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Original Article

The Safety and Side Effects of Amide-Based Local Anesthetics in Rats with Acetaminophen-Induced Hepatic Injury

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What's Known

- Amide-based local anesthetics are extensively used for pain management in outpatient surgeries.
- To date, most studies have mainly focused on the neurological side effects caused by amide-based local anesthetics.

What's New

- This is the first experimental study comparing the safety and side effects of five commonly used amide-based local anesthetics in rats.
- Articaine and prilocaine with felypressin induced the least effect on hepatic enzymes of rats with abnormal hepatic function. However, in rats with normal hepatic function, lidocaine caused the least hepatic damage.

Abstract

Background: The use of amide-based local anesthetics is generally considered to be safe. However, the literature on their safety in patients with hepatic injury is scarce. For the first time, the present study aimed to evaluate the effect and safety of five commonly used amide-based local anesthetics in the setting of hepatic failure.

Methods: A total of 96 Sprague-Dawley rats were studied from September 2015 to September 2016 in the Animal Laboratory Center, Shiraz University of Medical Sciences, Shiraz, Iran. They divided into three groups, namely a control, induced liver failure (LF), and non-LF groups. The rats were administered local anesthetic agents (lidocaine, prilocaine with felypressin, lidocaine with epinephrine, mepivacaine, articaine, and prilocaine). The effect of these drugs was evaluated by comparing the liver enzyme levels of the rats. The data were analyzed using SPSS software. The independent *t* test, one-way ANOVA, and the *post hoc* tests were used to compare groups. A $P<0.05$ was considered statistically significant.

Results: In non-LF rats, mepivacaine, lidocaine, and lidocaine with epinephrine caused a significant increase in aspartate aminotransferase (AST) level compared with the effect of prilocaine with felypressin and articaine. In non-LF rats, only mepivacaine resulted in a significant increase in AST level compared with lidocaine ($P=0.007$) and prilocaine with felypressin ($P=0.044$). In this group, only mepivacaine caused a significant increase in alanine transaminase (ALT) level compared with lidocaine ($P=0.016$). Whereas in the LF group, mepivacaine caused an increase in ALT level compared with the effect of both prilocaine with felypressin ($P=0.009$) and articaine ($P<0.001$). The use of mepivacaine in the LF group caused a significant increase in gamma-glutamyl transpeptidase level compared prilocaine with felypressin ($P=0.039$).

Conclusion: Articaine and prilocaine with felypressin local anesthetics induced the least change in hepatic enzyme levels in rats with abnormal hepatic function.

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Keywords ● Anesthetics ● Local drug-related side effects and adverse reactions ● Safety ● Liver failure ● Rats, Sprague-Dawley

Introduction

Amide-based local anesthetics are extensively used for pain

management in outpatient surgeries.[1] In comparison with ester local anesthetics, amide-based local anesthetics are preferred by medical practitioners because of their rapid and stable anesthesia.[2] All types of anesthetics have certain adverse side effects and cause a wide range of symptoms such as neurological signs (mental disorientation), seizures, and cardiac effects (hypotension and cardiac depression).[3-5] Following the administration of these anesthetics, their concentration in the bloodstream rises and subsequently, the nervous system is depressed. Since the main metabolism of amide-based local anesthetics is through the hepatic system,[6] a major concern surrounds the safety of these anesthetics in patients with hepatic injury. In individuals with insufficient liver function, the metabolic activity is impaired, resulting in an inability to process these anesthetics. Consequently, the concentration of the drugs in their blood flow remains high; leading to possible toxic levels.[7] However, despite the risk of toxicity, amide-based anesthetics remain the most commonly used anesthetics; underscoring the importance of safety measures before their administration.

To date, most studies have mainly focused on the neurological side effects caused by amide-based local anesthetics.[8, 9] Although the use of these types of anesthetics is generally considered to be safe,[2] literature on their safety in patients with hepatic injury is scarce. Therefore, the present study aimed to evaluate the hepatic effects of commonly used amide-based local anesthetics in rats with and without induced hepatic failure. To the best of our knowledge, this is the first experimental study that compares five of the most common local anesthetics in order to determine their effect and safety in the setting of hepatic failure.

Materials and Methods

The present randomized experimental study was conducted from September 2015 to September 2016 at the Animal Laboratory Center affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. A total of 96 male Sprague-Dawley rats aged 8 weeks and weighing 140±10 g were obtained from the animal laboratory. Initially, all rats were weighed to inhibit any discrepancies. The rats were housed in standard cages with 12-hour daylight (starting at 8:00 am) at an ambient temperature of 22±2 °C with 55% relative humidity. The rats were given a 5-day acclimatization period with free access to standard chow and water.[10] The usage of and care for rats were in accordance with the Guidelines for Laboratory Animal Care.[11] The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (IR.SUMS.REC.1396.48).

Based on a simple random sampling method, the rats were divided into three groups. The first group (n=16) served as the control group and was randomly divided into two equal subgroups. Half of the rats received intraperitoneal administration of paracetamol to induce liver failure without local anesthesia. The other half received no medication. The second group (n=40) included rats with induced liver failure that were subjected to local anesthesia. All rats in this group received intraperitoneal administration of paracetamol and were then randomly divided into five equal subgroups. Each subgroup was administered a different type of amide local anesthetic, namely lidocaine, prilocaine with felypressin, lidocaine with epinephrine, mepivacaine, or articaine. The third group (n=40) included rats without liver failure that were subjected to local anesthesia. The rats in this group were divided into five equal subgroups and were administered a local anesthetic agent similar to the second group. All of the drugs were made by EXIR Inc., Tehran, Iran.

Liver failure was induced by administration of 1 g/kg body weight paracetamol intraperitoneally using insulin syringes.[12] After four hours, the first blood samples were obtained to evaluate the severity of induced liver damage based on liver enzyme levels, namely aspartate aminotransferase (AST), alanine transaminase (ALT), and gamma-glutamyl transpeptidase (GGT). Then, based on the pharmacological toxicity, the rats were administered the maximum recommended doses of the local anesthetic agents through the oral mucosa.[13-15] The dosages for each type of amide local anesthetic were: 5 mg/kg lidocaine, 8 mg/kg prilocaine with felypressin, 7 mg/kg lidocaine with epinephrine, 5 mg/kg mepivacaine, 7 mg/kg articaine, and 7 mg/kg prilocaine. Second blood samples were obtained four hours after the first blood samples to compare liver enzyme levels.

Statistical Analysis

The data were analyzed using SPSS software for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to examine the normal distribution of data. The independent *t* test and one-way ANOVA test were used to compare the means of normally distributed quantitative data between the different groups. Tukey's HSD *post hoc* test was used to evaluate differences between the subgroups. Data were presented as mean and standard deviation (SD). $P<0.05$ was considered statistically significant.

Results

Baseline and pre-intervention hepatic enzyme levels in the rats of each subgroup are presented in table 1. There was no significant difference in the initial levels of liver enzymes between different anesthetic agents in both groups (with and without liver failure). After the administration of anesthetics, there was an increase in enzyme levels in all groups. The extent of change in AST, ALT, and GGT levels was significantly higher in rats with induced liver failure compared with those without induced liver failure (P<0.001).

The results showed that the extent of change in the AST level caused by the administration of each anesthetic agent was significantly different in both groups; rats without liver failure (P=0.006) and those with liver failure (P<0.001). The use of mepivacaine in rats with liver failure and lidocaine with epinephrine in rats without liver failure caused the highest AST level change. The *post hoc* tests in rats without liver failure showed that only mepivacaine caused a significant increase in the AST level compared with lidocaine (P=0.007) and prilocaine with felypressin (P=0.044). However, the administration of mepivacaine, lidocaine, and lidocaine with epinephrine in rats with liver failure showed a significant increase in the AST level compared with prilocaine with felypressin and articaine (table 2).

The extent of change in the ALT level caused by the administration of each anesthetic agent was significantly different in both groups; rats without liver failure (P=0.007) and those with liver failure (P<0.001). In both groups of rats, similar to the changes in AST level, mepivacaine and lidocaine with epinephrine caused the highest ALT level change. *post hoc* tests in rats without liver failure showed that only mepivacaine caused a significant increase in the ALT level compared with lidocaine (P=0.016). However, the administration of mepivacaine in rats with liver failure showed a significant increase in ALT level compared with prilocaine with felypressin (P=0.009). Articaine caused the least increase in ALT level compared with all other administered anesthetics (table 2).

The extent of change in the GGT level caused by the administration of each anesthetic agent was only significantly different in rats with liver failure (P<0.001). In this group, similar to changes in AST and ALT levels, mepivacaine and lidocaine with epinephrine caused the highest

Table 1: Baseline and pre-intervention hepatic enzyme levels in rats

		None	Lidocaine (mg)	Lidocaine with epinephrine (mg)	Prilocaine with felypressin (mg)	Mepivacaine (mg)	Articaine (mg)	P value*
Without liver failure	AST	41.86±2.79	41.87±2.85	41.85±2.85	42.02±2.56	41.78±2.83	41.80±2.75	0.981
	ALT	29.87±3.56	29.85±3.67	29.86±3.56	29.87±3.50	29.88±3.56	29.87±3.67	0.990
	GGT	6.50±0.77	6.54±0.79	6.56±0.78	6.55±0.80	6.52±0.79	6.50±0.78	0.251
With liver failure	AST	1957.62±101.92	1834.87± 293.90	1978.62± 298.55	1869.37± 101.15	2061.61± 174.90	1968.50± 150.15	0.290
	ALT	750.75±94.20	786.75±92.73	772.50±60.19	739.00±64.21	775.50±55.66	785.85±66.36	0.736
	GGT	6.62±0.74	6.37±0.51	6.87±0.84	6.12±0.64	6.37±0.51	6.62±0.74	0.211

*One-way ANOVA test (Tukey *post hoc*), AST: Aspartate aminotransferase, ALT: Alanine transaminase, GGT: Gamma-glutamyl transpeptidase, Data are presented as mean±SD

Table 2: Comparison of the difference in hepatic enzyme levels between different intervention groups

Hepatic enzymes		Medication use					P value*
		Lidocaine	Mepivacaine	Prilocaine with felypressin	Articaine	Lidocaine with epinephrine	
ΔAST† (units/L)	Without LF	138.00±8.87^{a}	216.37±65.77^{b}	153.50±32.65^{a}	183.12±44.05^{ab}	197.42±41.61^{ab}	0.006
	With LF	618.00±130.19^{a}	746.00±92.62^{a}	406.37±91.90^{b}	290.37±49.12^{b}	661.42±110.96^{a}	<0.001
	P value**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ΔALT (units/L)	Without LF	63.50±10.10^{a}	125.87±59.47^{b}	73.50±27.11^{ab}	102.87±31.17^{ab}	117.41±39.84^{ab}	0.007
	With LF	238.25±48.27^{ab}	289.00±37.87^{a}	222.62±40.01^{b}	141.50±23.04^{c}	259.57±29.49^{ab}	<0.001
	P value**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ΔGGT (units/L)	Without LF	2.00±0.75^{a}	2.00±0.53^{a}	2.25±0.46^{a}	2.37±0.91^{a}	2.00±1.15^{a}	0.813
	With LF	16.25±0.88^{ab}	17.25±0.77^{a}	15.75±1.28^{b}	12.50±1.06^{c}	16.42±0.97^{ab}	<0.001
	P value**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*One-way ANOVA test (Tukey *post hoc*), **Independent *t* test, †Post-intervention minus pre-intervention levels, $^{a, b, c}$ Significantly different change in enzyme level compared to the next following alphabet, LF: Liver failure, AST: Aspartate aminotransferase, ALT: Alanine transaminase, GGT: Gamma-glutamyl transpeptidase, Data are presented as mean±SD

GGT level change. However, in rats without liver failure, the administration of articaine and prilocaine with felypressin caused the highest GGT level change. However, this difference was not significant between different anesthetic agents. *post hoc* tests in rats with liver failure showed that mepivacaine caused a significant increase in GGT level compared with prilocaine with felypressin (P=0.039). Articaine showed the least increase in GGT level compared with all other administered anesthetics (P<0.001).

Discussion

The extent of change in hepatic enzyme levels caused by the administration of anesthetics varied in each group of rats. Lidocaine induced the least change in AST and ALT levels in rats without hepatic failure. However, in rats with hepatic failure, articaine and prilocaine with felypressin induced the least change in AST, ALT, and GGT levels. On the other hand, in both groups, mepivacaine and lidocaine with epinephrine induced the most changes in AST, ALT, and GGT levels; except for GGT level in rats without liver failure.

It was found that lidocaine administration in rats without liver injury caused the least change in hepatic enzyme levels. In other words, in rats with no history of liver injury, the more classic anesthetic lidocaine seemed to render the least change in hepatic enzymes. Lidocaine is extensively used as local anesthesia in North America.[2] However, prilocaine is the second choice after articaine when dealing with hepatic failure.

Clinical trials comparing existing local anesthetics and articaine vary significantly depending on the study design and outcome.[16] A previous study compared the efficacy of articaine and lidocaine in a sample of 30 patients in a randomized controlled trial.[17] They reported no significant difference in the onset and duration of anesthesia between these local anesthetic drugs. However, more recent studies have reported that articaine was more effective on different sites of action than other anesthetics. Some studies have reported that articaine was more effective in terms of its anesthetic effects and duration of induced anesthesia.[8, 18] In the present study, articaine caused the least changes in hepatic enzymes in rats with induced hepatic injury. Articaine is among the most common local anesthetics used in dental surgery. Its structure contains a thiophene ring instead of a benzene ring, which makes it more lipophile. Moreover, the drug renders minimal systemic toxicity due to its rapid hydrolization.[19]

Toxicity is one of the main concerns of using anesthetics.[7] Much controversy exists concerning the role of local anesthetics, mainly articaine, in relation to the incidence of neurological symptoms, more specifically paresthesia.[9] An older narrative review of a few case reports, evaluating the safety and side effects of local anesthetic myotoxicity, reported that bupivacaine seemingly caused the majority of side effects.[20] A recent review on systemic toxicity of local anesthetics reported that administration of a single dose of local anesthetics may not necessitate dose adjustment in the presence of liver dysfunction.[21] However, they suggested that caution should be advised among patients receiving multiple doses or infusions of local anesthetics; since aminoamide local anesthetics undergo first-pass metabolism by the liver P450 enzyme, which differs according to the specific drug and its pharmacological attributes. This shows the importance of our findings that different local anesthetics caused different extents of changes in liver enzyme levels. After articaine, prilocaine presented the least hepatic changes in those rats with induced hepatic failure. Perhaps a reason for its lower hepatic toxicity was that prilocaine is a secondary amide and is excreted via the liver as well as the kidneys; only a small fraction is excreted in urine.[19]

Systemic toxicity related to local anesthetics is reported to be mainly dose-dependent.[22] In the present study, we evaluated a maximum dose of local anesthetics in the settings of acetaminophen-induced liver failure in rats. Considering that our results showed acute hepatic effects of local anesthetics, these findings may be significant in clinical practice when dealing with patients with a history of hepatic injury.

The main limitation of the present study was that we only evaluated the hepatic effects of local anesthetics using an experimental study method. As stated in a previous study, other aspects of each anesthetic agent should be included in clinical assessments.[23] Future studies should include a variety of investigations when evaluating the efficacy and safety of local anesthetics. In addition, research studies on human subjects with hepatic failure are required to support our findings. The main strength of our study was evaluating the changes in hepatic enzymes caused by different local anesthetics in acetaminophen-induced hepatic failure rats. However, we only measured liver enzyme levels (AST, ALT, and GGT), which may not have been accurate indicators for the evaluation of the severity of the liver injury. It is recommended that future studies include measurement of additional factors such as albumin and prothrombin time.

Conclusion

Articaine and prilocaine with felypressin local anesthetics induced the least change in hepatic enzyme levels of rats with abnormal hepatic function. However, in the case of normal liver function, the more commonly used lidocaine seemed to render safer results.

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