Skin Response to Delayed Hypersensitivity Testing in Persons With Unilateral Stroke-related Paresis: Implications for People With Spinal Cord Injury

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Abstract

Background: Vaccination rates among individuals with spinal cord injury (SCI) could be improved if it can be shown that vaccination performed on insensate areas is effective. This would eliminate the the risk of discomfort and soreness at the injection site.

Objective: To determine whether immune responsiveness varies between areas with intact and impaired innervation in patients with stroke-related paresis.

Design: Prospective trial in which each subject served as his or her own control.

Setting: Rehabilitation wards and long-term care units at a Veterans Affairs Medical Center.

Patients: Individuals with a history of cerebrovascular accident (CVA) affecting 1 side of the body.

Methods: The Multitest cell-mediated immunity (CMI) and purified protein derivative (PPD) of tuberculin were administered intradermally to each arm of each subject.

Main Outcome Measures: Total millimeters of induration in response to either test and positive vs negative responses to either test were compared between the 2 arms of each subject.

Results: Response to delayed hypersensitivity testing did not differ between the arms affected and unaffected by CVA in each subject, and the time since CVA also did not affect the magnitude of the skin response.

Conclusions: Skin testing for delayed hypersensitivity can be effectively administered in the paretic arms of persons who have experienced CVA. Although this study was performed in patients with stroke-related impairment, it has implications for vaccine administration in individuals with SCI-related neurologic deficits.

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Key Words: Cell-mediated immunity; Delayed hypersensitivity; Cerebrovascular accident; Purified protein derivative of tuberculin; Vaccination; Vaccines, pneumococcal, influenza

INTRODUCTION

A growing body of evidence has documented the communication pathways between the central nervous

system and the immune system (1). Lymphoid tissue in the spleen and lymph nodes is innervated by autonomic nerve fibers, and lymphocytes possess receptors for neuropeptides and neurotransmitters (2). Whether immune responsiveness varies between areas with intact and impaired innervation is a question that has been studied in subjects with unilateral cerebrovascular accident (CVA) (2–5). In these European studies, purified protein derivative (PPD) of tuberculin was administered in both arms of each subject, and the cutaneous delayed-

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type hypersensitivity (DTH) response was used as a measure of antigen-specific T-lymphocyte reactivity. An induration response to PPD involves both T-lymphocyte activation and the autonomous nervous system, because tissue infiltration by T-lymphocytes, increased blood flow, and edema all contribute (6,7). Lateralization of the DTH response, defined as a greater DTH response on 1 limb than the other, was observed in some but not all studies. Furthermore, whether the response was greater in the paretic or unaffected limb was influenced by the infarction size (major or minor), age of infarction (recent or distant), and neuroanatomic location of the CVA. In contrast, the same researchers found that the antibody response to inactivated influenza vaccine was equivalent regardless of whether the vaccine was administered in a paretic or normal limb (4). All 4 studies were performed in a single European center and involved the same group of approximately 80 subjects with CVA.

Whether vaccines and skin tests can be administered in insensate areas of the body is highly relevant to current medical practice. The Centers for Disease Control (CDC) recommends that all patients who have experienced either spinal cord injury (SCI) or stroke receive annual vaccination for influenza (8), and many have risk factors that should prompt PPD testing as well. Several small clinical trials have shown that persons with SCI mount a normal antibody response to vaccination with either the pneumococcal 23-valent polysaccharide vaccine or the inactivated influenza vaccine (9–13). However, the current vaccination rate for influenza in the SCI population is less than 60%, and many people with SCI report that concern for a sore arm or dislike of injections in general are some of the factors that prevent them from accepting annual influenza vaccination (14,15). In the 2002 National Health Interview Survey, self-reported vaccination rates for influenza in persons with stroke were even lower, at 31.4% (16). If vaccines, PPD testing, and other percutaneous procedures could be routinely be performed in insensate areas, acceptance rates might increase. To allow comparisons with the prior studies of DHT lateralization in stroke performed at a single European center, we sought to study this phenomenon in a stroke population at our Veterans Affairs Medical Center. We used 2 measures of delayed hypersensitivity as a marker for T-lymphocyte response: (a) the Multitest cell-mediated immunity (CMI) (Pasteur-Mérieux, Lyon, France), a battery of 7 ubiquitous antigens and a negative control, and (b) the standard PPD of tuberculin. Our specific objective was to compare the intensity of the skin reactions to various antigens in the arm affected by CVA vs the arm unaffected by CVA, with each subject serving as his or her own control.

METHODS

Subjects

Patients hospitalized at the Michael E. DeBakey Veterans Affairs Medical Center (MEDVAMC) who had a history of

unilateral CVA (either recent or distant) were eligible for enrollment. All subjects had impaired perception of sensory stimuli in the arm affected by CVA. Subjects were recruited from rehabilitation wards and long-term care units. Exclusion criteria included inability to obtain consent from either the patient or guardian, presence of underlying diseases known to cause anergy, presence of diffuse skin disease that could interfere with interpretation of the test results, a history of positive PPD reaction, and a history of systemic reaction to other antigens included in the Multitest CMI (such as diphtheria or tetanus). Demographic information collected included age, sex, underlying medical conditions, and neuroanatomic localization of the CVA. Approval for the protocol was obtained from the Baylor College of Medicine Institutional Review Board and the Research and Development Committee at the MEDVAMC. The conduct of all studies conformed to the protocol and to the ethical and humane principles of research.

Skin Testing

Skin testing was performed on the volar surface of each arm, 1 arm immediately after the other. The investigator applying and interpreting the skin tests was not informed which arm had been affected by the CVA and did not do any neurologic assessment of the arms. Both the Multitest CMI and PPD antigen were administered intradermally in each arm. The Multitest CMI has 8 test heads preloaded with the following test substances: tetanus toxoid, diphtheria toxoid, streptococcus antigen, old tuberculin antigen, glycerin negative control, candida antigen, trichophyton antigen, and *Proteus* antigen. The Multitest CMI provides a standardized, reproducible means to test cell-mediated immunity (17,18). For the PPD test, 0.1 mL of the PPD antigen (5-unit strength) was applied intradermally. All tests were interpreted by the same investigator (SAS) at 48 hours by measuring induration diameters. For each of the CMI battery antigens, induration of at least 2 mm constituted a positive reaction. The percentage positive of the CMI battery was defined as the number of tests at least 2 mm/7 tests $\times$ 100. The negative control prong did not produce a response in any subject and was not included in this measure. The CMI response was also recorded as the sum of induration diameters for all test sites on a given arm. Anergic response to CMI testing was defined as all responses less than 2 mm. For the purposes of our study, an at least 10-mm induration to the PPD antigen was regarded as positive. This cut-off was based on the CDC criteria for tuberculin positivity, because all subjects were hospitalized during the time of the study and none were severely immunosuppressed (19).

Statistical Analysis

All statistical comparisons were performed using the SAS software programs (SAS Institute, Cary, NC). Two types of comparisons were made between the affected and

Table 1. Bilateral Response to Skin Testing in 30 Subjects With Unilateral Paresis

	Overall	Affected Arm	Unaffected Arm
PPD (mm induration)	6.8 ± 8.3	6.3 ± 7.6	7.2 ± 9.1
CMI (mm induration)	6.3 ± 8.7	5.5 ± 6.1	7.1 ± 10.8
CMI (% positive)	15 ± 15	13 ± 14	17 ± 16

Results are shown as mean ± SD.

unaffected arms: (a) absolute response to testing (positive vs negative for PPD, and percent positive on each arm for CMI) and (b) magnitude of response to testing (millimeters of induration diameter). Responses to the PPD and the CMI were calculated and compared separately. The Wilcoxon signed rank test was used to compare the percentage positive of the CMI battery between the affected and unaffected sides and the magnitude of the difference in induration between the affected and unaffected arms for both the PPD and the CMI. The positive vs negative comparison for the PPD between the affected and unaffected arms was done using the McNemar χ^2 test. The Wilcoxon rank sum test was used to compare magnitude of the difference in induration between the arms in terms of whether the CVA was recent (≤ 30 days) or distant (> 30 days).

RESULTS

Thirty subjects were enrolled in the study. All subjects were men, and their mean age was 63 years (range, 44–77 years). The median time since CVA was 26 days (range, 2 days to 72 months), although in the 13 subjects with distant CVA, the median time since CVA was 17 months. Of 60 PPD tests administered, 23 had a positive response: 11 on the affected side and 12 on the unaffected side. Mean responses to PPD and CMI testing are shown in Table 1. Interestingly, anergic response to CMI testing did not seem to predict response to PPD testing, because 5 of 30 subjects had an anergic response to the CMI Multitest and a positive response to PPD in the same arm. No subject had any measurable response to the glycerin control prong of the CMI test.

No comparison suggested any statistically significant difference in DTH response between the affected and unaffected arms. In response to PPD testing, 2 patients had a positive PPD on the unaffected arm and a negative PPD on the affected arm, vs 1 patient with a positive PPD on the affected arm and a negative PPD on the unaffected arm ($P = 1.0$, McNemar χ^2 test). In 4 cases, the area of induration in response to the PPD was larger on the affected side, whereas in 11 cases, the induration in response to PPD was larger on the unaffected side ($P = 0.09$, Wilcoxon signed rank test).

Results were similarly negative when comparing responses to the Multitest CMI between the affected arm and unaffected arm on each subject. The percentage of positive responses to the CMI battery was higher in the

unaffected arm in 8 cases and higher in the affected arm in 5 cases ($P = 0.32$, Wilcoxon signed rank test). The total millimeters of induration in response to the CMI battery on each arm was likewise similar ($P = 0.56$, Wilcoxon signed rank test). Anergic responses to CMI testing were seen in 13 of 30 affected arms and 11 of 30 unaffected arms ($P = 0.68$, χ^2 test).

Time elapsed since CVA did not affect the DHT response. Subjects with distant CVA (> 30 days) were compared with subjects with recent CVA (930 days) in terms of the difference in induration response to either PPD or the CMI battery between their 2 arms. Whether the CVA was recent or distant did not have a significant effect on this difference ($P = 0.20$ for PPD and $P = 0.40$ for CMI battery, Wilcoxon rank sum test). Redefining “recent” as no more than 60 days did not change these results. However, the mean millimeters of induration in response to PPD testing was slightly lower in the affected arm in subjects who had experienced CVA more than 30 days before (5.15 mm) than on the unaffected arm in these same subjects (7.23 mm). This difference was not statistically significant. PPD responses were similar in the affected arm (7.23 mm) and unaffected arm (7.18 mm) of subjects who had experienced a recent CVA.

DISCUSSION

Our study addresses the important clinical question of whether CVA affects the skin response to DHT. We did not find any significant difference in the response to either PPD or CMI testing between a subject’s affected and unaffected arms. Whether the CVA was distant or recent likewise did not seem to affect the skin response to DHT. These results are at variance with those of Tarkowski et al (2–5,13). However, the lateralization of the PPD response in the studies of Tarkowski et al mainly appeared in subgroup analyses. When they analyzed a group of 80 patients with recent stroke as a whole, no significant lateralization of the PPD response was noted (2). The protocol of Tarkowski et al differed from ours in that their subjects initially received 2 U of PPD, and subjects who failed to respond to initial PPD testing were retested with 5 U of PPD. Their subjects in general had a greater induration response than ours (mean induration of 16.2 mm in comparison with 6.8 mm in diameter), and their cut-off for a positive PPD response was 6 mm rather than 10 mm of induration. Some of the

discordance between their results and ours may reflect differences in the study populations or study protocols.

Our study suggests a possible trend toward a slightly smaller response on the affected side. This difference is unlikely to be of clinical significance, because in many cases, the response was equivalent or greater on the affected side. The main limitations of our study are the small sample size and the lack of female subjects in our institution, because we studied only 30 male subjects. Another constraint is the limited information about neurologic deficits in our subjects, because we did not confirm the reported sensory deficits. However, in a small sample, subgroup comparisons based on motor vs sensory deficit or complete vs incomplete deficits would be unlikely to yield meaningful results.

Our study does not specifically address whether vaccination can be performed in areas affected by CVA or SCI, but it has relevance to this question. Prior studies in the United States of vaccinating subjects with SCI with either the 23-valent pneumococcal polysaccharide vaccine or the inactivated influenza vaccine did not specify if the vaccine was given in an insensate or sensate area (7–10). However, in 2 of these studies (8,10), a similar antibody response to vaccination was mounted in tetraplegia and paraplegia, and presumably subjects with tetraplegia were vaccinated in insensate areas. A subgroup of 30 CVA subjects in 1 of the studies of Tarkowski et al (4) received inactivated influenza vaccine in either paretic or nonparetic arms, and no differences were observed in antibody response. If the local T-cell responses do not differ appreciably between arms affected and unaffected by CVA, as we found in this study, it seems likely that response to vaccination would be similar regardless of the site of vaccination.

CONCLUSIONS

We conclude from these results that DHT can be performed without detriment on the arm affected by CVA. If the patient has diminished sensation as a result of the CVA in 1 limb, the patient can thus be spared the discomfort associated with skin testing. Whether vaccination can also be effectively performed in areas affected by CVA or SCI is an important question for future studies.

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