SPINAL MANIPULATIVE THERAPY REDUCES INFLAMMATORY CYTOKINES BUT NOT SUBSTANCE P PRODUCTION IN NORMAL SUBJECTS

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ABSTRACT

Objective: To examine the effect of a single spinal manipulation therapy (SMT) on the in vitro production of inflammatory cytokines, tumor necrosis factor α, and interleukin (IL) 1β, in relation to the systemic (in vivo) levels of neurotransmitter substance P (SP).

Methods: Sixty-four asymptomatic subjects were assigned to SMT, sham manipulation, or venipuncture control group. SMT subjects received a single adjustment in the thoracic spine. Blood and serum samples were obtained from subjects before and then at 20 minutes and 2 hours after intervention. Whole-blood cultures were activated with lipopolysaccharide (LPS) for 24 hours. Cytokine production in culture supernatants and serum SP levels were assessed by specific immunoassays.

Results: Over the study period, a significant proportion ($P \leq .05$) of sham and control subjects demonstrated progressive increases in the synthesis of tumor necrosis factor α and IL-1β. Conversely, in a comparable proportion of cultures from SMT-derived subjects, the production of both cytokines decreased gradually. Normalization of the observed alterations to reflect the changes relative to self-baselines demonstrated that, within 2 hours after intervention, the production of both cytokines increased significantly ($P < .001$ to .05) in both controls. In contrast, a significant ($P < .001$ to .05) reduction of proinflammatory cytokine secretion was observed in cultures from SMT-receiving subjects. In all study groups, serum levels of SP remained unaltered within 2 hours after intervention.

Conclusions: SMT-treated subjects show a time-dependent attenuation of LPS-induced production of the inflammatory cytokines unrelated to systemic levels of SP. This suggests SMT-related down-regulation of inflammatory-type responses via a central yet unknown mechanism. (J Manipulative Physiol Ther 2006;29:14-21)

Key Indexing Terms: *Manipulation; Spinal; Cytokines; Substance P; Inflammation; Spinovisceral Effect*

Although the exact biologic mechanism(s) of systemic effects of spinal manipulative therapy (SMT) remain unclear, the current model of somatoautonomic or spinovisceral reflex provides a critical link between spinal manipulation, the autonomic nervous system, and various visceral functions.[1] Animal experiments have demonstrated that somatoautonomic reflex effects are apparent in multiple nonmusculoskeletal systemic responses, including those of the cardiovascular, digestive, urinary, endocrine, and the immune system.[2,3] On the other hand, similar studies in humans are still limited particularly with respect to the effect of SMT on the integrated biologic activities of the nervous and immune systems.

Neural immunoregulation that sustains immune homeostasis is based on the reciprocal communication between the immune and the nervous systems. It is executed primarily by biologic actions of numerous soluble mediators such as

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Sources of support: This study was supported by the Canadian Memorial Chiropractic College and a Public Health Service grant no. U24 AR45166 through the Consortial Center for Chiropractic Research. The contents of this article are solely the responsibility of the authors.

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Paper submitted July 12, 2005; in revised form September 20, 2005.
0161-4754/$32.00

doi:10.1016/j.jmpt.2005.10.002

Table 1. *Demographic data of subjects*

Group	Age (y)	Sex (female/male)
VC, n = 20	23.7 ± 1.32	13/7
SHM, n = 20	25.6 ± 1.06	12/8
SMT, n = 24	24.9 ± 1.89	11/13

cytokines and neurotransmitters.[4,5] The close association of autonomic nerve terminals with macrophages and lymphocytes also facilitates a chemically mediated transmission between nerves and immune cells.[6] These observations suggest strongly that spinovisceral reflex effects might include alterations in the functional activity of cells in the immune and/or inflammatory responses.

Inflammation, which is a normal response to disturbed homeostasis caused by infection, injury, or trauma, is associated with augmented production of numerous proinflammatory and immunoregulatory cytokines and neurotransmitters. Among the principal mediators of inflammation, tumor necrosis factor α (TNF-α), interleukin (IL) 1, and the neurotransmitter, substance P (SP), play pivotal roles in the regulation of both local and systemic inflammatory responses.[7-9] Recent studies have shown that the production of such mediators is highly increased in patients with discogenic back pain, implicating their biologic activity in the pathogenesis of this condition.[10]

The relationship between SMT and the synthesis of biologic regulators of physiological responses by inflammatory cells remains unexplored. Earlier studies by Brennan et al[11,12] also suggested that peripheral blood lymphocytes from SMT-treated normal subjects may produce higher concentrations of biologically active TNF-α possibly induced by SP. The aim of the present study was, therefore, to examine if a single spinal manipulation has an effect on the lipopolysaccharide (LPS)–induced in vitro production of TNF-α and IL-1β. Because the production of these cytokines may be regulated by biologic actions of SP,[9] the effect of SMT on the systemic (in vivo) release of SP was also examined.

Methods

Subjects

All subject-handling procedures were approved by the Research Ethics Committee of the Canadian Memorial Chiropractic College. The study included 64 age- and sex-matched normal (asymptomatic) subjects who had not received a spinal manipulation for a minimum of 6 months before enrolment in the study (Table 1). The subjects were accepted into the study according to strict inclusion/exclusion criteria. They were screened by a single clinician for restrictions in segmental motion in the upper thoracic spine (T1-T6) and assigned randomly to one of three groups: SMT, sham manipulation (SHM), or venipuncture control (VC). SMT consisted of a single bilateral hypothenar (Carver-Bridge)–type "adjustment" applied to the involved vertebral segment and was evidenced by an audible cavitation. The adjustment is applied with the medial borders of the fifth metacarpal of both hands placed over the involved area with the medial aspect of the pisiform contacting the transverse processes of the involved motion segment. A high-velocity low-amplitude thrust is applied in an anterior and superior direction. Sham manipulation consisted of an identical setup using similar force but with positioning and line of drive that did not cavitate the joint. Subjects in the VC group were treated similarly to the SMT and SHM groups in every way except for the thrust. Blood samples were obtained from all subjects before any intervention and then at 20 minutes and 2 hours after treatment. For determination of serum SP levels, 5 mL of nonheparinized blood was drawn into a separation tube and placed in ice. After 1 hour, specimens were centrifuged at 4°C, and sera were aliquoted to be frozen at −78°C until use. A coding system was used to identify samples with a view of blinding the laboratory investigator(s) to the three groups. In every subject, samples collected before intervention served as a self-control (baseline) to which posttreatment responses were compared. Aliquots of sera and corresponding culture supernatants were stored at −78°C and were thawed immediately before testing.

Culture System

A whole-blood culture system, similar to that described by Wilson et al,[13] was used. Briefly, heparinized blood samples were diluted 10-fold with tissue culture medium, RPMI 1640, supplemented with 5×10^{-5} mol/L 2-mercaptoethanol and antibiotics. LPS from *Escherichia coli* serotype 055:B5 (Sigma Chemical Co, St Louis, Mo) was selected for the induction of TNF-α and IL-1β production. To ascertain that all pathways of LPS recognition were activated, cultures were stimulated, at initiation, with LPS at doses of 1, 5, and 10 μg/mL. Cultures were maintained for 24 hours at 37°C, in a humidified 5% CO_2 incubator. At the conclusion of the incubation period, culture supernatants were collected, aliquoted, and frozen at −78°C until further analysis. Routinely, the surface phenotypes of mononuclear cells (CD3, CD14, CD19, and CD56) in the studied blood samples were determined by direct immunofluorescent staining with an appropriate monoclonal antibody marker and flow cytometry analysis.

Determinations of Cytokine Production

The levels of TNF-α and IL-1β in supernatants from whole-blood cultures were determined by specific enzyme-linked immunosorbent assays using DuoSet ELISA development system for natural and recombinant human cytokines (R&D Systems, Minneapolis, Minn). Briefly, Immulon 4 HBX flat-bottom microtiter plates (Thermo Labsystems, Franklin, Mass) were coated with a predeter-

Table 2. *Effect of SMT on LPS-induced synthesis of TNF-α*[a]

Group	LPS (μg/mL)	TNF-α level, mean ± SEM (pg/mL) Baseline	20 min	2 h
VC	1	1481 ± 134	1658 ± 141	1744 ± 369
	5	1892 ± 135	2111 ± 141	2218 ± 164
	10	2255 ± 146	2446 ± 155	2621 ± 196
SHM	1	1498 ± 149	1656 ± 129	1854 ± 195
	5	1833 ± 171	1949 ± 163	2201 ± 218
	10	2332 ± 198	2559 ± 208	2912 ± 231
SMT	1	1518 ± 182	1374 ± 187	1304 ± 137
	5	1942 ± 112	1784 ± 97	1794 ± 163
	10	2319 ± 144	2088 ± 149	1821 ± 191

[a] The levels of TNFα were determined in supernatants from whole-blood cultures initiated before (baseline) and then at 20 minutes and 2 hours after SHM or SMT manipulation. The VC group includes individuals who were subjected to repeated venipuncture only. All cultures were activated with LPS at the indicated concentrations and cultivated for 24 hours.

mined concentration of an appropriate mouse antihuman capture antibody (anti–TNF-α or anti–IL-1β). After washing and blocking the wells with bovine serum albumin, duplicate dilutions of standards (defined amounts of a given cytokine) or of the tested supernatants were added to the wells and incubated for 2 hours. Plates were then washed and incubated with a specific detection antibody (biotinylated goat antihuman anticytokine antibody). After 20-minute incubation and multiple washing, the plates were incubated for 20 minutes with streptavidin-horseradish peroxidase solution and then substrate solution (mixture of H_2O_2 and tetramethylbenzidine). After the development of the color, the absorbance was measured at 450 nm using a Titertek Multiscan (Flow Laboratories, Helsinki, Finland). Concentrations of a given cytokine were calculated from the linearized (best fit) standard curve determined by regression analysis. Each of the studied samples was tested at least twice at two to four different dilutions.

Determination of Serum SP

Measurements of SP in intact (not extracted) sera of the studied subjects were carried out using a recently developed competitive enzyme immunoassay (R&D Systems) strictly as recommended by the manufacturer. The assay used the competitive binding between SP in a tested sample and the fixed amount of alkaline phosphatase–labeled SP for sites on a rabbit polyclonal anti-SP antibody. The antibody-antigen complexes were then immobilized by goat antirabbit immunoglobulin bound to microplate wells, and a substrate solution (pNPP) was added to determine the immobilized enzyme concentration. Immediately after color development, the absorbance was read at 405 nm using the Titertek Multiscan. Serum concentrations of SP were calculated from a standard curve constructed by plotting the mean absorbance for each standard on a linear y-axis against its concentration on a logarithmic x-axis. The absorbance (color intensity) was inversely proportional to the concentration of SP in the tested sample.

Statistics

Results are presented, except where indicated, as mean values ± SEM. It has been well established for values of human cytokine secretion induced in vitro to have skewed distribution.[14,15] Thus, statistical significance of differences was determined using Student paired *t* test (self control vs posttreatment at 20 minutes and vs posttreatment at 2 hours) or using nonparametric statistics. Analysis of proportions in independent samples was performed using χ^2 with Yates correction.[16] Statistical significance was recognized if the *P* value was less than .05. Data were analyzed using Statistica Software, release 6.0 (StatSoft, Inc, Tulsa, Okla).

Results

Peripheral Blood Mononuclear Cells

Phenotypic analysis showed that no significant quantitative changes within the subpopulations of peripheral blood mononuclear cells (lymphocytes, monocytes, and natural killer cells) transpired in any of the study groups over the postintervention period (data not shown). These results ascertained that blood cultures used in the present study were comparable with respect to their cellular contents.

LPS-Induced Production of TNF-α

Before sham or SMT treatment, the mean values of this cytokine production were comparable in all studied groups and showed a dose-dependent type of response that ranged from approximately 1500 to 2300 pg/mL (Table 2, baseline). Compared with their respective baselines, the levels of TNF-α were consistently higher (1600-2500 pg/mL) in cultures established from VC and SHM subjects at 20 minutes after intervention. Further augmentation in the release of TNF-α was apparent in supernatants from cultures initiated 2 hours after intervention (range, 1700-2800 pg/mL). Despite these alterations, the magnitude of TNF-α production remained dose-dependent in the controls (Table 2).

Conversely, in cultures from SMT-treated subjects, the mean production of TNF-α declined within 20 minutes of SMT. This effect was apparent across all groups, regardless of the dose of the inducer (LPS; Table 2). The levels of TNF-α in cultures exposed to low and moderate (1 and 5 μg/mL) doses of LPS remained unaltered when examined at 2 hours after treatment (Table 2). However, secretion of TNF-α decreased further in parallel cultures exposed to the highest dose of LPS (10 μg/mL). The mean concentration of TNF-α in these preparations was reduced by close to 35% compared with VC and SHM cultures stimulated with the same dose of endotoxin and by more than 20% compared with the mean baseline level (Table 2; Fig. 1A). Thus,

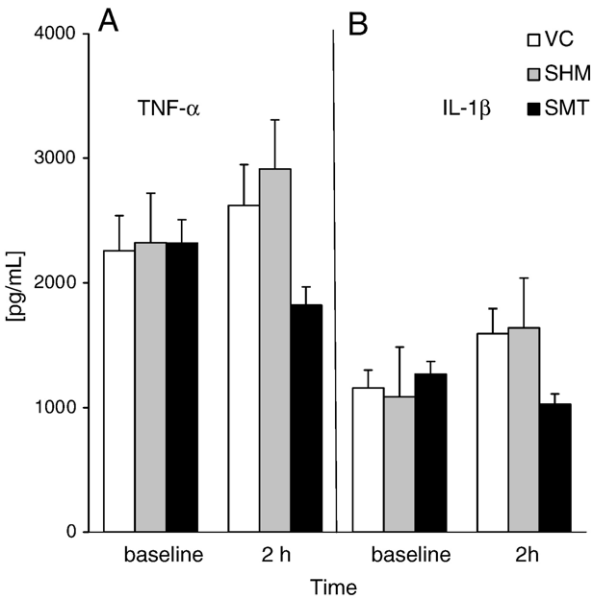

Fig 1. *Synthesis of TNF-α (A) and IL-1β (B) in LPS-activated (10 μg/mL) whole-blood cultures from control (VC and SHM) and experimental (SMT) subjects studied before (baseline) and at 2 hours after intervention.*

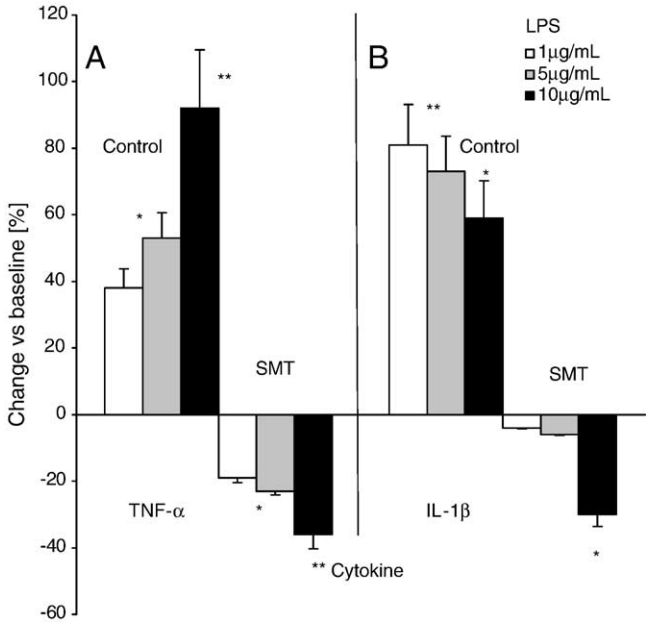

Fig 2. *The effect of SMT on the synthesis of TNF-α (A) and IL-1β (B) in whole-blood cultures activated with three different doses of LPS and examined at 2 hours after intervention. The bars represent the mean values (±SEM) of increases (controls) or decreases (SMT) in the production of a given cytokine expressed as a percentage of the subjects' respective baselines. **P <.001, *P <.05.*

although most cultures from VC and SHM subjects showed progressive increases in the level of inducible TNF-α, the opposite effect was apparent in cultures from individuals receiving SMT.

Comparison of proportions within each group (unaltered vs reduced and unaltered vs increased TNF-α pro- production) revealed that frequencies of cultures showing increased levels of TNF-α production in VC- and SHM-treated subjects were significantly higher ($P < .001$ to .05) than those in SMT-treated individuals. In contrast, frequencies of cultures in which the synthesis of TNF-α was reduced were significantly higher ($P < .001$) in subjects receiving SMT.

Due to the variability in the level of normal human response to endotoxin, the analysis of alterations in absolute levels of TNF-α in the studied cultures was not feasible. Thus, in all subjects, changes in the level of TNF-α production were normalized to be expressed as the percentage of their respective self-baselines and then compared. In VC and SHM subjects studied at 20 minutes after intervention, increases in the production of TNF-α were significant ($P < .05$) only in cultures stimulated with the highest dose (10 μg/mL) of endotoxin (not shown). However, at 2 hours, control preparations demonstrated a significant ($P < .05$ to .001) augmentation of TNF-α synthesis across all concentrations. No significant differences with respect to the previously mentioned alterations were apparent between VC and SHM groups. In contrast, in all cultures from SMT-treated subjects, the levels of TNF-α were significantly ($P < .05$) reduced at both 20 minutes and 2 hours after treatment.

Fig 2A illustrates typical postintervention changes in the level of TNF-α production observed in the control (cumulative data for VC and SHM) and SMT cultures activated with LPS at a concentration of 10μg/mL.

Production of IL-1β in LPS-Stimulated Cultures

The levels of IL-1β were determined in freshly thawed aliquots of supernatants corresponding to those used for the determination of TNF-α production. The mean values of baseline level synthesis of this cytokine were comparable in all groups (Table 3) ranging from 700 to close to 1200 pg/mL, depending on the dose of the inducer. Overall, the profile of time-related alterations in the level of IL-1β determined in the control (VC, SHM) or experimental (SMT) cultures paralleled closely that in the production of TNF-α. In both control groups, the mean levels of IL-1 β increased markedly (1100 to more than 1600 pg/mL) after 2 hours after intervention/initial venipuncture, depending on the LPS dose used. Relative to baseline, the production of IL-1β in both controls increased over the study period by 30% to more than 60% (Table 3; Fig 1B). Moreover, the frequencies of cultures exhibiting augmented IL-1β production at 2 hours after intervention were significantly ($P < .001$) higher than those showing unaltered or decreased production of this cytokine. On the other hand, in a significant proportion ($P < .05$) of cultures from SMT-treated subjects activated with 1 or 5 μg/mL of LPS,

Table 3. *Effect of SMT on LPS-induced synthesis of IL-1 β*[a]

Group	LPS (μg/mL)	IL-1 β level, mean ± SEM (pg/mL) Baseline	20 min	2 h
VC	1	723 ± 85	798 ± 93	1140 ± 106
	5	967 ± 125	1037 ± 127	1375 ± 114
	10	1157 ± 137	1233 ± 141	1593 ± 177
SHM	1	701 ± 93	781 ± 91	1116 ± 137
	5	908 ± 134	988 ± 184	1294 ± 167
	10	1088 ± 135	1300 ± 155	1640 ± 212
SMT	1	795 ± 176	743 ± 85	725 ± 188
	5	969 ± 142	981 ± 130	951 ± 112
	10	1270 ± 164	1077 ± 147	1028 ± 155

[a] The levels of IL-1β were determined in supernatants from whole-blood cultures initiated before (baseline) and then at 20 minutes and 2 hours after SHM or SMT manipulation. The VC group includes individuals who were subjected to repeated venipuncture only. All cultures were activated with LPS at the indicated concentrations and cultivated for 24 hours.

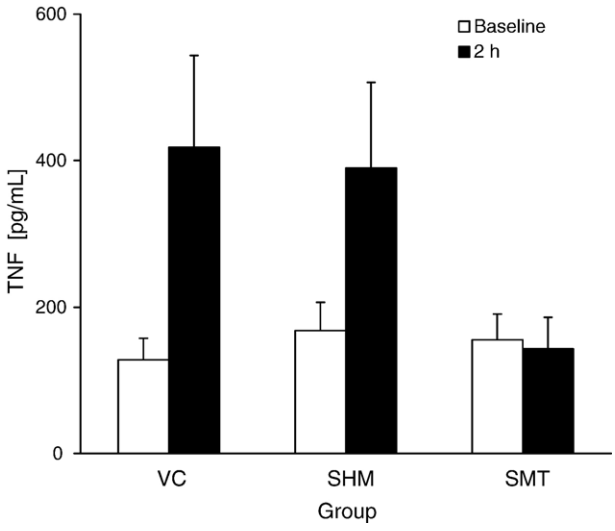

Fig 3. *Production of TNF-α in LPS-free (unstimulated) cultures from the studied subjects. The levels of TNF-α were examined in cultures established before (baseline) and at 2 hours after intervention.*

IL-1 production remained unaltered. In cultures stimulated with the highest dose of the inducer (10 μg/mL), the mean level of IL-1 β was reduced by close to 35% and 20% compared with both controls and the baseline, respectively (Table 3; Fig 1B). Fig 2B illustrates alterations in the production of IL-1β observed 2 hours after intervention and normalized to represent treatment-related intrasubject changes. The production of IL-1 β increased significantly ($P < .05$ to .001) in control cultures with the maximal effect observed in preparations activated with LPS at a concentration of 1 μg/mL. A significant ($P < .05$) decrease, in IL-1β secretion was apparent in SMT preparations activated by LPS at 10μg/mL.

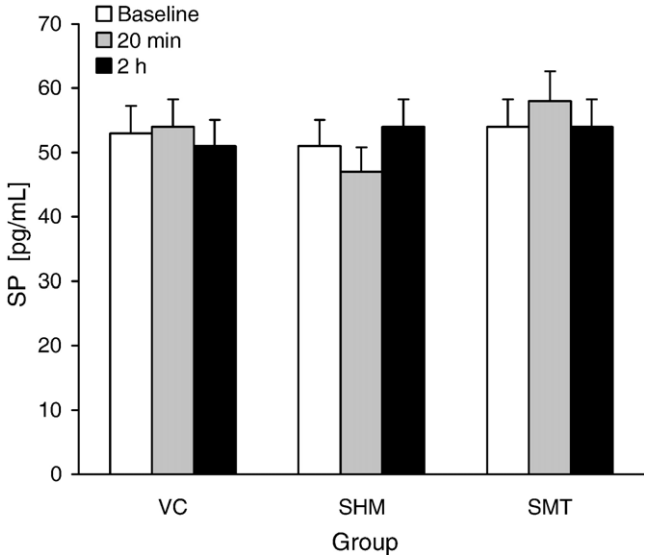

Fig 4. *Serum levels of SP in the studied subjects. The concentrations of SP were determined in samples collected from the control (VC and SHM) and experimental (SMT) groups at baseline and at 20 minutes and 2 hours after treatment.*

Constitutive Cytokine Secretion

Before treatment, most unstimulated (LPS-free) cultures contained no detectable concentrations of TNF-α. A few preparations (7/20 in VC, 8/20 in SHM, and 10/22 in SMT groups) showed comparable levels of spontaneous TNF-α synthesis (Fig 3). At 2 hours after intervention, the constitutive production of this cytokine increased (by twofold to threefold) in both controls but not in cultures from SMT-treated subjects. Furthermore, the number of cultures demonstrating spontaneous release of TNF-α was reduced in the SMT group (6/22, Fig 3). On the other hand, none of the LPS-free cultures contained IL-1β that could be detected within limits of the assay's sensitivity (>50 pg/mL).

Serum Levels of SP

Determinations were performed using freshly thawed serum samples collected at the time of blood withdrawal for the in vitro studies. Fig 4 compares data for VC and SHM controls and SMT subjects. There were no significant differences in SP levels across all groups at any of the given study time. Thus, baseline levels of serum SP were comparable in VC, SHM, and SMT subjects (55, 51, and 54 pg/mL, respectively) and showed no significant alterations within 2 hours after intervention.

Discussion

The present study supports the hypothesis that the spinovisceral reflex effect can encompass functional activity of the immune system. We believe this to be the first report to demonstrate that a single manipulative thrust

to an aberrant vertebral motion segment in the upper thoracic spine of asymptomatic subjects results in down-regulation of the capacity of human leukocytes for the production of proinflammatory cytokines induced by LPS. The study design used in the present report allowed us to create a model in which the LPS-induced inflammatory response in vitro, in control subjects submitted to multiple venipunctures, became augmented. The progressive augmentation of the production of both TNF-α and IL-1β observed in cell preparations from VC and SHM subjects was not unexpected. It has been shown that even minor surgical intervention and local blood clotting may lead to increased expression of messenger RNA for inflammatory cytokines such as TNF-α and IL-6 and thereby result in increased secretion of both cytokines.[17] Furthermore, immunological and inflammatory changes may be induced by a number of factors such as crush, cut, contusion, biopsy, or stress, and the biologic function of circulating blood cells can be altered by the impact of events in local tissues.[18,19] Several of these factors, without doubt, contributed to increased levels of constitutive secretion of TNF in LPS-free controls. Interestingly, such increases were not observed in SMT-treated subjects. The SMT group was exposed to the same number of venipuncture procedures as the control groups. Thus, attenuation of TNF-α and IL-1β in cultures from SMT-receiving normal subjects suggests that spinal manipulation effectively ameliorated the subsequent physiological responses of the peripheral blood cells to inflammatory stimuli.

Furthermore, cultures from SMT-treated individuals displayed reduced sensitivity to otherwise highly effective concentrations of LPS (Tables 2 and 3). LPS-induced activation of mammalian cells involves the interaction of LPS with serum LPS-binding protein (LBP) and recognition of LBP-LPS complexes by a membrane-bound receptor, CD14, on human monocytes.[20] In addition to CD14, LPS interacts with transmembrane receptor(s) responsible for activating signal transduction.[21] It has been well documented that the LPS-induced inflammatory cascade manifested by the production of TNF-α, followed by subsequent induction of IL-1, may be initiated by CD14/LBP-dependent or independent binding determined by endotoxin concentration.[22] Thus, diminished response to higher concentrations of LPS observed after SMT may suggest that one of the activation pathways was affected adversely. In addition, the present study used whole-blood cultures. High (1-10μg/mL) concentrations of LPS induce proinflammatory cytokine production by both mononuclear cells and polymorphonuclear neutrophils.[23] Attenuated proinflammatory cytokine synthesis observed after SMT may therefore reflect altered activity of polymorphonuclear neutrophils. As reported earlier by Brennan et al,[11,12] the biologic responses of these cells may be indeed altered by spinal manipulation.

However, results of the present report do not confirm the earlier observations of Brennan et al[12] concerning the effect of SMT on the synthesis of TNF-α by peripheral blood cells and its relation to the plasma level of SP. In contrast to data presented in this report, Brennan et al[11,12] observed enhanced production of TNF-α in mononuclear cell cultures from subjects receiving spinal manipulation accompanied by elevation in plasma levels of SP. It should be noted that the study design and experimental protocol described by Brennan et al[12] cannot be compared directly to ours. The former study used fractionated mononuclear blood cell cultures obtained at 15 minutes after treatment and activated briefly by a single dose of LPS.[12] This approach did not allocate for the effect of SMT on delayed and sustained LPS activation which occurs 6 to 12 hours after exposure and reflects the capacity of a given cell population for the cumulative synthesis of inflammatory mediators.[24]

The present report used a whole-blood culture system in which cytokine-releasing cells were maintained in the donor's (autologous) serum. Thus, cytokine-producing cells were cultivated in the presence of all serum-associated, potentially regulatory polypeptides, including SP. Generally, this neurotransmitter is implicated in the induction of inflammatory mediators' production.[9] Although the synthesis of TNF-α and IL-1β was attenuated in a significant proportion of the SMT-receiving subjects and the opposite effect was observed in both controls, no apparent fluctuations in serum concentrations of SP were observed in any of the studied groups (Fig 1). It is, therefore, unlikely that SP could have contributed to the observed modulation of cytokine production.

In the present study, the manipulative thrust was directed to the thoracic spinal segment determined to exhibit aberrant segmental movement. An audible cavitation was used as our guide for successful manipulation. However, as determined by Ross et al,[25] such cavitation does not necessarily occur specifically at the manipulated segment. Furthermore, multiple cavitations might occur, although a single audible cavitation is perceived by the clinician. Thus, no claim can be made of the specificity of the effect of SMT relative to the level of spinal segment involved.

The nature of anti-inflammatory effect observed after spinal manipulation is presently unknown. Recent recognition of a neural immune circuit as instrumental in the control of inflammation[26,27] may suggest that activation of endogenous anti-inflammatory pathways should be considered as a systemic consequence of SMT. Recent investigations have shown that the vagus nerve may control central inflammatory responses.[28,29] Surgical or chemical vagotomy can render animals sensitive to endotoxin shock.[30] Conversely, systemic inflammatory responses to endotoxin are attenuated after the direct electrical or pharmacological stimulation of the vagus nerve.[31] Investigations in vitro have shown that LPS-induced human macrophage production of TNF-α, IL-1β, and IL-6 was inhibited after the exposure of these cells to the

principal neurotransmitter of parasympathetic nervous system, acetylcholine (Ach).[32,33] Of interest is that Ach does not inhibit the synthesis of IL-10, an anti-inflammatory cytokine produced simultaneously with TNF, IL-1, and IL-6 by LPS-activated macrophages.[31] The Ach-mediated reactions, described now as "cholinergic anti-inflammatory pathway,"[32,33] occur after vagus nerve stimulation and the subsequent interaction of Ach with the nicotinic receptor $\alpha 7$ subunit expressed by many cells of the immune system.[34]

Direct experimental evidence that the spinovisceral reflex effect on the immune system may be mediated by vagus nerve activation is currently not available. However, the vagus nerve provides innervation to the principal body organs as well as the reticuloendothelial system. It has been postulated that, in humans, the vagus output can be regulated through conditioning, acupuncture, and other therapies targeting the cholinergic anti-inflammatory pathway.[29] It is now feasible that such therapies could include SMT, and experimental work in this area should yield the supporting evidence.

The clinical significance of observations presented in this study are also related to studies on the pathophysiology of discogenic low back pain and sciatica. Investigations have revealed that local inflammatory response and the associated production of proinflammatory mediators, such as TNF-α, may represent a major factor in the genesis of pain and functional changes in the neural activity of spinal nerve roots.[10] Hypoalgesic effects of SMT have been already reported.[35] Furthermore, a preliminary report by Mohammadian et al[36] has demonstrated that a single spinal manipulation treatment may lessen capsaicin-induced local hyperalgesia and allodynia, suggesting that a central anti-inflammatory mechanism might be indeed activated by manipulative therapy.

Recent clinical studies have shown that TNF-α blockade by anti–TNF-α monoclonal antibodies was highly effective in reducing sciatic pain.[37] Based on these and related results, development of drugs targeting the production and/or action of proinflammatory cytokines is now suggested as critical for pain management in patients with low back pain and sciatica.[38] In contrast to pharmacological interventions, SMT is likely to present a noninvasive and efficacious alternative to such therapies. Future studies are now necessary to address the issue of the effect of SMT on the magnitude and duration of inflammatory responses in chiropractic patients.

Conclusion

SMT-treated subjects show a time-dependent attenuation of LPS-induced production of the inflammatory cytokines unrelated to systemic levels of SP. This suggests SMT-related down-regulation of inflammatory-type responses via a central yet unknown mechanism.

Acknowledgment

The authors are grateful to Ms Janet Hayes, RN, for help with venipuncture, Dr Janet Ho for technical assistance, and Dr Judy Waalen for her expert assistance with statistical analyses.

Practical Application

- This was a novel experimental design to explore somatovisceral effects of SMT through experimental assessment of the inflammatory-type responses using cytokine secretion as an outcome measure.
- The findings suggest that SMT may be a potential therapeutic modality to reduce inflammatory response.

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