Does Spinal Block Through Tattooed Skin Cause Histological Changes in Nervous Tissue and Meninges?

An Experimental Model in Rabbits

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Background and Objectives: Although there is no documented evidence that tattoo pigments can cause neurological complications, the implications of performing neuraxial anesthesia through tattooed skin are unknown. In this study, we aimed to assess whether spinal puncture performed through tattooed skin of rabbits determines changes over the spinal cord and meninges. In addition, we sought to evaluate the presence of ink fragments entrapped in spinal needles.

Methods: Thirty-six young male adult rabbits, each weighing between 3400 and 3900 g and having a spine length between 38.5 and 39 cm, were divided by lot into 3 groups as follows: GI, spinal puncture through tattooed skin; GII, spinal puncture through tattooed skin and saline injection; and GIII, spinal puncture through skin free of tattoo and saline injection. After intravenous anesthesia with ketamine and xylazine, the subarachnoid space was punctured at S1-S2 under ultrasound guidance with a 22-gauge 2½ Quincke needle. Animals in GII and GIII received 5 μL/cm of spinal length (0.2 mL) of saline intrathecally. In GI, the needle tip was placed into the yellow ligament, and no solution was injected into the intrathecal space; after tattooed skin puncture, 1 mL of saline was injected through the needle over a histological slide to prepare a smear that was dyed by the Giemsa method to enable tissue identification if present. All animals remained in captivity for 21 days under medical observation and were killed by decapitation. The lumbosacral spinal cord portion was removed for histological analysis using hematoxylin-eosin stain.

Results: None of the animals had impaired motor function or decreased nociception during the period of clinical observation. None of the animals from the control group (GIII) showed signs of injuries to meninges. In GII, however, 4 animals presented with signs of meningeal injury. The main histological changes observed were focal areas of perivascular lymphoplasmacyte infiltration in the pia mater and arachnoid. There was no signal of injury in neural tissue in any animal of both groups. Tissue coring containing ink pigments was noted in all GI smears from the spinal needles used to puncture the tattooed skin.

Conclusions: On the basis of the present results, intrathecal injection of saline through a needle inserted through tattooed skin is capable of producing histological changes over the meninges of rabbits. Ink fragments were entrapped inside the spinal needles, despite the presence of a stylet.

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Tattoos have become fashionable worldwide.[1] In the last decade, tattooing of unconventional sites such as the lumbar and sacral area has become popular.[2,3] As a result, anesthesiologists who perform neuraxial blocks are faced with the dilemma of whether to place the needle through the tattooed skin.

Several carcinogenic aromatic amides have been detected in the tattoo ink, but it is not clear whether the compounds embedded in the skin are biologically active.[4] Theoretical concerns have been raised about the possibility of a hollow needle entrapping tissue fragments containing such pigments when the needle is advanced to the deeper structures.[5] These pigments potentially can be deposited in the subarachnoid space when injection is made for spinal anesthesia. Chemicals in the tattoo ink could induce arachnoiditis. Similar reaction has been described following the injection of local anesthetics with preservatives in the epidural space.[6] Large-bore needles favor loading tissue deposits, but even 25-gauge Quincke point and Whitacre needles used for spinal anesthesia have been shown to produce tissue coring.[7]

To date, there are no reports of complications following neuraxial blocks placed in the lumbar area when the needle was placed through tattooed skin.[8] Theoretical risks may be debated, but experiments should be performed to determine the safety of placing a needle through the tattooed skin for a neuraxial block. A survey was conducted in Portugal regarding this matter. Of 162 anesthesiologists participating in the survey, 56.8% of the respondents said they preferred to place the neuraxial block through the clear skin, as they were not sure what the effects of the pigment would be when placed in the spinal canal.[9] Unlike Portuguese anesthesiologists, 57% of French anesthesiologists who were surveyed favored placing the epidural needle through the tattooed skin, and 70% felt that there was no consensus on this matter.[10]

As there are no studies in the literature describing the effects of tattoo pigment introduced into the intrathecal space by needle puncture, we conducted this study in an animal model to determine the effects of such pigments on the meninges and the neural tissue and to infer the safety of such a practice. We hypothesized that tattoo ink fragments may cause inflammatory injury over nervous tissue and meninges when they are inadvertently injected into the intrathecal space.

METHODS

The Botucatu Medical School Ethics Committee on animal experiment approved the study. Thirty-six adult male rabbits were obtained from the Experimental Animal Center at the State University of São Paulo at Botucatu campus. The mean weight of the animals was 3.5 (SD, 0.3) kg (group I [GI]) and 3.4 (SD, 0.3) kg (group II [GII]) for the experimental groups and 3.9 (SD, 0.3) kg

for the control group (group III [GIII]). The mean length of the vertebral column was 39 (SD, 1) cm for both experimental groups and 38.5 (SD, 0.9) cm for the control group. All tests were performed in accordance with the guidelines of the International Association for the Study of Pain.[11]

Rabbits were randomized to 1 of the 3 groups based on the injection performed through the tattooed skin. Each group had 12 rabbits. All intrathecal needle punctures were performed with ultrasound guidance using a linear transducer with 6- to 13-MHz frequency. The 3 groups were as follows:

GI: Spinal puncture was made through tattooed skin, but no solution was injected. Needle was advanced up to the yellow ligament and then removed.

GII: Spinal puncture was made through tattooed skin, and 0.9% normal saline was injected.

GIII: Spinal puncture was made through clear skin with no tattoos, and 0.9% normal saline was injected.

The volume of saline injected into the spinal fluid was 5 μL/cm length of the vertebral column of the animals.

Group I was evaluated for the presence of tissue coring and/or ink pigments when the needle was placed through the tattooed skin. Group II was evaluated for the effects of tissue coring containing pigments on the meninges and the neural tissue. Finally, in GIII, the effect of saline injected into the intrathecal space was studied. It was crucial to ensure that the volume of saline injected in a very small intrathecal space in rabbits did not trigger any neural injury due to an increase in intrathecal pressure jeopardizing medullary blood flow.[12] This group was also studied to exclude possible intraneural injection. Intraneural injection of volume as little as 0.05 mL can produce degeneration in the peripheral nerve of the rabbit.[13]

Experiments were performed in 2 phases:

1. tattoo protocol
2. spinal puncture protocol

Tattoo Protocol (Phase 1)

In phase 1, animals had the skin over intervertebral spaces S1-S2 (diameter = 2 cm) tattooed with a red pigment and underwent the following experimental sequence. The animals fasted for 12 hours before the procedure, with water ad libitum. All rabbits were anesthetized with intravenous xylazine (3 mg/kg) and ketamine (10 mg/kg). An area 10 cm around the site of the spinal puncture at S1-S2 intervertebral space level was washed with soap and water. This was then followed by removal of the hair, and the skin was cleansed with 0.9% normal saline. The naked skin was then prepared and draped in a sterile manner with chlorhexidine gluconate. Tattoo was done by a professional tattoo artist. Animals were evaluated for 30 days after the tattooing procedure and before spinal puncture. This time was divided into 3 phases: inflammatory reaction and necrosis, formation of basement membrane, and normal epidermis and dermis. According to the literature, once the skin shows normal ultrastructure when analyzed using an electron microscope, ink particles are found only in dermal fibroblasts.[14]

Intrathecal Puncture Protocol (Phase 2)

Spinal puncture was performed 1 month after the tattooing. The animals fasted 12 hours before the procedure, with water ad libitum. All rabbits were once again anesthetized with intravenous xylazine (3 mg/kg) and ketamine (10 mg/kg). A 20-cm area around the site of the spinal puncture at S1-S2 intervertebral space level was washed with soap and water. Hair was removed, and the skin was cleaned with 0.9% normal saline. The area was prepared and draped in a sterile manner. Ultrasound-guided (M-Turbo; SonoSite, Bothell, Washington) subarachnoid puncture was made using a linear transducer (6- to 13-MHz frequency). The transducer was draped in a sterile Tegaderm (3M Health Care), and sterile ultrasound gel was applied. The puncture was performed through the midline, approximately 45 degrees to the skin with a 22-gauge Quincke point needle. Difficulties during the procedure were recorded. If a traumatic spinal tap was identified, as defined by the need for more than 1 attempt of puncture, the animal was immediately excluded from the study, and no solution was administered into the subarachnoid space. Once the needle was properly located and identified by ultrasound image, the stylet was removed from the needle, and 5 μL/cm of spinal length (0.2 mL) of saline was injected over 10 seconds using 1-mL disposable syringes (GII and GIII). No solution was administered in GI into the intrathecal space. In GI, after lumbar puncture, the needle was withdrawn from the animal skin; then, the stylet was removed from the needle, and saline (1 mL) was injected through the needle over a histological slide to prepare a smear that was dyed by the Giemsa method to enable tissue identification if present.

Evaluation and Outcomes

Animals were evaluated 1 hour after intrathecal puncture and once daily for 6 months after the lumbar puncture. All animals received prophylactic antibiotic therapy every 15 days to prevent infections. Each animal was assessed for the following secondary outcomes: motor deficit and response to nociception.

Motor deficit was evaluated according to Drummond and Moore criteria,[15] as follows: 0 = paraplegic, with no lower-extremity motor function; 1 = poor lower-extremity motor function (flicker of movement or weak antigravity movement only); 2 = some lower-extremity function with good antigravity strength but inability to draw legs under body and/or hop; 3 = ability to draw legs under body and hop but not normally; and 4 = normal motor function.

Nociception was assessed by reaction to painful pressure in lower and upper extremities and ears. To prevent possible interference due to visual perception of the stimuli by the animals, one researcher was responsible for masking the animals with a nontransparent cloth comfortably positioned around their neck. Nociceptive pressure stimuli were applied by the bilateral pinch of a skin fold over sacral, lumbar, and thoracic dermatomes, as well as the interdigital membranes of limbs and ears by a surgical clamp. The presence of pain was defined by the following: limb withdrawal, vocalization, and facial expression. Nociception was classified dichotomously into absent or present.

Spinal Cord Preparation and Staining

Histological analysis of the spinal cord and meninges of the rabbits was performed after the 6-month observation period. Animals were then killed by decapitation after intravenous sodium pentobarbital. Thereafter, the lumbar and sacral segments of the spinal cord with the surrounding meninges were removed quickly within 3 minutes to minimize the risk of injuries to those tissues from ischemia and apoptosis. The anatomical specimens were fixed in a 10% formalin solution. After a 7-day incubation period, 0.5-cm-thick histological sections were prepared starting at 10 cm above the level of the intrathecal puncture to the end of the cauda equina. The histological sections were stained by hematoxylin-eosin (H&E). Two researchers (E.M.G. and M.A.M.) experienced in histological neurotoxicity assessment independently classified each of the sections according to the presence or absence of histological injury. If any kind of lesion was identified, it was further specified. To investigate the possible dose-related gradient

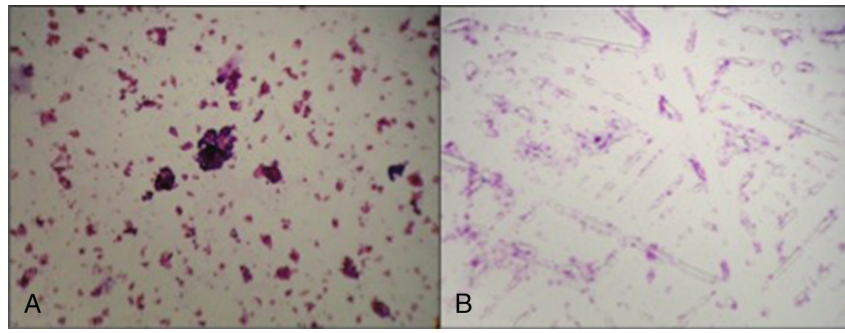

FIGURE 1. A, Red ink fragments. B, The dyed NaCl crystals observed under optical microscopy (original magnification ×40).

effect, injuries were stratified according to the severity and extent as ascertained by consensus. Pathologists who performed the histological evaluation were blinded to the study protocol. The histological results were classified as "normal," when no changes were observed in the histological sections. When the "injury" was presented, the histological changes were classified based on the following criteria:

(*a*) injury type: 1 = arachnoiditis, 2 = arachnoiditis + spinal cord injury, 3 = spinal cord injury
(*b*) injury site: 1 = dorsal, 2 = lateral, 3 = ventral
(*c*) injury extent: 1 = <10%, 2 = >10% and <50%, 3 = >50%
(*d*) injury depth: 1 = white matter, 2 = gray matter, 3 = white and gray matter
(*e*) blood vessels: 1 = normal, 2 = wall thickening, 3 = thrombosis

Statistical Analysis

The sample size was calculated according to Fleiss et al,[16] estimating a proportion of histological neurotoxicity of 1% and 70%[17] in the control and tattooed groups, respectively, so as to obtain a power value of 90% while setting the 1-sided α level for statistical significance at 0.05.

Data Analysis

The R software version 2.3.4 (R Foundation, GNU project, MIT, Boston, Massachusetts, and University of Auckland, New Zealand) was used for the performance of statistical analysis. In order to evaluate the effectiveness of the randomization procedure and the comparability of the 2 study groups, we performed 1-way analysis of variance comparing group differences regarding animals' weights and the length of their vertebral column. One-sided Fisher exact test was selected to compare the frequencies of the findings on primary and secondary outcomes between the experimental and the control groups. α Level for statistical significance was set at 0.05.

RESULTS

Groups I and II were similar in weight, whereas animals in GIII were heavier ($P = 0.001$). Length of the vertebral column was similar in all groups ($P = 0.06$). None of the animals were excluded from the study because of traumatic puncture or death. The time of recovery from anesthesia in all groups was approximately 30 minutes. During the 6 months of observation, none of the animals had impaired motor function or decreased nociception.

The smear from GI needle (after spinal puncture through the tattoo) showed red ink fragments under optical microscopy (Fig. 1A). We also performed a smear with 1 mL of saline dyed by the Giemsa method (Fig. 1B). It showed only normal saline crystals.

Histological Results

None of the animals from GIII (puncture through clear skin + spinal injection) showed macroscopic signs of direct injuries such as hemorrhage, infarct, vacuolization, necrosis, or meningeal thickness (Figs. 2A, B). In GII (tattoo puncture + saline spinal injection), however, 4 animals had signs of meningeal injury. The main histological changes observed were areas with lymphoplasmacyte infiltration in meninges and in blood vessels (Table 1; Figs. 3A–D).

DISCUSSION

A strong evidence-based guideline for performing a neuraxial block through a tattooed skin remains an unfulfilled goal.[18] Although tissue coring has been well documented after puncture with different needles,[7,19] coring of tissue by spinal needles with specific tattoo pigment transfer or migration has not been reported.

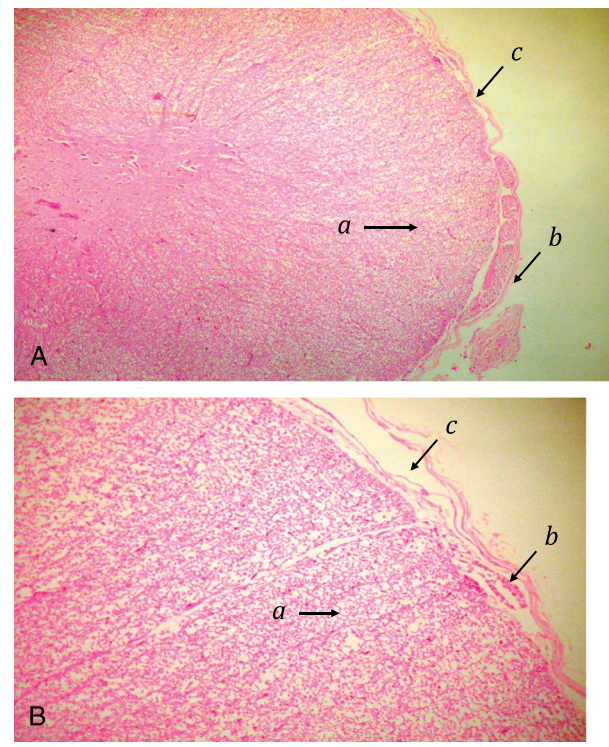

FIGURE 2. Group III (figures A and B – no tattoo + saline spinal injection): segment of lumbar nerve tissues (arrow *a*), blood vessels (arrow *b*), and meninges (arrow *c*) showing no abnormalities (H&E stain, original magnifications × 40 [A], × 100 [B]).

TABLE 1. Findings Observed in GII (Tattoo + Saline Spinal Injection) During the Review of Histological Sections

	Meninges and Blood Vessel			
Histological Changes	Fibrous Thickening	Inflammatory Infiltration	Trabeculae Formation (Adherence Among Pia, Arachnoid, and Dura Mater)	Spinal Cord Necrosis
Animal 1	Absent	Absent	Absent	Absent
Animal 2	Absent	Absent	Absent	Absent
Animal 3	Absent	Absent	Absent	Absent
Animal 4	Absent	Absent	Absent	Absent
Animal 5	Absent	Focal area of limphoplasmocitary perivascular infiltration in pia mater (posterior area; <5%)	Absent	Absent
Animal 6	Absent	Absent	Absent	Absent
Animal 7	Absent	Focal area of limphoplasmocitary infiltration in pia mater (posterior area; <5%)	Absent	Absent
Animal 8	Absent	Absent	Absent	Absent
Animal 9	Absent	Focal area of limphoplasmocitary perivascular infiltration in arachnoid (posterior area; <5%)	Absent	Absent
Animal 10	Absent	Focal area of limphoplasmocitary perivascular infiltration in pia mater and arachnoid (posterior area; <5%)	Absent	Absent
Animal 11	Absent	Absent	Absent	Absent
Animal 12	Absent	Absent	Absent	Absent

This study is the first to show that intrathecal injection of saline through a needle inserted through tattooed skin produces histological changes in the meninges of rabbits. The main histological changes consisted of lymphocytic infiltrates in the blood vessels in the pia and arachnoid mater. Moreover, our results demonstrate the presence of ink fragments into the spinal needle when a puncture is made through a tattooed skin. The presence of a stylet did not prevent the entrapment of ink fragments into the needle.

Hollow needles, with or without stylet, entrap tissue fragments (cores) in their bore as they pass to the deeper structures. Campbell et al,[7] during a study of the needle tips of Quincke and Whitacre needles, found that the 25-gauge spinal needles currently used in anesthesia could produce tissue coring after failed attempts to identify the intrathecal space. In a cadaveric study, fluorescein tissue particles were seen in all 3 needles on microscopic evaluation after the 27-gauge modified Quincke, Sprotte, and Whitacre needles placed through a fluorescein scrubbed

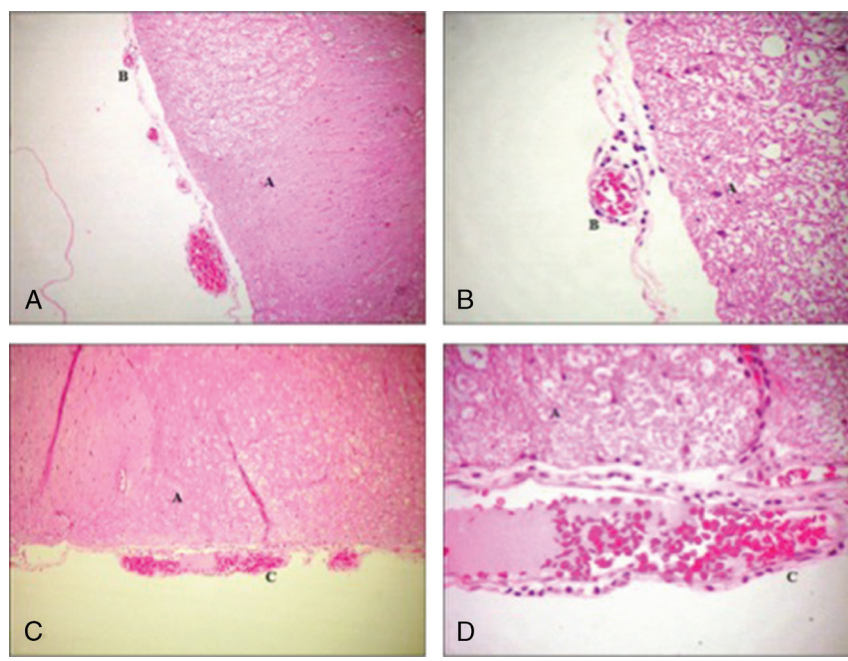

FIGURE 3. Group II (tattoo + saline spinal injection): lumbar nervous tissue with no abnormalities (A). A and B, Animal 5 shows blood vessels into the pia mater presenting with lymphoplasmacyte inflammatory infiltration (B) (H&E stain, original magnifications ×100 [A], ×400 [B]). C and D, Animal 9 shows perivascular inflammatory infiltration into the arachnoids (H&E stain, original magnifications ×100 [C], ×400 [D]).

back into the subarachnoid space.[19] In the present study, after intrathecal puncture through tattooed skin, tissue impregnated with red ink fragments was observed in the smear under optical microscopy.

Tattoos containing red ink are most often associated with skin disorders.[20] Based on this fact, we chose the red pigment to tattoo the skin of the animals. Tattoo compounds, in comparison with cosmetics, for example, are not officially controlled. Therefore, the origin and chemical structure of the coloring agents are not known. Consequently, neither the tattoo artist nor the tattooed patient has any information about the compounds in the skin. In vitro studies have provided some evidence that tattoo colors—industrial pigments, which have been produced by the chemical industry—were never intended for human use. They may indeed contain hazardous, toxic, and/or carcinogenic compounds.[21,22] Moreover, in 2005, Jack et al[23] reported a case of axillary lymphadenopathy clinically mimicking metastatic melanoma 30 years after a decorative tattoo.

The prolonged clinical observation of 6 months enabled us to evaluate chronic effects of ink fragments on the meninges. Results of previous studies using the same methodology, however, in other animal model (dogs) with shorter time of clinical evaluation (21 days), showed that the exogenous substance primarily damages the nervous tissue, where clinical and histological alterations are immediately observed after the agent administration.[17,24] However, when the meninges are damaged, a longer interval of the inflammatory reaction causes nervous tissue lesion. Nevertheless, another study using the same methodology with subarachnoid amitriptyline injection depicted extensive adhesive arachnoiditis 21 days after spinal injection.[25] It is possible that the lymphocyte infiltration found in GII was the beginning of an inflammatory process that would cause additional histological changes if a longer time interval of clinical evaluation were chosen. Additional studies with different time intervals of killing the experimental animals after intrathecal injection through tattooed ink would need to be conducted to answer this question.

As a limitation of the present study, no red ink fragments were found in the neural tissue during the optical microscopic analysis. However, the present study was a blinded (for the histological analysis), controlled trial in which potential confounding issues due to lesions induced by the spinal puncture procedure in the tattooed group were controlled by comparison to the control group.

The experimental model selected (single intra-spinal puncture) is similar to the usual spinal anesthesia procedures done in humans. This technique has less risk of complications than other models in which implantable intrathecal catheters are used. Furthermore, fast removal and fixation of the anatomical specimens, as well as the comparison with controls submitted to the same procedure, make it unlikely that the histological findings in our study were due to cord extraction–related ischemic injuries or other procedure-related mechanisms. It is possible that a foreign substance that entered the neural tissue and meninges could cause the histological changes found in the animals of GII. In this case, ink fragments entrapped at the tip of the spinal needle are likely to be the foreign substance that caused this damage. Accordingly, we believe that future studies are needed to elucidate the earlier and later effects of tattoo pigments over the meninges and nervous tissue.

In conclusion, our results indicate that intrathecal injection of saline through a needle inserted through tattooed skin is capable of producing histological changes in the meninges of rabbits. To date, there are no reports of spinal complications from inserting a needle through tattooed skin. However, one should not assume the safety of this practice because of the lack of reported complications. The number of patients having neuraxial anesthesia through tattooed skin is likely to be small. In addition, arachnoiditis and other neuropathies do not occur immediately after exposure to the noxious agent. It may be too early to detect any long-term consequences. Further studies are necessary to elucidate the mechanism of the meningeal toxicity observed and to evaluate the clinical long-term outcome in rabbits and in different animal models to determine the safety of this practice. Based on the results of the present study, we suggest that more research is needed to determine the safety of inserting a spinal needle through tattooed skin. Our clinical recommendation is that the spinal needles should preferably be placed through areas free of pigments in the presence of lumbar tattoos because of the possibility of tissue coring.

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