Clinical Trial of an Aldose Reductase Inhibitor in Diabetic Neuropathy

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SUMMARY

A single-blind, nonrandomized, placebo crossover clinical trial of an aldose reductase inhibitor, Alrestatin (AY 22, 284) was performed over a 4-mo period in nine patients with diabetic peripheral neuropathy. Most patients had subjective benefit, but objective measures of conduction were essentially unchanged. Substantial toxicity was evident, particularly photosensitive skin rash. DIABETES *30*:459–464, June 1981.

Peripheral neuropathy is a common and disabling complication of diabetes mellitus.[1,2] Of all diabetic complications, it has the most direct relationship with poor glycemic control, and considerable evidence has now emerged to support a metabolic pathogenesis.[1–3]

Typical diabetic peripheral neuropathy is distal, symmetrical, and mixed sensorimotor in type.[2] Both sensory and motor nerve conduction is decreased.[1,4,5] Histologically, segmental demyelination is characteristic[6] but Schwann cell, axonal, and vascular abnormalities are all well described.[1] The primary site of abnormality is not well defined and may be within the Schwann cell, axons, or in the perineural space.[1]

Considerable evidence suggests that improvement in nerve function follows better glycemic control.[1,3,4,7,8] Conduction abnormalities in conduction remain, however,[1,4,7,9] in all but the newly diagnosed diabetics commenced on insulin.[8] Residual defects in conduction may be attributable to deficiencies in the quality of glycemic control ordinarily achieved with conventional diabetic management.[10] Recent advances in monitoring of long-term glycemic control by measurement of glycosylated hemoglobin (GHb)[13] allow clinical trials to be monitored objectively for the quality, and variability in, diabetic control.[14] This is particularly important in diabetic peripheral neuropathy where improved glycemic control is an important variable.[3,4,7,8]

Studies in diabetic and galactose-fed rats have shown that the polyols sorbitol and galactitol accumulate in peripheral nerves and lens, respectively, at levels proportional to the ambient concentrations of blood glucose and galactose.[12,16] Furthermore, administration of Alrestatin, an aldose reductase inhibitor, resulted in lowered polyol levels and retarded development of both cataract and abnormal motor nerve conduction.[12]

Development of orally active inhibitors of aldose reductase have allowed testing of the hypothesis that the metabolic effects of hyperglycemia on nerves are mediated by excessive accumulation of sorbitol and perhaps other polyols.[11,12] A small study of Alrestatin in four patients with severe peripheral neuropathy has appeared.[15]

We report a clinical study of Alrestatin in severe diabetic peripheral neuropathy in nine patients, during which subjective benefit was demonstrated without any improvement in conduction except for one patient. Considerable toxicity, particularly photosensitive skin rash, was evident.

PATIENTS, MATERIALS, AND METHODS

PATIENT SELECTION

Patients had severe typical diabetic peripheral neuropathy.[2] In each case, peripheral neuropathy was present for over 1 yr, and led to severe debilitating pain, regular analgesic usage, and at least one hospitalization before the trial, principally due to symptomatic neuropathy. Diabetics with marked renal failure (creatinine clearance < 40 ml/min) or a history of alcohol abuse, malnutrition, or neurotoxic medication were excluded. Patient data are summarized in Table 1. In addition to peripheral neuropathy, eight had autonomic neuropathy, two had amyotrophy, and one had radiculopathy. Other diabetic complications included retinopathy (8) [background (6) and proliferative (2)], hypertension (3), cataract (2), ischemic heart disease (2), and peripheral vas-

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TABLE 1
Patient characteristics

Patient	Sex	Age	Duration diabetes (yr)	Duration peripheral neuropathy (yr)	Insulin dose (U/day)
HM	M	56	26	1	72
LD	M	70	25	6	56
AF	M	47	40	5	112
CC	M	84	30	1	100
JH	F	35	12	12	36
JB	F	26	6	3	96
EI	F	52	14	9	32
DW	F	42	32	13	60
SD	F	31	11	7	120
Mean ± SEM		49 ± 6	22 ± 4	6.3 ± 1.5	76 ± 11

cular disease (2). None had congestive cardiac failure or cerebrovascular disease.

TRIAL DESIGN

All patients were commenced on the active drug during in-hospital stay, so a randomized design was not possible. A nonrandomized single (patient)-blind, placebo-controlled crossover design was employed. Patients were stabilized on the maximum tolerated dose orally within the first of 3 wk in hospital. After this, a further 5 wk of active drug treatment in outpatient care was followed by an 8-wk placebo period. Before entry into the trial, diabetic control was optimized for at least 4 mo by the referring physicians. After entry into the trial, no specific measures to improve glycemic control were employed. No changes in diet or insulin were initiated by the trial physicians. Preexisting medication [hypertension (2) and recurrent urinary tract infection (1)] were continued without change throughout the trial apart from one patient who ceased fludrocortisone (see below). All used oral analgesics, and a patient record of weekly use was maintained.

Pulse, temperature, blood pressure, and urinalysis were estimated five times daily during the in-hospital phase. Before and during the in-hospital stay, detailed hematologic and serum biochemical screening was performed weekly. Following discharge, these tests were repeated at 8 and 16 wk.

Creatinine clearance, uric acid clearance, and 24 h urinary protein excretion were estimated weekly during the in-hospital phase.

Ophthalmologic assessment (visual acuity, ophthalmoscopy), glycosylated hemoglobin (GHb), full neurologic examination, and nerve conduction studies were performed by the same observers before treatment and at 3, 8, and 16 wk afterward.

During the active drug period, blood samples were taken immediately before next dose to determine steady-state plateau drug levels.

Subjective responses were evaluated by symptoms, and patient records of analgesic usage were examined by the investigators. Responses were recorded as worse (−), no change (0), some improvement (+), marked improvement (++), and return to normal (+++).

Placebo tablets identical in appearance to active drug were administered between 8 and 16 wk at a dose equivalent in tablets per day to the final tolerated Alrestatin dose. Patients were informed that placebo tablets would be substituted for active drug at some stage but no other details were given. Tablets were changed at 2–4-wk intervals. Informed consent was obtained from each patient before entry into the study.

LABORATORY METHODOLOGY

Hematology. Hematologic assessment included hemoglobin, white cell, and platelet count, red and white cell indices by Coulter-Counter S. Blood film, reticulocyte count, ESR, prothrombin time, and partial thromboplastin time were determined by routine methods.

Biochemistry. Routine serum biochemical measurements including electrolytes, calcium, phosphate, urea, creatinine and liver function tests (albumin, bilirubin, alkaline phosphatase, SGOT, SGPT, and γGT) were measured by automated methods (SMAC-20).

Twenty-four-hour collections of urine were examined for volume, total creatinine, uric acid, and protein excretion by standard methods.

Serum Alrestatin levels were determined by fluorescence after high pressure liquid chromatography. Serum samples (100 μl) were diluted 1:10 with distilled water, deproteinized with 5 ml 5% perchloric acid, vortexed for 30 s, and centrifuged (2000 rpm, 5 min.). Aliquots of supernatant (20 μl) were injected into a C18 reverse phase column with a solvent system of methanol:water:acetic acid (700:600:6) at a flow rate of 1 ml/min. Quantitation was by fluorescence at 389 nm after activation at 346 nm. Blanks (serum and buffer) were consistently less than 0.5 μM.

Glycosylated hemoglobin was determined by a cation exchange resin technique.[17]

NERVE CONDUCTION STUDIES

Motor conduction studies were performed on the right median and lateral popliteal nerves with surface recording electrodes applied to the abductor pollicis brevis and extensor digitorium brevis muscles, respectively. The stimulus was a 0.2-ms-duration square wave derived from Disa Mini Stim, and the amplitude was adjusted to obtain a maximal contraction from the muscle. Stimuli were applied to the median nerve at the wrist and antecubital fossa and to the lateral popliteal nerve at the ankle and knee. The amplitude of the evoked muscle action potential obtained on stimulation at each site was measured, together with both the terminal latency and the velocity of conduction of the fastest fibers between the points of stimulation. The surface temperature of the limb was measured with a Thermistor and the velocity was adjusted over the serial studies by 2.4 m/s for each degree centigrade change.

A sensory action potential was recorded with surface recording electrodes from the median nerve of the wrist on supramaximal stimulation of the index finger with ring electrodes. The latency to peak and amplitude of the evoked response was measured. Similarly, a mixed nerve action potential was recorded from the lateral popliteal nerve at the neck of the fibula with needle recording electrodes on supramaximal stimulation of the nerve at the ankle.

The electrical responses were amplified and displayed on a Medelec M-scope following pre-amplification, using Tektronix FM 122 amplifiers.

TABLE 2
Symptomatic responses

Patient	Alrestatin dose g/day	Peripheral neuropathy 3 wk	8 wk	16 wk	Autonomic neuropathy	Other neuropathy
HM	6	++	++	0	Unimproved	Nil
LD	7.5	++	+	0	Unimproved	Amyotrophy worse
AF	6	++	++	0	Unimproved	Nil
CC	4.5	++	++	+	Unimproved	Amyotrophy unimproved
JH	4	+	+	+	Improved	Nil
JB	4	+	+†	*	Nil	Nil
EI	3	++	+‡	*	Partial improvement	Nil
DW	3	++	*	*	Unimproved	Nil
SD	—	*	*	*	*	

* Not evaluated due to termination.
† Terminated at 7 wk (rash).
‡ Terminated at 6 wk (nausea).

Normal control values were obtained from a group of hospital and university employees and convalescent surgical patients without history of neuropathy. None were on potentially neurotoxic medication, and full neurologic examinations were normal.

STATISTICS

All results were expressed as mean ± 1 SEM (standard error of mean). Statistical analysis was by unpaired T test and analysis of variance. A P value of less than 0.05 was considered significant.

NERVE FUNCTION

Peripheral neuropathy—subjective. Subjective responses to Alrestatin in 3 wk were seen in six patients with marked (++) benefit and two patients with some (+) benefit (Table 2). At 8 wk, five maintained the same and two maintained less benefit than at 3 wk, and after placebo for 8 wk, two were still better than before the trial while three returned to pretrial levels.

Peripheral neuropathy—objective. Motor nerve conduction (latency, amplitude, and conduction velocity) and sensory nerve conduction (latency and amplitude) parameters were unchanged in the group overall (Table 3). In no case did nerve conduction become detectable in nerves where it had been undetectable before Alrestatin therapy. For statistical purposes where conduction was undetectable [motor (2), sensory (3)] these results were omitted from calculation of mean conduction parameters. An analysis of variance did not show any significant effect of time or treatment on any nerve conduction parameters, whether these were analyzed as raw data or normalized (to 100% as baseline value). Normalization of raw data was performed in view of the large between-patient variance.[30]

Glycosylated hemoglobin values were correlated inversely with right median nerve motor conduction latency ($r = -0.44$, $P < 0.02$) but no other nerve conduction parameters.

One patient, illustrated in Figure 1, did appear to have some reproducible improvement on Alrestatin twice accompanied by subjective benefit. Some lowering of glycosylated hemoglobin was also evident.

Other neuropathy. Impotence did not change in any of the four men during Alrestatin or placebo therapy. Two patients experienced some improvement in autonomic symptoms; in one (EI), marked decrease in diarrhea without improvement in severe postural hypotension, sweating, or dysphagia were considered to reflect a drug side effect rather than improved autonomic function. In another (JH), marked improvement in postural hypotension and diarrhea on Alrestatin allowed complete cessation of fludrocortisone (0.3 mg daily), which was not previously possible. This response, however, was sustained throughout the placebo period and for over 6 mo after completion of the study.

Amyotrophy was not influenced in two patients. In one (LD), severe pain with amyotrophy worsened while some improvement in symptoms of peripheral neuropathy was noted. There were no neurologic or ophthalmologic changes noted throughout the study.

GLYCOSYLATED HEMOGLOBIN

Glycosylated hemoglobin levels did not change significantly throughout the trial, though a gradual reduction in mean values (baseline versus 16 wk; 12.0 ± 0.9 versus 9.9 ± 0.7, $P < 0.09$) was attributed to the dropping out of

TABLE 3
Nerve conduction parameters

Time (wk)	Sensory (R Median) Latency (ms)	Sensory (R Median) Amplitude (μV)	Motor R Median Latency (ms)	Motor R Median Amplitude (μV)	Motor R Median Velocity (m/sec)	Motor R Lateral popliteal Latency (ms)	Motor R Lateral popliteal Amplitude (μV)	Motor R Lateral popliteal Velocity (m/s)
Baseline	3.2 ± 0.2	4.4 ± 0.6	3.7 ± 0.3	3.9 ± 0.7	48.9 ± 2.3	5.9 ± 0.6	2.8 ± 1.2	37.3 ± 1.8
3	3.2 ± 0.3	6.0 ± 1.7	3.6 ± 0.4	4.2 ± 0.9	47.4 ± 2.5	5.2 ± 0.5	2.5 ± 1.2	36.0 ± 1.9
8	3.2 ± 0.2	6.8 ± 1.6	3.7 ± 0.3	5.3 ± 1.0	51.2 ± 1.6	5.7 ± 1.0	2.5 ± 0.7	35.8 ± 2.1
16	3.4 ± 0.2	4.2 ± 0.9	4.3 ± 0.2	7.8 ± 3.2	47.8 ± 1.4	6.0 ± 0.5	1.0 ± 0.3	36.4 ± 2.6
Control A(15) Age 31.7 ± 2.6 yr	2.7 ± 0.1	22.4 ± 2.1	3.3 ± 0.1	7.2 ± 0.5	60.4 ± 1.3	4.4 ± 0.2	5.0 ± 0.6	49.6 ± 1.0
B(20) Age 63.4 ± 2.2 yr	3.4 ± 0.1	11.5 ± 0.9	3.8 ± 0.2	6.6 ± 0.6	52.2 ± 0.9	5.1 ± 0.3	3.0 ± 0.4	44.1 ± 1.1

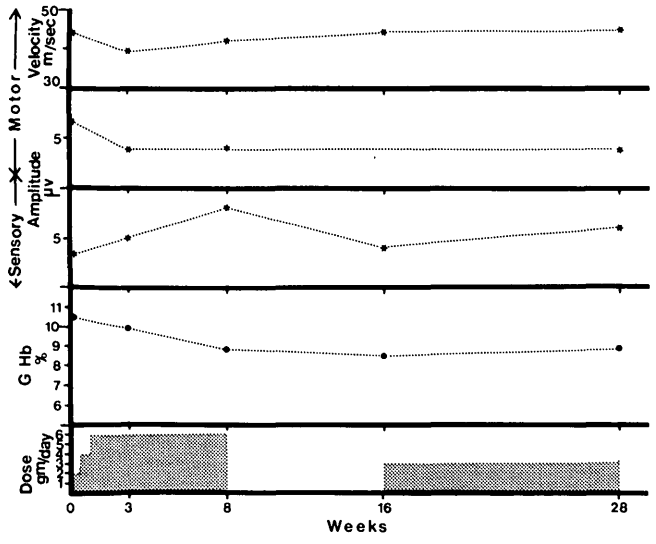

FIGURE 1. Changes, over time, in motor nerve conduction (latency, amplitude, and conduction velocity) and sensory nerve conduction (latency and amplitude) with Alrestatin therapy in one patient.

the four patients with drug toxicity. No change in hemoglobin or reticulocyte counts occurred.

TOXICITY AND SIDE EFFECTS

Nausea was the most common side effect and this limited tolerated dose in each case. The one patient who terminated the study due to intractable nausea (EI) had the highest plateau drug level (755–981 μM).

Constipation was noted in three patients. One patient developed tinnitus on placebo.

Skin rash was observed in four patients. Two men had minor photosensitivity with blistering lesions on the dorsum of the hands resembling porphyria cutanea tarda. No evidence of liver disease or abnormalities of porphyrin metabolism were demonstrated. In both cases, the lesions were minor and transient, and had resolved within a week of cessation of Alrestatin. Two women (DW and JB) had severe generalized photosensitivity requiring cessation of active drug and withdrawal from the study. These occurred at 3 and 7 wk and at total Alrestatin doses of 56 and 158 g, respectively. Plateau drug levels in the one (JB) were not excessive (318 μM). In this woman, provocation by sunlight filtered through window glass occurred, suggesting photosensitivity to short wavelength light consistent with the absorption and emission spectra of Alrestatin. Both women had residual photosensitivity for several months following cessation of Alrestatin, but full resolution had occurred by 6 mo. Biochemical evidence of hepatotoxicity was evident in one case (DW) resolving within 6 mo.

One patient (SD) developed septicemia and acute renal failure on the second day of drug administration. Sepsis had begun in an ingrown toenail. This episode was considered to be coincidental and a similar episode of acute renal failure had occurred 2 yr previously following infection.

Apart from skin rash and one case of hepatitis, presumably drug-induced, no other biochemical or hematologic changes occurred. Four of nine patients entered were unable to complete the trial due to toxicity [rash (2), nausea (1), and acute renal failure, possibly unrelated (1)]. These four women were significantly younger (37.8 ± 5.8 versus 58.4 ± 8.6, $P < 0.05$) and had poorer metabolic control (higher GHb 13.6 ± 1.3 versus 10.7 ± 1.0, $P < 0.06$; lower serum bicarbonate 20.8 ± 1.7 versus 25.8 ± 0.8, $P < 0.01$; lower hemoglobin 11.6 ± 0.4 versus 15.0 ± 0.6, $P < 0.001$; higher serum phosphate 1.26 ± 0.07 versus 0.92 ± 0.01, $P < 0.01$). All other clinical, hematologic, and biochemical criteria in the four subjects not completing the trial were the same as the rest of the group.

PHARMACOKINETICS

In view of the short half-life of Alrestatin in serum (approximately 60 min,)[15] doses were administered 4 times daily. Maximum tolerated doses varied from 3 to 7.5 g/day (mean 4.8 ± 0.6 g/day), and steady-state plateau drug levels ranged from 318 to 981 μM. No significant correlation between steady-state plateau levels of Alrestatin and daily dose, creatinine clearance, or serum albumin was evident.

Prompt oral absorption was confirmed[15] in three patients following an initial 0.5-g dose with peak plasma levels of 110–214 μM occurring between 45 and 150 min after administration.

DISCUSSION

Our study did not show any objective benefit from the administration of Alrestatin to patients with severe peripheral neuropathy. Subjective benefit was noted in most patients, although this was not consistent with a drug effect in all cases. Furthermore, none of the nerve conduction parameters were significantly altered and no consistent benefits were demonstrated subjectively or objectively in autonomic neuropathy or amyotrophy.

Subjective improvement without changes in nerve conduction parameters may be due to a placebo effect, an analgesic effect, or insensitive parameters of nerve conduction studied. The frequency of subjective responses was unusually high for a placebo response, as under painful conditions, these are usually seen in about a third of subjects.[28] In some cases, an apparent beneficial effect lasted long after cessation of Alrestatin, and since Gabbay et al.[15] recovered greater than 99% of ingested drug unchanged in urine, this seemed unlikely to be a drug effect. Nevertheless, the severe photosensitive drug eruptions, presumably due to accumulation of Alrestatin and/or its metabolites in skin, lasted for many weeks after cessation of drug ingestion consistent with some accumulation of the drug within the body.

An analgesic effect seemed unlikely in view of inconsistencies in responses between patients. In one patient (LD), a marked increase in the pain associated with amyotrophy occurred while the pain of the peripheral neuropathy abated.

Evaluation of nerve function posed considerable problems. Heterogeneity of patients in terms of age, duration of diabetes and neuropathy, and manifestations of neuropathy are inevitable in such trials and may obscure small but definite beneficial effects. Subjective evaluation of pain is notoriously liable to placebo response. Crossover to placebo, preferably double-blind, can overcome some of these difficulties, though the placebo effect would still tend to diminish apparent benefit from an effective drug. Randomized trial design may have partly overcome this difficulty, although the risks of introduction of a new drug were felt to warrant the initial in-hospital phase, making a randomized

design impractical. On the other hand, objective measures of motor and sensory conduction have limitations, including sensitivity to extraneous factors such as age,[9] temperature, metabolic factors, and day-to-day variability of no known cause.[19] Motor nerve conduction velocity reflects only the function of the largest, most rapidly conducting nerve fibers, while in diabetic neuropathy, large and small myelinated as well as unmyelinated fibers are affected.[1] Functional testing of all but the largest nerve fibers remains unsatisfactory and the function of smaller and unmyelinated fibers remains undetermined.

Discrepancies between changes in sensory and motor nerve conduction have been noted previously.[5,22,30] For example, in one study, blood sugar and glycosylated hemoglobin levels were correlated with motor but not sensory nerve conduction velocities,[5] and in another, greater improvement in sensory rather than motor nerve conduction velocities was seen after *myo*-inositol therapy.[22] Greater improvement in sensory rather than motor nerve conduction may have contributed to the frequency of subjective responses seen in our study.

The failure of Alrestatin to improve apparent nerve function may have been due to inadequate degree of blockade of aldose reductase, irreversible structural damage to nerves, insensitive methods of evaluation, or an alternative pathogenic mechanism of metabolic neuropathy apart from polyol accumulation. Since no bioassay of aldose reductase activity is available, it was not possible to confirm a significant degree of enzyme blockade. The enzyme, aldose reductase, is not present in erythrocytes.[12] Hepatic conversion of sorbitol to fructose[20] is mediated by enzymes other than aldose reductase as well, and consequently, it is not an appropriate marker of degree of enzyme inhibition. Nevertheless, dose-related nausea limited the amount of drug tolerated, so higher dosage would not be possible. Serum levels achieved in our study were comparable with those attained by Gabbay et al.[15] following i.v. injection. In their study, subjective but not objective improvement in nerve function was noted after i.v. administration, while oral doses of 4 g/day resulted in lower serum levels and neither subjective nor objective benefit. Despite lowering of sorbitol and fructose levels in sciatic nerve of streptozotocin-diabetic rats[16] and improvement in nerve conduction of galactose-fed rats[12] by AY 22, 284 administration, no evidence has yet emerged to confirm an effect of aldose reductase inhibition on nerve conduction in human or animal models of diabetes.

Improved metabolic control may result in improved nerve function, although some deficit remains except in newly diagnosed diabetics treated with insulin.[8] This suggests that in long-standing diabetics, some of the defect in nerve function may be irreversible even with improved metabolic control. Further trials may be more rewarding in early diabetic neuropathy where structural damage is less and where a closer analogy to the successful animal models of aldose reductase inhibition holds.[12]

Glycosylated hemoglobin measurement revealed an apparent (nonsignificant, $P < 0.09$) downward trend attributable to the dropping out of four patients due to drug toxicity. No change in hemoglobin or reticulocyte count occurred to suggest a change in mean red cell life span.[14] Random and postprandial blood glucose values were not different on Alrestatin or placebo. Consequently, the effect of Alrestatin in augmenting endogenous glucose-stimulated insulin release[18] seemed unlikely to have altered glycemic control in long-standing insulin-dependent diabetics. Since glycemic control is a known factor in diabetic nerve function, long-term clinical trials should be monitored for alteration in glycosylated hemoglobin or other glycosylated protein levels.[29]

Finally, other mechanisms in the pathogenesis of the metabolic neuropathy of diabetes may be important. These include *myo*-inositol deficiency,[1,21,22] altered permeability of the blood-nerve barrier,[23] abnormal myelin lipid synthesis,[24] impaired axonal flow,[25] or nerve ischemia.[26] Whether these are cause or effect or even whether blockade of aldose reductase has effects on these abnormalities will await further clinical trials of more potent aldose reductase inhibitors currently under development.[27]

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