

# An aldose reductase inhibitor reverses early diabetes-induced changes in peripheral nerve function, metabolism, and antioxidative defense<sup>1</sup>

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## SPECIFIC AIMS

Aldose reductase inhibitors (ARIs) prevent peripheral nerve dysfunction and morphological abnormalities in animal models of diabetes. However, some experimental intervention studies and clinical trials of ARIs on diabetic neuropathy (DN) appeared disappointing because of either 1) their inadequate design and, in particular, insufficient correction of the sorbitol pathway activity or 2) the inability to reverse established functional and metabolic deficits of DN by AR inhibition in general. Moreover, it has recently been hypothesized that the key physiological role of AR is the detoxification of lipid peroxidation products, and thus inhibition of the enzyme under diabetic conditions could actually be detrimental rather than beneficial. The present study was therefore designed 1) to evaluate whether diabetes-induced neurovascular dysfunction, nerve conduction deficits, and the key metabolic abnormalities can be reversed by treatment with an adequate dose of ARI, i.e., a dose that completely inhibits the increased sorbitol pathway activity, and thus to assess the rationale for continued development of ARIs for treatment of DN; and 2) to identify the relation between AR and oxidative stress in the diabetic nerve by evaluating an ARI on parameters of lipid peroxidation and antioxidative defense.

## PRINCIPAL FINDINGS

### 1. Treatment with an adequate dose of ARI reverses diabetes-induced neurovascular dysfunction and nerve conduction deficits

The experiments were performed in control rats and streptozotocin-diabetic rats treated with or without the ARI sorbinil at 65 mg kg<sup>-1</sup>d<sup>-1</sup>, the dose that completely corrected diabetes-induced increase in the sciatic nerve sorbitol pathway activity, for 2 wk after 4 wk of untreated diabetes. ARI treatment partially corrected diabetes-induced decrease in sciatic endoneurial nutritive blood flow (Fig. 1A) as-

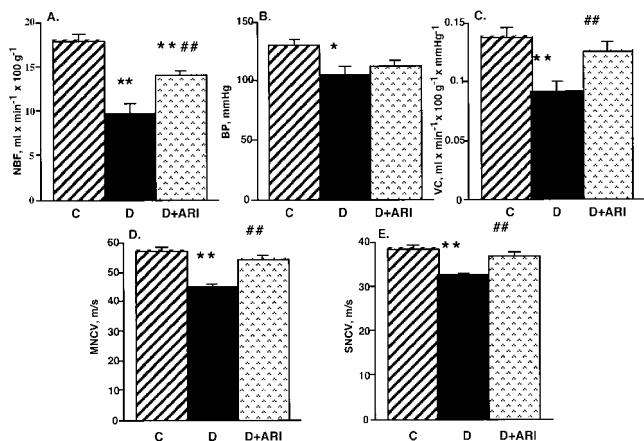
sessed by microelectrode polarography and hydrogen clearance, did not affect mean systemic blood pressure (Fig. 1B), and essentially corrected decreased endoneurial vascular conductance (Fig. 1C) and motor (Fig. 1D) and sensory (Fig. 1E) nerve conduction deficits.

### 2. Treatment with an adequate dose of ARI reverses diabetes-induced key metabolic abnormalities such as mitochondrial and cytosolic NAD<sup>+</sup>/NADH redox imbalances and energy deficiency in peripheral nerve

Free mitochondrial NAD<sup>+</sup>/NADH ratio, a metabolic measure of tissue oxygenation and mitochondrial oxidative capacity, was calculated from the steady-state metabolite concentrations and the equilibrium constant of the  $\beta$ -hydroxybutyrate dehydrogenase system. This ratio was decreased in the sciatic nerve of diabetic rats compared with nondiabetic controls and was partially corrected in the ARI-treated diabetic group (Fig. 2A). In a similar fashion, free cytosolic NAD<sup>+</sup>/NADH ratio calculated from the steady-state metabolite concentrations and the equilibrium constant of the lactate dehydrogenase system was decreased in the sciatic nerve of diabetic rats compared with nondiabetic controls, and this decrease was partially corrected in the ARI-treated diabetic group (Fig. 2B). Administration of ARI to diabetic rats reversed clearly manifested energy failure (decrease in phosphocreatine/creatine ratio) in peripheral nerve (Fig. 2C).

<sup>1</sup> To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.01-0603fje>; to cite this article, use *FASEB J.* (November 14, 2001) 10.1096/fj.01-0603fje

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**Figure 1.** Evaluation of an ARI on diabetes-induced changes in peripheral nerve function. *A*) Sciatic endoneurial NBF. *B*) Blood pressure (BP). *C*) Endoneurial vascular conductance (VC). *D*) MNCV. *E*) SNCV (mean  $\pm$  SE,  $n=10-12$ ). C, controls; D, untreated diabetic group; D+ARI, diabetic group treated with ARI. \* \*\*Significantly different vs. controls ( $P<0.05$  and  $<0.01$ , respectively); ## significantly different vs. untreated diabetic group ( $P<0.01$ ).

### 3. Treatment with an adequate dose of ARI counteracts rather than exacerbates diabetes-induced lipid peroxidation and antioxidant loss in peripheral nerve

Increased nerve lipid peroxidation manifested by accumulation of malondialdehyde plus 4-hydroxyalkenals was revealed in diabetic rats compared with nondiabetic controls, and this activation was completely arrested by an ARI treatment (Fig. 2D). The concentrations of two major nonenzymatic antioxidants, GSH (Fig. 2E) and ascorbate (Fig. 2F) were reduced in the peripheral nerve of diabetic rats compared with nondiabetic controls, and the deficiency in both antioxidants was essentially reversed in the ARI-treated diabetic group.

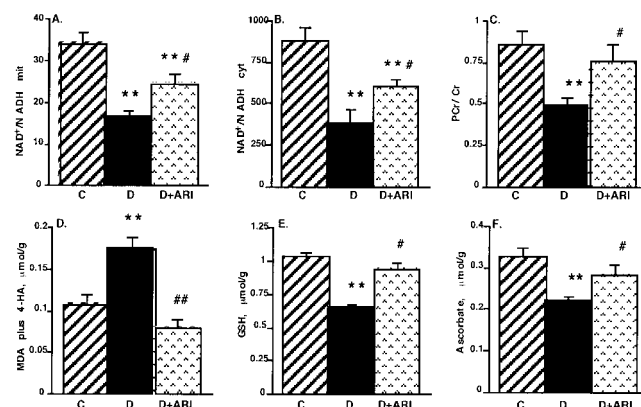
## CONCLUSIONS

The role for AR in the pathogenesis of peripheral DN is supported by at least five lines of evidence: 1) similarity of a number of functional, metabolic, and morphological abnormalities in animal models of diabetes and galactose feeding; 2) demonstration of preventive effects of structurally diverse ARIs on functional, biochemical, and structural changes of DN; 3) potentiation of galactose-induced neuropathy in the transgenic mice expressing human AR and the absence of functional deficits of DN in the AR knockout (AR<sup>-/-</sup>) mice; 4) identification of high AR protein level as an independent risk factor for DN in patients with type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus; and 5) the finding of a 30.2% increase in the frequency of the Z-2 allele of the AR gene, known to be associated with two- to threefold AR expression, in

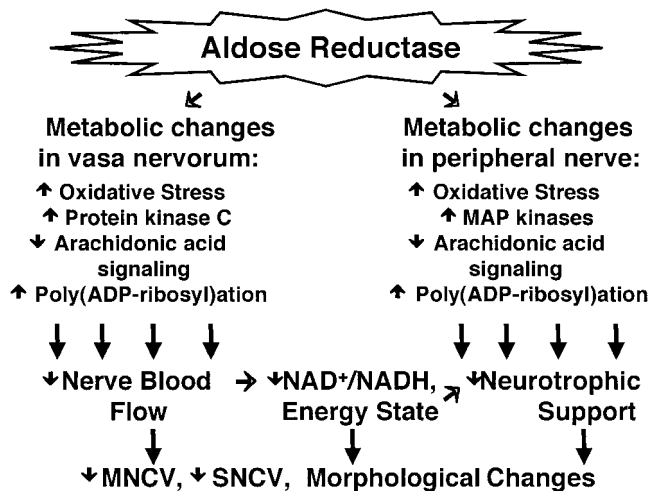
patients with DN compared with uncomplicated group. Despite such strong support of the AR concept of DN from experimental and human studies, the contradictory results of intervention animal studies and the inefficacy of ARIs in clinical trials raised the question of reversibility of existing abnormalities of peripheral DN with AR inhibition. To address this question, we performed experiments with the well-studied ARI sorbinil administered at a dose known to entirely inhibit diabetes-induced increase in the sorbitol pathway activity for 2 wk after 4 wk of untreated diabetes. We have previously demonstrated that the rat model with 3 wk of streptozotocin diabetes displays clearly manifested peripheral nerve blood flow and conduction deficits, NAD<sup>+</sup>/NADH redox imbalances, energy deficiency, and evidence of enhanced oxidative stress.

The present study clearly indicates that treatment with adequate doses of ARIs, i.e., doses that completely inhibit increased sorbitol pathway activity, is the most effective approach for reversal of at least early diabetic neuropathy, and that all major functional (nerve blood flow and conduction deficits) and metabolic (mitochondrial and cytosolic NAD<sup>+</sup>/NADH redox imbalances, energy failure) changes are reversed by an ARI therapy.

In the last several years, the debate about a 'primary mechanism' of diabetic complications has centered on oxidative stress and its relationship with other hyperglycemia-initiated factors. It has recently been suggested that three pathways leading to diabetic complications—increased sorbitol pathway activity, nonenzymatic glycation/glycooxidation, and PKC activation—originate from oxidative stress, particularly production of superoxide anion radicals in mitochondria. This concept, at least the part related to the sorbitol pathway, is not supported by experimental studies



**Figure 2.** Evaluation of an ARI on diabetes-induced changes in the key metabolic variables and enhanced oxidative stress in peripheral nerve. *A*) Free mitochondrial NAD<sup>+</sup>/NADH ratios; *B*) free cytosolic NAD<sup>+</sup>/NADH ratios; *C*) PCR/Cr ratios; *D*) lipid peroxidation product concentrations; *E*) GSH concentrations; *F*) ascorbate concentrations (mean  $\pm$  SE,  $n=6-10$ ). C, controls; D, untreated diabetic group; D+ARI, diabetic group treated with ARI. \* \*\*Significantly different vs. controls ( $P<0.01$ ); #, ## significantly different vs. untreated diabetic group ( $P<0.05$  and  $<0.01$ , respectively).



**Figure 3.** The key primary role for AR in functional, signal transduction, metabolic, neurotrophic, and morphological abnormalities characteristic for peripheral diabetic neuropathy.

demonstrating the absence of any suppression of tissue sorbitol pathway activity by antioxidants, i.e., those neutralizing superoxide anion radicals (DL- $\alpha$ -lipoic acid, taurine, and probucol) in diabetic animal models. Recently, several groups have put forward the hypothesis that the key physiological role of AR is the detoxification of lipid peroxidation products and thus AR inhibition in the diabetic conditions is detrimental rather than beneficial. However, numerous experimental studies with administration of ARIs to control animals did not reveal an appearance of oxidative stress or diabetes-like complications as observed with pro-oxidants. The effects of ARIs and antioxidants on diabetic complications (neuropathy) are unidirectional, i.e., both classes of agents prevent or delay diabetes-associated aberrations in nerve function, metabolism, neurotrophic support, and morphology. The present study clearly demonstrates that treatment with adequate

doses of ARIs counteracts the most important manifestations of oxidative injury, and that in relation to oxidative stress, AR inhibition is beneficial rather than harmful.

In conclusion, diabetes-induced neurovascular dysfunction, nerve conduction deficits, key metabolic changes, and oxidative injury in early DN are reversed by treatment with an adequate dose of ARI, i.e., the dose that completely inhibits the increased sorbitol pathway activity. Our findings support the key primary role for AR in the pathogenesis of DN (Fig. 3) and thus the need for continued development of new potent and well-tolerated ARIs. Analysis of past ARI clinical trials indicates their failure resulted from either inadequate doses and insufficient inhibition of the sorbitol pathway activity or unacceptably high systemic toxicity of the inhibitors rather than inapplicability of the AR concept for DN in humans. Robust inhibition of AR with Zenarestat in diabetic human nerve has yielded dose-dependent efficacy on nerve structure and function; 80% lowering of nerve sorbitol concentration appeared to be required for improvement in nerve electrophysiology and fiber density. Whereas experimental studies in animal models indicate the reversibility of early DN, clinical trials in the U.S. have been performed in patients with nerve structural changes, i.e., far more advanced DN, a stage of the disease that may be highly resistant to any drug therapy. An approach more likely to meet with success may be a prevention or early intervention modeled on the experiments reported here. The ARIs are widely used for treatment of patients with DN in Japan and are prescribed to patients with newly diagnosed diabetes mellitus, together with insulin or hypoglycemic agents, or with early small fiber neuropathy. This approach is justified considering that tight blood glucose control with the complete lack of intermittent hyperglycemia is difficult, often impossible, to achieve and has not been shown to reverse established neuropathy. FJ