

Original Article

Postmortem redistribution of lidocaine after epidural injection in beagle dogs

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Abstract: Objectives: An animal model using beagle dog has been established to investigate the postmortem redistribution of lidocaine. Materials and methods: 18 dogs were euthanized and injected lidocaine (13 mg/kg) via epidural immediately. An autopsy was performed at 0, 12, 24, 48, 72, 96 hours after drug administration. All animals were stored in supine position at room temperature. For the other groups, lidocaine was given via epidural 6, 12, 24 hours after dogs were euthanized. Followed treatments were as above described. All samples were treated for detection of the concentration of lidocaine. Results: It was found that lidocaine could diffuse via blood vessel rapidly post administration. And the concentration of lidocaine in the blood from ventriculus sinister increased obviously in a time-dependent manner. Meanwhile, the postmortem tissue distribution of lidocaine was significantly different. However, the process of postmortem redistribution of lidocaine was obviously delayed in dogs which were given drugs after death. Conclusions: Together results revealed the process of postmortem redistribution of lidocaine via epidural injection, and provided the method to distinguish the lidocaine-induced death and drug administration after death.

Keywords: Lidocaine, anesthesia accidents, postmortem redistribution

Introduction

As a common local anesthetic drug, lidocaine is also used in intraspinal anesthesia. However, because of the specific structure of canalis vertebralis and the physicochemical property of lidocaine, drug-induced anesthetic death occurs frequently [1-5]. Therefore, whether the death in surgery is associated with anesthesia has become a focus in forensic research. The pharmacokinetic/pharmacodynamic properties of lidocaine and drug combination were often used to explain the lidocaine-induced anesthetic accidents. However, the postmortem redistribution of lidocaine was studied less, only some qualitative and quantitative detection had been performed [6-9]. Meanwhile, there was less specific forensic criterion on medicolegal expertise of anesthetic death except clinical characterization and autopsy studies.

The studies on postmortem redistribution were mainly focused on concentration gradient diffu-

sion: the diffusion from drug-enriched organs to peripheral tissues. Postmortem diffusion generally refers to drug diffusion in a concentration gradient manner after death [10]. For example, drugs diffused from an area of high concentration (reservoir), such as gastrointestinal tract, liver, lung and cardiac muscle to peripheral tissues which has a lower concentration of drugs [11]. Postmortem diffusion is the main mechanism of redistribution [12, 13], and multiple drugs could diffuse in corpse followed this manner. As an efficient and easy diffused drug, lidocaine might easily diffuse to different tissues after death.

We had established the method for detection of lidocaine in biological samples and multiple animal models. In this study, postmortem redistribution dog model was used to investigate the characterization of diffusion of lidocaine after epidural injection, which could provide novel feature for lidocaine-induced anesthetic accidents in medicolegal expertise.

Postmortem redistribution of lidocaine after epidural injection

Table 1. The postmortem diffusion of lidocaine in dogs

Samples	The concentration of lidocaine (mean \pm SE, $\mu\text{g/g}$ or $\mu\text{g/mL}$; n=3)					
	0 h	12 h	24 h	48 h	72 h	96 h
Heart	0	0.9 \pm 0.3*	1.09 \pm 0.4*	1.5 \pm 0.2*	1.3 \pm 0.5*	1.9 \pm 0.9*
Liver	0	0.8 \pm 0.4*	3.3 \pm 0.9*	0.9 \pm 0.2*	1.5 \pm 0.4*	1.3 \pm 0.5*
Spleen	0	0	0	0	0	0
Lung	23.3 \pm 7.23	24.7 \pm 7.88	28.2 \pm 8.82	23.4 \pm 4.41	22.7 \pm 6.7	18.7 \pm 5.23
Kidney	0	0.9 \pm 0.4*	1.6 \pm 0.3*	1.1 \pm 0.1*	1.9 \pm 0.7*	1.0 \pm 0.4*
Temporalis muscle (for control)	0	0	0	0	0	0
Injection site of muscle	0	1.3 \pm 0.5*	3.4 \pm 0.7*	2.4 \pm 1.0*	5.4 \pm 1.7*	4.0 \pm 1.7*
Blood in ventriculus dexter	34.3 \pm 6.7	33.7 \pm 7.4	23.8 \pm 7.5	19.1 \pm 6.1	24.7 \pm 7.7	16.7 \pm 5.5
Blood in ventriculus sinister	4.7 \pm 1.8	8.6 \pm 4.1	15.3 \pm 6.5*	15.5 \pm 3.8*	21.0 \pm 5.4*	15.8 \pm 6.4*
Blood in postcava	24.3 \pm 6.9	34.6 \pm 11.2	30.0 \pm 4.7	28.5 \pm 3.1	27.2 \pm 4.6	24.7 \pm 7.2
Blood in precava	24.3 \pm 6.1	25.6 \pm 7.7	21.9 \pm 7.9	29.1 \pm 6.5	23.2 \pm 6.5	17.6 \pm 6.5
Bile	0	0	0	0	0	0
Urine	0	0	0	0.21 \pm 0.07*	0.09 \pm 0.01*	0.16 \pm 0.05*
Vitreous humor	0	0	0	0	0	0
Brain	11.5 \pm 3.1	8.7 \pm 3.5	5.8 \pm 3.1	6.2 \pm 3.3	7.3 \pm 4.0	7.2 \pm 3.8
Cervical spinal cord	233.3 \pm 60.5	278 \pm 73.8	179.3 \pm 43.4	113.7 \pm 59.5	132.3 \pm 36.3	128.7 \pm 22.0
Segmental spinal cord	374.4 \pm 134.6	389.6 \pm 67.1	289.6 \pm 110.1	235.9 \pm 77.4	218.5 \pm 24.1	198.2 \pm 34.1
Lumbar spinal cord	834.3 \pm 80.1	765.3 \pm 111.7	645.4 \pm 72.0	531.7 \pm 103.0	542.1 \pm 118.5	464.2 \pm 61.7
Sacral segment of spinal cord	884.3 \pm 63.8	807.2 \pm 108.2	651.2 \pm 55.0	551.3 \pm 167.2	390.3 \pm 100.1	521.4 \pm 64.0

* $P < 0.05$ compared to 0 h group.

Materials and methods

Animals

Adult male beagle dogs (10-14 months) weighing 15-20 kg were from animal center of Shanxi Medical University. Animals were individually housed followed the guidelines of the animal committee. Before experiments, dogs were fasted overnight. General anesthesia was induced with propofol and maintained with isoflurane in oxygen circle system as described [14]. Animals were positioned in lateral on table throughout the experiment, and the BL-biological function experimental system (Taimeng, Chengdu, CN) was used to monitor the electrocardiogram, breathing changes and blood pressure following its protocol. Lidocaine solution (2%, Fuxing, Shanghai, CN) at a dose of 13 mg/kg was prepared for epidural injection. The epidural punctures were performed at the L1-L2 or L2-L3 intervertebral spaces. Then the animals were killed by gas embolism. While the electrocardiogram, breathing changes and blood pressure disappeared, the lidocaine solution was injected via epidural punctures immediately within 5 mins. The animals were stored in supine position at room temperature. And the autopsy was performed at 0, 12, 24,

48, 72, 96 hours after drug administration. For the other groups, lidocaine was given via epidural 6, 12, 24 hours after dogs were executed, and the followed experiments were performed as above described. All studies were permitted by the committee for animal experiments of the first hospital of Shanxi Medical University.

Sample collection

After epidural injection, the autopsy was performed at different times. Brain, different spinal sections (cervical/segmental/lumbar/sacral segment of spinal cord), heart, lung, liver, spleen, kidney, muscle of injection site and temporalis muscle for control were removed. And the bile, urine, blood from heart and peripheral blood were also collected for detection.

Sample preparation and GC/MS methods

Samples were homogenized in water. The internal standard SKF525A were added into the homogenate, then the PH was adjusted to 2-3 by 1% HCl. After oscillation and centrifugation, the acid layer was removed to another tube. The PH was adjusted to 14 by 2 M NaOH. Then samples were extracted by diethyl ether for two times. The diethyl ether was evaporated and

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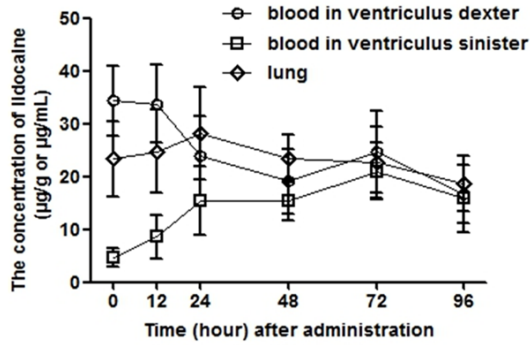


Figure 1. The concentration of lidocaine in lung, blood in ventriculus dexter and ventriculus sinister after different postmortem time (n=3).

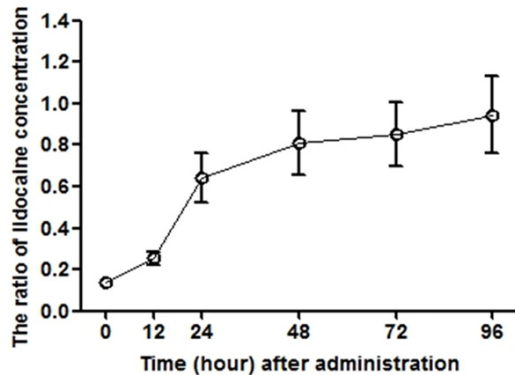


Figure 2. The ratio of concentration of lidocaine in ventriculus sinister to that in ventriculus dexter (n=3).

the residual was dissolved with 50 µL ethanol. For control, standard lidocaine was added to the blank control samples.

GC/MS were used to detect the concentration in each sample. The structure of compounds was confirmed by mass spectrometry (MS, Finnigan, US). The concentration of lidocaine was approved by gas chromatography (GC, Finnigan, US. DB-5 capillary column, 30 m × 0.25 mm × 0.25 µm; EI source: 70 eV; mass range: 50-650; column temperature: 150°C, maintaining 1 min, then to 280°C at a speed of 10°C per min for another 2 min; the temperature of ion source: 200°C; the carrier gas: helium with a speed of 1.5 ml/min).

Statistical analysis

All data were expressed as mean ± SE. One-Way ANOVA analysis followed by Student Newman Keuls test (SNK) was used to compare differences between groups.

Results

The postmortem diffusion of lidocaine in dogs

After epidural injection, lidocaine could diffuse from epidural to spinal cord, brain and canalis vertebralis, the venous plexus. Then the concentration of lidocaine in precava, postcava, blood of heart and lung increased immediately. Although the increasing of lidocaine in urine and heart, liver, kidney were not as obvious as above tissues and blood, lidocaine could still diffuse to these organs. However, there was no detectable lidocaine in spleen, temporalis muscle, bile and vitreous humor, suggested that these tissues could not be affected by post-mortem diffusion (**Table 1**).

It should be noticed that the concentration of lidocaine in blood from ventriculus sinister increased significantly in a time-dependent manner after drug administration (**Figure 1**). Meanwhile, the concentration of lidocaine in ventriculus dexter decreased with time. The ratio of concentration of lidocaine in ventriculus sinister to ventriculus dexter approached 1 at 72 h after death (**Figure 2**).

The concentration of lidocaine in animals that were given drugs after death

After the animals were killed for 6, 12, 24 hours, lidocaine was given via epidural injection. It was found that there was no difference in initial concentration of lidocaine between each group (**Table 2**). However, the postmortem redistribution, especially the increasing in concentration of lidocaine from the blood of ventriculus sinister was suppressed obviously in these groups time-dependently (**Figure 3**).

Discussion

Postmortem redistribution has been widely studied in postmortem diagnosis of drug poisoning and experimental animal researches [15, 16]. However, lidocaine, one of important anesthetic drugs which often induce anesthetic accidents, has been investigated less. It has been only reported that lidocaine could diffuse from weasand to the blood of heart during a cardiopulmonary resuscitation surgery [17]. Using a postmortem redistribution dog model, lidocaine was injected via epidural to investigate the possible toxicokinetics mechanism of lidocaine-induced anesthetic events. During

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Table 2. The postmortem diffusion of lidocaine in dogs received drugs at different time after death

Samples	The concentration of lidocaine (mean \pm SE, $\mu\text{g/g}$ or $\mu\text{g/mL}$; n=3)			
	0 h	6 h	12 h	24 h
Heart	0	0	0	0
Liver	0	0	0	0
Spleen	0	0	0	0
Lung	23.3 \pm 7.23	12.9 \pm 3.9	13.5 \pm 3.1	12.4 \pm 3.7
Kidney	0	0	0	0
Temporalis muscle (for control)	0	0	0	0
Injection site of muscle	0	0	0	0
Blood in ventriculus dexter	34.3 \pm 6.7	24.1 \pm 7.0	20.6 \pm 7.8	20.1 \pm 6.8
Blood in ventriculus sinister	4.7 \pm 1.8	5.0 \pm 1.1	4.4 \pm 1.9	2.4 \pm 0.7
Blood in postcava	24.3 \pm 6.9	30.3 \pm 7.3	27.3 \pm 4.7	29.7 \pm 5.7
Blood in precava	24.3 \pm 6.1	26.8 \pm 6.9	22.4 \pm 7.5	24.4 \pm 6.0
Bile	0	0	0	0
Urine	0	0	0	0
Vitreous humor	0	0	0	0
Brain	11.5 \pm 3.1	10.4 \pm 1.4	9.3 \pm 2.1	7.9 \pm 1.7
Cervical spinal cord	233.3 \pm 60.5	319.7 \pm 162.3	301.3 \pm 114.8	274.2 \pm 70.3
Segmental spinal cord	374.4 \pm 134.6	458.3 \pm 197.2	415.2 \pm 130.4	379.0 \pm 136.2
Lumbar spinal cord	834.3 \pm 80.1	783.2 \pm 139.0	759.5 \pm 212.8	753.2 \pm 231.9
Sacral segment of spinal cord	884.3 \pm 63.8	863.1 \pm 278.3	852.9 \pm 231.6	874.3 \pm 172.9

* $P < 0.05$ compared to 0 h group.

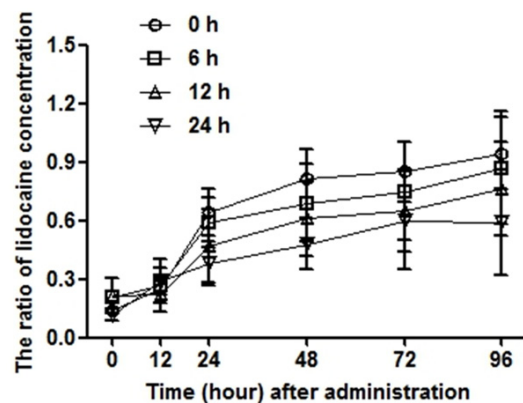


Figure 3. The ratio of concentration of lidocaine in ventriculus sinister to that in ventriculus dexter at different time after euthanasia (n=3).

postmortem diffusion, the biological membrane plays an important role. As lipid bilayer, the permeability to drugs is strongly related to the lipid solubility of drugs, the distribution between sides, integrality of membrane, temperature and other factors. In this study, we found that after epidural injection, lidocaine could rapidly distribute to lung, the blood of heart, postcava and precava. As an easy dif-

fused drug with high lipid solubility, lidocaine might be easily diffused after death. Meanwhile, there were plenty of venous plexus in the two side of vertebral canal in the periphery of epidural, which were important galleries linked postcava and precava. Thus, lidocaine could penetrate the basilar membrane of capillary to venous plexus around vertebral canal, thereby transfer to the blood in postcava, precava and ventriculus dexter. It was observed that the concentration of lidocaine in lung was high, the diffusion of lidocaine from ventriculus dexter to lung via pulmonary artery and the abundant vessels and alveolar membrane in lung could both contribute to this phenomenon.

As it was known, the postmortem diffusion was strongly associated with the time after death. Our results also proved evidence. It was showed that the concentration of lidocaine in the blood of ventriculus sinister increased obviously time-dependently. Moreover, the ratio of lidocaine concentration in ventriculus sinister to ventriculus dexter also increased to 1 with time. Interestingly, the concentration of lidocaine in lung was declined after 24 hours. It might speculate that the increased lidocaine in the blood

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of ventriculus sinister could be from lung via diffusion after death. Together results showed that the postmortem diffusion of lidocaine mainly affected the tissues nearby the injected position, such as spinal cord, brain. The peripheral tissues including heart, liver and kidney were also affected. However, the tissue far away from the injection position, like spleen, temporalis muscle and bile was not participated in this process. It has reported that the concentrations of drugs administrated postmortem were obviously different. In our study, the rapidly diffusion of lidocaine postmortem in the dogs which were injected with drugs different time after death were similar to that in animals received drugs immediately after execution. Thus, there was a normal diffusion while the internal environment changed less in body after death. However, while the decomposing occurs and the PH changes after death, the diffusion manner was different from normal postmortem. The diffusion of lidocaine from ventriculus dexter to ventriculus sinister could be delayed while the drug was administrated long term after death. This interesting appearance might be useful in medicolegal expertise to distinguish whether the drug was given after death or it directly induces anesthetic accidents. In conclusion, the postmortem diffusion of lidocaine was strongly related to the physicochemical property of the compound and the change of internal environment in body after death. The most important redistribution of lidocaine could be through diffusion from ventriculus dexter to ventriculus sinister thereby affected peripheral tissues. This study could contribute to the medicolegal expertise on identification of lidocaine-induced anesthetic accidents and whether the drug was administrated after death.

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Disclosure of conflict of interest

None.

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