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Tocopherol long chain fatty alcohols decrease the production of TNF-α and NO radicals by activated microglial cells

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Abstract—The synthesis of a series of Tocopherol long chain Fatty Alcohols (TFA) and their biological activities on the modulation of microglial activation are described. Specifically, the 2-(12-hydroxy-dodecyl)-2,5,7,8-tetramethyl-chroman-6-ol, the TFA bearing 12 carbon atoms on the side chain ($n = 12$), shows the most potent inhibition of secretion on nitric oxide (NO) and tumour necrosis factor-α (TNF-α) by lipopolysaccharide (LPS)-activated microglia.

1. Introduction

Microglial cells, the brain resident monocyte–macrophage cell population, derive from the bone marrow mononuclear phagocyte cell lineage and enter the brain parenchyma during development. In the normal brain tissue, microgliocytes are found as quiescent cells throughout the brain parenchyma, where they represent around 15% of the cell population.[1] Upon appropriate stimulation, these cells continue the previously halted differentiation process to become immunocompetent phagocytic cells. This type of activation is observed after brain injury or infection, as well as during the development of neuropathies like Alzheimer's disease, stroke or demyelinating diseases such as multiple sclerosis. After a rapid change in cell morphology, activated microgliocytes produce pro-inflammatory cytokine like tumour necrosis factor-α (TNF-α)[2] as well as the free radicals nitric oxide ($NO^{\bullet}$)[3] and superoxide anion ($O_2^{\bullet -}$).[4] These radicals combine to form the very reactive peroxynitrite radical ($ONOO^-$). We have previously shown that the in vitro production of these radicals along with TNF-α secretion induces neurodegenerative events comparable to those observed in Alzheimer's disease.[5] The appearance of phagocytic vesicles finally completes the microglial activation process.[3]

In an attempt to modulate these neurotoxic properties of activated microglial cells, we have undertaken the synthesis and the characterization of the pharmacological properties of small molecules, which are based on a chemical structure likely to present biological activities.

Previous studies have shown the neurotrophic activity of *n*-hexacosanol, a long chain primary alcohol containing 26 carbon atoms. Both, the length of the chain and the ω-hydroxyl function are crucial factors for the biological activity.[6] As we are dealing with neurodegeneration, we are interested in compounds presenting a neuroprotective as well as a neuroregenerative activity. Our approach is the investigation of hybrid compounds combining a neurotrophic ω-alkanol structure and an antioxidant moiety. Former studies led to the synthesis of 3-(15-hydroxy-pentadecyl)-2,4,4-trimethyl-2-cyclohexen-1-one (tCFA-15).[7] This trimethyl cyclohexenonic long chain fatty alcohol induces the differentiation of foetal neurons and neural stem cells.[8]

Here we report the synthesis and biological activities of long chain fatty alcohols bearing an α-tocopherol moiety, the chroman ring as functionalized nucleus in addition to an ω-alkanol side chain. α-Tocopherol, a

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member of the vitamin E family, is the major lipid soluble antioxidant in vivo[9] and has been shown to have neuroprotective effects, which act, at least partially, through suppression of microglial activation.[10]

Here, we report that the in vitro production of nitric oxide and TNF-α by activated microglial cells is modulated by a co-incubation with the tocopherol long-chain fatty alcohols (TFAs).

2. Chemistry

Starting from either commercially available tetradecane-1,14-dioic acid **2c** and oxacycloheptadecan-2-one **2d** or decane-1,10-diol **3a** and dodecane-1,12-diol **3b**, the corresponding TFAs **1a**–**d** are synthesized through a four, respectively, five step procedure.

According to Scheme 1, reduction of diacid **2c** and lactone **2d** with lithium aluminium hydride affords the corresponding alcohols **3c** (80%) and **3d** (95%). Diols **3a**–**d** are then submitted to a monoiodation using a 57% aqueous solution of hydroiodic acid to give ω-iodo-alkanols **4a**–**d** (61–78%), which are readily protected with *tert*-butyldimethylsilyl chloride in the presence of imidazole to provide the corresponding silylated iodo-alkanols **5a**–**d** (96–99%).[11]

The key intermediates **6a**–**d** are obtained in a single step procedure starting from compounds **5a**–**d**. Primary alkyllithiums are prepared by lithium–iodine exchange using *tert*-butyllithium.[12] A subsequent in situ addition of but-3-en-2-one provides the corresponding allylic alcohols **6a**–**d** (45–58%).[13] The chroman moieties are formed racemically at carbon 2 by condensation of the different allylic alcohols with trimethylhydroquinone in the presence of aluminium chloride and nitromethane.[14] Deprotection of the terminal alcohol occurs in situ to afford the 2-(*n*-hydroxy-alkyl)-2,5,7,8-tetramethyl-chroman-6-ols **1a**–**d** (36–53%).

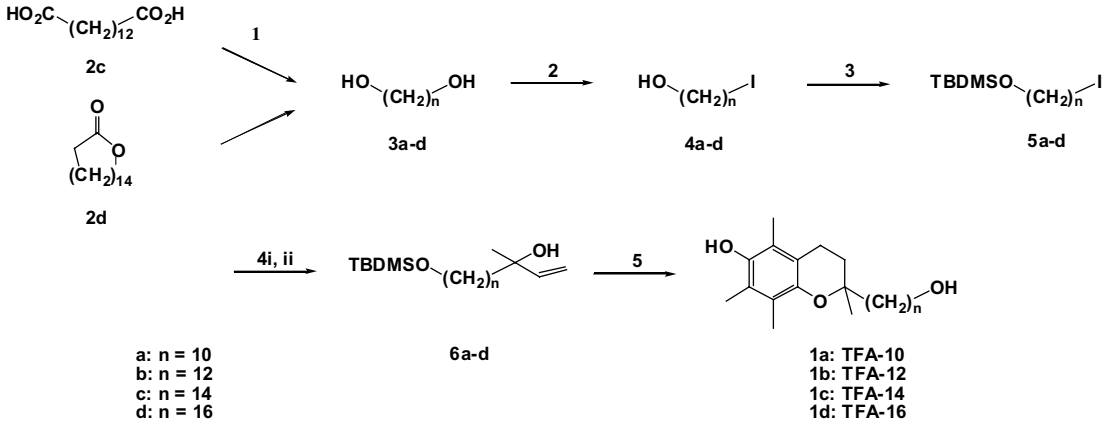
3. Biological activities

The mouse microglial cell line MMGT12 (a generous gift of Dr. E. Vanmechelen, Gent)[15] was activated by exposure to lipopolysaccharide (LPS 0.01 μg/mL) for 24 or 48 h in the presence or absence of different concentrations of TFAs or α-tocopherol. The secretion of TNF-α after 24 h was measured using an ELISA assay (R&D System), the production of nitric oxide after 48 h was monitored by quantifying the nitrite concentration (the final degradation product of NO in buffered solutions) using a microplate adapted assay of the Griess method.[16] The expression levels of the NO-synthase type-II, the enzyme responsible for the induced production of NO, were examined using immunoblotting after SDS-PAGE separation. Major histocompatibility class II molecules expression was examined by flow cytometry, using the rat anti-mouse MHC class II monoclonal antibody IBL-3/5 from Serotec. The secondary fluorescent antibody (goat anti-rat) was also from Serotec.

Figure 1 shows the modulation of the induced TNF-α production after 24 h of exposure to LPS as well as the modulating molecules. Final concentrations of TFAs and α-tocopherol range from 10^{-5} to 10^{-7} M. α-Tocopherol and TFA-10 **1a** show no effect. A slight concentration-dependent inhibition of TNF-α release is induced in the presence of 10^{-5} M TFA-16 **1d**. TFA-12 **1b** and TFA-14 **1c** show a major inhibition of the secretion at 10^{-5} M as well as at 10^{-6} M.

Figure 2 shows the modulation of the nitrite accumulation after a 48 h incubation in the presence of LPS. α-Tocopherol and TFA-10 **1a** show no effect. TFA-16 **1d** shows a mild inhibition at 10^{-6} M. Strong inhibitions are obtained with TFA-12 **1b**, TFA-14 **1c** and TFA-16 **1d** at 10^{-5} M.

The abundance of the enzyme responsible for the NO production, NO-synthase-II (NOS-II) is strongly down regulated by TFA-12 whereas no effect is observed after TFA-10 treatment (Fig. 3).



Scheme 1. General synthesis of TFA's **1a**–**d**. Reagents and conditions: (1) $LiAlH_4$, THF, 0 °C to rt, 15 h, 80–95%; (2) HI aq 57%, toluene, 90 °C, 6 h, 61–78%; (3) TBDMSCl, imidazole, CH_2Cl_2, rt, 3 h, 96–99%; (4) (i) *t*-BuLi, ether, −78 °C, 5 min; (ii) but-3-en-2-one, ether, −78 °C, 15 min, 45–58%; (5) trimethylhydroquinone, $AlCl_3$, nitromethane, CH_2Cl_2, −20 °C to rt, 3 h, 36–53%.

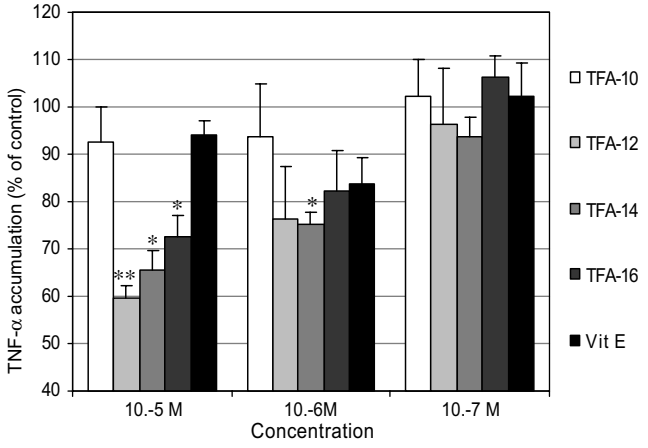


Figure 1. Effect of TFA-10–TFA-16 **1a–d** and vitamin E on TNF-α production of MMGT12 cells in the presence of LPS (0.01 μg/mL). Culture supernatants were collected after a 24 h incubation. TNF-α production data are mean ± SEM (bars) values obtained from three experiments, each conducted in independents cultures ($n = 3$). Results are expressed as a percentage of control (0.01 μg/mL LPS; 100% = 3264 pg/mL TNF-α). *$p < 0.05$, **$p < 0.01$, TNF-α expression levels are significantly different from the corresponding control levels.

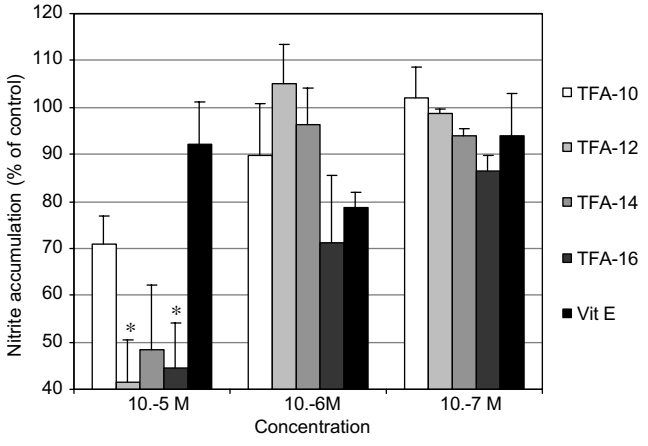


Figure 2. Effect of TFA-10–TFA-16 **1a–d** and vitamin E on NO_2^- accumulation of MMGT12 cells in the presence of LPS (0.01 μg/mL). Culture supernatants were collected after a 48 h incubation. NO_2^- accumulation data are mean ± SEM (bars) values obtained from three experiments, each conducted in independents cultures ($n = 3$). Results are expressed as a percentage of control (0.01 μg/mL LPS; 100% = 8.34 nmol/mL NO_2^-). *$p < 0.05$, NO_2^- expression levels are significantly different from the corresponding control levels.

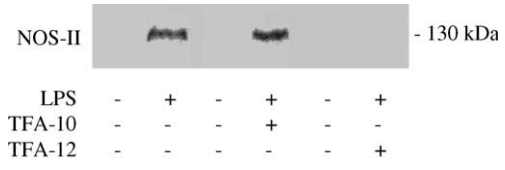


Figure 3. Effect of TFA-10 **1a** and TFA-12 **1b** on NOS type II protein expression of MMGT12 cells in the presence or absence of LPS (0.01 μg/mL). Cell lysates were prepared for immunoblotting after a 24 h incubation.

LPS activation induces a minor rise in the expression levels of the major histocompatibility class II molecules. However, none of the molecules has an effect on the expression levels of MHC class II molecules on the microglial cells (results not shown).

4. Discussion and conclusion

The microglial cells are readily activated after in vitro stimulation with the Gram-negative bacterial wall component lipopolysaccharide (LPS). Besides changing cell morphology, the cells start to release pro-inflammatory products like TNF-α and nitric oxide free radicals, which are known to induce neuronal death.[5] Since epidemiological studies document the importance of these inflammatory components during the course of Alzheimer's disease,[17,18] the search for pharmacological tools to modulate the pro-inflammatory activation of central microglial cells appears as a necessary step towards a more complete treatment of the disease. Two of the molecules of the TFA family appear to be able to influence significantly the activation of the microglial cells. TFA-12 **1b**, TFA-14 **1c** and to a lesser extend TFA-16 **1d** down-regulate two of the activation parameters studied in this work. The activity clearly depends on the length of the side chain, TFA-10 **1a** having no effect and TFA-16 **1d** showing only minor effects. The most potent length seems to be obtained with TFA-12 **1b**.

The chroman ring composed of α-tocopherol, does not appear to be the sole responsible agent for this effect, since under our experimental conditions, α-tocopherol alone has no significant effect on the microglial response.

No effect was observed on the expression levels of the MHC class II molecules. This aspect of our results seems to hint at an effect of the TFA molecules on the signal transduction pathway of the pro-inflammatory stimulations. TNF-α secretion and NOS-II production depend on the NF-*k*B pathway.[19,20] Class II expression however is regulated via different pathways.[21]

TFA molecules modulate, but have no inhibiting effect on the pro-inflammatory activation of microglial cells. Under pharmacological aspects, this property is particularly important. Microgliocytes are important cells in the maintenance of cerebral integrity. These cells play a crucial role in the clearance of amyloid-β depositions during the course of Alzheimer's disease.[3] A complete inhibition of their activation would therefore most probably present a deleterious effect on the course of the disease. Further studies on the biological properties, and especially on the signalling cascades affected, of the molecules of the TFA family are necessary to fully characterize their pharmacological potentials.

In a first attempt, we decided to synthesize racemic TFAs because most synthetic supplements of α-tocopherol used in the food industry are a mixture of eight possible stereoisomers arising from three chiral centres. On the other hand, the side chains bear an even number of carbon atoms because most naturally occurring

aliphatic chains derive from acetyl-CoA (two carbon atoms) condensation.

Nonetheless, it would be interesting to synthesize TFAs with a fixed configuration at carbon atom 2 and/or an odd number of carbon atoms at the side chain. This would allow comparing TFA-11 and TFA-13 with the most active compound TFA-12 **1b** and determining whether there is a difference in the modulation of microglial activation depending on the enantiomer at carbon atom 2.

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References and notes

1. Dickson, D. W.; Lee, S. C.; Mattiace, L. A.; Yen, S. H.; Brosnan, C. *GLIA* **1993**, *7*, 75.
2. Lee, S. C.; Liu, W.; Roth, P.; Dickson, D. W.; Berman, J. W.; Brosnan, C. F. *J. Immunol.* **1993**, *150*, 594.
3. Weldon, D. T.; Rogers, S. D.; Ghilardi, J. R.; Finke, M. P.; Cleary, J. P.; O'Hare, E.; Esler, W. P.; Maggio, J. E.; Mantyh, P. W. *J. Neurosci.* **1998**, *18*, 2161.
4. Colton, C. A.; Gilbert, D. L. *FEBS Lett.* **1987**, *223*, 284.
5. Hemmer, K.; Fransen, L.; Vanderstichele, H.; Vanmechelen, E.; Heuschling, P. *Neurochem. Int.* **2001**, *38*, 557.
6. Borg, J.; Toazara, J.; Hietter, H.; Henry, M.; Schmitt, G.; Luu, B. *FEBS Lett.* **1987**, *213*, 406.
7. (a) Girlanda-Junges, C.; Keyling-Bilger, F.; Schmitt, G.; Luu, B. *Tetrahedron* **1998**, *54*, 7735; (b) Luu, B.; Schmitt, G.; Keyling, F.; Girlanda, C.; Yamada, M.; Suma, Y. Int. Patent: WO 99/08987, February 25, 1999; (PCT/JP98/03560); (c) Girlanda-Junges, C.; Lutz-Bucher, B.; Gonzalez de Aguilar, J.; Loeffler, J.; Luu, B. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2537; (d) Luu, B.; Gonzalez de Aguilar, J.; Girlanda-Junges, C. *Molecules* **2000**, *5*, 1439.
8. Luu, B.; Mohier, E.; Suzuki, H.; Suma, Y.; Yamada, H. Int. Patent: WO 02/29014, April 11, 2002; (PCT/JP01/08136).
9. (a) Lei, H.; Atkinson, J. *J. Org. Chem.* **2000**, *65*, 2560; (b) Pope, S.; Burtin, G.; Clayton, P.; Madge, D.; Muller, D. *Bioorg. Med. Chem.* **2001**, *9*, 1337.
10. Li, Y.; Liu, L.; Barger, S.; Mrak, R.; Griffin, S. *J. Neurosci. Res.* **2001**, *66*, 163.
11. Goulaouic-Dubois, C.; Guggisberg, A.; Hesse, M. *Tetrahedron* **1995**, *51*, 12035.
12. (a) Bailey, W.; Punzalan, E. *J. Org. Chem.* **1990**, *55*, 5404; (b) Negshi, E.; Swanson, D.; Rousset, C. *J. Org. Chem.* **1990**, *55*, 5406.
13. Cheng, D.; Knox, K.; Cohen, T. *J. Am. Chem. Soc.* **2000**, *122*, 412.
14. Cohen, N.; Scott, J.; Bizzaro, F.; Lopresti, R.; Eichel, W.; Saucy, G. *Helv. Chim. Acta* **1978**, *61*, 837.
15. Briers, T. W.; Desmaretz, C.; Vanmechelen, E. *Neuroimmunology* **1994**, *52*, 153.
16. Morga, E.; Faber, C.; Heuschling, P. *Neuroimmunology* **1998**, *87*, 179.
17. McGeer, P. L.; McGeer, E. G. *Neurobiol. Aging* **2001**, *22*, 799.
18. Fernandez-Vizarra, P.; Fernandez, A. P.; Castro-Blanco, S.; Encinas, J. M.; Serrano, J.; Bentura, M. L.; Munoz, P.; Martinez-Murillo, R.; Rodrigo, J. *Neurobiol. Dis.* **2004**, *15*, 287.
19. Akama, K. T.; Albanese, C.; Pestell, R. G.; Van Eldik, L. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5795.
20. Nick, J. A.; Avdi, N. J.; Young, S. K.; Lehman, L. A.; McDonald, P. P.; Frasch, S. C.; Billstrom, M. A.; Henson, P. M.; Johnson, G. L.; Worthen, G. S. *J. Clin. Invest.* **1999**, *103*, 851.
21. Figueiredo, F.; Koerner, T. J.; Adams, D. O. *J. Immunol.* **1989**, *143*, 3781.