All-trans retinoic acid induces nerve regeneration and increases serum and nerve contents of neural growth factor in experimental diabetic neuropathy

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Local diminution of the neural growth factor (NGF) contributes to the apparition of diabetic neuropathy. All-trans retinoic acid (RA) increases the expression of neural growth factor and its receptor participating in translation pathways. This study evaluates RA as a treatment of diabetic neuropathy: 120 mice were assigned randomly to 4 groups. Group A (n = 30) was taken as control; group B (n = 30) received 50 mg/kg intraperitoneal streptozotocin (STZ); group C (n = 30) received STZ, and after diabetic neuropathy developed, they were treated with subcutaneous RA 20 mg/kg daily during 60 days; and group D (n = 30) only received RA. Plasma glucose, thermosensitive tests, serum, and the nerve contents of NGF were measured in all animals. Evaluation by electron microscopy was performed in search of morphologic changes secondary to neuropathy and nerve regeneration. Diabetic mice had an increased threshold to pain. Treatment with RA in diabetic mice reverted changes in sensitivity as compared with diabetic mice that received placebo ($P < 0.001$). No differences in pain threshold among controls, RA, and diabetes mellitus (DM) + RA groups were found. Glucose levels were not affected by the treatment with RA. NGF diminished significantly in the sciatic nerve in diabetic mice as compared with controls and with the RA group. Animals with DM + RA had a significant increase of NGF in nerves as compared with the other groups. RA also regressed the ultrastructural changes induced by diabetes that showed increased neural regeneration. RA can revert functional and ultrastructural changes and induce neural regeneration after the establishment of diabetic neuropathy, possibly because of the increased of NGF concentrations in nerve terminals. (Translational Research 2008;152:31–37)

Abbreviations: DM = diabetes mellitus; DRG = dorsal root ganglion; NGF = neural growth factor; PPAR = peroxisome proliferator-activated receptor; RA = retinoic acid; RAR = retinoic acid receptor; RXR = retinoic X receptor; STZ = streptozotocin

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AT A GLANCE COMMENTARY

Background

Retinoic acid may foster the protective effect from NGF and other neutrophines that share the same intracellular pathways related with growth and neuronal survival.

Translation Significance

Our results suggest that RA has a potential therapeutic effect in diabetic neuropathy, which improves physiologic mechanisms such as the stimulation of NGF production.

Neuropathy is the most common complication of diabetes mellitus (DM); it occurs in 60% of patients and affects their quality of life.[1,2] Diabetic neuropathy is characterized by a progressive loss of nervous myelinic and amyelinic fibers. Diabetic neuropathy progresses symmetrically and follows a fiber-length–dependent pattern that affects sensory and autonomic fibers. Patients present pain, atrophic changes, and autonomic disturbances. Multiple physiopathologic mechanisms have been proposed; among these mechanisms are microvascular damage of the nerve, increased activity of aldose reductase, and free radicals generation.[2,3] We have previously reported a decrease of neural growth factor (NGF) in serum related to the severity of neuropathy in human and experimental diabetes models.[4,5] In experimental diabetes models, the administration of NGF in diabetic mice prevents the development of neuropathy; however, its use in diabetic patients is very expensive and has shown discouraging results.[6,7]

All-trans RA is an essential molecule for cellular differentiation and an important morphogen in somatic development by binding to nuclear receptors and transcription complexes. These complexes include a pair of ligand-activated transcription factors, such as the retinoic acid receptor (RAR)–retinoic X receptor (RXR) heterodimer.[8] The heterodimeric pair binds to a DNA sequence called a retinoic acid-response element. Induction or repression of gene transcription requires phosphorylation of these receptors and recruitment of coactivators or corepressors. More than 500 genes, which include cytokines and cytokine receptors, are RA responsive.[9] Multiple studies have shown a supportive effect of RA in axonal growth and neuronal survival, which suggests transcriptional activation of genes for neurotrophins and its receptors.[9-11] Among the genes regulated by RA, 2 of the most important are NGF and its receptor.[12] Additionally, RA has a synergic neuroprotective effect over NGF on intracellular pathways related with neuronal growth and neuronal survival.[10,13,14] However, RA can induce the expression of RARβ.[15] Retinoic ligand activation of RARβ induces gene transcription by interacting with distinct promoter consequences in target genes[16] and promotes functional regeneration of sensory axons in the spinal cord.[17]

The administration of RA in diabetic mice before the development of neuropathy prevents the morphologic and sensitive alterations. Neuropathy represents an injury that causes RA-induced gene transcription and intracellular translocation of retinoid receptors as well as increases transcription of the latter. The role of RA in the injured nervous system is still under investigation, having probably principal roles in neuroprotection and axonal growth, modulation of inflammation, and differentiation.[18] The drastic depletion of NGF in diabetic mice is prevented by the administration of RA, which increased its contents to even higher levels than those found in healthy controls.[19]

MATERIAL AND METHODS

The protocol was approved by the laboratory animals' care and handling review board and conformed to the policies, recommendations, and guidelines of the National Institutes of Health. One hundred and twenty male Swiss albino mice aged 2 months old, with an average weight of 20 g, were used in this study. They were maintained in a climate-controlled room on a 12-h light–dark cycle and were allowed food and water *ad libitum*.

The mice were randomized in 4 groups. Group A (control; n = 30) received intraperitoneal saline solution from days 1 to 5, and then a subcutaneous vehicle (corn oil from days 30 to 90); group B (DM; n = 30) received intraperitoneal 50 mg/kg streptozotocin (STZ; Sigma Chemicals Co. L. St Louis, Mo) during 5 days to induce DM[5,20] and then received corn oil subcutaneously, in the same manner to group A. Group C (DM + RA; n = 30) received STZ as in group B. After sensitive neuropathy was established and confirmed with sensibility tests (30 days after STZ), mice from this group received a daily subcutaneous dose of 20 mg/kg of an all-trans RA isoform (Sigma & Aldrich Parque Industrial, Toluca, Mexico) suspended in corn oil during 90 days. Mice from group D (RA; n = 30) received saline solution from days 1 to 5 and then received 20 mg/kg of all-trans RA subcutaneously during days 30–90.

Glucose levels were measured at the beginning and at the end of the treatment with RA in animals administrated with STZ. The blood sample was obtained from the tail and was quantified with a commercial glucometer and reactive bands (BAYER, Cervantes Saavedra, Mexico). Individual body weight was measured at the beginning and at the end of the RA treatment.

Nociceptive tests were performed on days 30 and 90 (before and after treatment with RA) in all animals, with a tail immersion test. The tail-flick latency of each animal was