig. 1a), which prevented oxidation of the thiol under synthesis conditions [19]. Prior to coupling TBTP (i.e. a thiol (5) which is the non-acetylated form of compound 8) to pantetheine (1) to produce the required disulfide 4, the acetyl group of 8 was removed by means of base hydrolysis in a one-pot synthesis procedure. This resulted in the successful synthesis and purification of TBTP-pantetheine (4, Fig. 1a). The overall yield of this simple three-step synthesis is low (overall yield of 5%), mainly due to two factors.

First, the reported preparation of 8 in this manner constitutes low yields (30%) [19] and we found similar results with an overall yield of 17%. An alternative route for the preparation of TBTP (5) has been reported and utilizes commercially available 4-bromobutyltriphenylphosphonium bromide in combination with hydrosulphide exchange resin to deliver TBTP in one step in a yield of 95% [20]. This method might prove much more effective in future strategies for TBTP-linked molecules. Secondly, synthesis involving pantothenate derivatives proves to be challenging if the 4′-hydroxyl group is not protected, since these compounds are usually water-soluble which impairs the use of aqueous work-up procedures before purification. This, in combination with the presence of water soluble TBTP and bis-TBTP in the reaction mixture, complicates purification of the final product. Nevertheless, despite these obstacles we were still successful in the preparation and purification of 4 and the final purity of the compound was determined as >83% pure by HPLC analysis (assuming identical -values of the contaminant(s) as compared to 4).

Fig. 1. Synthesis and stability of TBTP-pantetheine (4).

a) Synthesis route for TBTP-pantetheine in three steps starting from 4-bromo-1-butene (6). b) Stability of TBTP-pantetheine in water at different temperatures (-20 °C in black, -4 °C in dark grey, 25 °C in light grey, 37°C in white) over two weeks. Experiment was performed in triplicate and error bars denote standard deviations. c) Pantothenate formation by the degradation of TBTP-pantetheine (triangles) vs. pantethine (circles) in 10% FCS over 60 min. Experiment was done in triplicate and error bars denote standard deviations.

Stability of TBTP-pantetheine

The aqueous stability of 4 was determined after 1, 7 and 14 days at various temperatures (-20°C, 4°C, 25°C and 37°C). No degradation was observed at low temperatures (i.e. -20°C and 4°C); however, mild decay (<15%) was observed for samples stored in water at 25°C and 37°C (Fig. 1b). Degradation mainly resulted in the formation of pantetheine and TBTP. The data therefore suggest that decay of 4, when measured in a biological matrix, is not due to aqueous instability of the compound.

Next, we set out to determine the stability of TBTP-pantetheine (4) in the presence of fetal calf serum which exhibits pantetheinase activity. Pantethine and TBTP-pantetheine were incubated in the presence of 10% fetal calf serum for 60 minutes, and the amount of pantothenate (2, the degradation product) formed was measured by HPLC at different time intervals. The stability of pantetheine bearing a TBTP-moiety (4) appeared not to be higher than the stability of pantethine. In fact, from Fig. 1c it is clear that more pantothenate (2) is formed in 60 minutes from 4 than from pantethine. This illustrates that, similar to previous studies, pantetheinases are very promiscuous in accepting pantetheine derivatives with modifications on the cysteamine moiety of the molecule.

Membrane Permeability of TBTP-pantetheine (4)

It is of particular clinical interest to study whether the derivatization of pantetheine (1) by attaching a TBTP-moiety (5) leads to an increase in membrane permeability. In general, it is assumed that passive diffusion is the most important mode of permeability involved in the blood brain barrier [24]. A Parallel Artificial Membrane Permeability Assay (PAMPA) was used to investigate the passive diffusion behavior of TBTP-pantetheine (4) compared to pantethine (the disulphide of 1) [24]. By using this assay, 4 and pantethine were tested for their ability to permeate through the artificial membrane and their mass (or membrane) retention was determined. The negative logarithm of permeability is shown (Fig. 2a) and indicates that compounds with a high tendency to permeate through the membrane will have a low value, such as caffeine which was used as positive control, whereas a high value will indicate little diffusion as seen for amiloride which was used as negative control for permeation [24]. The permeability of pantethine and TBTP-pantetheine (4) was found to be similar (Fig. 2a), indicating poor diffusion through the membrane by both these compounds. However, the mass retention of TBTP-pantetheine (4, 20%) was found to be significantly higher than that for pantethine (~2%) (Fig. 2b, Student's t-test, two-sided, $P = 0.0075$). These
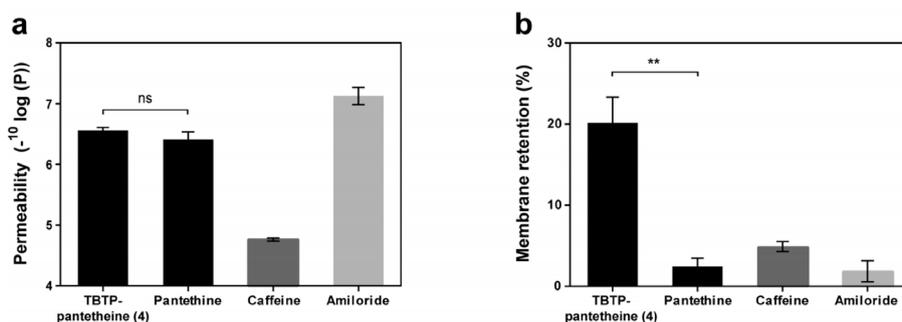

Fig. 2. Membrane diffusion of TBTP-pantetheine (4) compared to pantethine (disulphide of 1).

a) Membrane permeability of TBTP-pantetheine (4) compared to pantethine (disulphide of 1) using a PAMPA test. Caffeine and amiloride are included as positive and negative control, respectively. No significant difference is denoted as "ns" and was determined with a Mann-Whitney U-test (two-sided, P = 0.4166). Note that the negative logarithm of permeability is shown. b) Membrane retention of TBTP-pantetheine (4) compared to pantethine (disulphide of 1) using the same experiment that generated data shown in Fig. 2a. The significant difference observed between TBTP-pantethine (4) and pantethine (disulphide of 1) is indicated by ** (Student's t-test, two-sided, P = 0.0075). Experiments were done in quadruplicate with error bars indicating standard error mean.

results show that TBTP-pantetheine (4) is indeed more lipophilic than pantethine (the disulphide of 1) but that passive permeability of the compound is not increased. This assay does not include the possibility of active transport of either TBTP-pantetheine (4) or pantethine by carriers present in the blood brain barrier. It is believed that the involvement of these active transport processes in permeability of drugs have been underestimated and might be processes which warrant further investigation [24].

CONCLUSION AND FUTURE OUTLOOK

Here we described the derivatization of pantetheine in an attempt to improve its pharmacological activity as a rescue agent for PKAN. We were successful in preparation and sufficient purification of TBTP-pantetheine (4) and showed that the TBTP-pantetheine shows increased membrane affinity, but not increased passive membrane permeability, as compared to pantethine. TBTP-pantetheine (4) is stable in aqueous solution, however it is degraded by pantetheinases present in fetal calf serum. This effect might be overcome when using this compound in combination with known pantetheinase inhibitors; although, pantetheinase inhibitors may introduce toxicity and alter some biological processes [17]. Even though TBTP-pantetheine (4) is being degraded faster than pantethine *in vitro*, future work will be of value to test this novel derivative of pantetheine *in vivo* on insect and human cells in a PKAN model to determine if it has superior rescue potential over pantethine. There is also potential to extend 4 further as a drug for other diseases. Among other uses, pantethine has been found to ameliorate MPTP induced toxicity in a murine model of Parkinson's disease. However, effective delivery to the drug target of the compound is still lacking [5]. The exact mode of action of pantethine is still unknown. Pantethine is reduced *in vivo* to pantetheine and subsequently the rescue potential of this molecule might be due to its metabolites cysteamine or pantothenate. Although cysteamine is being used to treat cystinosis, it has also been found to have side effects when treating this disease [25]. In contrast a study on the toxicity of pantetheine shows that this compound has low toxicity in mice [26,27]. Therefore, treatment with pantethine or pantetheine might be a safer alternative for cysteamine, because pantethine is less toxic and still will be reduced to pantetheine (1) and then further degraded by pantetheinases to cysteamine, which is the active rescue compound for cystinosis treatment. It is currently unclear why cysteamine shows increased toxicity compared to pantethine. Addition of the TBTP-moiety (5) to pantetheine (1), as presented and investigated here, could possibly act as carrier to either deliver pantetheine or cysteamine to its specific target in various diseases. The applicability of TBTP (5) in this regard however needs to be further investigated.

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

3

REFERENCES

Rumberger JA, Napolitano J, Azumano I, Kamiya T, Evans M. Pantethine a derivative of vitamin B5 used as a nutritional supplement, favorably alters low-density lipoprotein cholesterol metabolism in low– to moderate–cardiovascular risk North American subjects: a triple-blinded placebo and diet-controlled investigation, Nutr Res (New York, N.Y). 2011;31:608-615.

Babizhayev MA. New Concept in Nutrition for the Maintenance of the Aging Eye Redox Regulation and Therapeutic Treatment of Cataract Disease; Synergism of Natural Antioxidant Imidazole-Containing Amino Acid-Based Compounds, Chaperone, and Glutathione Boosting Agents: A Systemic Perspective on Aging and Longevity Emerged From Studies in Humans, Am J Ther. 2010;17:373-389.

Penet MF, Abou-Hamdan M, Coltel N, Cornille E, Grau GE, de Reggi M, et al. Protection against cerebral malaria by the low-molecular-weight thiol pantethine, Proc Natl Acad Sci USA. 2008;105:1321-1326.

Sun L, Xu S, Zhou M, Wang C, Wu Y, Chan P. Effects of cysteamine on MPTP-induced dopaminergic neurodegeneration in mice, Brain Res. 2010;1335:74-82.

Cornille E, Abou-Hamdan M, Khrestchatisky M, Nieoullon A, de Reggi M, Gharib, B. Enhancement of L-3-hydroxybutyryl-CoA dehydrogenase activity and circulating ketone body levels by pantethine. Relevance to dopaminergic injury, BMC Neurosci. 2010;11:51.

Gibrat C, Cicchetti F. Potential of cystamine and cysteamine in the treatment of neurodegenerative diseases, Prog Neuropsychopharmacol Biol Psychiatry. 2011;35:380-389.

Stack EC, Ferro JL, Kim J, Del Signore SJ, Goodrich S, Matson S, et al. Therapeutic attenuation of mitochondrial dysfunction and oxidative stress in neurotoxin models of Parkinson's disease, Biochim Biophys Acta. 2008;1782:151-162.

Rana A, Seinen E, Siudeja K, Muntendam R, Srinivasan B, van der Want JJ, et al. Pantethine rescues a Drosophila model for pantothenate kinase-associated neurodegeneration, Proc Natl Acad Sci USA. 2010;107:6988-6993.

Garcia M, Leonardi R, Zhang YM, Rehg JE, Jackowski S. Germline deletion of pantothenate kinases 1 and 2 reveals the key roles for CoA in postnatal metabolism, PLoS One. 2012;7:e40871.

Wittwer CT, Gahl WA, Butler JD, Zatz M, Thoene JG Metabolism of pantethine in cystinosis, The Journal of Clinical Investigation. 1985;76:1665-1672.

Kaskow BJ, Michael Proffit J, Blangero J, Moses EK, Abraham LJ. Diverse biological activities of the vascular non-inflammatory molecules – The Vanin pantetheinases, Biochem Biophys Res Comm. 2012;417:653-658.

Dupre S, Graziani MT, Rosei MA, Fabi A, Del Grosso E. The enzymatic breakdown of pantethine to pantothenic acid and cystamine, Eur J Biochem. 1970;16:571-578.

Wittwer CT, Burkhard D, Ririe K, Rasmussen R, Brown J, Wyse BW, et al. Purification and properties of a pantetheine-hydrolyzing enzyme from pig kidney, J Biol Chem 1983;258:9733-9738.

Wittwer C, Wyse B, Hansen RG. Assay of the enzymatic hydrolysis of pantetheine, Anal Biochem. 1982;122:213-222.

Spry C, Macuamule C, Lin Z, Virga KG, Lee RE, Strauss E, et al. Pantothenamides are potent, on-target inhibitors of Plasmodium falciparum growth when serum pantetheinase is inactivated, PLoS One. 2013;8:e54974.

De Villiers M, Macuamule C, Spry C, Hyun YM, Strauss E, Saliba KJ. Structural Modification of Pantothenamides Counteracts Degradation by Pantetheinase and Improves Antiplasmodial Activity, Acs Med Chem Lett. 2013;4:784-789.

Jansen PAM, van Diepen JA, Ritzen B, Zeeuwen PLJM, Cacciatore I, Cornacchia C, et al. Discovery of Small Molecule Vanin Inhibitors: New Tools To Study Metabolism and Disease, ACS Chemical Biology. 2012;8:530-534.

Jansen PAM, Hermkens PHH, Zeeuwen PLJM, Botman, PNM, Blaauw RH, Burghout P, et al. Combination of pantothenamides with vanin inhibitors: a novel antibiotic strategy against gram-positive bacteria, Antimicrobial Agents and Chemotherapy. 2013;57:4794-4800.

Burns RJ, Smith RA, Murphy MP. Synthesis and characterization of thiobutyltriphenylphosphonium bromide, a novel thiol reagent targeted to the mitochondrial matrix, Arch Biochem Biophys. 1995;322:60-68.

Mehiri M, Caldarelli S, Di Giorgio A, Barouillet T, Doglio A, Condom R, et al. A ready-to-use fluorescent-labelled-cysteine-TBTP (4-thiobutyltriphenylphosphonium) synthon to investigate the delivery of non-permeable PNA (peptide nucleic acids)-based compounds to cells, Bioorg Chem. 2007;35:313-326.

Smith RA, Porteuous CM, Gane AM, Murphy MP. Delivery of bioactive molecules to mitochondria in vivo., PNAS. 2003;100:5407-5412.

Gane EJ, Weilert F, Orr DW, Keogh GF, Gibson M, Lockhart MM, et al. The mitochondria-targeted anti-oxidant mitoquinone decreases liver damage in a phase II study of hepatitis C patients, Liver International. 2010;30:1019-1026.

Snow BJ, Rolfe FL, Lockhart MM, Frampton CM, O'Sullivan JD, Fung V, et al. A double-blind, placebo-controlled study to assess the mitochondria-targeted antioxidant MitoQ as a disease-modifying therapy in Parkinson's disease, Movement Disorders. 2010;25:1670-1674.

Mensch J, Melis A, Mackie C, Verreck G, Brewster ME, Augustijns P. Evaluation of various PAMPA models to identify the most discriminating method for the prediction of BBB permeability, Eur J Pharm Biopharm. 2010;74:495-502.

Corden BJ, Schulman JD, Schneider JA, Thoene JG. Adverse reactions to oral cysteamine use in nephropathic cystinosis, Dev Pharmacol Ther. 1981;3:25-30.

Schwartz E, Bagdon RE Toxicity Studies of Some Derivatives of Pantothenic Acid, Toxicol Appl Pharmacol. 1964;6:280-283.

Knott RP, Tsao DP, McCutcheon RS, Cheldelin VH, King, TE. Toxicity of pantetheine, Proc Soc Exp Biol Med. 1957;95:340-341.