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The novel steroidal alkaloids dendrogenin A and B promote proliferation of adult neural stem cells

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ABSTRACT

Dendrogenin A (DDA) and dendrogenin B (DDB) are new aminoalkyl oxysterols which display re-differentiation of tumor cells of neuronal origin at nanomolar concentrations. We analyzed the influence of dendrogenins on adult mice neural stem cell proliferation, sphere formation and differentiation. DDA and DDB were found to have potent proliferative effects in neural stem cells. Additionally, they induce neuronal outgrowth from neurospheres during *in vitro* cultivation. Taken together, our results demonstrate a novel role for dendrogenins A and B in neural stem cell proliferation and differentiation which further increases their likely importance to compensate for neuronal cell loss in the brain.

1. Introduction

Neural stem cells are found in the subventricular zone lining the lateral ventricles and in the hippocampus. They are persistent, and have the unique ability to generate neurons in the adult brains of humans and other mammals [1]. The development of cell-based therapies for replacing neurons that have been lost to neurodegenerative diseases has been a central goal for several years [2]. The proliferative capacity of the neural stem cells and their ability to form neurons, astrocytes and oligodendrocytes make them excellent candidates for this purpose [3]. Epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) [4], and neurosteroids such as pregnenolone have been shown to affect neural stem cells in various ways [5]. However, the search of new compounds inducing neural stem cell proliferation and differentiation is a major challenge in the field of regenerative therapies.

Neurosteroids have been suggested to be important for organ development, cell differentiation and cell survival [6]. Furthermore, the neurosteroids and various other oestrogen receptor modulators are present in the extracellular environments of diverse organs, including the brain, cardiovascular system and adipose tissue [7]. It has recently been demonstrated that neurosteroids are key regulators of cholesterol homeostasis [8]. Interestingly, several lines of evidence indicate that cholesterol metabolism influences cell proliferation [9,10,18]. Allopregnanolone was the first neurosteroid that was proven to trigger neuronal proliferation and differentiation in the central nervous system. It has also been shown that the brains of patients with neurodegenerative conditions such as Alzheimer's disease (AD) contain unusually low levels of pregnenolone, the precursor of all neurosteroids [11,12]. Other naturally occurring steroids that have been linked to central nervous system (CNS) disorders include 22R-hydroxycholesterol, 17-β-estradiol, and solasodine [13–15].

Dendrogenins constitute a new family of oxysterol compounds that trigger cell differentiation in various tumor cell lines including tumour cells of neuronal origin [16]. Dendrogenins were named for their ability to induce dendrite outgrowth. Two of the most effective dendrogenins identified to date are 5α-hydroxy-6β[2-(1*H*-imidazol-4-yl)ethylamino]cholestan-3β-ol, which is known as dendrogenin A (DDA), and 5α-hydroxy-6β-[3-(4-aminobutylamino)propylamino]cholest-7-en-3β-ol, or dendrogenin B (DDB) (Fig. 1A). DDA and DDB are formed by the stereospecific coupling of 5,6α-epoxy-cholesterol with histamine and spermidine,

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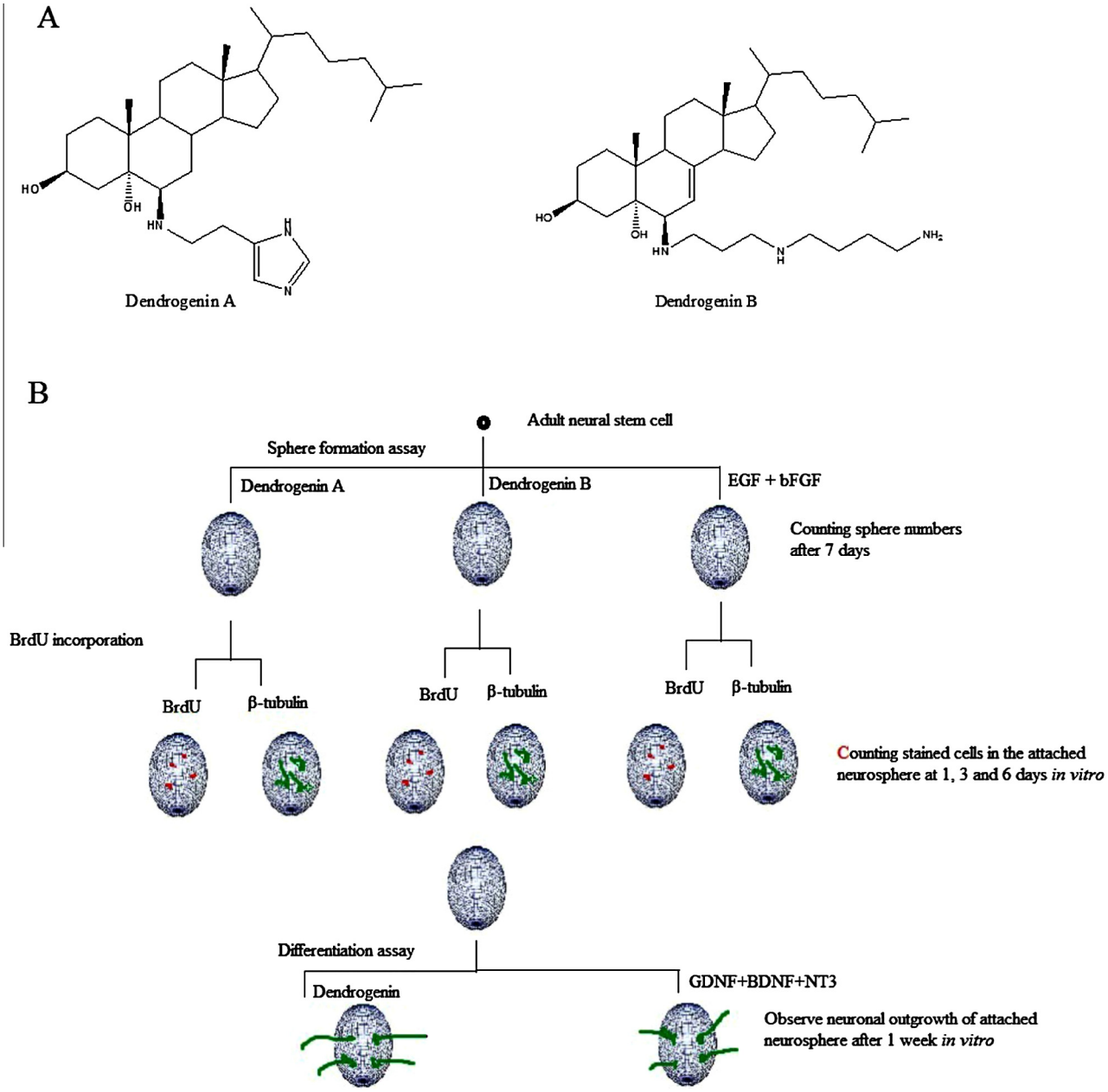

Fig. 1. (A) Chemical structure of dendrogenin A (DDA) and dendrogenin B (DDB). (B) Schematic illustration of the experimental protocol describing the different assays used including the sphere-formation assay, BrdU incorporation assay and differentiation analysis (β-tubulin labeling was used to indicate if the differentiated cells co-localized with BrdU).

respectively, at the C6 position on ring B of the steroid backbone of cholesterol [17]. Because they feature a basic side chain on a steroid backbone, they are steroidal alkaloids. We recently demonstrated that DDA is a natural metabolite of cholesterol that can be found in various mammalian tissues including brain [19]. DDA and DDB exhibit different biological properties. DDA was reported to induce tumour cell differentiation and death in cancer cell lines of different origins [19]. In addition, exposure to DDA and DDB at nanomolar concentrations induced dendrite outgrowth on tumour cells of neuronal origin (SH-SYSY, SK-NS-H, Neuro2A, U87) and triggered the differentiation of pluripotent carcino-embryonic P19 cells into neurons [16]. Interestingly, we found that both DDA and DDB promoted normal motor neuron survival at low doses [16]. As opposed to DDA, redifferentiation activity of DDB was selective to tumour cell lines of neuronal origin stimulated cell survival at concentrations in the micromolar range [16]. This established that the nature of the basic side chain plays a crucial role in determining the specific biological effects of dendrogenins. To date, the effects of dendrogenins have only been examined in tumour cell lines. The aim of the present study is to investigate dendrogenin effects on the growth and differentiation of primary neural stem cell cultures (Fig. 1B).

2. Material and methods

2.1. Preparation of neural stem cells from adult mice

All animal procedures were performed according to the ethical standards of the Karolinska Institute and Sweden's national regulations (approval No. 464/03). Six- to eight-week old mice (female, line C57BL/6; B&K laboratories) were sacrificed by cervical

dislocation. The brain was carefully rinsed in HBSS medium. Two longitudinal cuts were made between the rhinal fissure and hippocampal area. The subventricular zone was exposed and micro-dissected. The subventricular zone layer was incubated for 20 min in HBSS with 2 mM glucose containing 0.7 mg/ml hyaluronic acid, 0.2 mg/ml kynurenic acid and 200 U/ml DNAse (Sigma). Trypsin activity was halted by adding 5 ml of EBSS-BSA-Hepes (0.04 mg/ml BSA) to the cell suspension. This was followed by mechanical trituration and centrifugation at 200*g* for 5 min. Suspensions of dissociated cells were seeded into flasks at densities of 5×10^5/ml. Neurospheres were harvested after 8 days, centrifuged, and dissociated mechanically using a polished Pasteur pipette for further expansion. The culture medium was Eagle's medium/F12 (DMEM/F12; Gibco, MD), containing L-glutamine, antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) and the B27 supplement (Gibco). The cells were cultured at 37 °C in an incubator under a 5% CO_2 atmosphere.

2.2. Neurosphere formation and proliferation

DDA and DDB were synthesized as described previously [16]. Sphere formation assays were performed by seeding neural stem cells at a density of 250,000 cells/well in uncoated 24-well dishes and incubating them for 7 days. The medium was supplemented with or without DDA (10 nM), DDB (10 nM) or growth factors EGF and bFGF (human recombinant, 20 ng/ml; Peprotech). The number of spheres in each well was counted after 6 days of incubation. Bromodeoxyuridine (BrdU) analysis was performed at 1, 3 and 6 days. Briefly, cultures were labelled with BrdU (Zymed, Burlingame, CA) for 18 h prior to plating on poly-L-ornithine coated dishes. BrdU incorporation was counterstained with an anti-β-tubulin antibody. Each experiment was repeated at least 3 times, and triplicate samples were counted for each set of conditions investigated.

2.3. Neurosphere differentiation and immunocytochemistry

The neurospheres were plated on poly-L-ornithine coated 24-well dishes and treated either with neurotrophins (Glial cell-derived neurotrophic factor + Brain-derived neurotrophic factor + Neurotrophin-3; GDNF + BDNF + NT-3, 20 ng/ml) or with 10 nM of DDA or DDB. The medium was changed and the differentiation-promoting factors were replenished every second day. The cell cultures were fixed in 4% paraformaldehyde for 10 min. Neurospheres were examined first with a phase-contrast microscope (Nikon, Tokyo, Japan), after which the immunohistochemical staining was verified using a fluorescence microscope (Zeiss Axiovert, Jena, Germany). Specimens were incubated with a primary antibody against β-tubulin (type III; 1:500 Sigma) overnight at 4 °C, followed by incubation with the Alexa Fluor 488 (Molecular Probes, Eugene, OR). Trypan blue staining was used to determine cell viability.

2.4. Statistical analysis

The cell or sphere counts from different treatment groups were calculated from 8 microscopic observational fields and analysed for each individual experiment (*n*). Statistical comparisons were performed using multiple-factor analysis of variance (two-way ANOVA) followed by a pair-wise Mann–Whitney *U* test.

3. Results

3.1. Neural stem cell proliferation

We first investigated the effect of DDA and DDB on neural stem cell proliferation in comparison to EGF + bFGF. We found that both DDA and DDB induced proliferation at 10 nM as observed to EGF + bFGF treatment (Fig. 2A). The stem cells exhibited some degree of proliferation and expansion in all treatment groups compared to control. The viability of the stem cells was greater than 90% based on Trypan blue staining. Inspection of the neurospheres under a light microscope revealed that they were round and consisted of a variable number of cells (Fig. 2B–D). The number of neurospheres in each well after 6 days of cultivation was counted and the mean number per well was plotted (Fig. 2A). While there was a difference between the sizes of the neurospheres in the EGF + bFGF- and dendrogenin-treated groups, there was no significant difference between the groups in terms of the total sphere count. DDA treatment produced an average of 367.3 ± 19.1 neurospheres ($n = 6$), while DDB treatment yielded 333.6 ± 17.3 and EGF + bFGF treatment yielded 409.3 ± 12.8. The average sphere sizes for the DDA- and DDB-treated cultures were 189.1 ± 24.3 and 192 ± 27.0 μm, respectively, while that for the EGF + bFGF cultures was 301.4 ± 45.9 μm. The average size of the spheres in the negative control cultures was 120.5 ± 16.8 μm.

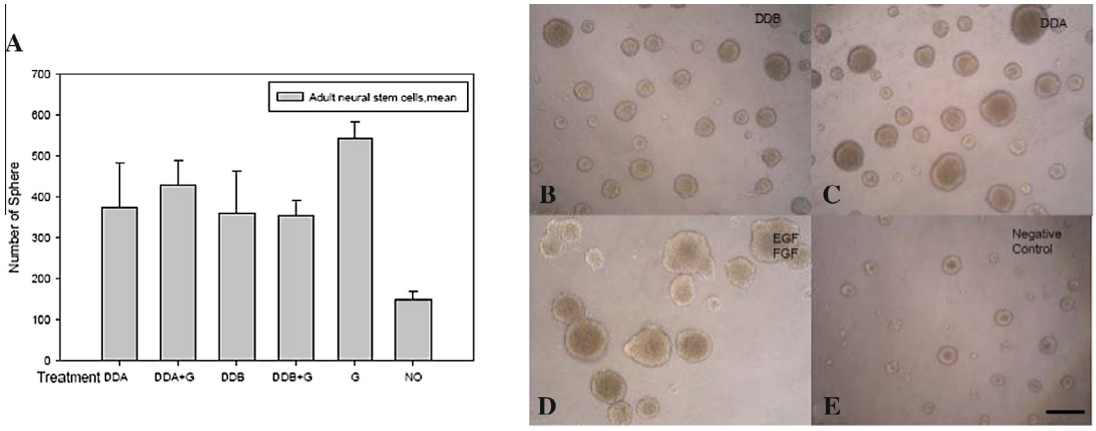

Fig. 2. Quantification of neuronal stem cell-derived sphere with different treatment groups: DDA, DDA + growth factors (DDA + G), DDB, DDB + growth factors (DDB + G), growth factors only (G), and negative controls with no addition (NO). The figures show the number of primary neurospheres formed per well after 6 days in serum-free medium in nonadherent Petri dishes. (A) One experimental data set showing the mean neurospheres number per well for each treatment group. (B–D) Photomicrographs illustrating the appearance of the neurospheres after 6 days in suspension. Note the differences in neurosphere size and number between the various treatments. (B) DDB-treated culture. (C) DDA-treated culture. (D) EGF + bFGF-treated culture. (E) Negative control culture. The scale bar in subfigure E corresponds to a distance of 200 μm and applies to panels (B–D).

3.2. Neural stem cell differentiation

To investigate further the mitogenic effect of dendrogenins on neural stem cells, we performed BrdU incorporation assay. Treatment with 10 nM DDA or DDB increased the number of BrDU positive cells to same extent as growth factors (EGF + bFGF) at days 1, 3 and 6 (Table 1). BrdU-positive cells had round or oval-shaped nuclei and their chromatin was condensed and strongly stained (not shown). The percentage of BrdU-labelled cells fell by 13% between days 1 and 6. BrdU-labelled cells were observed in the cultures shortly after plating and their abundance decreased at day 6 as the extent of β-tubulin staining increased (Table 1). By day 6, there was no difference in the numbers of BrdU-labelled cells in the DDA-, DDB- and EGF + bFGF-treated cultures. This decrease was accompanied by a gradual increase in β-tubulin staining. The number of cells positive for β-tubulin in each culture increased by approximately a factor of two between days 1 and 6 after plating, reaching mean values of 48.2 ± 1, 45.5 ± 1, and 48.3 ± 1 ($n = 6$) for the DDA-, DDB- and EGF + bFGF-treated groups, respectively, at day 6. β-Tubulin labelling was used as a marker of neuronal differentiation in order to confirm the effects of the dendrogenins on the regenerative capacity of the stem cells. At days 1 and 3, more than 60% of the adult neural stem cells that had been treated with dendrogenins or EGF + bFGF were actively dividing, indicating that they had retained their proliferative ability (Fig. 1A). Additionally, more than 40% of the cells were expressing β-tubulin on day 6, revealing that a considerable proportion of the replicated cells became neurons (Table 1).

Table 1
Mean numbers of cells exhibiting BrdU incorporation and β-tubulin labelling. The values are means ± standard deviation for three independent experiments covering the period between days 1 and 6 after plating. In addition, sphere numbers are the mean number of spheres per dish.

	BrdU positive cells			β-Tubulin positive cells			Sphere numbers
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6	Day 6
DDA	76.6 ± 5.3	63.5 ± 3.7	58.5 ± 3.4	30.3 ± 3.1	39.5 ± 3.4	49.9 ± 4.3	367.3
DDB	79.6 ± 5.1	60.2 ± 3.2	62.0 ± 3.7	28.6 ± 3.3	38.6 ± 3.1	42.0 ± 4.6	333.6
Trophic factors	74.8 ± 3.4	70.6 ± 3.7	64.3 ± 5.4	26.5 ± 4.3	38.4 ± 3.4	59.7 ± 3.1	409.5
Control	68.1 ± 3.4	57.2 ± 4.4	41.4 ± 3.8	21.4 ± 3.1	25.7 ± 3.8	18.7 ± 4.3	132.5

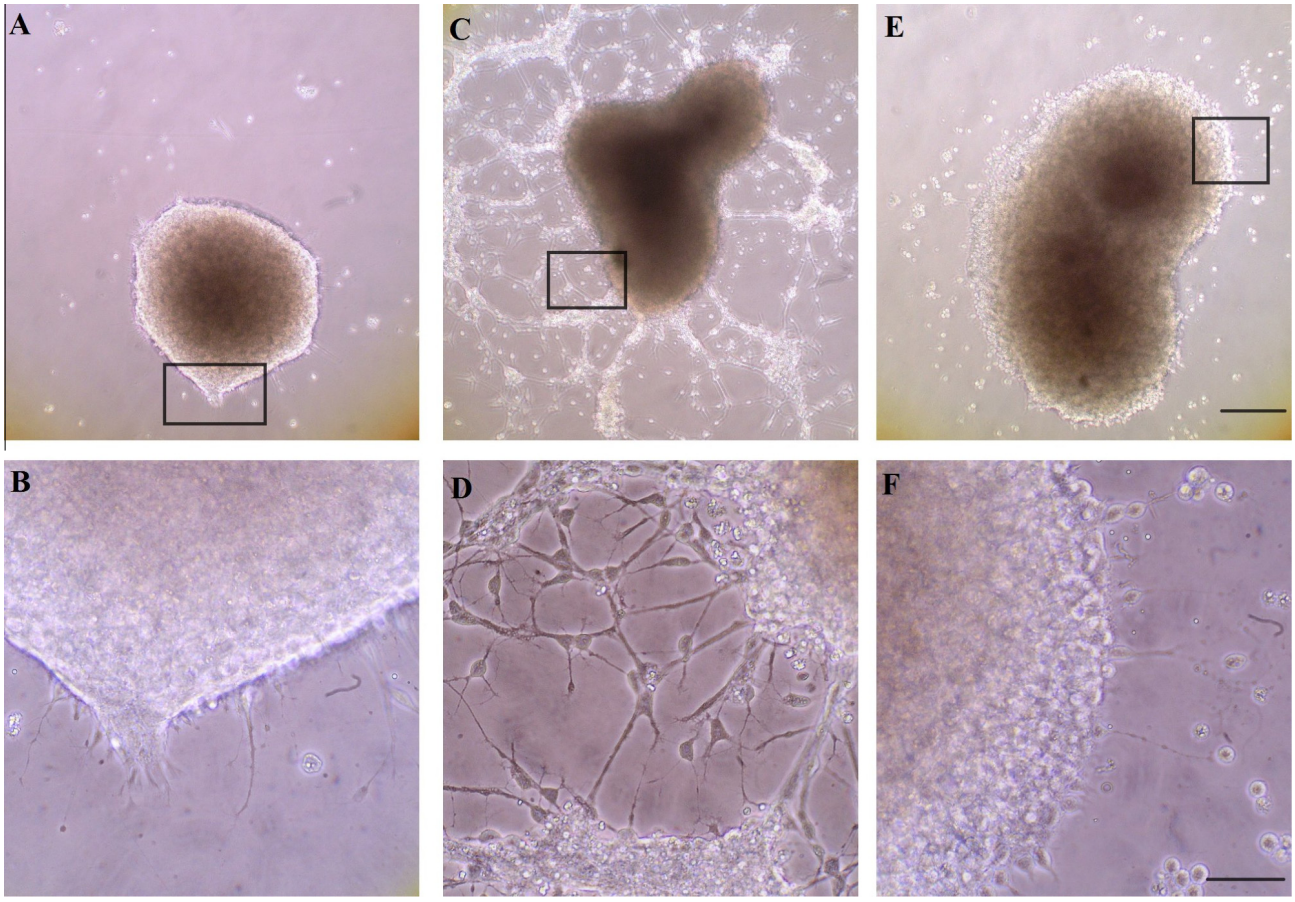

Fig. 3. Light transmission images of neurospheres grown on uniformly coated dishes for 7 days. The spheres were propagated for a week prior to differentiation and then cultured in media containing a dendrogenin (DDB), neurotrophins (BDNF + GDNF + NT-3), or no added substances. (A) The dendrogenin (DDB) treated spheres appear to be intact and compact with little evidence of cell migration and sprouting. (B) A higher magnification view of the area inside the dark rectangle of subfigure A, showing neuronal processes emerging from the neurospheres. Neuronal outgrowth was seen in the vicinity of the sphere zone in the dendrogenin (DDB) treated spheres. (C) Neuron-like cells with fine lengthy processes were abundant in the regions surrounding the neurotrophin-treated spheres. Neurons migrating from the neurotrophin-treated spheres were distributed across the surface. (D) A higher magnification view of the boxed area in subfigure C. (E) No neuronal processes were seen in the vicinity of the untreated neurospheres. (F) A higher magnification view of the boxed area in subfigure E. Images B, D, and F are shown at identical magnification. The scale bar in the upper row of panels (A, C, and E) represents 100 μm while that in the lower row (panels B, D, and F) represents 30 μm.

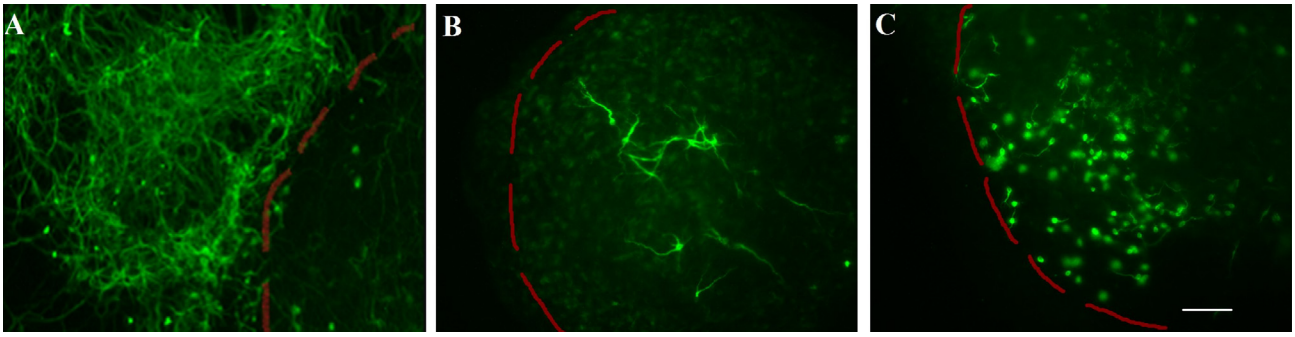

Fig. 4. Staining with the neuronal marker revealed the morphology and distribution of neuron cells and their processes. Neurospheres derived from neural stem cell were allowed to differentiate by culturing them on coated surface for 7 days. After differentiation, neurons appeared inside and outside the neurosphere edges (dotted red line). (A) Fluorescence micrograph of neurotrophin-treated spheres showing a clear differentiation profile and the emission of elongated neuronal processes in the region surrounding the spheres. Note that the cells were immunopositive for β-tubulin (as indicated by their green fluorescence after staining with Alexa Fluor 488). (B) Fluorescence micrograph of dendrogenin-treated spheres (DDB). Neuron-like cells that were immunopositive for the neuron-specific β-tubulin with fine short processes were observed, along with other weakly-stained cells. The neurons were randomly distributed and did not protrude from the sphere zone. (C) β-Tubulin positive cells were located in the core of the neurosphere and did not invade or emerge from the non-treated sphere border. Note the number and distribution of the neuron-like cells in the different groups. The scale bar in panel (C) corresponds to a distance of 30 μm and also applies to panels (A) and (B).

3.3. Neurosphere differentiation

To further explore the regenerative capacity of dendrogenin on the stem cells, the neurospheres were allowed to attach and grow in differentiation medium supplemented with either dendrogenin or a cocktail of neurotrophins (GDNF + BDNF + NT3; Fig. 1B). Phase contrast microscopy was used to assess the viability of the neurospheres and to determine their outgrowth patterns in both groups. The mean process number for the negative control group was 2.5 ± 0.4 cells with neurite with an average length of 8.8 ± 2.6 μm based on a total of 102 neurons from 28 observational fields. The mean number of processes for the neurotrophin-treated group was 20.4 ± 6.6, average length of 36.7 ± 11.8 μm based on a total of 247 neurons from 28 observational fields. These data confirmed that peptidic neurotrophins induced neurite outgrowth in neurospheres (Fig. 3) consistently with data from the literature [20]. Interestingly 10 nM treatment of neurospheres with DDB induced neurite outgrowth and sprouting. The degree of neural outgrowth observed among dendrogenin-treated spheres was lower than that for those treated with neurotrophins (Fig. 3). While neuronal processes were observed to protrude from the edge of the sphere, they did not sprout extensively or disrupt the sphere architecture with DDB. These findings were verified by fluorescence microscopy, which revealed that the dendrogenin-treated spheres contained fewer and shorter neurons within the sphere zone. The mean number of neuronal processes for the dendrogenin treated group was 7.7 ± 2.1, average length of 17.2 ± 5.4 μm based on a total of 116 neurons from 28 observational fields. Finally, the β-tubulin antibody stained the neuron-bearing spheres evenly and specifically. In addition, the dendrogenin-treated neurospheres remained in a relatively condensed form after a week in culture. Conversely, the neurotrophin-treated β-tubulin-positive neurons formed processes that extended an appreciable distance beyond the sphere zone. In general, the dendrogenin-treated spheres had lower neuron numbers, shorter neuron lengths, and lower neuron densities than those treated with neurotrophins at each time point, while the negative control neurospheres exhibited only limited neuronal outgrowth (Fig. 4).

4. Discussion

The sphere-formation assay results clearly indicate that DDA and DDB act as proliferative factors, causing the expansion of adult neural stem cells in culture in a similar way to the commonly used trophic factors EGF and bFGF. However, we did not observe any additive effect on proliferation in cultures treated with EGF + bFGF and either DDA or DDB. To confirm that the increased sphere formation was due to stem cell proliferation, BrdU (a thymidine analogue) was added 18 h prior to fixation. Subsequent fluorescent examination showed that most of the cells within each culture were labelled with BrdU, indicating that the spheres contained dividing cells. By quantifying the number of BrdU-positive cells, it was shown that the presence of DDA and DDB caused the percentage of cells incorporating BrdU to be maintained at a relatively high level compared to the untreated controls. The plated cells exhibited strong BrdU incorporation between days 1 and 3, but BrdU incorporation declined between days 3 and 6 due to the transition from proliferating cells to postmitotic neurons. This decline was accompanied by an increase in the expression of the neuronal marker β-tubulin. These results do not necessarily indicate that the dendrogenins caused significant increases in the number of stem cells, but it is clear that the dendrogenins affected the maintenance of neural progenitor cells and their self-renewal properties.

Treatment with 10 nM of DDA and DDB induced neuronal outgrowth from plated neurospheres. Although β-tubulin immunohistochemistry indicated the positive expression of a neuronal marker in the differentiated neurospheres, the outgrowing neurons did not exhibit dendritic projection, arborisation or sprouting outside the sphere zone. It has previously been shown that neurotrophin treatment increase neuronal survival, outgrowth, and sprouting [21], and that neurotrophins such as GDNF, BDNF and NT3 improve neuronal recovery both *in vitro* and *in vivo* [22]. It appears that dendrogenins did modulate the neurogenic properties of the neurospheres but that their effects on neuronal outgrowth at a concentration of 10 nM were less potent than those of GDNF, BDNF and NT3. Previous studies have shown that dendrogenins have powerful regenerative effects on different neuronal cell lines. The effects of DDA and DDB have previously been investigated in tumour cell lines, which are more commonly used to test the biological activity of new drugs. The adult neural stem cells used in this study represent a more appropriate *in vitro* model for targeting studies related to regenerative strategies.

There is considerable scientific and clinical interest in identifying external factors that can be used to promote neural stem cell division and the differentiation of stem cells into neurons [23]. In recent years, a number of important breakthroughs have been made in this area, raising hopes that stem cell-based therapies could be developed to restore brain functions that have been lost due to neurodegeneration. However, the development of effective and safe regeneration treatments will require the ability to precisely control the differentiation, proliferation and survival of

neural stem cells. Neurosteroids are known to have neuroprotective effects and to rescue cells from programmed cell death [24–26]. It has been demonstrated that their receptors are expressed in the epithelial and nonepithelial cells, including neurons [27], and they share cellular signalling pathways with other trophic factors [28–30], which further increases their likely importance.

Our results demonstrate that DDA and DDB treatment causes increased proliferation in the neural stem cell pool. The effects of DDA and DDB on neurosphere formation and proliferation were comparable to those of EGF + bFGF. The fact that dendrogenin treatment increased the incidence of BrdU incorporation and sphere formation suggests that dendrogenins can act as mitogens in neural stem cells as well as the conventional growth factors. The effects of dendrogenins on stem cell regeneration are consistent with the results presented by Brinton [31], which showed that neurosteroids induce cytoarchitectural regression in cultured fetal hippocampal neurons. However, further studies will be required to fully understand this effect.

Dendrogenins could provide powerful therapeutic options for the treatment of neurogenic disorders i.e., Alzheimer's disease, Parkinson's disease and Huntington's disease in which the establishment of committed neural stem cells with a high proliferative capacity and increased neurogenesis would be desirable. Lost or damaged brain cells do not normally regenerate *in vivo*, but they have been regenerated *in vitro*. It is possible that the successful regeneration of even a small percentage of nerve fibers might restore functions lost due to neurodegenerative diseases. The major challenge in the development of such therapies will be to ensure that the cells used to achieve this replacement, i.e., the neural stem cells, divide in the same way as they would in undamaged tissue. In this context, it would clearly be desirable to investigate the interactions between dendrogenins and the stem cells that naturally exist in the brain. We have demonstrated that dendrogenin treatment at a concentration of 10 nM induces proliferation and neuronal outgrowth in such cells. However, further *in vivo* studies will be required to determine the mechanism of action and to identify optimal doses and methods of dendrogenin application in living tissue.

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References

[1] L.P. Deleyrolle, B.A. Reynolds, Isolation, expansion, and differentiation of adult mammalian neural stem and progenitor cells using the neurosphere assay, Methods Mol. Biol. 549 (2009) 91–101.
[2] F. Barnabé-Heider, J. Frisén, Stem cells for spinal cord repair, Cell Stem Cell 3 (2008) 16–24.
[3] S. Weiss, B.A. Reynolds, A.L. Vescovi, C. Morshead, C.G. Craig, D. van der Kooy, Is there a neural stem cell in the mammalian forebrain?, Trends Neurosci 19 (1996) 387–393.
[4] E. Moyse, S. Segura, O. Liard, S. Mahaut, N. Mechawar, Microenvironmental determinants of adult neural stem cell proliferation and lineage commitment in the healthy and injured central nervous system, Curr. Stem Cell Res. Ther. 3 (2008) 163–184.
[5] R.C. Melcangi, P. Froelichsthal, L. Martini, A.L. Vescovi, Steroid metabolizing enzymes in pluripotential progenitor central nervous system cells: effect of differentiation and maturation, Neuroscience 72 (1996) 467–475.
[6] P. Taupin, Nootropic agents stimulate neurogenesis. Brain Cells, Expert Opin. Ther. Pat. 19 (2009) 727–730.
[7] J.L. Turgeon, M.C. Carr, P.M. Maki, M.E. Mendelsohn, P.M. Wise, Complex actions of sex steroids in adipose tissue, the cardiovascular system, and brain: insights from basic science and clinical studies, Endocr. Rev. 27 (2006) 575–605.
[8] S. Chen, J.M. Wang, R.W. Irwin, J. Yao, L. Liu, R.D. Brinton, Allopregnanolone promotes regeneration and reduces β-amyloid burden in a preclinical model of Alzheimer's disease, PLoS One 6 (2011) 24293.
[9] M.R. Paillasse, P. de Medina, G. Amouroux, L. Mhamdi, M. Poirot, S. Silvente-Poirot, Signaling through cholesterol esterification: a new pathway for the cholecystokinin 2 receptor involved in cell growth and invasion, J. Lipid Res. 50 (2009) 2203–2211.
[10] S. Silvente-Poirot, M. Poirot, Cholesterol metabolism and cancer: the good, the bad and the ugly, Curr. Opin. Pharmacol. 12 (2012) 673–676.
[11] J.C. Naylor, J.D. Kilts, C.M. Hulette, D.C. Steffens, D.G. Blazer, J.F. Ervin, J.L. Strauss, T.B. Allen, M.W. Massing, V.M. Payne, et al., Allopregnanolone levels are reduced in temporal cortex in patients with Alzheimer's disease compared to cognitively intact control subjects, Biochim. Biophys. Acta 1801 (2010) 951–959.
[12] O. Lazarov, M.P. Mattson, D.A. Peterson, S.W. Pimplikar, H. van Praag, When neurogenesis encounters aging and disease, Trends Neurosci. 33 (2010) 569–579.
[13] Z.X. Yao, R.C. Brown, G. Teper, J. Greeson, V. Papadopoulos, 22R-hydroxycholesterol protects neuronal cells from beta-amyloid-induced cytotoxicity by binding to beta-amyloid peptide, J. Neurochem. 83 (2002) 1110–1119.
[14] W. Mayo, V. Lemaire, J. Malaterre, J.J. Rodriguez, M. Cayre, M.G. Stewart, M. Kharouby, G. Rougon, M. Le Moal, P.V. Piazza, D.N. Abrous, Pregnenolone sulfate enhances neurogenesis and PSA-NCAM in young and aged hippocampus, Neurobiol. Aging 26 (2005) 103–114.
[15] L. Lecanu, A.I. Hashim, A. McCourty, I. Giscos-Douriez, I. Dinca, W. Yao, S. Vicini, G. Szabo, F. Erdélyi, J. Greeson, V. Papadopoulos, The naturally occurring steroid solasodine induces neurogenesis *in vitro* and *in vivo*, Neuroscience 183 (2011) 251–264.
[16] P. de Medina, M.R. Paillasse, B. Payré, S. Silvente-Poirot, M. Poirot, Synthesis of new alkylaminooxysterols with potent cell differentiating activities: identification of leads for the treatment of cancer and neurodegenerative diseases, J. Med. Chem. 52 (2009) 7765–7777.
[17] M. Poirot, S. Silvente-Poirot, Cholesterol-5,6-epoxides: chemistry, biochemistry, metabolic fate and cancer, Biochimie 95 (2013) 622–631.
[18] P. de Medina, M.R. Paillasse, G. Segala, M. Poirot, S. Silvente-Poirot, Identification and pharmacological characterization of cholesterol-5,6-epoxide hydrolase as a target for tamoxifen and AEBS ligands, Proc. Natl. Acad. Sci. U S A 107 (2010) 13520–13525.
[19] P. de Medina, M.R. Paillasse, G. Segala, M. Voisin, L. Mhamdi, F. Dalenc, M. Lacroix-Triki, T. Filleron, F. Pont, T.A. Saati, et al., Dendrogenin A arises from cholesterol and histamine metabolism and shows cell differentiation and anti-tumour properties, Nat. Commun. 4 (2013) 1840–1850.
[20] B.Y. Chen, X. Wang, Z.Y. Wang, Y.Z. Wang, L.W. Chen, Z.J. Luo, Brain-derived neurotrophic factor stimulates proliferation and differentiation of neural stem cells, possibly by triggering the Wnt/β-catenin signaling pathway, J. Neurosci. Res. 91 (2013) 30–41.
[21] S.B. Rangasamy, K. Soderstrom, R.A. Bakay, J.H. Kordower, Neurotrophic factor therapy for Parkinson's disease, Prog. Brain Res. 184 (2010) 237–264.
[22] N. Weishaupt, A. Blesch, K. Fouad, BDNF: the career of a multifaceted neurotrophin in spinal cord injury, Exp. Neurol. 238 (2012) 254–264.
[23] B.A. Reynolds, R.L. Rietze, Neural stem cells and neurospheres: re-evaluating the relationship, Nat. Methods 2 (2005) 333–336.
[24] J.M. Wang, P.B. Johnston, B.G. Ball, R.D. Brinton, The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression, J. Neurosci. 25 (2005) 4706–4718.
[25] R.D. Brinton, J.M. Wang, Preclinical analyses of the therapeutic potential of allopregnanolone to promote neurogenesis *in vitro* and *in vivo* in transgenic mouse model of Alzheimer's disease, Curr. Alzheimer Res. 3 (2006) 11–17.
[26] S.C. Correia, R.X. Santos, S. Cardoso, C. Carvalho, M.S. Santos, C.R. Oliveira, P.I. Moreira, Effects of estrogen in the brain: is it a neuroprotective agent in Alzheimer's disease?, Curr Aging Sci. 3 (2010) 113–126.
[27] D. Le Menuet, S. Viengchareun, M. Muffat-Joly, M.C. Zennaro, M. Lombès, Expression and function of the human mineralocorticoid receptor: lessons from transgenic mouse models, Mol. Cell Endocrinol. 217 (2004) 127–136.
[28] M.A. Arevalo, M. Santos-Galindo, M.J. Bellini, I. Azcoitia, L.M. Garcia-Segura, Actions of estrogens on glial cells: implications for neuroprotection, Biochim. Biophys. Acta 1800 (2010) 1106–1112.
[29] A.A. Pieper, S. Xie, E. Capota, S.J. Estill, J. Zhong, J.M. Long, G.L. Becker, P. Huntington, S.E. Goldman, C.H. Shen, et al., Discovery of a proneurogenic, neuroprotective chemical, Cell 142 (2010) 39–51.
[30] F.D. Porter, G.E. Herman, Malformation syndromes caused by disorders of cholesterol synthesis, J. Lipid Res. 52 (2011) 6–34.
[31] R.D. Brinton, The neurosteroid 3 alpha-hydroxy-5 alpha-pregnan-20-one induces cytoarchitectural regression in cultured fetal hippocampal neurons, J. Neurosci. 14 (1994) 2763–2774.