Sural Nerve Sorbitol in Patients With Diabetic Sensorimotor Polyneuropathy

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OBJECTIVE — Nerve sorbitol levels have been measured in sural nerve biopsy samples from patients with diabetic sensorimotor polyneuropathy in several studies using different methods and measurement units. In this study, we compared the results of sorbitol assays to determine the required sensitivity of analytical methods for nerve sorbitol measurements.

RESEARCH DESIGN AND METHODS — We performed a literature search using PaperChase for reports of nerve sorbitol in diabetic patients and selected those with nerve conduction studies to delineate the severity of nerve damage.

RESULTS — In patients who had undergone a nerve conduction study, the standardized nerve sorbitol levels were 0.034–0.300 nmol/mg wet nerve.

CONCLUSIONS — Our results showed the level of sensitivity required in laboratory methodology to perform this assay in the target population and aid in the planning of clinical research trials of aldose reductase inhibitor agents.

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Peripheral neuropathy is a major complication of diabetes, with a prevalence rate >50% (1). Although the pathophysiology of diabetic peripheral sensorimotor polyneuropathy (DSP) is complex and controversial, it is known that the polyol pathway is an intrinsic element in the evolution of this disorder (2). In response to elevated blood glucose, aldose reductase enzyme activity increases, resulting in an increased conversion of glucose to sorbitol, one of the alcohol sugars. This in turn leads to an accumulation of sorbitol in erythrocytes and within nerves. Laboratory diabetic animal studies have shown that increased nerve sorbitol is associated with nerve damage (3). Abnormal activity of the polyol pathway is associated with multiple pathophysiological changes in peripheral nerve (2).

If the aldose reductase enzyme system can be inhibited, then nerve sorbitol accumulation should be avoided, thus potentially preventing or even reversing the nerve damage in patients with DSP. Several aldose reductase inhibitors have been developed and studied in human patients with DSP, but success with these agents has been elusive. Pfeifer and Schumer (4) described the difficulties in clinically evaluating an aldose reductase inhibitor. Experience has shown that sorbitol levels in human nerve should be determined with sural nerve biopsy before exposing large numbers of patients to potentially toxic compounds in long and expensive clinical trials as not all aldose reductase inhibitors penetrate human peripheral nerve (5).

Knowing the expected nerve sorbitol levels in patients with DSP is important in clinical research for two reasons. First, the proposed laboratory methodology must be sufficiently sensitive to measure sorbitol levels at the nanomole concentrations typically found in DSP. This resembles chasing a molecule through a nerve. Second, knowing the expected range of nerve sorbitol provides guidance when planning studies of aldose reductase inhibition therapy. An inappropriate study population could be selected that may not have elevated nerve sorbitol because of either an unconfirmed diagnosis or severely diseased nerve; in those cases, the pharmacodynamic evaluation becomes difficult and a good compound may be discarded for the wrong reasons.

We present here the results of a literature survey concerning nerve sorbitol levels in patients with DSP conducted while planning a sural nerve biopsy study of a novel aldose reductase inhibitor.

RESEARCH DESIGN AND METHODS

— We performed a literature search using PaperChase with these search terms: sorbitol, diabetic neuropathy, diabetes, and nerve biopsy. PaperChase includes MEDLINE, HealthSTAR, and OLDMEDLINE. We found eight reports in which nerve sorbitol levels were measured in diabetic patients (6–13). Of these eight, nerve conduction studies (NCSs) were performed in six, but the results were described in only four of the reports (9,11–13). These electrophysiological measurements identify the presence of diabetic neuropathy and reliably reflect the severity of the disease process (14). If the polyol pathway theory is correct, then patients with electrophysiologically confirmed DSP would be expected to have elevated nerve sorbitol levels. NCSs thus provide an electrophysiological confirmation of the DSP diagnosis and bolster the clinical signs and symptoms. Another reason to require the presence of a sural nerve response is that NCS results then ensure that the biopsied nerves con-

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Abbreviations: DSP, diabetic peripheral sensorimotor polyneuropathy; NCS, nerve conduction study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Patient demographic characteristics and NCSs*

	n	Age (years)	Diabetes duration (years)	DSP duration (years)	HbA_{1c} (%)	Amplitude (μV)	CV (m/s)
1. Dyck et al. 1980 (6)	—	56	—	—	—	—	—
2. Sima et al. 1988 (9)	16	49.1 ± 4.4	10.6 ± 1.6	2.8 ± 1.0	8.2	2.5 ± 1.1	37.3 ± 1.3
3. Dyck et al. 1988 (10)	25	49 (range 24–64)	13.9 ± 9.6	—	—	—	—
4. Sima et al. 1993 (11)	14	54.3 ± 10.2	18.4 ± 10.4	6.2 ± 2.5	6.7	2.4 ± 2.2	28.6 ± 8.1
5. Greene et al. 1999 (12)	50	52.0 ± 1.7	10.5 ± 1.2	3.1 ± 0.5	10.3	8.02 ± 0.78	43.0 ± 0.8
6. Sundkvist, et al. 2000 (13)	10	62 ± 2	—	—	7.5	3.7 ± 3.5	41.0 ± 6

Data are means ± SD, unless otherwise indicated. Amplitude represents sural nerve potential amplitude. CV, sural nerve conduction velocity.

tain sufficient residual nerve fibers to permit sorbitol measurements. Of the eight studies, four met the criterion of reporting NCS results, but nerve sorbitol was reported in variable units in these four.

To compare the results, the nerve sorbitol measurements were standardized as nanomoles per milligram of wet nerve. The sorbitol concentration per protein content was converted to concentration per weight of wet nerve in milligrams, using Mayhew's measurement of 52.3 mg protein/g wet nerve in diabetic patients (7). Therefore sorbitol was measured as

(sorbitol in nmol/mg protein ×
52.3 mg protein/g nerve) ÷
1,000 = sorbitol in nmol/mg wet nerve

RESULTS — The demographic characteristics and NCS results for the patients from the four reports are chronologically described in Table 1, and each study is numbered for ease of discussion. Two additional reports, studies 1 and 3 (6,10), are included in Table 1 because NCSs were performed, although the results were not provided. The demographic characteristics show that study 4 (11) had older patients with a longer duration of diabetes and DSP and the most severe DSP, as demonstrated by sural nerve amplitude and conduction velocity parameters. The demographics for this report are described in a separate article (15). Study 5 (12) selected patients with milder DSP, as demonstrated by the sural nerve conduction parameters, although glycemic control based on HbA_{1c} was the worst in this group and the best in study 4. Studies 2 (9) and 4 had similar electrophysiological changes with low sural nerve potential amplitudes, although study 4 had slower conduction velocities, indicating more advanced neuropathy due to greater loss of large nerve fibers in study 4 patients. Study 5 showed relatively normal sural nerve parameters, indicating that this population had mild neuropathy. The patients in study 6 (13) were slightly older than those in studies 2, 4, and 5, but otherwise the nerve conduction results were similar to those in the other studies.

The sorbitol levels in peripheral nerves from patients with DSP are shown in Table 2. The results demonstrate the sensitivity of the gas chromatography–mass spectrometry method for detecting nerve sorbitol. In studies 1 and 3, the results of NCSs were not provided, so the severity of neuropathy could not be reliably assessed in these patients. Study 3 reported the highest sorbitol levels by gas chromatography, but comparison with the other studies was limited by the lack of data from NCSs.

In the two studies not listed in the tables for reasons stated above, Mayhew et al. (7) reported postmortem nerve sorbitol concentrations of 0.39 ± 0.49 nmol/mg wet nerve in diabetic patients, but did not indicate the time from death to postmortem examination. In the second study, Hale et al. (8) found nerve sorbitol levels of 0.028 (0.012–0.496) nmol/mg wet nerve in nerve samples obtained from the proximal stump during leg amputation, but NCSs were not performed. Both of these studies are often cited in the scientific literature, so they are described here to provide a complete review despite their lack of electrophysiological DSP measurements.

CONCLUSIONS — This report showed that patients with DSP and viable nerve, both established by NCS, will accumulate sorbitol in sural nerve, as is demonstrable in biopsy samples. Studies 2 and 4–6 showed that this accumulation can be expected in the range of 0.034–0.300 nmol/mg wet nerve. This wide

Table 2—*Nerve sorbitol levels*

	Reported sorbitol levels	Standardized sorbitol levels (nmol/mg wet nerve)	Method used to determine levels
1. Dyck et al. 1980 (6)	0.06 (0.1–0.77) mmol/kg wet nerve	0.06 (0.01–0.77)	GC/MS
2. Sima et al. 1988 (9)	0.1–0.03 μmol/kg wet nerve	0.10–0.30	FEZ
3. Dyck et al. 1988 (10)	0.76 (0.1–2.2) nmol/mg wet nerve	0.76 (0.1–2.2)	GC
4. Sima et al. 1993 (11)	2.15 ± 1.41 nmol/mg protein	0.11 ± 0.07*	GC, FD
5. Greene et al. 1999 (12)	800 ± 100 nmol/g protein	0.042 ± 0.005*	GC/MS
6. Sundkvist et al. 2000 (13)	643 ± 412 pmoles/mg protein	0.034 ± 0.022*	GC/MS

Data are value (range) or means ± SD. *Sorbitol concentration per protein content was converted to concentration per weight of wet nerve in milligrams as follows: (sorbitol in nmol/mg protein × 52.3 mg protein/g nerve) ÷ 1,000 = sorbitol in nmol/mg wet nerve. FD, flame detector; FEZ, fluorometric enzyme assay; GC, gas chromatography; MS, mass spectrometry.

range may be explained by differences in laboratory techniques and the variable nature of DSP. Thus, any laboratory planning to perform this assay needs to have methods sufficiently sensitive to demonstrate similar levels of sorbitol accumulation. This accumulation was consistent across the four reports cited above (9,11–13), with another four reports supporting this finding (6–8,10). Although Mayhew et al. (7) reported sorbitol accumulation in postmortem nerve samples of patients with DSP, glucose metabolism is probably altered after death, thereby limiting comparability of those results to the results in other reports. Furthermore, it is probable that Hale et al.'s report (8) included patients with advanced DSP because of the requirement for amputation, although NCSs were not available to document the severity of disease (8). Nonetheless, sorbitol accumulation was demonstrable in this group as well, although less than in the other studies, perhaps because of the more severe neuropathy and greater loss of nerve fibers. Both investigations provide additional important information concerning nerve sorbitol in subjects with DSP.

We were not able to explain the sorbitol concentrations of 0.76 nmol/mg wet nerve in study 3 by Dyck et al. (10). This level is higher than that found in the other studies. A possible explanation might be that nerve fascicles were desheathed and the assay was performed on the endoneurium. However, the same desheathing was performed in study 1, also by Dyck et al. (6), in which lower levels of sorbitol were reported. Myoinositol measurements reported by both Dyck et al. (10) and Hale et al. (8) showed no difference between subjects with and without diabetes.

This report has several limitations. The number of studies in which nerve sorbitol was measured in biopsy specimens with NCSs is small, as were the number of patients in each study. Study 5 by Greene et al. (12) is the most reliable because the patients were carefully selected, the range of neuropathy was narrow and well defined, and the number of patients was the largest of all four studies. The results of study 6 by Sundkvist et al. (13) closely supports the findings of study 5. Thus, it is likely the levels of sorbitol accumulation are the most accurate in these two studies. Nerve sorbitol assays in studies 5 and 6 were performed in the same laboratory, the University of Michigan Nerve Biopsy Laboratory.

It is unlikely that many more studies of this nature will be done because they are difficult to perform; they require an invasive procedure, namely nerve biopsy, with its attendant complications of sensory loss, pain, delayed wound healing, and infection (16) Because diabetic neuropathy is essentially a length-dependent axonopathy that first affects the largest and most distal nerves, such as the sural nerve, and because the sural nerve is relatively accessible, it is commonly used in biopsy studies to assess the pathophysiology of DSP. In diabetic patients with moderate-to-severe neuropathy, sural nerve function may be already lost due to axonal damage and loss. Consequently, determining the severity of neuropathy with the aid of NCSs helps in interpreting published reports of nerve sorbitol assays. Finally, because of the variability in patient populations and testing methods, a meta-analysis in which the data are combined does not seem reasonable.

The purpose of this report was not to identify an optimal assay procedure or assay sensitivity. Sensitivity when applied to an assay generally refers to the lower limit of quantification. Any assay sensitivity would need to exceed the range found here if an investigator wished to evaluate the ability of an aldose reductase inhibitor to lower nerve sorbitol by 80–90%, as suggested by Greene et al. (12).

Although nerve sorbitol levels have been quantified in rat tissue, applying this technique to human peripheral nerve is difficult. The amount of tissue available from a human biopsy is less than that from rats. Furthermore, the concentration of sorbitol in human nerve appears to be less than that found in rat nerve, perhaps because of differences in tissue levels of aldose reductase and sorbitol dehydrogenase or differences in the severity of diabetic hyperglycemia.

Two questions inherent in any therapeutic clinical trial are *1*) What are the expected baseline values? and 2) What are the expected values in the control group? This report confirmed that patients with DSP and viable nerve, as established by NCSs, have an accumulation of sorbitol in the biopsied sural nerve of 0.034–0.300 nmol/mg wet nerve. This information is crucial in trials of aldose reductase inhibitors as laboratory analytic methods must be adequately sensitive to detect similar sorbitol levels and the study patients should represent the target population.

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