Bioorganic & Medicinal Chemistry Letters 18 (2008) 6119–6121

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

In situ blood–brain barrier permeability of a C-10 paclitaxel carbamate

Carlo Ballatore [a,b,*], Bin Zhang [a], John Q. Trojanowski [a], Virginia M.-Y. Lee [a], Amos B. Smith III [b]

[a] Center for Neurodegenerative Diseases Research, Institute on Aging, University of Pennsylvania, 3600 Spruce Street, Philadelphia, PA 19104-6323, USA
[b] Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, PA 19104-6323, USA

ARTICLE INFO

Article history:
Received 17 September 2008
Revised 1 October 2008
Accepted 3 October 2008
Available online 10 October 2008

Keywords:
Taxane
Paclitaxel C-10 carbamate
Blood–brain barrier
Mouse brain perfusion
Neurodegenerative diseases

ABSTRACT

We report the synthesis and blood–brain barrier (BBB)-permeability of ^{14}C-CNDR-29, a paclitaxel C-10 carbamate derivative shown to be devoid of P-glycoprotein (Pgp)-interactions, in an in situ mouse brain perfusion model, in comparison with ^{14}C-paclitaxel. The results presented reveal a 3- to 4-fold higher BBB-permeability for the C-10 modified taxane compared to paclitaxel. These results support the notion that circumvention of Pgp-mediated efflux can lead to higher BBB-permeability. Further studies however are needed to evaluate the therapeutic potential of the C-10 carbamates paclitaxel derivatives for the treatment of CNS diseases.

Over the past several years, there has been growing interest in the development of brain-penetrant microtubule stabilizing (MT)-agents for the treatment of different central nervous system (CNS) conditions, including cancer as well as non-proliferative diseases such as Alzheimer's disease (AD) and related disorders.[1] Among the various classes of MT-stabilizing natural products, taxanes have been arguably one of the most intensely studied.

Indeed, paclitaxel[2] (Fig. 1), the first chemotherapeutic MT-stabilizing agent to receive FDA approval for the treatment of cancer, was found to be a potential clinical candidate for other disorders, including neurodegenerative diseases.[3] However, the issue of limited brain uptake due to poor blood–brain barrier (BBB)-permeability prevented further development of this compound for CNS indications. Paclitaxel, as well as several related compounds, is known to be a good substrate for the ATP-binding cassette (ABC) active transporter P-glycoprotein (Pgp),[4] which is highly expressed in the kidneys, liver, intestine and the BBB.[5] Previous studies[6,7] have demonstrated that Pgp-knockout mice exhibit higher paclitaxel cerebral-uptake compared to wild type animals and that pharmacological modulation of Pgp in wild type mice can increase paclitaxel brain levels by 3- to 6-fold compared to animals non-treated with Pgp-modulators. Furthermore, unlike control animals, when Pgp-knockout mice or wild type mice treated with Pgp-inhibitors were implanted with a tumor in the brain, peripheral administration of paclitaxel was found to produce significant anti-neoplastic effects. Taken together, these findings highlight the importance of Pgp in limiting paclitaxel's entry in the CNS, and suggest that circumvention of Pgp-mediated efflux can produce therapeutically relevant paclitaxel brain levels.[6,8] However, since Pgp-function comprises an important defense mechanism, protecting the CNS from toxic substances, ideal candidate compounds for brain diseases should be able to reach therapeutically effective brain-concentrations without causing a significant disruption in Pgp-function. This is particularly important in the context of chronic neurodegenerative diseases, where lack of Pgp-function, and/or other BBB-dysfunctions, have been clearly linked to the pathology of these diseases.[9,10]

Importantly, recent studies have shown that selected chemical modifications at the C-10 position of taxanes can result in analogues that are neither substrates nor inhibitors of Pgp.[11,12] These

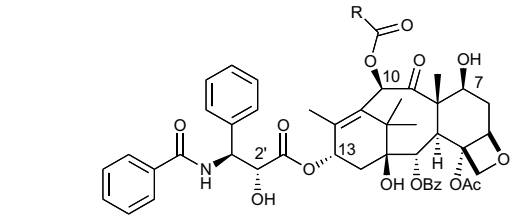

Figure 1. Paclitaxel and representative structures of C-10 modified derivatives known to be devoid of Pgp-interactions.

* Corresponding author.
E-mail address: bcarlo@sas.upenn.edu (C. Ballatore).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.10.024

compounds are typified by the C-10 succinimidyl ester TX-67 (Fig. 1),[13] which notably was found to have an apparent permeability coefficient 6–10 times higher than paclitaxel, in a rat brain perfusion model.[11] Other examples of C-10 modified taxanes lacking Pgp-interactions include a series of C-10 carbamates (e.g., CNDR-3 and CNDR-29, Fig. 1) which were found to be comparatively more stable than C-10 esters like TX-67, and therefore potentially more desirable for further clinical development.[12] Preliminary SAR for the C-10 carbamate series suggested that efficient circumvention of Pgp-mediated efflux in vitro could generally be achieved by derivatives bearing a polar moiety, typically a carboxylic acid moiety, at the C-7 or C-10 position. Interestingly, among the C-10 carbamates found to be devoid of Pgp-interactions, CNDR-29 was the only example that lacked the carboxylic acid residue. This relatively more lipophilic compound was found to be more cell-permeable than the corresponding carboxylic acid containing analogues, as suggested by cell-cytotoxicity studies.[12] Thus, because of the more favorable combination of lipophilicity and lack of Pgp-interactions, CNDR-29 was selected for BBB-permeability studies in a mouse brain perfusion model. Towards this end [^{14}C]-labeled CNDR-29 was prepared and tested in comparison with [^{14}C]-paclitaxel.

[^{14}C]-CNDR-29 was prepared by reacting an appropriately protected paclitaxel-10-deacetyl-10-carbonyl-*N*-methylimidazol-inium iodide salt **A**[14] with [1,2 ^{14}C]-ethanolamine (5–10 mCi/mmol), to furnish carbamate **B**, which was directly treated with HF/Py obtaining [^{14}C]-CNDR-29 (8.5 mCi/mmol; Fig. 2).[15] The brain perfusion experiment[16] was conducted in triplicate by infusing 0.1 μCi of the test compound (or [^{14}C]-paclitaxel), in 500 μL of buffer, into the carotid artery of an appropriately prepared mouse over a 60-s time period. Following the infusion, the vasculature was washed for an additional 60 s with buffer containing non-radiolabeled compound and finally the animal was sacrificed and the brain collected for quantification of radioactivity in a scintillation counter. A brain perfusion experiment was also conducted with [^{14}C]-sucrose to verify the physical integrity of the BBB.

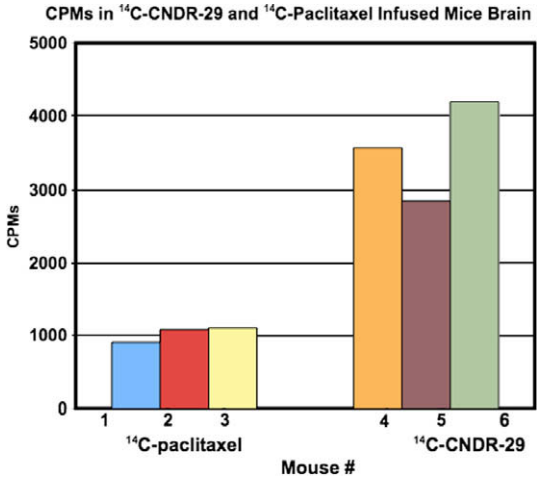

Figure 3. CPMs in [^{14}C]-CNDR-29 and [^{14}C]-paclitaxel infused mice brain.

The results, summarized in Figure 3, reveal that the total counts observed in brain samples of [^{14}C]-CNDR-29-treated mice was approximately 3–4 times higher than that found in the corresponding paclitaxel-treated animals, demonstrating that CNDR-29 exhibits higher BBB-permeability than paclitaxel, and thus suggesting that CNDR-29 may be able to reach comparatively higher brain levels than paclitaxel, after peripheral administration. It should be noted, however, that brain perfusion models are relatively controlled experimental systems that do not consider other potentially limiting factors such as metabolism and plasma protein binding, which may greatly influence the overall brain uptake. Thus, although these results clearly suggest a higher permeability for CNDR-29, further studies are needed to evaluate whether the observed relative increase of 3- to 4-fold in BBB-permeability in the in situ mouse brain perfusion model may translate into thera-

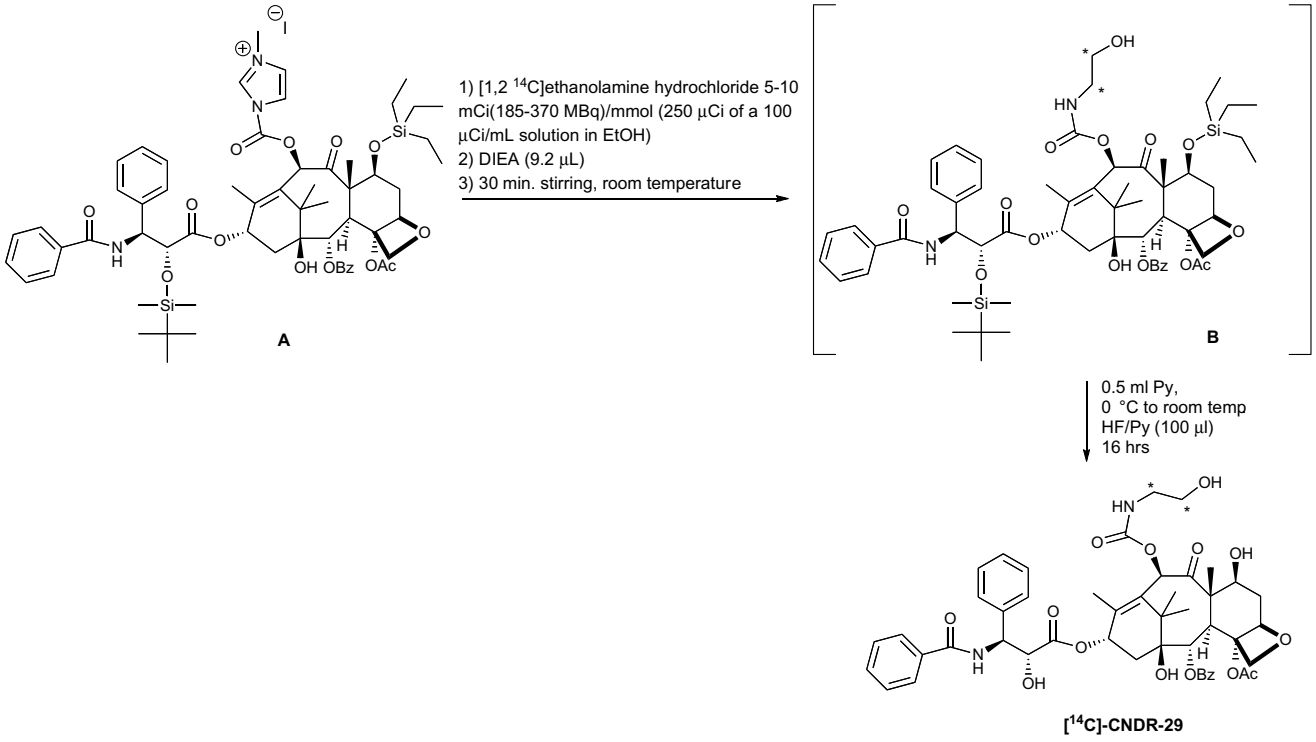

Figure 2. Synthesis of [^{14}C]-CNDR-29. [^{14}C]-labels are indicated with an asterix.

peutically useful brain concentrations after peripheral administration. Nonetheless it is interesting to note that, bearing in mind the experimental differences, these brain perfusion data appear to be in agreement with reports that co-administration (ip) of paclitaxel with a range of Pgp-modulators produce a relative increase of 3–6 times in paclitaxel mouse brain concentrations.[6,8] These results are also consistent with the notion that circumvention of Pgp-mediated efflux can lead to higher BBB-permeability. Finally, it should be noted that the prototype compound TX-67, under similar brain perfusion experimental conditions, was reported to have an even more pronounced relative enhancement in BBB-permeability (6- to 10-fold) compared to paclitaxel. The relatively higher BBB-permeability of TX-67 may support the hypothesis of the involvement of an active transport mechanism (e.g., monocarboxylic acid transporters).[11] To evaluate this possibility, and in order to select the most plausible candidate compound(s) for efficacy studies in a transgenic animal model of neurodegenerative taoupathies, a comparative evaluation in mouse PK models of C-10 modified paclitaxel analogues, including TX-67, as well as other MT-stabilizing agents from different classes of natural products is ongoing in our laboratories.

Acknowledgments

Financial support for this work was provided by the NIH (U01 AG029213). We thank Professor Donna Huryn and Dr. Hugo Geerts for helpful discussions.

References and notes

1. Trojanowski, J. Q.; Smith, A. B., III; Huryn, D.; Lee, V. M.-Y.. *Expert Opin. Pharmacother.* **2005**, *6*, 683.
2. Rowinsky, E. K.; Donehower, R. C. *N. Engl. J. Med.* **1995**, *332*, 1004.
3. Zhang, B.; Maiti, A.; Shively, S.; Lakhani, F.; McDonald-Jones, G.; Bruce, J.; Lee, E. B.; Xie, S. X.; Joyce, S.; Li, C.; Toleikis, P. M.; Lee, V. M.-Y.; Trojanowski, J. Q. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 227.
4. Sparreboom, A.; van Asperen, J.; Mayer, U.; Schinkel, A. H.; Smit, J. W.; Meijer, D. K. F.; Borst, P.; Nooijen, W. J.; Beijnen, J. H.; van Tellingen, O. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2031.
5. Girardin, F. *Dialogues Clin. Neurosci.* **2006**, *8*, 311.
6. Kemper, E. M.; van Zandbergen, A. E.; Cleypool, C.; Mos, H. A.; Boogerd, W.; Beijnen, J. H.; van Tellingen, O. *Clin. Cancer Res.* **2003**, *9*, 2849.
7. Gallo, J. M.; Li, S.; Guo, P.; Reed, K.; Ma, J. *Cancer Res.* **2003**, *63*, 5114.
8. Fricker, G.; Miller, D. S. *Pharmacology* **2004**, *70*, 169.
9. Cirrito, J. R.; Deane, R.; Fagan, A. M.; Spinner, M. L.; Parsadanian, M.; Finn, M. B.; Jiang, H.; Prior, J. L.; Sagare, A.; Bales, K. R.; Paul, S. M.; Zlokovic, B. V.; Piwnica-Worms, D.; Holtzman, D. M. *J. Clin. Invest.* **2005**, *115*, 3285.
10. Zlokovic, B. V. *Neuron* **2008**, *57*, 178.
11. Rice, A.; Liu, Y.; Michaelis, M. L.; Himes, R. H.; Georg, G. I.; Audus, K. L. *J. Med. Chem.* **2005**, *48*, 832.
12. Ballatore, C.; Hyde, E.; Deiches, R. F.; Lee, V. M.-Y.; Trojanowski, J. Q.; Huryn, D.; Smith, A. B., III *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3642.
13. Yanbin Liu, F.; Ali, S. M.; Boge, T. C.; Georg, G. I.; Victory, S.; Zygmunt, J.; Marquez, R. T.; Himes, R. H. *Comb. Chem. High Throughput Screen.* **2002**, *5*, 39.
14. Ballatore, C.; Aspland, S. E.; Castillo, R.; Desharnais, J.; Eustaquio, T.; Sun, C.; Castellino, A. J.; Smith, A. B., III *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2477.
15. Detailed experimental procedure for the synthesis of [^{14}C]-CNDR-29: to a previously prepared imidazolinium iodide salt **A** (14.4 mg, 0.011 mmol) in a 20 mL scintillation vial (screw cap) fitted with a magnetic stir bar, an addition of [1,2 ^{14}C]-ethanolamine hydrochloride (250 μCi; 5–10 mCi/mmol; 0.0333 mmol in 2.5 mL of ethyl alcohol; Moravek Biochemicals and Radiochemicals) is made using a disposable 5 mL syringe, followed by an addition of diisopropylethyl amine (DIEA; 9.2 μL; ~0.066 mmol). The reaction vessel is then capped and stirred for 30 min at room temperature to give compound **B**, which is directly used for the next step without any purification or work-up. Thus, the crude reaction mixture is diluted with 0.5 mL of pyridine, cooled to 0 °C, and finally added with 100 μL of HF/pyridine. The reaction mixture is then allowed to stir for 16 h (overnight) allowing the temperature to rise to room temperature. After 18 h the reaction is quenched by addition of 100 μL of a saturated solution of $CuSO_4$, then the mixture is concentrated via a stream of nitrogen (3–4 h). The residue is re-dissolved in ~5 mL of ethyl acetate and the resulting mixture is washed with 1 mL of a saturated solution of $CuSO_4$ directly in the same scintillation vial used for the synthesis. The top organic layer is then collected with a Pasteur and transferred into a new scintillation vial. After removal of the volatiles via a stream of nitrogen the residue is re-dissolved in the minimal amount of dichloromethane/methyl alcohol 9:1 (ca. 100 μL). Finally, the compound is purified by preparative TLC (dichloromethane/methyl alcohol, 9:1) obtaining the desired compound (4.2 mg; 42% yield; 8.5 mCi/mmol).
16. The brain perfusion experiment was adapted from previously described procedures[17]: brain perfusion experiments were conducted using B6C3 mice. A 0.5-in. incision was made in the skin of anesthetized animals in order to expose the carotid triangle. After separation of superficial structures, such as thyroid and salivary glands, non-blood vessel soft tissue and fat, the carotid sheath was exposed and carefully dissected to expose the internal and external branches of the carotid artery. A single suture was then tied to the external carotid branch, while two additional sutures were set loosely in the proximal and distal region of the common carotid artery, respectively. The proximal ligature was then gently tighten and the distal portion of the artery was clamped off. One end of the P10 tubing was then inserted near the proximal ligature to a depth of ~2 mm, while the other end was connected to a 1-mL syringe filled with saline. The distal clamp was then released and a small amount of saline was injected into the internal branch of the carotid artery to check for signs of leaking or bleeding. The P10 tubing was then connected to a perfusion pump with 1 mL syringe filled with the test compound. The heart was then exposed, stopped and the right atrium cut to allow blood outflow and finally infusion of the test compound was made at a 0.5 mL/min for a total volume of 0.5 mL. An isotonic solution (500 μL saline with 100IU heparin) containing non-radiolabeled test compound was then infused at 0.5 mL/min to rinse the vasculature and then the brain was immediately collected and cut in small pieces which were dissolved in 5 mL of Solvable overnight at 60 °C. The samples were then added with 0.1 mL of 30% H_2O_2 and incubation continued for an additional 30 min until discoloration. A 1 mL of brain sample was then added with 5 mL of Ultima Gold and the radioactivity measured in a scintillation counter.
17. Audus, K.; Rose, J. M.; Wang, W.; Borchardt, R. T. Brain microvessel endothelial cell culture systems. In *An Introduction to the Blood–Brain Barrier: Methodology and Biology*; Pardridge, W. M., Ed.; Cambridge University: Cambridge, 1998; pp 86–93.