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Local anesthetic effect of some benzoyl amino acids and phthalyl amino acids using hot-plate technique and pin pricking technique

Abstract

Alkyl and phenyl side chain amino acid were reacted with benzoyl chloride and phthalyl anhydride to give benzoyl amino acids and phthalyl amino acids respectively.

Materials and methods: These compounds were tested for their local anesthetic effect as ointment using Hot-Plate technique in mice and as sodium salt solution using Pin-Pricking technique in rabbits.

Results showed that the local anesthetic effect of the prepared ointment using a Hot-plate test on mice revealed that all benzoyl amino acids were significant anesthetic effect. For Phthalyl amino acids, a hot-plate test for the local anesthetic effect showed no significant effect for Phthalylanine and Phthylvaline and significant effect for Phthylalglycine, Phthalyl methionine and Phthalylphenyl alanine compared with Vaseline. All studied Phthalyl amino acids showed a lowered local anesthetic effect than lidocaine ointment. For Pin Pricking test all benzoyl and Phthalyl amino acid exhibit anesthesia but lower than lidocaine solution. Conclusion: Prepared of benzoyl amino acids and phthalyl amino acids have significant local anesthetic effects, benzoglycine ointment showed a significant effect compared with other a significant benzoyl valine, Benzoyl leucine and Benzoyl phenylalanine. All studied Phthalyl amino acids showed a lowered local anesthetic effect than lidocaine ointment. And significant effect for Phthalylglycine, Phthalylmethionine and Phthalyl phenyl alanine compared with Vaseline (negative control).

Keywords: bezoylamino acids, phthalyl amino acids, local anesthetic effect, hot-plate technique, pin-pricking technique

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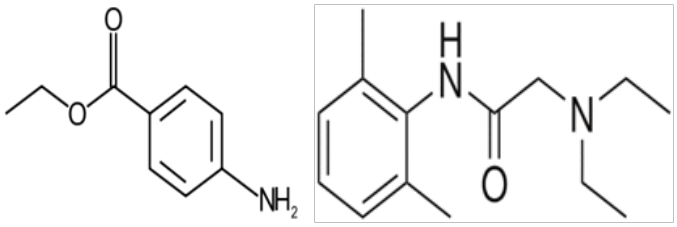
Introduction

Since the discovery of cocaine, several compounds related to benzocaine, lidocaine and isogramine were known as local anesthetic agents.[1,2] But despite their effect they show side effects such as addiction and allergy.[3,4] The general structure of benzocaine and lidocaine illustration in Figure 1, in which the phenyl (Ph) or aryl (Ar) ring is directly or indirectly attached to carbonyl group. The amino alkyl side group for all agents is for solubility purposes: Benzocaine structure contains a carbonyl group attached directly to benzene ring and through oxygen to amino alkyl group. BAA contains a carbonyl group attached directly to benzene ring through nitrogen to carboxy alkyl group, whereas PAA contain two carbonyl groups attached directly to benzene ring and through nitrogen to carboxy alkyl group. Local anesthetics are agents that reversibly block nerve conduction when applied to a circumscribed area of the body.[4] This present work aim to prepare and evaluate the local anesthetic effect of some derivative namely benzoyl amino acids (BAA.I) and phthalyl amino (acids PA A, II) in which benzoyl group or phthalyl group is attached directly to the alpha- amino group of the amino acid. These compounds are structurally related to benzocaine (III), because numerous improvements in the manufacture of local anesthetic solutions have been made. The present work aims to prepare and evaluate the local anesthetic effect of some N-benzoyl amino acids and N- phthalyl amino acids with aliphatic side chain; which are structurally related to benzocaine.



Benzocaine structure Lidocaine structure

Figure 1 Benzocaine (Ph.CO.O. amino alkyl) and Lidocaine (Ar. NH. CO. CH_2. amino alky) structure.

Materials and methods

All chemicals were purchased from Fluka chemicals and were used without further purification. Recorded melting points were uncorrected FTIR – spectra were recorded using.

Animals

Either sex albino mice (from Animal Care House, College of Dentistry, Mosul University) weighing (25–35g) were used. The mice were housed in rodent plastic cages under normal 12hr light/dark cycle with free access to tap water and food pellets. Ambient temperature and relative humidity were maintained at 22±1°C and 45±5 respectively.

Preparation of benzoyl amino acids

The amino acid (0.02mol) was dissolved in 80 ml N NaOH solution and then cooled to 0°C. The reaction mixture was maintained at a pH at 8-9. After the addition was completed the stirring was continued for further 1h. The reaction mixture was then acidified with concentrated HCl until pH become 2 was reached. The precipitated product was extracted with 2*25ml ethyl acetate. The combined extract was dried over dry Na_2SO_4. Filtered and solvent evaporated under vacuum to give oily product. The oil was dissolved in 20ml ethyl acetate with stirring and cooled to 0°C and 20ml of n-Hexane was added to give the benzoyl amino acid as white crystals.[5–7]

Preparation of phthalyl amino acids: (General Procedure)

Equimolar amount of phthalic anhydride and the recommended amino acid were mixed in a dry beaker and heated in sand bath with mixing until 134-140°C where effervesce commences and gradually increase in vigor after 10-20minutes. The mixture suddenly froths up and reaction temperature increases to 150-160°C and becomes almost solid. The heat was discontinued cooled, water was added to disintegrate the solid, filtered and precipitate washed with cold water, recrystallized from water\ ethanol. The UV spectra and FTIR spectra were recorded.[8]

Preparation of the ointments

2% (W\W) of lidocaine (Mosul drug industry) were prepared by grinding the control and studied compounds in Vaseline (Samara drug industry).

Preparation of solution

2% (W\V) of the sodium salt of benzoyl amino acid and phthalyl amino acid were prepared by mixing equivalent amount of benzoyl amino acid and phthalyl amino acid with sodium hydroxide in distilled deionized water.

Measurements of local anesthetic activities

Hot- plate technique in mice[9]

The mice were randomly divided into 2 main groups (AB) group A was divided to six groups, groups, five animals for each group. Group B were divided to seven groups, five animals for each group. The response was assessed for all animals in group A and B after 5minutes of topical ointment application. Each mouse was placed individually on a hot-plate maintained at 55±10C. The response latency was evaluated based on either animal licking of its feet or jumping out following plate contact. The experiment was repeated three times for each animal. The percentage increased in reaction time of local anesthetic Maximal Possible effect (%MPE) was recorded.[10]

% increase in reaction time (antinociceptive) MPE = (T1-T0 / 30-T0)×100

T0 = mean time for the control group (second)

T1 = mean time for the test group (second)

30= cut off time (second)

The prolongation of latency times compared with the values of the control was used for comparison.

The following groups were examined:

Benzoyl amino acids group (Group A):

The mice were divided into six groups (5mice\group) and treated as follows:

Group 1: Vaseline as negative control.

Group 2: 2% lidocaine ointment as positive control.

Group 3: 2% Benzoyl valine

Group 4: 2% Benzoyl leucine

Group 5: 2% Benzoyl phenylalanine

Group 6: 2% Benzoyl glycine

Phthalylamine amino acids group (Group B):

The mice were divided into six groups (5mice\group) and treated as the following:

Group 1: Vaseline (as negative control)

Group 2: lidocaine ointment 2% (as positive control

Group 3: 2% Phthalyl valine

Group 4: 2% Phthalyl glycine

Group 5: 2% Phthalyl methionine

Group 6: 2% Phthalyl alanine

Group 7: 2% Phthalyl phenyl alanine

Pin-Pricking technique in rabbits: (Elise et al 1979)

The local anesthetic activity of sodium salts of some benzoyl amino or phthalylamino acid (2% w/v) compared with lidocaine solution (2% w/v) were assessed by Pin-Pricking the area prepared for surgical procedure as a following: Stimulation by needle was used to allow easy measurement of antinociceptive effect of prepared solution. After 3 minutes of local injection the solution, evaluated the local anesthetic effect by Pin-Pricking technique.[11]

A total of 24 healthy (1.5-2 kg) rabbits of either sex, aged (5-6) months were obtained from local market. They were housed under control condition 23±2 ^{0}C. Restricted cycle area on the dorsal of all rabbits was prepared for test. Prepared solution of group A and B were injected on diameter of restricted cycle to infiltration of prepared solution. After 3minutes of solution injection, the tips of 0.2mm diameter needle gently touched to the restricted cycle on the dorsal lateral skin without penetrated the skin). Thus, positive values indicate relative anesthesia or analgesia and negative values indicate relative hyperalgesia and no anesthesia.[12] The test was repeated every 10min. The following groups were assessed:

Benzoyl amino acids group (4rabbits\group):

Group 1: 2% lidocaine hydrochloride as control

Group 2: 2% Benzoyl phenyl alanine

Group 3: 2% Benzyl glycine

Phthalyl amino acids group (4rabbits\group):

Group 1: 2% lidocaine hydrochloride as control

Group 2: 2% Phthalyl alanine

Group 3: 2% Phthalyl glycine

Statistical analysis

The data were expressed as mean±SD, difference between experimental groups was statistically analyzed by one-way analysis of variance (ANOVA) followed by the least significant difference test. The data as a percentage analyzed by fisher exact probability test. The level of significance was at $p<0.05$.

Results

The FTIR spectra of the prepared benzoyl amino acid showed –NH stretching vibration at 3370-3400cm^{-1}, 3050-3100cm^{-1} for aromatic –CH stretching, 2940-2980cm^{-1} for aliphatic -CH stretching vibration, 1740-1765cm^{-1} for carbonyl group of carboxylic groups, 1540-1570cm^{-1} for aromatic C=C stretching, 1170-1209 for –C-N stretching. For phthaloylation acid the FTIR spectra of all studied compounds showed stretching band at 3450-3550cm^{-1} for –OH, 3040-3110cm^{-1} for aromatic –CH 2930-2920 cm^{-1} for aliphatic –CH ,1740-1765 for aromatic CO, 1710-1730 for carboxylic carbonyl group and 1550-1580 for aromatic C=C. The local anesthetic effect of the prepared ointment using a hot-plate test on mice revealed that all benzoyl amino acids showed a significant anesthetic effect compared with Vaseline (negative control) but less effective than lidocaine ointment (positive control). For benzoyl amino acid, benzoglycine showed a significant effect compared with other a significant benzoyl valine, Benzoyl leucine and Benzoyl phenylalanine (Figure 2). For Phthalyl amino acids, a hot-plate test for the local anesthetic effect showed no significant effect for Phthyl alanine and Phthyl valine and significant effect for Phthalyl glycine, Phthalyl methionine and Phthalyl phenyl alanine compared with Vaseline (negative control). All studied Phthalyl amino acids showed a lowered local anesthetic effect than lidocaine ointment. In general benzoyl amino acids showed a better local anesthetic effect than Phthalyl amino acids (Figure 3).

In Pin-Pricking technique for lidocaine solution following topical administration, all experimental rabbits showed unresponsive to pinprick and the response to needle stimulation were abolished. This result obtained after 3 minutes from topical application and last up to 80minutes. Unresponsive to a pinprick; consider as duration of anesthesia (Figure 2). In Phthalyl phenyl alanine and Phthalylglycine produce local anesthetic effect after 5 and 10minutes respectively and last up to 30 minutes but the intensity of local anesthetic effect is less than lidocaine solution (Table 1). Whereas in benzoglycine and benzoyl phenylalanine solution produce weak anesthetic effect with lowest intensity effect than lidocaine and Phthalyl amino acids groups (Table 2).

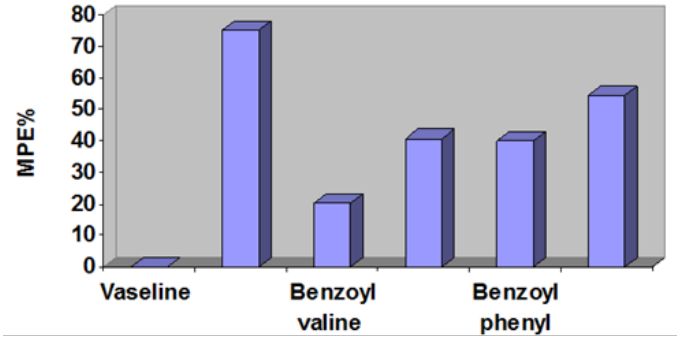


Figure 2 Effect benzoyl amino acids group (Group A) on antinociceptive maximum pain.

-Values are mean+SD.

Animal number: number: 5mice/group

*Significantly different from the control value P< 0.05.

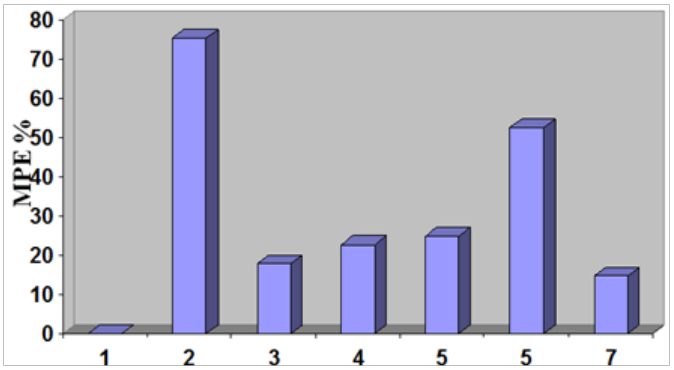


Figure 3 Effect Phthalyl amino acids group (Group A) on antinociceptive maximum pain

- Values are mean+SD.

Animal: number: 5mice/group

*Significantly different from the control value P< 0.05.

*Vaseline (1), Lidocaine (2, Phthalyl Valine (3),

Phthalyl Glycine (4), Phthalyl methionine (5),

Phthalyl alanine (6) phenylalanine (7)

Table 1 Percentage of local anesthetic effects of Phthalyl alanine and Phthalyl glycine in comparison to lidocaine during 60minutes in rabbits.

Groups	Time after drug injection (minute)											
	5	10	15	20	25	30	35	40	45	50	55	60
Lidocaine	100%*	100%*	100%*	100%*	100%*	100%*	100%*	100%	100%*	100%*	100%	100%*
Phthaloyl Alanine	50%*	50%*	75%*	100%*	75%*	75%*	50%*	50%*	25%	zero	zero	Zero
Phthaloyl Glycine	zero	50%*	100%*	100%*	100%*	75%*	50%*	25%	zero	zero	zero	

-Values are mean+SD, Animal: number: 4 Rabbits/group, *Significantly different from the control value P < 0.05.

Table 2 Percentage of local anesthetic effects of % Benzoyl phenyl alanine and Benzyl glycine in comparison to lidocaine during 60minute in rabbits

Groups	Time after drug injection (minute)											
	5	10	15	20	25	30	35	40	45	50	55	60
Lidocaine	100%*	100%*	100%*	100%*	100%*	100%*	100%*	100%	100%*	100%*	100%	100%*
Benzoyl phenylalanine	25%	25%	25%	25%	25%	25%	zero	zero	zero	zero	zero	Zero
Benzoyl glycine	50%*	50%*	50%*	25%	25%	zero	zero	zero	zero	zero	zero	

- Values are mean percentage for 4 Rabbits /group

*Significantly different from the control value P< 0.05

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Discussion

Local anesthetic solutions have been utilized in clinical dentistry to alleviate or eliminate pain associated with invasive procedures as early as the 19th century.[13] Local anesthetics are agents that reversibly block nerve conduction when applied to a circumscribed area of the body.[2,4]

In general, structural formula of benzocaine and lidocaine illustrated in Figure 1 showed the required of sp^2 carbon (C=O, C=C or C=N) attached directly or indirectly to benzene ring from one side and to tertiary amino alkyl group on the other side. The presence of amino alkyl group is for water solubility purposes but may cause irritation (Gisvold 1989). The present work replaces amino alkyl group by carboxyl alkyl group for two reasons. The first is for its intermediate solubility in water since the carboxyl group (Pka 3.5-4) is almost completely ionized at physiological pH (7-7.4). The second reason is that biological hydrolysis of the amide link may give benzoic acid (in case of benzoyl amino acid) or Phthalyl acids (in case of Phthalyl amino acids) an amino acid. All Compounds are either biological intermediate or known for pharmaceutical or used in food industry. For benzoyl amino acid the replacement of oxygen by nitrogen seems to lower the local anesthetic effect of all benzoyl amino acids. Benzoyl glycine give better anesthetic effect when compared with other benzoyl amino acids which raise the question of Chirality since only benzoyl glycine doesn't contain chiral carbon and the rest benzoyl amino acids are benzoyl derivative of racemic amino acid.

Hot- plate technique in mice for Phthalyl amino acids showed the Phthalyl alanine is significantly more active than Vaseline negative control but still less active as an anesthetic effect than lidocaine. This may be due to the incorporation of second carbonyl group (group (sp^2 hybridized) ortho to the first carbonyl group attached to benzene ring. This incorporation lowers the local anesthetic effect of Phthalyl amino acids compared with benzoyl amino acid. This result accepted with other study that found drugs with sodium channel blocking actions preferentially suppressed thermal nociception, that sodium channel blocking agents have a preferential antinociceptive action against thermal stimulation that is likely to be attributed to their local anesthetic action.[14,15] In Pin-Pricking technique in rabbits we showed that the Phthylal amino acids benzoyl amino acids solution produced local anesthetic effect. Needle stimulation was used to elicit the cutaneous trunci reflex (a needle was gently touched to the dorsal lateral skin and intensity of the reflex was observed, therefore in the present study we found that the reaction to pinprick technique is positive after five minute, this result agreement with previous study suggested that positive values in pinprick technique indicate relative anesthesia or analgesia and negative values indicate relative hyperalgesia and no anesthesia.[8,16]

Local anesthetics block the sensation of pain by interfering with the propagation of peripheral nerve impulses. The generation and the conduction of action potentials are inhibited. Nerves generate impulses by creating changes in membrane permeability. This change in permeability allows for a substantial inflow of sodium ions across the nerve membrane, which creates an action potential. The generated action potential then becomes part of the information relay along the nerve, allowing communication between the peripheral and central nervous systems. Restricting the action potential can therefore block this communication, and if a long enough portion of the nerve can become involved, anesthesia will result. In the simplest of terms, local anesthetics bind to receptors near the sodium channel on the nerve membrane. As the amount of the local anesthetic accumulates, the sodium channels become obstructed. Impulses along the nerve are slowed, the strength and propagation of the action potential are diminished, and communication along the fiber is blocked.[1,2] Although local anesthetics are often used as analgesics, it is their ability to provide complete loss of all sensory modalities that is their distinguishing characteristic. The contrast with general anesthesia should be obvious, but it is perhaps worthwhile to emphasize that with local anesthesia the drug is delivered directly to the target organ, and the systemic circulation serves only to diminish or terminate its effect.[17]

Conclusion

According to our findings, benzoyl amino acids showed a better local anesthetic effect than Phthalyl amino acids as ointment but when used as solution we found that Phthalyl amino acids showed a better local anesthetic effect than general benzoyl amino acids.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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