Study of Local Anaesthetic Effect on Lignocaine and Bupivacaine in Experimental Animal Models

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Abstract
Local anesthetics prevent or relieve pain by interrupting nerve conduction. They bind to a specific receptor site within the pore of Na⁺ channels in nerves and block ion movement through this pore. In general, their action is restricted to site of application and rapidly reverses on diffusion from site of action in the nerve. This study was carried out to evaluate the local anesthetic action of lignocaine and Bupivacaine. To evaluate surface anesthetic action rabbit cornea method was used and intracutaneous wheel test in guinea pigs for infiltration anesthesia. It was found that both the drugs demonstrated local anesthetic action but bupivacaine acts for more duration as compared to lignocaine and its duration increases with increase in concentration.

Keywords: Lignocaine, bupivacaine, local Anesthetic and Rabbit

Introduction
One of the major developments which have contributed to the advancement of surgery is the facility of operating without pain to patient. Prior to 1846 attempts to provide comfort during operative procedures were minimally effective and the development of surgery was necessarily limited. [1] William T.G. Morton’s public demonstration of ether in that year revolution medical care throughout the world. [2] The evolution of anesthesiology was a medical specialty with surgical procedures. Beyond the obtundation of consciousness and creation of quiescent surgical field, anesthesiology applies principles of physiology, pharmacology to assess and reduce surgical risk, maintain homeostasis, attenuate the surgical stress response and provide analgesia.

Local anesthetics prevent or relieve pain by interrupting nerve conduction. They bind to a specific receptor site within the pore of Na⁺ channels in nerves and block ion movement through this pore.[3] In general, their action is restricted to site of application and rapidly reverse on diffusion from site of action in the nerve. Local anesthetics can be administered by variety of routes including topical, infiltration, field or nerve block, IV regional spinal or epidural. [4] Local anesthetics block conduction by decreasing or preventing the large transient increase in the permeability of excitable membrane to sodium that normally is produced by a slight depolarization of the membrane. [5] This action of local anesthetics is due to their direct interaction with voltage-gated sodium channels. As the anesthetic action progressively develops in a nerve the threshold for electrical excitability gradually increases, the rate of rise of action potential declines, impulse conduction slows and the safety factor for conduction decreases. These factors decrease the probability of propagation of action potential and nerve conduction eventually fails.[6]
In addition to sodium channels anesthetics can bind to other membrane proteins in particular potassium channels. Since interaction of local anesthetics with potassium channel requires higher concentration of drug, blockade of conduction is not accompanied by any large or consistent change in resting membrane potential.[7] Biochemical, biophysical and molecular biological investigation during past two decades have led to a rapid expansion of knowledge about structure and function of Na+ channel and other voltage-gated ion channels.[8] The Na+ channel are heterotrimetric complexes of glycosylated proteins with an aggregate molecular size in excess of 300,000 Daltons. The individual subunits are designated α (260,000 daltons) β1 (36,000 daltons) β2 (33,000 daltons). The large α subunit of Na+ channel contains four homologous domain (I to IV) each domain is thought to consist of six trans membrane segments in α – helical conformation and an additional, membrane pore of the channel is presumed to reside in the center of nearly symmetrical structure formed by the four homologous domains. [9] The voltage dependence of channel opening is hypothesized to reflect conformational changes that result from the movement of gating charges in response to changes in the transmembrane potential.[10] The gating charges are located in S4 transmembrane helix, the S4 helices are both hydrophobic and positively charged, containing lysine and Arginine residues move perpendicular to the plane of the membrane under the influence of the trans membrane potential, initiating a series of conformational changes in all four domains which lead to the open state of the channel.[11] After it opens, the Na+ channel inactivates within the few milliseconds due to closure of an inactivation gate. This functional gate is formed by the short intracellular loop of protein that connects homologous domains III and IV. [12] The loop folds over the intracellular mounts of the Trans membrane pore during the process of inactivation. It may bind to an in activation gate “receptor” formed by intracellular mouth of the pore. Amino acid residues that are important for local anesthetic binding are found in S6 segments in domain IV. Hydrophobic amino acid reduces near the centre and intracellular end of S6 segment may interact directly with bound local anesthetics.

Lidocaine or lignocaine is a common local anesthetic and antiarrhythmic drug. Lidocaine is used topically to relieve itching, injected as a dental aesthetic, and in minor surgery. The most commonly encountered lidocaine preparations are marked by Abraxis Pharmaceutical Products under the brand names Xylocaine and Xylocard, and as ‘Lanacane’ topical ointment in the UK, though lidocaine is also found in many other proprietary preparations.[13]

Materials and Methods

The study was conducted for a period of 18 months, in Department of pharmacology, Rajiv Gandhi Institute of Medical science, Srikakulam, AndhraPradesh, India. The study was approved by Institutional Ethics committee (IEC), Rajiv Gandhi Institute of Medical science. Chemicals and solutions used for the study include, Lignocaine, Bupivacaine, Double distilled water, Normal saline and Albino Guinea pigs, Albino Rabbit.

1. SURFACE ANESTHESIA (Rabbit’s cornea method): The standard method of Sollmann (1918) was followed with minor modifications. Albino rabbits in group of four each drug concentration was taken and eye lashes carefully clipped. Eyes were tested for touch and light reflexes and the diameters of the pupils were measured. Right conjunctival sac was opened to make a pocket and 1-2 drops of the test drug were instilled so that the corneal surface was in contact with the solution for 60 seconds. The left eye was taken as control. All the drugs were tested in concentrations of 0.5, 1.0 and 2%. At intervals of minute reflexes were tested. The time of onset (when the corneal reflex disappeared) and the time of recovery (when the corneal reflex reappeared) were noted. The time interval between the disappearance and reappearance of the corneal reflex gave an idea of duration of action. The animals were kept under observation for four days for signs of irritation if any, such as blepharospasm, oedema of the conjunctiva and pitting or opacity of the cornea.

2. INTRA CUTANEOUS WHEAL TEST IN GUINEAPIGS: The technique followed was that of Bulbring and Wajda (1945). Fully grown albino guinea pigs weighing 300-400gms were used for this test. On the day preceding the experiment hair on the dorsal midline of each guinea pig was clipped and two areas of skin diameter 4-5 cm were shaved without abrading the skin. The sensitivity of skin is greatest in the midline and slightly more in front area than in back area on the day of experiment animals were placed in a suitable restrained position and oriented so that their backs can be conveniently reached for intradermal injection. The test drug was injected in concentration of 0.25, 0.5 and
1% intracutaneously with a 26 gauge, 3/8n long needle fitted to 1ml capacity tuberculin syringe. 0.25ml volume of drug was injected and the time noted. The wheals were identified by drawing a circle around each with a marking pen. Four guinea pigs with a total of 16 wheals were made available for each concentration. After observing the normal reaction to pin prick outside the wheal, the reaction of the animal to pin prick applied inside the wheal was tested every minute over all the injected areas, and the time was noted when the animal failed to respond and also when the animal started responding again to pin prick. This gave an idea of duration of anesthesia. The mean time of duration was calculated. The animal was observed for 4 days for signs of in duration, necrosis and ulceration if any.

**Results**

From our study, it was clear that both lignocaine and bupivacaine demonstrated local anesthetic action but Bupivacaine produced local anesthetic action for more duration as compared to lignocaine for every concentration and the duration of local anesthesia increases correspondingly with increase in concentration.

**Figure 1: INFILTRATION METHOD [DRUG CONC-0.25ML]**

**TEST-1**: Indicates the mean duration of action of Lignocaine by infiltration method at concentration 0.25 ml -18.5 Min. No of Animals present; 4 Guinea pig. Drug Administer in this group; T1 [Lignocaine], route of administration is subcutaneous.

**TEST-2**: Indicates the mean duration of action of Bupivacaine by infiltration method at concentration 0.25 ml -43.5 Min. No of Animals present; 4 Guinea pigs. Drug administer in this group; T2 [Bupivacaine] and route of administration is subcutaneous.

**Figure 2: DRUG CONC-0.50ML**

Test-1 Indicates the mean duration of action of Lignocaine by infiltration method at concentration 0.5 ml -30 Min. No of animals present; 4 Guinea pigs. Drug administer in this group; T1[Lignocaine] and route of administration is subcutaneous.

Test-2 Indicates the mean duration of action of Bupivacaine by infiltration method at concentration 0.5ml -54.5 Min. No of Animals present; 4 Guinea pigs. Drug administer in this group; T2 [Bupivacaine] and route of administration is subcutaneous.

**Figure 3: DRUG CONC-1ML**

**TEST-1** Indicates the mean duration of action of Lignocaine by infiltration method at concentration 1.0 ml -45.5 Min. No of animals present; 4 Guinea pigs. Drug administer in this group; T1[Lignocaine] and route of administration is subcutaneous.

**TEST-2** Indicates the mean duration of action of Bupivacaine by infiltration method at concentration 1.0 ml -65.75 Min. No of Animals present; 4 Guinea pigs. Drug administer in this group; T2 [Bupivacaine] and route of administration is subcutaneous.

**Figure 4: SURFACE ANESTHESIA [DRUG CONC-0.50ML]**

**Test-1:** Indicates the mean duration of action of Lignocaine by Surface Anesthesia Method at concentration 0.5 ml-6.5 Min. No of Animals present; 4 Albino Rabbits. Drug administer in this group; T1 [Lignocaine] and route of administration is drops instillations.

**Test-2** Indicates the mean duration of action of Bupivacaine by surface anesthesia method at concentration 0.5ml -18.25 Min. No of Animals present; 4 Albino Rabbits. Drug administer in this group; T2 [Bupivacaine] and route of administration is drops instillations.

**Figure 5: DRUG CONC. 1ML**

Test-1: Indicates the mean duration of action of Lignocaine by surface anesthesia method at concentration 1 ml -12.25 Min. No of Animals present; 4 Albino Rabbits. Drug administer in this group; T1 [Lignocaine] and route of administration; Drops Instillations

Test-2: Indicates the mean duration of action of Bupivacaine by surface anesthesia method at concentration 1 ml-35.75 Min. No of Animals present; 4 Albino Rabbits. Drug administer in this group; T2 [Bupivacaine] and route of administration; Drops Instillations.

**Table 1:** Comparison of Mean responses at different dosages with the effective dosage of the drug for Infiltration Method. P value < 0.05; P value between T1 & T2 [Conc-0.25ml] - 7.1459, P value between T1 & T2 [Conc-0.50ml] - 10.893 and P value between T1 & T2 [Conc-1ml] - 4.359.

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<th>Name of the Drug</th>
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<th>Mean (min)</th>
<th>Std Dev</th>
<th>Std Error</th>
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<td>T2</td>
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**Table 2:** Comparision of mean responses at different dosages with the effective dosage of the drug for surface anesthesia. P value < 0.05; P value between T1 & T2 [Conc-0.50ml] - 5.6083, P value between T1 & T2 [Conc-0.50ml] - 10.893 and P value between T1 & T2 [Conc-1ml] - 4.359.

<table>
<thead>
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<th>Name of the Drug</th>
<th>Conc (ml)</th>
<th>Mean (min)</th>
<th>Std Dev</th>
<th>Std Error</th>
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<td>T2</td>
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Table 1 & Table 2: Comparison of Mean responses at different dosages with the effective dosage of the drug for infiltration and surface anesthesia. P value < 0.05; P value between T1 & T2 [Conc-0.50ml] - 5.6083, P value between T1 & T2 [Conc-0.50ml] - 10.893 and P value between T1 & T2 [Conc-1ml] - 4.359.
Discussion

Surgery without anesthesia cannot be imagined. Be it a local surgical procedures or major surgical operation. Anesthetic drugs had been a very important discovery. Anesthetic drugs can be general anesthetic drugs or local anesthetic drugs. Local anesthetic drugs do not produce loss of consciousness and they have localized action. Local anesthetics are classified as amide type and ester type. Amide type local anesthetics include intermediate acting Lignocaine and longer acting Bupivacaine. In this study the duration of local anesthetic action of Lignocaine is compared with that of Bupivacaine. The study was carried out in both rabbit and guinea pigs. Four rabbits were included in each group to evaluate the surface anesthesia in different concentrations such as 0.5, 1.2% of both lignocaine and bupivacaine. The time interval between disappearance and reappearance of corneal reflex denotes the duration of action. Similarly four guinea pigs were included in each group to evaluate the infiltration anesthesia in the concentration of 0.25, 0.5, 1% of both lignocaine and bupivacaine. Intracutaneous wheal were made by using the test drugs in different concentration and reaction of animal to pin prick was tested every minute and the time interval between disappearance of response to pin prick gives idea about the duration of anesthesia.

There are various screening techniques to assess local anesthetic action of a drug. For initial screening of a drug, rabbit is one of the best animals as it is easy to handle, can be used repeatedly since the animal is not sacrificed. The second choice of animal for initial screening is guinea pig. It is good especially for infiltration or regional anesthesia.

In the present study it is found that the mean duration of action of lignocaine is 18.5 min with 0.25 ml, 30 min with 0.5 ml and 45.5 min with 1 ml when infiltration method was used, whereas it is 43.5 min with 0.25 ml, 54.5 min with 0.5 ml and 65.75 min with 1 ml of Bupivacaine when assessed by infiltration method.

By using surface anesthesia method the mean duration of local anesthesia is 6.5 min with 0.5 ml, 12.25 min with 1 ml and 18 min with 2 ml of lignocaine where as it is 18.25 min with 0.5 ml, 35.75 min with 1 ml and 43.25 min with 2 ml of Bupivacaine.

In one of the study by Akerman S.B, who conducted surface local anesthetic property of Lignocaine found that it produces local anesthesia of high intensity, rapid onset and with a comparatively long duration of action using corneal, intranasal and intratracheal application. But the surface anesthetic activity of lidocaine was substantially lesser than Bupivacaine.

D Wight et.al studied the local anesthetic effect of Lignocaine and Bupivacaine and found that Bupivacaine as a longer duration of action as compared to that of lignocaine which has application in complex wounds that require long repair times.

J.H. Me Clure compared lignocaine and Bupivacaine by subcutaneous administration in guinea pig. Adrenaline 5 micro gram / ml when given along with both the drugs decreased the blood flow increasing the duration of action of both the drugs.[14] In another study by Lim TK et. al it was found that the quaternary lidocaine derivative produced local anesthesia for a longer duration when assessed by guinea pig intradermal wheal assay[15]. Roberto et.al compared lignocaine and bupivacaine by using intra cameral irrigation they found that lignocaine in the 4.0% concentration acts for a duration of 15-20 min whereas Bupivacaine in 0.5 - 2 % concentration as per 20-30 min.[16]

Thus local anesthetic are warranted whenever a clinical procedure causes pain which could be eliminated by their use. It increases patient comfort and facilitates patient cooperation that is needed during surgical procedures. Local anesthetic reversibly blocked nerve impulses by disrupting permeability to sodium during an action potential. The onset of action is largely dependent on the pharmacokinetics and dosage given. In the structure of local anesthetic there is presence of hydrophilic and hydrophobic moieties that is separated by an intermediate ester or amide linkage. The hydrophobic moiety is aromatic whereas the hydrophilic moiety usually is tertiary mine but can also be a secondary amine. The nature of the linkage group determine some of the pharmacological properties for e.g. local anesthetics with an ester link are readily hydrolyzed by plasma esterase. Moreover the more a local anesthetic drug is hydrophobic, greater is the potency and longer is the duration of action. This arises because association of drug at Hydrophobic sites enhances the partitioning of drug to its site of action and decreases the rate of metabolism by plasma esterases and liver enzymes. In addition, the receptor sites for these drugs on sodium channels are thought to be hydrophobic so that receptor affinity for anesthetic agents is increased for more hydrophobic...
Conclusion

The present study was carried out to compare the local anesthetic action of lignocaine and bupivacaine given in different concentration in experimental models. The experimental models were surface anesthesia (Rabbits cornea method) and intracutaneous wheal test in guinea pigs. Both lignocaine and bupivacaine were given in concentration of 0.25, 0.5 and 1 % in infiltration method and both of the drugs were given in concentration of 0.5, land 2% in surface anesthesia method.

It was found that both lignocaine and bupivacaine produced local anesthetic action when assessed for surface anesthesia and infiltration anesthesia. On inter drug comparision of lignocaine and bupivacaine it was found that as the concentration of drug increases the duration of action also increases when assessed by both the methods.

Conflict of interest: None declared

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References