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Lack of support for bexarotene as a treatment for Alzheimer's disease

Eugene O'Hare [a, *], Ross Jeggo [b], Eun-Mee Kim [c], Bridgeen Barbour [a], Jean-Sebastien Walczak [d], Philip Palmer [a], Taylor Lyons [a], Deaglan Page [a], Donncha Hanna [a], Jolyon R. Meara [e], David Spanswick [b, d, f], Jian-Ping Guo [g], Edith G. McGeer [g], Patrick L. McGeer [g], Peter Hobson [e]

[a] School of Psychology, Queen's University, University Road, Belfast BT7 1NN, Northern Ireland, UK
[b] Neurosolutions Limited, P.O. Box 3517, Coventry CV4 7ZS, UK
[c] School of Psychology, University of Ulster, Cromore Road, Coleraine BT52 1SA, UK
[d] Cerebrasol Limited, P.O. Box 63534, Montreal Quebec H3W1JO, Canada
[e] Institute of Primary Care and Public Health, Cardiff University CF10 3XQ, UK
[f] Department of Physiology, Monash University, Clayton VIC 3800, Australia
[g] Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver V6T1Z3, BC, Canada

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ABSTRACT

Bexarotene has been reported to reduce brain amyloid-β (Aβ) levels and to improve cognitive function in transgenic mouse models of Alzheimer's disease (AD). Four groups failed to fully replicate the primary results but the original authors claimed overall support for the general conclusions. Because of its potential clinical importance, the current work studied the effects of bexarotene using two animal species and highly relevant paradigms. Rats were tested for the ability of bexarotene to prevent changes induced by an Aβ challenge in the form intracerebroventricular (i.c.v) administration of 7PA2 conditioned medium (7PA2 CM) which contains high levels of Aβ species. Bexarotene had no effect on the long-term potentiation of evoked extracellular field excitatory postsynaptic potentials induced by i.c.v. 7PA2 CM. It also had no effect following subcutaneous administration of 2, 5, 10 and 15 mg/kg on behavioral/cognitive impairment using an alternating-lever cyclic-ratio schedule of operant responding in the rat. The effects of bexarotene were further tested using the APPSwFILon, PSEN1*M146L*L286V transgenic mouse model of AD, starting at the time Aβ deposits first begin to develop. Mice were sacrificed after 48 days of exposure to 100 mg bexarotene per day. No significant difference between test and control mice was found using a water-maze test, and no significant difference in the number of Aβ deposits in cerebral cortex, using two different antibodies, was apparent. These results question the potential efficacy of bexarotene for AD treatment, even if instigated in the preclinical period prior to the onset of cognitive deficits reported for human AD.

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1. Introduction

Cramer et al. (2013) reported that bexarotene (Bex) administration rapidly decreased $A\beta_{1-40}$ and $A\beta_{1-42}$ interstitial fluid levels in the brains of two month old PPswe/PS1Δe9 (APP/PS1) transgenic (Tg) mice – a well-studied Tg mouse model of Alzheimer's disease (AD). The effect was seen within 6 h of administration, and there was a 25% reduction of Aβ by 24 h. A single dose of 100 mg/kg orally was reported to maintain the effect for 70 h, and a similar outcome was found in normal (non-Tg) C57B1/6 mice. It was further reported that acute administration of Bex resulted in the rapid elimination of both diffuse and compact Aβ plaques in the cortex

* Corresponding author.Tel.: +44 (0)2890 975445.
E-mail addresses: e.ohare@qub.ac.uk (E. O'Hare), r.jeggo@neurosolutionsltd.com (R. Jeggo), e.kim@ulster.ac.uk (E.-M. Kim), bmcmahon09@qub.ac.uk (B. Barbour), jswalczak@cerebrasol.com (J.-S. Walczak), ppalmer01@qub.ac.uk (P. Palmer), tlyons03@qub.ac.uk (T. Lyons), d.page@qub.ac.uk (D. Page), donncha.hanna@qub.ac.uk (D. Hanna), jolyon.meara@wales.nhs.uk (J.R. Meara), david.spanswick@monash.edu (D. Spanswick), mcgeerpl@mail.ubc.ca (P.L. McGeer), peter.hobson@wales.nhs.uk (P. Hobson).

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and hippocampus of the APP/PS1 mice. In 11 month old APP/PS1 mice, Bex administration for 7 days produced significantly reduced levels of soluble and insoluble $A\beta_{1-40}$ and $A\beta_{1-42}$, resulting in a 50% reduction in Aβ plaque numbers. Behaviorally, Bex was reported to restore cognition and memory in APP/PS1, APPPS1-21 and Tg2576 Tg mice. In addition, 90 days of Bex administration in APP/PS1 mice, and 20 days of administration in APPPS1-21 mice induced improved hippocampal function, as measured using a contextual fear conditioning task and a water maze task. Also, nest construction in the Tg2576 Tg mouse was restored after 72 h of Bex administration, and odor habituation behavior was improved after 9 days of Bex administration when given to 12 and 14 month old mice. These findings implied that even acute administration of Bex to AD patients might be effective in treating both the early and late stages of AD.

Four independent research groups have already questioned these findings. Fitz et al. (2013), while confirming the observation of reversal of memory deficits and the decrease in interstitial fluid Aβ levels following Bex administration, found no effect on brain Aβ deposition. Veeraraghavalu et al. (2013) also found no effect of Bex in reducing the Aβ plaque burden in APP/PS1, 5XFAD, and APPPS1-21 mice. Price et al. (2013), using an almost identical treatment regimen to Cramer et al. (2013) were unable to find any evidence for positive effects of Bex. Tesseur et al. (2013) using mice and dogs, also could not fully replicate the reported effects of Bex administration. All of these studies involved administering single doses or very short term daily doses of Bex by oral gavage. Shortly after, a further group using an almost identical treatment regimen was unable to find any evidence for positive effects of Bex (LaClair et al., 2013). In response to these reports, the original authors stated their data "replicate and validate our central conclusion that bexarotene stimulates the clearance of soluble β-amyloid peptides and results in the reversal of behavioral deficits in mouse models of Alzheimer's disease" (Landreth et al., 2013).

Because of the obvious lack of unambiguous confirmation of the effects of Bex relative to the treatment of AD, and the potential that the original report might have for clinical applications, we have further investigated the effects of Bex using more thorough paradigms. Two animal species and different but highly relevant models of AD were employed. One of the species utilized was normal male Sprague–Dawley rats. We administered into freely moving normal rats conditioned medium (CM) from 7PA2 cells by intracerebroventricular (i.c.v.) injection. 7PA2 CM expresses human amyloid precursor protein and secretes Aβ oligomers, dimers, trimers and tetramers (Podlisny et al., 1995, 1998; Walsh et al., 2002; Walsh et al., 2005). The CM contains a total human Aβ concentration in the $pg.ml^{-1}$ range, which is similar to that found in human CSF (Podlisny et al., 1998). The i.c.v. 7PA2 CM injection model has consistently been shown to impair LTP in experimental animals (e.g., Walsh et al., 2002; Townsend et al., 2006; O'Hare et al., 2013), and is therefore highly suitable to test any ameliorative effects of Bex on synaptic transmission. Effects were measured by recording long-term potentiation (LTP) of evoked extracellular field excitatory postsynaptic potentials (fEPSPs) in the CA1 region of hippocampal slices. The i.c.v. 7PA2 CM injection model has also consistently been shown to detrimentally affect memory-related behavior in the rat under an alternating-lever cyclic-ratio (ALCR) schedule of food reinforcement (e.g., Cleary et al., 2005; Poling et al., 2008; Reed et al., 2011). Consequently, this method was used to assess the effect of Bex on behavior/cognition following subcutaneous (s.c.) administration of 2, 5, 10 and 15 mg/kg Bex in the rat.

The second species used was Tg mice. Tg AD mouse models have become a standard for screening potential pharmacological agents to treat AD. They have in common the introduction of one or more transgenes that enhance the production of human Aβ species. In view of the dramatic effects of Bex as reported by Cramer et al. (2013), it seemed appropriate to test the effects of Bex over time using an aggressive Tg mouse model of AD. The Jackson Laboratory Tg model of AD, APPSwFILon, PSEN1*M146L*L286V (Jackson Laboratory, Bar Harbor, ME, USA; B6SJSL) was employed as it is the most aggressive Tg mouse model of AD so far developed. Bex treatment was instigated at 7–8 weeks of age, when Aβ deposits first begin to develop in this model of AD. This would be analogous to treating AD cases in the preclinical phase of the disease, before cognitive deficits appear. Chronic administration of a very high dose of Bex (ca. 100 mg/kg/d) was used, as early treatment with such a high dose would be the best way of determining if Bex could have a preventative effect on Aβ accumulation and subsequent AD pathology. No ameliorative effects of Bex was found in any of the rat models (electrophysiological and behavioral), or mouse models (behavioral and histopathological) employed in the studies conducted.

2. Methods

2.1. 7PA2 cells

7PA2 cells are stably transfected Chinese hamster ovary (CHO) cells which incorporate the cDNA for APP (APP751). This is specific for the familial AD mutation Val1717Phe (Podlisny et al., 1998; Shankar et al., 2011). The cells secrete $A\beta_{1-40}$ and $A\beta_{1-42}$ (Shankar et al., 2011). These cells were grown to just below confluence in DMEM containing 10% FBS and 200 μg/ml G418. They were briefly washed in DPS and incubated at 37 °C with 5% CO_2 for 18 h with a sufficient volume of DMEM to cover the cells. After incubation, the medium was centrifuged at 3000 g for 15 min and either used directly or snap frozen and stored at −20 °C for later use. Using ELISA methodology, the concentration of total Aβ in the 7PA2 CM was in the range of 2–5 nM.

2.2. Animals

All animals were held in *vivaria* with automatically controlled temperature maintained at 23 °C under a 12 h light/dark schedule (lights on at 0800 h). Specific details are given below for each manipulation. The animal experiments were conducted with the approval of the appropriate institutional ethics committees, and approved by the Animal Use Protocol 09-AOU-E-033 Canada, and license from the United Kingdom Home Office.

2.3. LTP

Evoked extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded from 400 μm para-sagittal hippocampal slices prepared from male Sprague–Dawley rats (Charles River, Canada). After a 1 h recovery period at room temperature in artificial cerebrospinal fluid (aCSF; composition in mM: NaCl 127, KCl 1.6, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ and D-glucose 10), the hippocampal slices were transferred to an interface chamber warmed to 30 ± 1 °C and perfused with aCSF. Schaffer collateral fibres were stimulated every 30 s using a concentric bipolar electrode (FHC, USA) and evoked fEPSPs were recorded from the stratum radiatum of the CA1 region of the hippocampus. Stimulation intensity was set to evoke fEPSPs of 30–40% of the maximum amplitude. A 10 min stable baseline was recorded and thereafter the test substances were administered in aCSF for a 30 min period, prior to high frequency stimulation (HFS; 100 Hz for 1 s) of the Schaffer collateral pathway. The test substances used were 7PA2 CM (challenge) and CHO CM (wild-type control). Bex (Sigma, UK) was the test substance and DMSO the control vehicle. Bex was diluted with aCSF immediately prior to application to the hippocampal slices, giving final concentrations of 20 μM Bex and 0.1% DMSO. fEPSPs were recorded for 60 min after HFS stimulation, and the final 10 min of recording (20 sweeps) was used for group comparisons of LTP magnitude using a one-way ANOVA and Dunnett's post hoc test.

2.4. Operant behavior test

Behavioral data were collected using the ALCR schedule. Male Sprague–Dawley rats, weighing 220–250 g at the beginning of the experiment were maintained at 90% of their free-feeding body weights and housed individually with water available ad libitum. The rats were trained and tested in two-lever rat test chambers (Med Associates Inc., USA) enclosed in sound attenuating compartments. Food reinforcers were 45 mg sucrose pellets (BioServ, USA), that were delivered into a tray situated midway between the two operant response levers. The training regimen has been previously reported (Cleary et al., 2005; Reed et al., 2011). Briefly, operant behavioral sessions were conducted 7 d/wk and the rats were trained to press both levers for food reinforcement. Over approximately 20–30 sessions, the ALCR schedule of food reinforcement was introduced. Under this behavioral assay, rats must alternate to the other lever after pressing the currently correct lever a sufficient number of times

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