An Ultrasonographic and Histological Study of Intraneural Injection and Electrical Stimulation in Pigs

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BACKGROUND: In this study we evaluated the minimum stimulating current associated with intraneural needle placement and sonographic appearance of intraneural injection.
METHODS: We inserted a needle 2 cm inside 28 pig nerves (brachial plexus *in vivo*), recorded the minimum current to elicit a motor response, and injected dye (5 mL) under ultrasound (US) imaging.
RESULTS: The minimum current to elicit a motor response was 0.43 mA (range: 0.12–1.8 mA). Nerve expansion was visualized by US in 24 of 28 nerves. Histology revealed penetration of the epineurium in these same 24 nerves. There was no evidence of dysplasia within the fascicle of any nerve.
CONCLUSIONS: US may prove useful to detect intraneural injection, whereas a motor response above 0.5 mA may not exclude intraneural needle placement. The correlation between intraneural injection and neurological dysfunction remains unclear.
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Our ability to detect unintentional needle puncture and/or injection inside a nerve is limited. Subjective reporting of paresthesiae or pain upon injection are not reliable warning signs, even in awake patients (1,2). During nerve stimulation, the minimum threshold current associated with intraneural needle placement has not yet been established (3). Indeed, intra and extraneural peripheral nerve stimulation appear clinically indistinguishable (4,5). Data from one study suggest that high injection pressures may predict nerve injury after intraneural injection (6), but the association between high injection pressure and intraneural injection is not consistent. Recent reports propose that ultrasound (US) imaging may be useful for detecting intraneural injection (7,8). Our objectives for this animal study were to evaluate the minimum stimulating current and sonographic appearance associated with intraneural injection.

METHODS

After Institutional Animal Care Committee approval, we studied five female Yorkshire-cross pigs (weight: 55–60 kg). Under general anesthesia, each animal underwent bilateral incisions in the upper chest to expose the segments of the brachial plexus (equivalent to the cords). Care was taken to dissect the skin and overlying muscles without disrupting the nerves. A 22-gauge, 5-cm insulated needle (Stimuplex®, B. Braun Medical, Bethlehem, PA) was inserted lengthwise into each nerve and directed 2 cm proximally to ensure secure intraneural needle placement. Electrical stimulation was then applied to elicit a motor response using a nerve stimulator (Stimuplex® HNS11, B. Braun Medical) set at 1 Hz and 100 μsec duration. The minimum threshold current required to elicit a distal motor response in the digits was determined in duplicate or triplicate and recorded by two independent investigators.

After intraneural electrical stimulation, 2.5 mL dextrose 5% combined with 2.5 mL of Sennelier Black Indian Ink was injected through the intraneural needle at a rate of 1 mL/5 s. To assess the appearance of intraneural injection under US, a linear 15-MHz probe was placed directly over each nerve segment and injection was observed in real time using a Philips HDI 5000 machine (ATL Ultrasound, Bothell, WA), while video images were captured for nerve diameter measurements by two independent investigators.

After injection, the ink-stained nerve segments were excised for histological examination and the animals subsequently euthanized.

RESULTS

Intraneural electrical stimulation and injection were performed in all 28 identifiable nerve segments. A total of 65 intraneural stimulations were performed in 28 nerves. The median minimum threshold current

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Table 1. Minimum Current Required to Elicit Muscle Twitch During Intraneural Stimulation

Current (mA)	N^a
<0.2	22 (34)
0.2–0.5	14 (21)
0.51–1.0	26 (40)
>1.0	3 (5)

Values in parenthesis indicate the percentage.

[a] $N = 65$, number of samples.

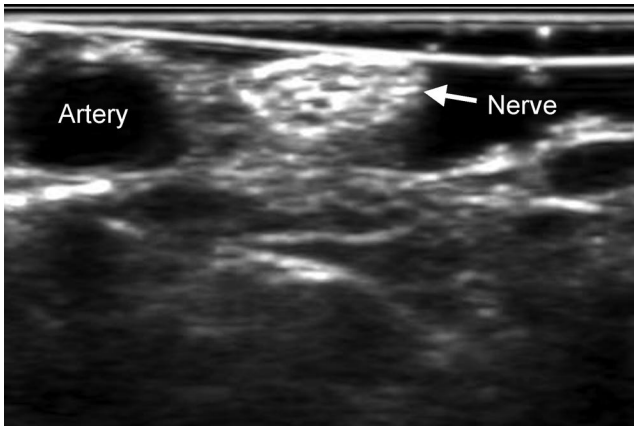

Figure 1. Preinjection sonogram. Transverse sonogram using a 15-MHz linear array transducer probe (Philips HDI 5000 system, Bothell, WA). The hypoechoic pulsatile axillary artery is readily identified. The adjacent nerve is seen as a distinct hyperechoic structure with internal hypoechoic punctuations.

required to achieve a motor response was 0.43 mA (range: 0.12–1.8 mA). The minimum threshold current was >0.5 mA in 45% of the intraneural stimulation samples, and >1 mA in 5% of the samples (Table 1).

Of the 28 nerves studied, ink-stained injectate was deemed completely intraneural in 24 nerves on the basis of real-time US visualization of nerve expansion (video). Injectate was deemed partially intraneural in one nerve, and completely extraneural in the remaining three nerves. For the 24 nerves in which injectate was deemed completely intraneural, the median increase in nerve diameter between pre and postinjection as measured by US was 57% (range: 14%–200%) (Figs. 1 and 2). For all 24 nerves where the injectate was deemed completely intraneural based on US imaging, direct inspection of the gross nerve specimens revealed tissue distension and thorough ink staining (Fig. 3).

In the remaining four nerves, limited (one nerve) or no (three nerves) expansion was seen on US. In these four nerves, sonographic evidence of extraneural fluid accumulation (hypoechoic collection outside nerve) appeared immediately after the initial 1 mL of injectate. Upon direct inspection of these four nerves, there was no obvious distension and ink staining was scant, appearing only to coat the nerve, as the ink readily washed off with saline irrigation.

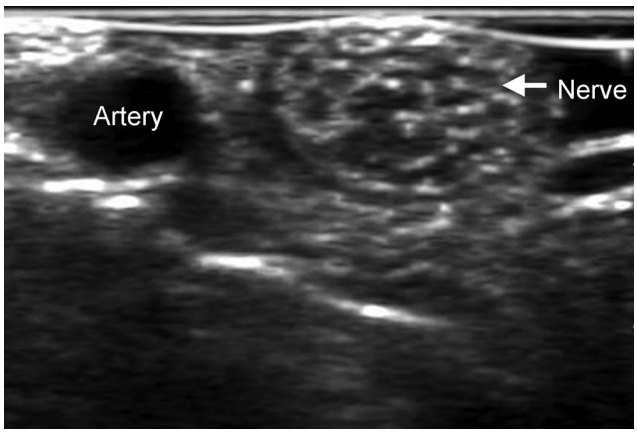

Figure 2. Postinjection sonogram. Transverse sonogram using a 15-MHz linear array transducer probe (Philips HDI 5000 system). During and after intraneural injection of 5 mL injectate, distension and expansion of the hyperechoic nerve structure are present.

Figure 3. Injected nerve specimens (gross). The two outside nerves reveal significant distension and thorough ink staining which suggests complete intraneural injection. The middle nerve reveals minimal distension and scant ink staining that is readily washed off, suggestive of extraneural injection.

Histological examination revealed evidence of intraneural injection in 24 of 28 nerves; that is, ink-stained injectate had penetrated the epineurium (Fig. 4). Each of these 24 nerves matched with those in which injectate was deemed completely intraneural by US. Among these 24 nerves, ink had minimally penetrated the perineurium (i.e., intrafascicular injection) in only two nerves (Fig. 5). In the four nerves where only limited (one nerve) or no (three nerves) expansion was visualized by US, there was scant or no histological evidence of intraneural injection. Finally, there was no histological evidence of dysplasia within the nerve fascicle in any of the 28 nerves studied (Fig. 6).

DISCUSSION

Under the present study conditions, we found US sensitive in detecting intraneural injection of as little as 1

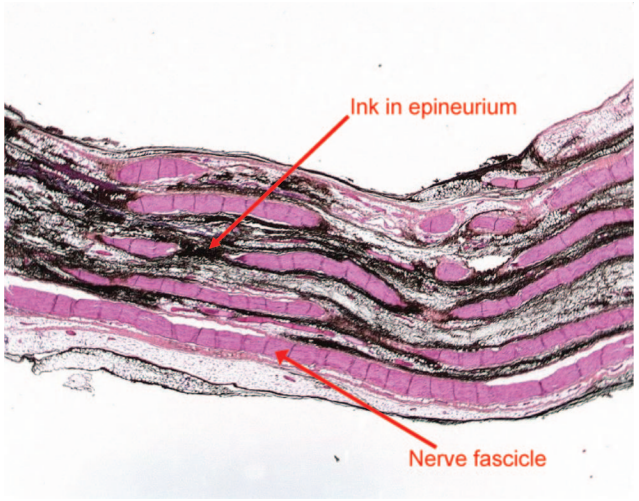

Figure 4. Postinjection histology. Longitudinal nerve section at ×15 magnification. This specimen demonstrates ink in the epineurium which is indicative of intraneural injection.

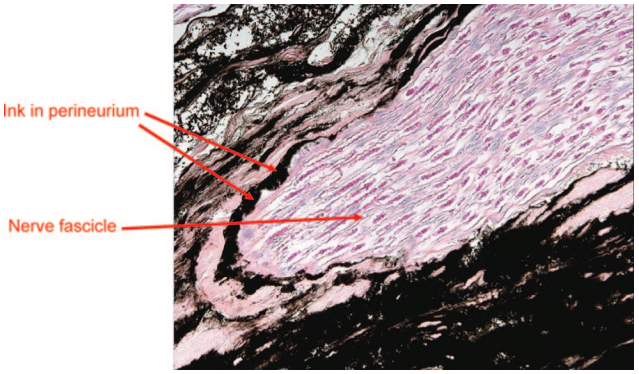

Figure 5. Postinjection histology. Longitudinal nerve section at ×200 magnification. This specimen demonstrates ink in the perineurium which is indicative of intrafascicular injection.

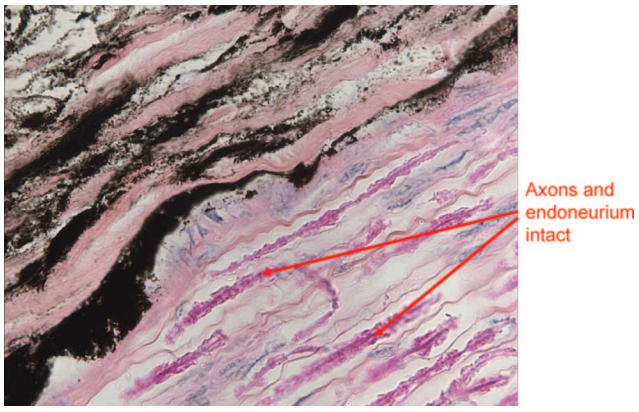

Figure 6. Postinjection histology. Longitudinal nerve section at ×600 magnification. This specimen demonstrates the normal architecture of the nerve fascicle following intraneural injection.

mL of injectate. The characteristic appearance of intraneural injection that we observed was quite different from that reported by Schafhalter-Zoppoth et al. (7). We observed tissue expansion by hypoechoic fluid within the predominantly hyperechoic nerve structure and not nerve compression by an expanding hypoechoic fluid collection as reported by Schafhalter-Zoppoth et al. (7). It is unclear if the difference in appearance can be explained by study conditions, or a complete intraneural injection in the present study versus subtotal injection in Schafhalter-Zoppoth et al. 's report (7).

Reports of accidental intraneural (1,2) and intraspinal (4,5) needle placement during nerve stimulator-guided block suggest that this method of localization is not fail-safe. Intraneural/spinal stimulation may not be associated with very low stimulating currents. In the present study, the minimum current required to achieve a motor response with intraneural needle placement was <0.2 mA in only one-third of cases. A lack of consistency in motor response during intraneural stimulation may be explained by topographical disparity of motor and sensory fibers within compound nerves (9). Another possible explanation for high current requirements is impaired nerve conduction secondary to mechanical nerve trauma after needle puncture (2 cm inside the nerve) in the present study.

Our study has several limitations. Importantly, we studied a very small sample of pigs. There may be differences in neural architecture and conduction properties between human and pig species, thus significantly undermining the generalizability of our data. Our study assessed the extent of neural damage after a 5-mL injection, not a typical injection volume in clinical practice. We reported histological findings after an acute intraneural injection and not long-term postinjection changes that might occur after days or weeks. We evaluated postinjection changes in histology and not in neurobehavior (e.g., paralysis). Thus, the clinical relevance of these findings remains unclear and is the subject of our continuing investigation. Finally, we inserted the needle 2 cm inside the nerve, a distance not likely reached in clinical practice.

In summary, our preliminary data suggest that US may be a useful tool to detect intraneural injection, and thus the subject of worthwhile future study. Further, a muscle twitch achieved above the conventional minimum stimulating threshold of 0.5 mA may not exclude intraneural needle placement. Finally, we found no association between intraneural injection and consequent nerve dysplasia upon early histological examination.

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