Original Article
Effects of glucose administered with lidocaine solution on spinal neurotoxicity in rats

Hanxiang Ma[1], Tingting Xu[2,3], Xiangsheng Xiong[2,4], Jingjing Mao[2,4], Fan Yang[2], Yonghai Zhang[1], Zhixia Bai[1], Xuexin Chen[1]

[1]Department of Anesthesiology, General Hospital of Ningxia Medical University, Yinchuan 750004, China; [2]Ningxia Medical University, Yinchuan 750004, China; [3]Department of Anesthesiology, Heze Municipal Hospital, Heze 274000, China; [4]Department of Anesthesiology, Huaiyin Hospital, Huaian 223300, China

Abstract: To investigate whether intrathecal administration of 10% glucose increases functional impairment and histologic damage in rats when mixed with 5% lidocaine. After implanted intrathecal catheter, 32 male Sprague-Dawley rats were randomly assigned to one of four groups: lidocaine group (Group L, n=8) received 5% lidocaine 20 μL, lidocaine with glucose group (Group LG, n=8) received 5% lidocaine with 10% glucose 20 μL, glucose group (Group G, n=8) received 10% glucose 20 μL and normal saline group received normal saline 20 μL (Group NS, n=8). Four days after intrathecal injection, sensory impairments of rats in the four groups were evaluated by using the tail-flick test. The histologic changes of spinal cord and nerve roots were observed by electron microscopy and light microscopy. There was no significant difference in baseline tail-flick latencies between the four groups (P=0.284). On the 4th day after intrathecal injection, the assessment result of sensory function was similar to baseline (P=0.217) in saline-treated animals. Sensory impairment occurred after intrathecal administration of 5% lidocaine, and 10% glucose with 5% lidocaine worsen this satiation (P=0.0001); histologic changes in 10% glucose with 5% lidocaine-treated group has differ significantly from lidocaine-treated group (P=0.001). Sensory function after intrathecal administration of 10% glucose was similar to baseline and did not differ from the saline group (P=0.995); histologic changes in 10% glucose-treated rats did not differ significantly from saline controls (P=0.535). These results suggest that 5% lidocaine can induce spinal neurotoxicity and 10% glucose with 5% lidocaine could worsen spinal neurotoxicity.

Keywords: Glucose, spinal neurotoxicity, lidocaine, intrathecal injection

Introduction

Spinal anesthesia as a safe technique, which has various advantages as Kettner et al. [1] described in a meta-analysis, is being widely used in anesthesia practice. However, concerns about potential neurotoxicity of intrathecal local anesthetics have been aroused great attention [2-4]. Recent clinical injuries during spinal anesthesia have provided some indirect evidences that local anesthetics have neurotoxic effect [5-7]. It is reported that a variety of spinal neurologic disorders have been described as hyperesthesia, motor weakness and paresthesia in the legs, backache, severe pain and burning in patients' perineal region and cauda equina syndrome [7-9]. Though ischemia, infection, physical trauma and local anesthetics themselves are speculated to underlie neurotoxicity after spinal anesthesia, yet the possible mechanism of that potential neurotoxicity are unclear [4, 7].

With its different proportion in mixture, glucose of various concentrations that act as a common component in anesthetic solution is being used for spinal anesthesia. It is reported [10] that neurons have a constantly high glucose demand. The quantity of glucose uptake by neurons relies on the extracellular glucose concentration, and the glucose presents highly in subarachnoid space during spinal anesthesia. However, Hashimoto and Sakura [11] have conducted a study that rats who had received 1-hour infusion of 10% glucose, 5% lidocaine or normal saline administered via implanted intrathecal catheter were evaluated in persistent sensory impairment, the result of
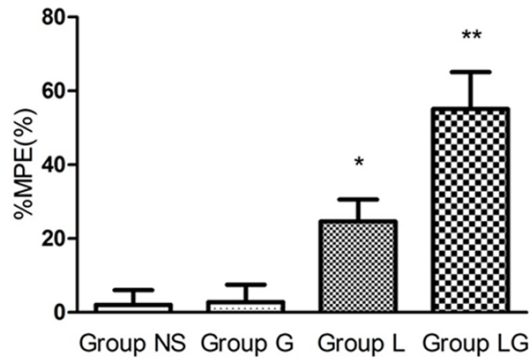
Figure 1. Sensory function 4 days after an intrathecal injection (20 µL/min) of normal saline, 10% glucose, 5% lidocaine or 5% lidocaine with 10% glucose in group NS, group G, group L and group LG, respectively. Group NS=normal saline group, Group G=glucose group, Group L=lidocaine group, Group LG=lidocaine with glucose group. Tail-flick latency values were calculated as the average of the tail and are expressed as percent maximum possible effect (%MPE), which %MPE=(tail-flick latency-baseline)/(cut-off-baseline) ×100. Data reflect the mean ± SD. *$P<0.05$ versus glucose or saline. **$P<0.05$ versus all other groups.

which indicated that glucose could not induce neurologic injury at clinical concentrations. Although the functional impairment and histologic damage of spinal cord or nerve roots were not induced by glucose in their study, 10% glucose increasing the risk of neurotoxicity is still being questioned when it is used with a hyperbaric local anesthetic in spinal anesthesia. Accordingly, we hypothesize 5% lidocaine mixed with 10% glucose may increase spinal neurotoxicity in spinal anesthesia. The purpose of this study is to investigate the histologic and functional changes of spinal cord and nerve roots after intrathecal 5% lidocaine mixed with 10% injection.

Materials and methods

Animals

With approval from the Committee on Animal Research of the Ningxia Medical University, 32 male Sprague-Dawley rats after catheterization (250-300 g, Experimental Animal Center of Ningxia Medical University, China) were included in this study. All of the procedures were performed in accordance with the National Institute of Health guidelines on animal care. Rats included in this study were individually removed to a plastic cage with wood chips.

Food and water were available ad libitum. The animal house was on 12 h light-dark cycles, and kept at a relative humidity between 45% and 65% and at a room temperature between 22-24°C. All rats were trained in the test situation several times before experiment.

Experimental protocol

The rats were randomly divided into four groups, 8 rats in each group: lidocaine group (Group L, n=8) received 5% lidocaine 20 µL, lidocaine with glucose group (Group LG, n=8) received 5% lidocaine with 10% glucose 20 µL, glucose group (Group G, n=8) received 10% glucose 20 µL and normal saline group received normal saline 20 µL (Group NS, n=8). The rats with any neurological deficit or other complications and with not appearing to bilateral lower extremity paralysis after intrathecal injection of 2% lidocaine 15 µL within 5 min were excluded from the study. Four days after intrathecal injection of the solution described above, the sensory function of animals were evaluated by using the tail-flick test. And then, the animals were sacrificed by injecting overdose of propofol and processed for examination of light microscopy and electron microscopy. The neurological changes of nerve roots and spinal cord were observed, and the injury degree of those was compared between groups through nerve injury score described in what follows in the passage.

Surgical preparation

A rat model with chronic intrathecal catheterization has been described in details by Saito et al. [12] the catheter was intrathecally implanted using an aseptic technique under anesthesia with intraperitoneal injection of pentobarbital sodium (50 mg/kg). The end of the small profiled catheter was introduced into the subarachniod space, with over a length of 1.3~1.5 centimeter in the L3-4 vertebra; the other end of the catheter was tunneled subcutaneously toward the occiput, with the distal tip exposed out of skin in the neck. Cefazolin sodium (100 mg) was injected intramuscularly to prevent infection. The rats recovered from the intrathecal catheterization three days before the beginning of experiments, and each rat was observed and evaluated during recovery period in order to ensure normal sensory responses, gait and motor.
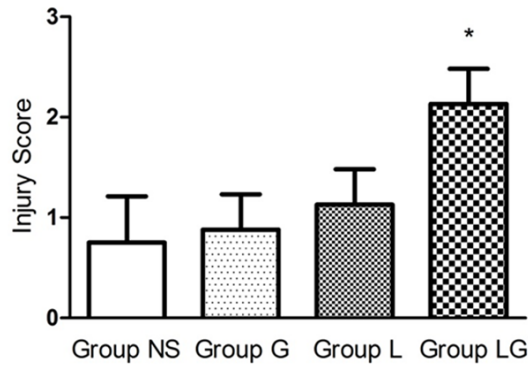
Figure 2. Nerve injury score for sections obtained 10 mm caudal to the conus 4 days after an intrathecal infusion of normal saline, 10% glucose, 5% lidocaine or 5% lidocaine with 10% glucose in group NS, group G, group L and group LG, respectively. Group NS=normal saline group, Group G=glucose group, Group L=lidocaine group, Group LG=lidocaine with glucose group. Nerve injury scores were based on all fascicles present in each nerve cross-section. Each fascicle was assigned an injured score of 0-3, where 0=normal (no edema or injured nerve fibers); 1=mild (edema, but little or no nerve fiber degeneration or demyelination); 2=moderate (demyelination or demyelination involving less than 50% of nerve fibers); and 3=severe (degeneration or demyelination involving more than 50% of fibers). Data are expressed as mean ± SD. *$P<0.05$ versus all other groups.

Measurement of sensory function

As Hashimoto and Sakura described [12, 13], the tail-flick test, which was performed at the distal, intermediate and proximal portions of the tail by using a device (Ugo Baseline, Italy), was used to assess sensory function. Unless the response occurred by 10 seconds, the heat stimulus was terminated to prevent tissue damage (cut-off value).

Neuropathologic examination

After evaluated the sensory impairment, the animals were sacrificed by injecting overdose of propofol, and after that, fixative solution was perfused in rats for nerve tissue fixation. The lumbar spinal cord and relevant nerve roots were dissected to get pathological tissues. The tissues positioned at 6 mm rostral and 12 mm caudal to the conus medullaris were sectioned for light and electron microscopic examination.

Data analysis

Functional assessment: Tail-flick latencies at distal, intermediate and proximal portions of

the tail for each rat were averaged to achieve mean latencies. The average tail-flick latency of each rat was converted to percent maximal possible effect, which calculated as (tail-flick latency-baseline)/(cut-off-baseline) ×100.

Histologic analysis: Sections obtained 6 mm rostral to the conus were used for qualitative evaluation of the spinal cord, and 12 mm caudal to the conus for quantitative analysis of nerve injury. Each fascicle present in the cross-section was assessed for an injury score of 0-3 (0=normal: no edema or injured nerve fibers; 1=mild: edema, but little or no nerve fiber degeneration or demyelination; 2=moderate: degeneration or demyelination involving less than 50% of nerve fibers; 3=severe: degeneration or demyelination involving more than 50% of fibers), as described previously [13-16]. The injury scores of all fascicles for each rat were then calculated to get an average injury score.

Statistics: Before administration of the test solutions, one-way analysis of variance was used to detect the group equivalent of baseline tail-flick latencies. A paired t-test was used to compare the baseline latencies with the latencies 4 days after bolus infusion in the saline group. Factorial analysis of variance (ANOVA 2X2) was used to compare the percent maximal effect data for tail-flick latencies, with comparisons of all pairs performed with the Tukey Kramer test. With Dunn correction for multiple comparisons, Mann-Whitney U test was used to evaluate nerve injury severity for lidocaine group versus lidocaine with glucose group, lidocaine group versus saline group and glucose group versus saline group. For all comparisons, P<0.05 was considered significant.

Results

After catheterization, 31 rats were included in the experiment, one rat exhibited catheter obstruction after intrathecal catheterization was excluded from data analysis. All catheters tips were dissected and found to lie approximately at the L4-6 lumbar vertebral level in subarachnoid space.

Neurologic function

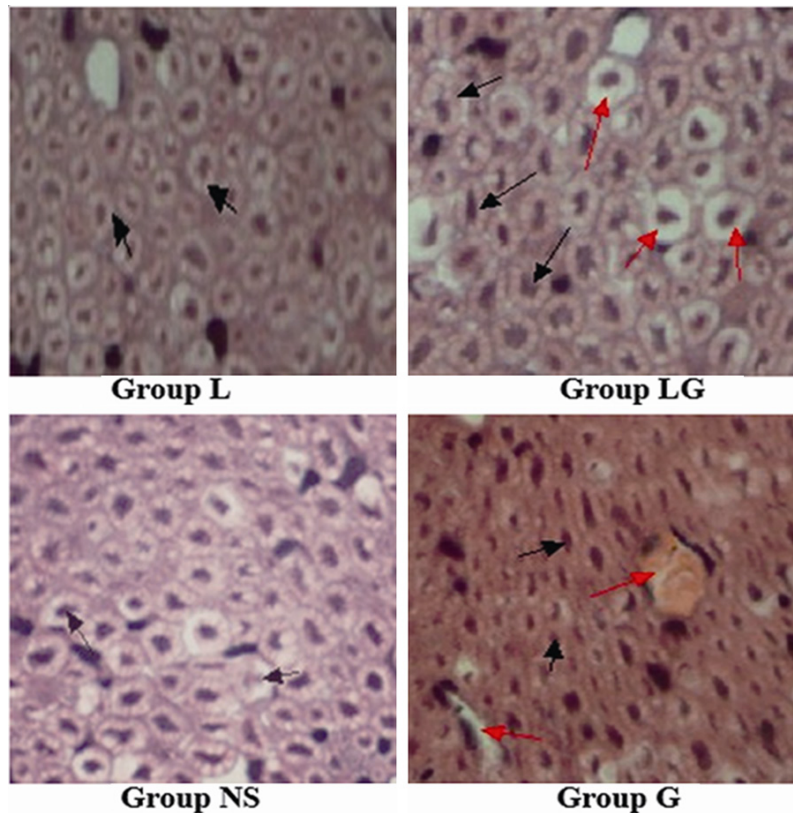There was no significant difference in baseline of tail-flick latencies among the four groups

Figure 3. HE ×400; observed by light microscopy four days after subarachnoid catheter the obvious nerve edema (black arrow) and demyelination phenomenon (red arrow). Group NS=normal saline group, Group G=glucose group, Group L=lidocaine group, Group LG=lidocaine with glucose group.

thologic evaluation revealed no significant difference in lidocaine-treated group versus saline-treated group and glucose-treated group versus saline-treated group (P=0.097, P=0.535) (**Figure 2**).

Damage fraction in the light microscope was more serious in group LG than that in group L, group G and group NS, and as far as the severity of damage fraction were concerned, there was no significant difference among group L, group G and group NS. The nerve histological changes were as follows: (1) group LG: edema and demyelination of nerve fibers or transgender; (2) group L: changes in the main to a small amount of edema and demyelination of nerve fibers nerve histological changes; (3) group G and group NS: mainly mild edema of histological changes (**Figures 3 and 4**).

(P=0.284). On the 4th day after intrathecal administration of saline, the assessment results of sensory function were similar to the baseline (P=0.217). The elevation in tail-flick latencies (percent maximal possible effect) in lidocaine with glucose-treated group differed significantly from latencies in lidocaine-treated group, glucose-treated group and saline-treated animals (P=0.0001, P=0.0001, P=0.0001). There was significant difference in tail-flick latency (percent maximal possible effect) for lidocaine-treated group compared that with glucose-treated group and saline-treated group (P=0.0001, P=0.0001) (**Figure 1**).

Neuropathologic evaluation

The spinal cord injury score revealed that it was more severe in lidocaine with glucose-treated group than that in lidocaine-treated group, glucose-treated group and saline-treated group (P=0.001, P=0.0001, P=0.0001). Neuropa-

Discussion

As a component of anesthetic solution, glucose was usually used to increase the density of anesthetic solution, which can be great benefit to cycle fluctuations inhibition in clinical anesthesia. Hyperbaric local anesthetics made with glucose produce effectiveness in controlling the level of anesthesia. However, this anesthesia practice is still being questioned because there were some studies that have reported that spinal neurotoxicity occurred after intrathecal administration of local anesthetic mixed with glucose [17, 18]. This is the reason why recent studies have drawn attention to the possibility of increasing the risk of potential neurotoxicity after adding glucose to the solution [19].

The results demonstrate that rats with intrathecal injection of 5% lidocaine have expressed more severe sensory impairment
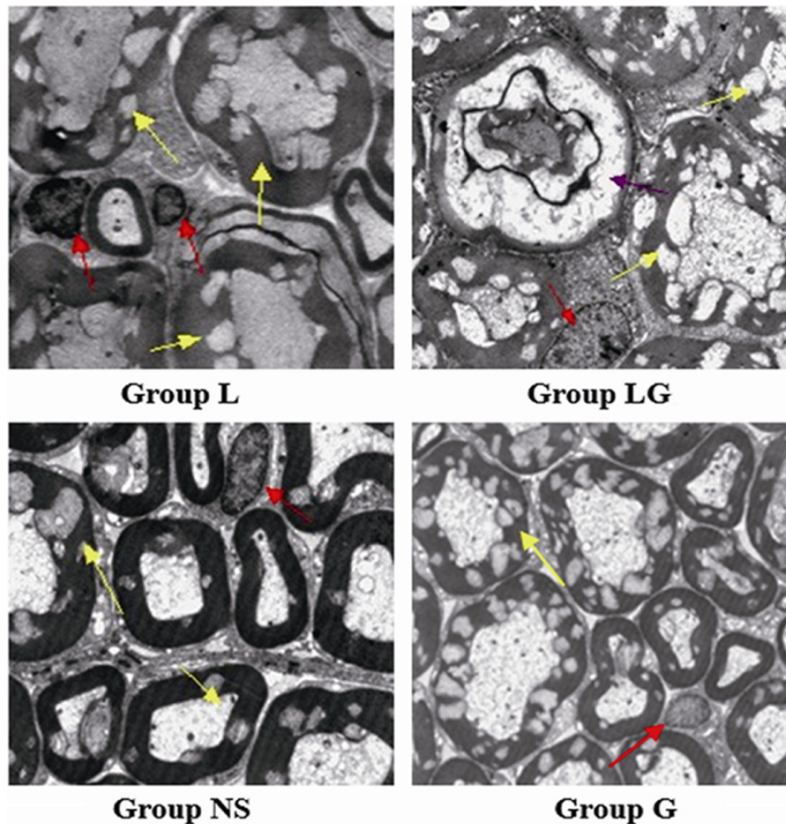
Figure 4. 1000×; Schwann cells (red arrow) can be clearly observed in the surface of the myelin nerve obvious edema (yellow arrow) and demyelination of nerve fibers phenomenon (purple arrow), no significant damage to the mitochondria in the cells. Group NS=normal saline group, Group G=glucose group, Group L=lidocaine group, Group LG=lidocaine with glucose group.

by Russell et al., [21] may increase the reactive oxygen species and mitochondrial swelling, which proceeds to neuronal apoptosis. On the other hand, intrathecal administration of hyperbaric solution (local anesthetic with high concentration of glucose) can lower the pH of cerebrospinal fluid, which has been postulated to contribute to the development of tachyphylaxis as well as to neurotoxicity [23]. Due to the hyperbaric solution with 10% glucose, more local anesthetics concentrated in spinal space than usual; this might increase the direct neurotoxic effect that is caused by local anesthetic.

However, sensory function and histologic changes 4 days after intrathecal administration of 10% glucose alone were similar to baseline and did not differ from the saline group in our study. In Hashimoto's study [11], the rats were divided into three groups to receive infusions of 5% lidocaine, 10% glucose, or normal saline. Their results demonstrated that persistent sensory impairment in rats with administration of 5% lidocaine occurred. But it did not exhibit in rats with administration of glucose and normal saline, which is consistent with our results.

Sakura's study [24] has reported that 5% lidocaine mixed 7.5% glucose did not alter the neurotoxicity in rats. In their investigation, the way that the rats received a single intrathecal infusion of 5% lidocaine mixed 7.5% glucose within 30 minutes to 4 hours at a lower rate of 1 µL/min is different to this study. Although 5% lidocaine mixed 7.5% glucose were used in spinal space, the intrathecal administration rate is low. The way that they use is not common in clinical spinal anesthesia that was performed by a bolus intrathecal injection of local anesthetics with glucose. The current study

than that of intrathecal injection of normal saline or glucose. Moreover, compared with intrathecal injection 5% lidocaine alone, the rats with intrathecal administration of 5% lidocaine with 10% glucose have induced more severe sensory impairment and morphologic damage. These findings indicate that injuries followed with subarachnoid block are led by the direct neurotoxic effect of local anesthetic, as is like the results observed in other studies [20-22]. And 10% glucose could worsen the potential neurotoxicity with intrathecal administration of 5% lidocaine in rats. Because of excessive anesthetic concentrations and certain local anesthetic formulations in previous studies, neurotoxicity of local anesthetic occurred after spinal anesthesia [15, 18]. Outside the local anesthetic, there are some other factors contributing to the neurotoxic effect. On the one hand, high glucose-induced oxidative stress and mitochondrial dysfunction in neurons, as reported

indicated that neurotoxicity might be related to the intrathecal administration of a larger cumulative dose of lidocaine with glucose.

There are several limitations to this present study. First of all, only one kind of concentration of glucose was chosen in this study. Different kinds of concentrations of glucose mixed with local anesthetics are used in clinical practice, and the highest concentration used in clinical practice is 10% glucose solution. However, the other lower concentrations of glucoses with local anesthetic have been studied [11, 24]. Secondly, four days instead of the day after intrathecal injection of the four test solutions, sensory impairment was evaluated with the term of withdrawal from a noxious thermal stimulus. But this time point was used to ensure the sensory dysfunction was not caused by residual anesthetic after intrathecal lidocaine.

We concluded that intrathecal injection of 5% lidocaine could induce spinal nerve sensory impairment, and 10% glucose could worsen the potential neurotoxicity of rats with intrathecal administration of 5% lidocaine. The present study suggests that the potential neurotoxicity should be considered when the local anesthetics mixed into high concentration of glucose are used in subarachnoid block.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xuexin Chen, Department of Anesthesiology, General Hospital of Ningxia Medical University, 804 Shengli Street, Yinchuan, China. Tel: +86-13995001031; Fax: +86-951-4082981; E-mail: Chenxuexin2637@163.com

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