

13-*cis* Retinoic Acid (Accutane) Suppresses Hippocampal Cell Survival in Mice

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ABSTRACT: Use of the acne drug Accutane (13-*cis* retinoic acid, [13-*cis* RA]) has been associated with severe depression. This association has been considered controversial because no causative link has been found between 13-*cis* RA and this disorder. A recent hypothesis has suggested that atrophy of the hippocampus can result in depression. We now show, in a mouse model, that endogenous RA generated by synthetic enzymes in the meninges acts on hippocampal granule neurons, and chronic (3-week) exposure to a clinical dose of 13-*cis* RA may result in hippocampal cell loss. In humans this may be conjectured to be the mechanism by which Accutane contributes to depression.

KEYWORDS: depression; vitamin A; neurogenesis; dentate gyrus; BrdU; subgranular zone; meninges; RALDH2

INTRODUCTION

Accutane is an effective antiacne drug for which, in the year 2000, nearly 2 million prescriptions were dispensed.¹ The active agent of the drug is 13-*cis* retinoic acid (13-*cis* RA). This is probably metabolized systemically to its all-*trans* isomer and binds to its specific nuclear receptor, which activates the transcription of a large number of genes.² In general, the action of RA is to induce differentiation of immature, proliferating cells to become mature cells.² This function likely results in many of the side effects of Accutane that are directed towards proliferating cells in the adult such as in the skin, gut, and bone.³ One controversial side effect, however, is on behavior. Several reports have suggested a link between Accutane use and severe depression with suicidal ideation.^{3–5} Given that RA's potent effect is on immature cells, it would be predicted that the predominant effect of this drug on the brain would be on the regions of proliferation and neural birth, which are limited to only two regions—the subventricular zones adjacent to the lateral ventricles and the dentate gyrus of the hippocampus. There has been great interest in the events of proliferation and neural birth in the hippocampus, in part because of a recent hypothesis proposing that a breakdown in such processes can contribute to depression.^{6–9} If 13-

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cis RA repressed the number of new neurons born in the hippocampus, this could result in a decline in hippocampal function and, in turn, contribute to depression. In this study we first show that RA is normally synthesized in the hippocampus, where it promotes transcription in the granule cells of the dentate gyrus. Three weeks' exposure to a clinical dose of 13-*cis* RA results in a change in this signal. We go on to show that this same regime of 13-*cis* RA exposure results in a significant decline in the survival of newly born granule cells, suggesting that chronic exposure to 13-*cis* RA may result in hippocampal cell loss.

RESULTS AND DISCUSSION

Hippocampal plasticity is under the control of several hormones that regulate gene transcription via nuclear receptors, including estrogen and the glucocorticoids. RA also regulates gene transcription via members of this receptor family and is probably important for the generation of synaptic plasticity.¹⁰ FIGURE 1a shows that a major source of RA for the hippocampus is likely to be from the RA-synthesizing enzyme RALDH2 that is present in the meninges overlaying the brain adjacent to the ventral (infrapyramidal) blade of the dentate gyrus. This would presumably create an asymmetrical distribution of RA across the hippocampus. This asymmetry can be seen using a transgenic RA reporter mouse line to detect RA-activated transcription in which RA signaling is visualized by expression of the beta-galactosidase reporter gene, identified by detection with a specific antibody (FIG. 1b).¹¹ The endogenous RA signal is predominantly localized to the infrapyramidal blade closest to the meningeal source of RA. Daily exposure to an exogenous source of RA, in the form of Accutane, would be expected to change this pattern. Mice were injected intraperitoneally with 13-*cis* RA at a dose of 1 mg/kg/day, the typical treatment dose used for acne. FIGURE 1c illustrates induction of the RA reporter gene in the dentate gyrus after 3 weeks of 13-*cis* RA treatment. The increase is most pronounced in the suprapyramidal (dorsal) blade of the dentate gyrus, changing the balance of RA signaling with a more uniform RA reporter response in the blades.

What are the consequences of this change in RA signaling after exposure to 13-*cis* RA? RA regulates neural differentiation in the developing CNS² and may be expected to normally control neurogenesis in the hippocampus. Excess amounts of RA would be predicted to deregulate these processes. We investigated the effects of 3 weeks' RA treatment on the survival of hippocampal cell precursors. These cells were labeled at the beginning of the 3-week period with bromodeoxyuridine

FIGURE 1. Normal RA signaling in the dentate gyrus of the hippocampus and its change as a result of 13-*cis* RA exposure. One source of RA for the hippocampus is likely to be meninges, which express the RA synthesizing enzyme RALDH2, as detected by immunohistochemistry (a). Normal RA signaling in the hippocampus is localized to the neurons of the dentate gyrus, as indicated by the induction of a RAREhsplacZ RA reporter gene, and is predominantly in the infrapyramidal blade, identified using antibodies against the beta-galactosidase RA reporter protein (b). The greater induction in this lower blade is likely due to the source of RA from RALDH2 in the meninges below. After 21 days of injection of 13-*cis* RA the number of cells in the dentate gyrus that express beta-galactosidase (indicating RA signaling) increased and was more uniformly distributed between the two blades (c).

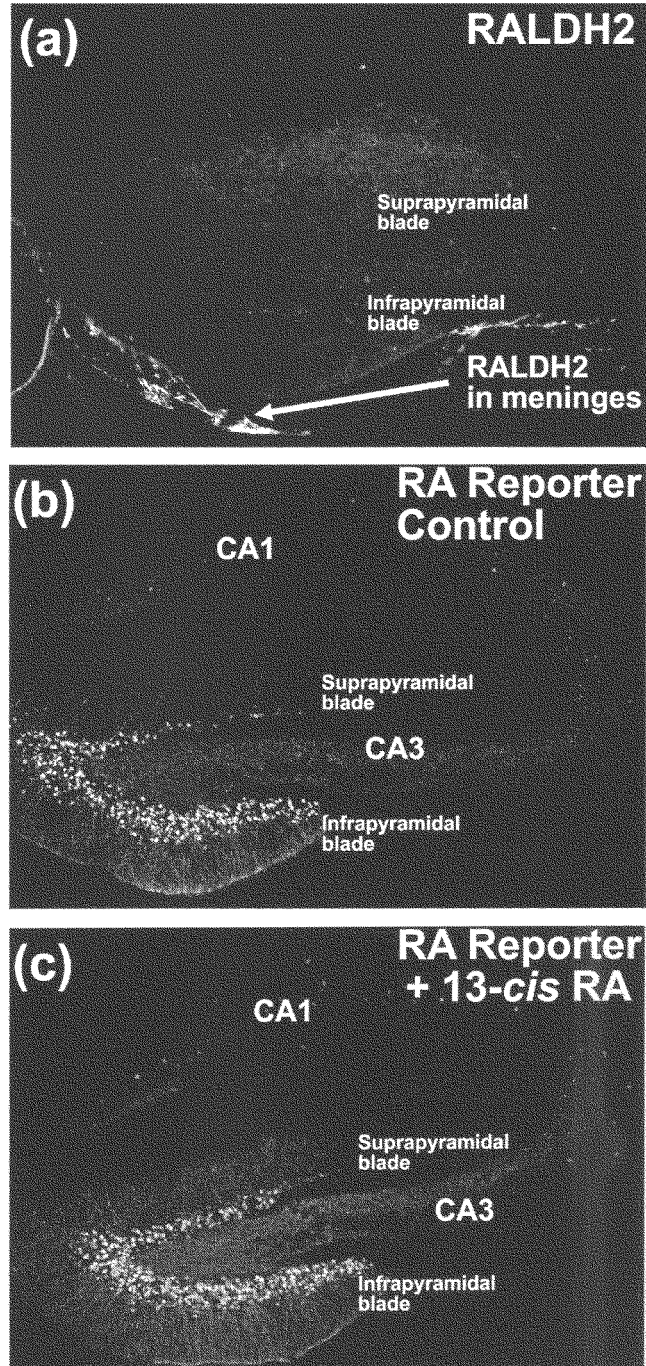


FIGURE 1. See previous page for legend.

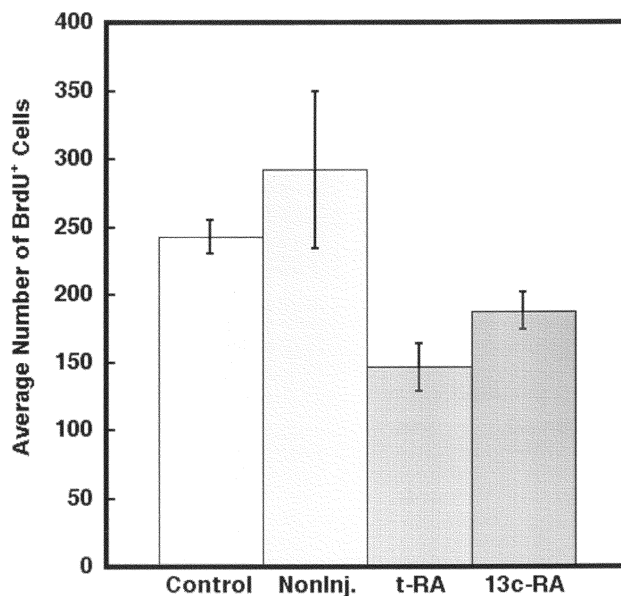


FIGURE 2. The decline in number of BrdU-positive cells remaining in the hippocampus and hippocampal SGZ after 21 days of exposure to 13-*cis* or all-*trans* RA. If proliferating cells are labeled with BrdU and RA treatment is initiated and continued over 21 days, then a significant reduction (23–40%) in the average number of BrdU-positive cells is evident throughout the hippocampal formation with either the all-*trans* or 13-*cis* isomer of RA. It is likely that both isomers act to reduce hippocampal cell survival via the same mechanism utilizing the RA receptor, implying that 13-*cis* RA is isomerized to the all-*trans* isomer that binds to the receptor to activate transcription. Average number of BrdU-positive cells \pm standard error of the mean are given below for each group. Control (vehicle-injected) = 243 ± 13 , noninjected control = 292 ± 58 , tRA = 146 ± 18 , $t = 4.56$, $P = 0.011$ compared to control (vehicle-injected) by two-tailed t -test 13*cis*-RA = 188 ± 14 , $t = 2.9$, $P = 0.044$ compared to control (vehicle-injected) by two-tailed t -test.

(BrdU),¹² and each day the animals were vehicle injected (50% DMSO, 50% saline), noninjected, or injected with all-*trans* or 13-*cis* RA. In humans, Accutane (13-*cis* RA) is used orally between 0.5–2.0 mg/kg/day over a 4-month treatment period, predominantly in a teenage population in whom the rate of neurogenesis would be predicted to be relatively high.¹³ To parallel these conditions, our studies were performed on young adult CD-1 mice, an age and strain with relatively high levels of neurogenesis.^{13,14} After 3 weeks the number of BrdU-labeled cells were counted throughout the hippocampal formation in every 12th 40- μ m section, taking the average of 3 mice per group (see FIG. 2). Although there was a slight decrease in survival over 3 weeks in the control-injected animals versus uninjected animals, the average number of BrdU-labeled neurons was comparable. However, both all-*trans* and 13-*cis* isomers of RA significantly decreased the number of surviving cells by 40% and 23%, respectively. Because all-*trans* RA is the isomer that activates the RA

receptors, this result suggests that 13-*cis* RA is isomerized to the all-*trans* isomer to have its suppressive effect on cell survival.

In short, our results suggest that RA may be a local regulator of cell birth in the hippocampus. Excess RA, in the form of Accutane (13-*cis* RA) or all-*trans* RA, reduces the survival of new cells born in the dentate gyrus of the hippocampus. The increasing evidence that deficits in hippocampal neurogenesis play a role in the etiology of depression^{6–9} implies that deregulation of the normal pathway of RA signaling may be an underlying cause of Accutane-induced depression.

[NOTE ADDED IN PROOF: Treatment of mice with 13-*cis* RA has recently been shown by Crandall *et al.*¹⁵ to result in a reduction in hippocampal cell proliferation, neurogenesis, and hippocampus-dependent learning.]

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