Impact of Local Anesthesia on Wound Healing

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Abstarct

Different local anesthetics produce multiple effects on wound healing, although this effect did not demonestrated clearly in experimental study. This study aimedto evaluate the effects of several local anesthetics (lidocaine, articaine, mepivacaine) on healing of oral mucosal wound healing in experimental rabbits by histological examination.The study was conducted on eight healthy adult male New Zealand white rabbits which divided into four group(n=2) control group which received no treatment group two (received lidocaine 2% with adrenalin) group three (received mepivacaine) and group four (received articaine 4%). oral mucosal wound were initiated experimentally in all group and each group received their treatment by local application one time daily for six day(except control group) in the third day half of the animal were sacrificed in each group and histological examination for the wound area were conducted , the remaining animal were sacrificed in the sixth day of treatment and histological examination were made for the wound area to compare the result between the four group. The mepivacaine group produce better result (enhance healing of the oral mucosal wound) followed by atricaine and lidocaine group compared with control one**.** This study showed that different type of local anesthesia produces different effects on wound healing manufisted histologically and clinically.

Key words: *lidocaine, mepivacaine, articaine, oral wound, mucosal wound.*

Introduction

Mucosa of the oral cavity consist of the mucous membrane lines the mouth from inside, it contains stratified squamous epithelium (oral epithelium), and connective tissue (lamina propria) [1]

Oral mucosa is either keratinized or non-keratinized, which is thicker and contain larger amount of elastic fiber than first one [2].

Wound can be occurred as result of any damage to oral mucosa which cause interruption of its integrity. [3], wound healing is a very complex method consist mainly of three stages: migration of inflammatory cell (neutrophil, microphage), proliferation of fibroblast and other repairing cells, then remodeling of newly formed tissue.[4]. Many factors either systemic (stress, age, drugs) or local (infection, oxygen) may affect healing process [5].

There is a certain relationship may exist between healing of oral wound in human and certain animals like rabbits [6], so this study employed healing in rabbits as model for healing in human.

Local anesthetic inhabits a significant role in controlling pain in dentistry, it's either applied topically or injected to oral mucosa [7]. Local anesthetic may pass antibacterial effects by inhibit or kill a large number of bacteria and fungus in oral cavity [8], therefore it is possible that local anesthesia by passing antimicrobial effects may add additional advantage as antiseptic in prevent wound infection post operatively [9].

However, the local anesthetic effects on wound healing are not exposed obviously yet, in spite of

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literature studies, because different results are shown in this studies [10-14].The aims of this study were to evaluate the effects of different local anesthetic agents (lidocaine with adrenalin, mepevecaine, and articaine) when applied locally on the healing of oral wound in rabbits and their antibacterial effects on oral microbes by comparing between them.

Methododolgy

The study was carried out in the depart. of Basic Science, College of Dentistry, University of Mosul. The study was approved by scientific committee/department of basic science/college, of Dentistry/University of Mosul.

Animals: eight healthy adult male New Zealand white rabbits weighting between 1.5-2 kg were included in this study. These animals were housed under 16h light/8h dark cycle at 26-32C with access free to water and food.

These animals were classified into four groups (n=2) as follow:

Group one: control group received no treatment

Group two: animals received lidocaine 2%with adrenalin

Group three: animals received mebevicaine

Group four: animals received articaine 4%

All groups were subdivided into A and B subgroup (n=1)

Subgroup A: include animals sacrificed in the 3rd day of treatment, while subgroup include animals sacrificed in the 7th day of treatment

Drugs:

Lidocaine HCl 2%with epinephrine1:80.000 carpole (Huons Co.Korea) for group two, Mepivacaine al 3% carpole (registro santario S.A.) for group three,Articaine4% with adrenaline1:100.000 carpole (registro santario. S.A), for group four.

All drugs applied topically on the wound one time daily from first day to day seven after incision.

Surgical procedure:

All animals were anesthetized by using intramuscular injection of Xylazine 2% (0.2 ml/kg) and ketamine 10%(1mg/kg) of body weight [1] then Standardized incision were made with equal length in the right side in the oral mucosa of inner check of each rabbit.

Histopathopathological examination

At the third day of treatment each animal from subgroup A were sacrificed for tissue samples of the induced wound, the tissue fixed processed and stained by hematoxylin and eosin.[15].

The procedure was repeated in the sixth day of treatment for subgroup B from each group.

Criteria of histopathological examination:

1. Criteria of inflammatory response parameter (scoring): [16,17]

Score 1: Nil No inflammatory cells seen in the field of operation (X10).

Score 2: Mild When inflammatory cells present in few numbers, less than ½ of the field (X10).

Score 3: Moderate Inflammatory cells could be seen in more than ½ of the field (X10).

Score 4: Severe or abundant when inflammatory cells present in huge numbers, more than ¾ of the field (X10).

2. Criteria for amount of granulation tissue formation (Scoring): [16,17]

Score 1: Absent of granulation tissue formation in wound.

Score 2: Quantity of granulation tissue formation in the wound gap is scanty.

Score 3: Amount of granulation tissue formation is moderate in tissues.

Score 4: Total amount of granulation tissue formation in the wound is profound.

3. Criteria of re-epithelization parameters (Scoring), [18]

Score 0: Re-epithelialization at the edge of the

wound.

Score 1: Re-epithelialization covering less than half of the wound.

Score 2: Re-epithelialization covering more than half of the wound.

Score 3: Re-epithelialization covering the entire wound, irregular thickness.

Score 4: Re-epithelialization covering the entire wound, normal thickness.

Results

Histopathological examination of oral mucosal wound (diag. 1)

1: GROUP 1:

The Number of inflammatory cells was increased gradually and continues to 6 day without regression (diag. 4), also there are marked increasing of re-epithelialization mainly after 3rd day (diag. 3). The granulation tissue formation increased gradually with continuous activation of fibroblasts (Table 1).

2: GROUP 2:

There are delay onsets of inflammation either due to infection or abnormal responses of tissue to adding material (diag. 3). The granulation tissue formation is stable during all time of experiment (dig. 4) while there are well re-epithelialization's in the first three days and continues with lower potential in second three days (dig. 4) (figs 1 and 2).

3: GROUP 3:

There are mild differences between 3rd and 6th day in the number of inflammatory cells responses (diag. 3&4) while stable increasing of granulation tissue formation during healing process (diag. 3&4). Good re-epithelialization shown in the two-time interval with increasing in the thickness and prominent sub epithelial papilla

4: GROUP 4:

Also, there are mild differences between 3rd and 6th day in the number of inflammatory cells responses (diag. 3)

Good re-epithelialization in the two-time interval (diag. 4) and mild granulation tissue formation without increasing during healing process (diag. 3)

Results comparisons

When we compared the result of four groups in the 3th day of treatment we can see:

In groups 2 and 4 there are mild inflammatory responses while there is moderate inflammatory reaction in groups 1and 3

The re-epithelialization is very high in group 3 with less responses in groups 2and 4 but minimal response in group one

Granulation tissue formation constant in groups 2 and 4 while there is gradual increase in groups 1 and 3(diag. 3&4)

When we compared the result of four groups in the 6^{th} day of treatment we can see:

Inflammatory cell infiltration is similar in group 1 and 3 but they are higher than that of group 2 and 4.

The re-epithelialization is very good in 3rd group with less activity in group 1 and 2.

The granulation tissue is nearly constant in all groups (diag. 3&4).

Discussion of Results

The normal process of wound healing is a major aim of any surgical procedure [19]. Lidocaine is one of the most commonly used local anesthetics in dentistry due to its moderate duration of action and reasonable onset [20]. The addition of vasoconstrictor (adrenaline) to local anesthetic provides additional advantages like reduce systemic absorption and reduce bleeding from surgical site [21].

According to the result of this study group 2(lidocaine+adrenaline) produce less inflammation when compared with control group in the third day while granulation tissue and re-epithelization showed the same results in the 6^{th} day of treatment this was in agreement with other study [22]. Lidocaine may interfere with mast cell arrival at the site of injury as signal from this cell will participate in the inflammatory process, matrix

reabsorption and angiogenesis. The effects of adrenaline were to potentiate the blocking effect of lidocaine [23]. This study was disagreement with other study [24] which showed that lidocaine has a negative (delay) effect of wound healing by increasing risk of secondary infection specially when combined with adrenalin while Waite et al [25] in his study showed that lidocaine with adrenaline did not cause any effects (negative or positive) on wound healing.

According to this study, group 3(mepivecaine) produce highly inflammatory responses in 3rd day with a good healing in 6th day (manifested by increased re-epithelization and granulation tissue formation) compared with another group.

Mepivacaine is used now a day to replace lidocaine with adrenaline local anesthetics in case where the use of adrenaline is somewhat contraindication like patient with cardiovascular disease or thyroid problem [26] so it has been selected in this study.

According to our knowledge this is the first study conducted to study the healing effects of mebivacaine local anesthetics. The increased in the inflammatory responses in the 3rd day may be due to the effects of mebivacaine on increasing cytokine production which enhance inflammatory responses [27] this will promote healing by epithelial cell migration and remodeling of matrices in extracellular place which enhance granulation tissue formation in 6th day of treatment [28]. The positive effects of mebevacaine on healing also may be due to its antibacterial effects (9) also it may affect the maturation and sequence of collagen fibers [29].

In the group4 (articaine) there is a mild inflammation in the 3rd day with small amount of granulation tissue and re–epithelization in 6th day compared with another group. Articaine was selected in this study due to the facts that articaine became so popular in many countries due to its good efficacy. The results of this study was in agreement with Dogan et al study(2003) who showed that histological finding and breaking strength in articaine group is less than in control group[30], but its disagreement with the study of Senes et al (2015) which showed that there is no significant different between lidocaine and articaine group[31].

The inhibitory effects of articaine on healing in this study may be due to its proliferating or inflammatory phase of healing [32] or it may affect the mechanism that cause cell membrane stability, calcium movement inside the cell or sodium conduction, or by acting as enzyme inhibitors to hydroxylase enzyme which is responsible for collagen synthesis[33].

Table 1: Scoring data of the study

A1	2	1	1
B1	3	3	2
A2	0	2	2
B2	2	3	2
A3	2	3	1
B3	3	4	2
A4	0	2	2
B4	2	2	2

Figure . 1: comparison the results of four groups in 3rd day of experiment.

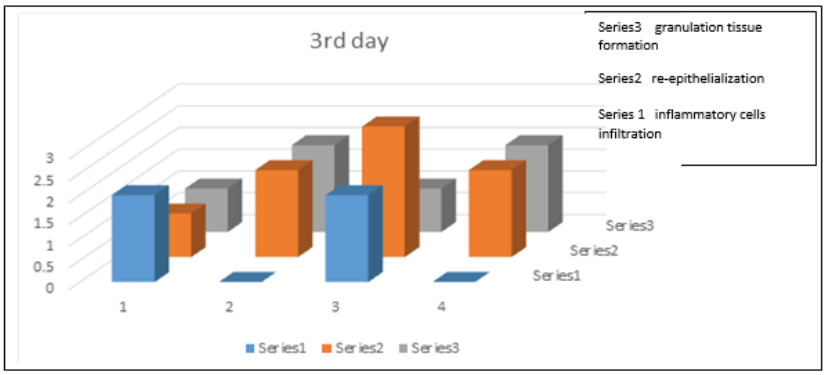

Figure 2: comparison the results of four groups in 6th day of experiment

Conclusion

In conclusion different types of local anesthetics may produce different effects on wound healing in mucosal membrane inside the oral cavity, present or absent of vasoconstrictor may provide additional advantage or disadvantage in this point although the concentration of local anesthetic is also an important point to note, this study demonstrate that mepivacaine provide a better result compared with control and other group but further study is required to demonstrate these effects in other animal and in other site are required.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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