LOCAL ANAESTHETIC ACTIVITY, IRRITANCY AND TOXICITY OF SEVEN NEW SYNTHETIC LIGNOCAINE ANALOGUES

By

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Lignocaine is an addition to the list of synthetic local anaesthetics by Lofgren and Lundquist (7). Since then attempts have been made to synthesise newer and better compounds, from the point of view of effectiveness and toxicity. Recently Trivedi and Dalal (10) synthesised a series of lignocaine analogues in order to find more effective all purpose local anaesthetic compounds. Some of these compounds were tested earlier by Patel and Jindal (8) who reported that one of these analogues—α-piperidenyl-N-(2:4 dimethyl benzyl) propionamide was found to be many times more potent than lignocaine, procaine and cocaine. This compound, though more potent than other analogues of lignocaine, was still less toxic than lignocaine. It was therefore thought proper to evaluate the local anaesthetic activity of some additional compounds of this series which were synthesised by Shah and Trivedi (9). The local tissue irritation often plays an important role in the evaluation of the pharmacological properties of parenteral preparations or when a preparation is designed for application on mucous membrane. Therefore, irritancy tests were performed. Acute toxicity of these compounds has also been determined in albino mice. The results of these investigations are presented in this paper.

MATERIALS AND METHODS

The following synthetic lignocaine analogues have been studied for their local anaesthetic activity, irritancy and toxicity :—

1. N [p- (4′-chlorobenzyloxy) phenyl]—α—diethenolamino propionamide hydrochloride (compound G).
2. N [m- (4′ - methylbenzyloxy) phenyl]—α—piperazino propionamide hydrochloride (compound H).
3. N [m-benzyloxyphenyl]—α—piperidinoacetamide hydrochloride (compound I).
4. Sym disubstituted N (m-benzyloxy-phenyl)—α—piperazino acetamide hydrochloride (compound J).
5. N [p- (4′ - chlorobenzyloxy) phenyl]—α—piperazino acetamide hydrochloride (compound K).
6. N [p-benzyloxyphenyl]—α—diethylamino propionamide hydrochloride (compound L).

7. N [p-benzyloxyphenyl]—α—piperidinoacetamide hydrochloride (compound M).

8. α—diethylamino-2, 6-acetoxylidide (lignocaine hydrochloride).

Local Anaesthetic Activity :

This has been determined with the help of two conventional methods *viz.* (*a*) surface anaesthesia in guinea-pigs (2). (*b*) intracutaneous wheal method in guinea-pig (1) with slight modification as described by Patel and Jindal (8). The local anaesthetic activity was compared with known reference drugs such as cocaine, procaine and lignocaine.

Local Irritancy :

Local irritancy was determined by the following methods :—

A. Mucous membrane irritation by rabbit eye test (3)

B. Intradermal irritation by the rabbit trypan blue test (5)

C. Subcutaneous irritation by the rabbit ear test (4)

Acute Toxicity :

Acute toxicity was determined in mice after intraperitoneal administration of drug solutions in graded doses. The animals were kept under observation for a period of 24 hours. During this period general symptoms were observed and the mortality was noted in each group. LD_{50} was determined according to Karber's method (6).

RESULTS

Local Anaesthetic Activity :

Surface anaesthesia : All the compounds studied have shown surface anaesthetic properties, in proportion to the concentrations employed. The individual compound generally showed uniform relationship between the effect and their concentrations. A linear dose response curve was obtained with all the compounds except cocaine (Fig. 1). Of the seven compounds L was the most potent. It was also found to be four times more potent than lignocaine and twice more potent than cocaine. The rest of the compounds G, H, I, J, K and M were 1, 1, 1/2, 1, 1/2, 1, times respectively as potent as lignocaine and 1/2, 1/2, 1/4, 1/2, 1/4 and 1/2 as potent as cocaine (Table I). The mean duration of anaesthesia with compounds G, H, I, J, K, L and M was 23.3, 24.5, 30.9, 28.8, 24.7, 23.3 and 28.3 minutes respectively. While the reference drugs cocaine and lignocaine had 20 and 15 minutes duration anaesthesia respectively (Table I).

Median effective doses and true fiducial limits at (P=0.95) for surface anaesthesia were worked out and on scrutinising these results it was observed that compound L is the most potent of all the drugs tested. Its potency is approximately 3.0 times that of cocaine or lignocaine. Compound M is nearly twice as potent as any of the reference drugs (Table II).

TABLE I

Surface anaesthesia on guinea-pig cornea with the lignocaine analogues and the reference drugs

%Conc. of drug Solu.		Cocaine	Lignocaine	Comp G.	Comp H.	Comp I.	Comp J.	Comp K.	Comp L.	Comp M.
1. 0.0125	a	..	..	..	..	..	..	..	35.0(8.3)	..
	b	..	..	..	..	..	..	..	23.0(1.4)	..
2. 0.025	a	25.0(9.57)	13.3(11.05)	..	..	..	...	...	56.0(5.1)	43.3(4.9)
	b	3.3(0.95)	3.6(2.15)	..	..	..	..	..	23.3(1.02)	18.3(1.0)
3. 0.05	a	40.0(8.10)	43.3(14.90)	31.7(7.4)	33.3(8.1)	..	31.7(4.0)	..	95.0(5.4)	61.7(4.1)
	b	6.5(2.50)	5.2(11.23)	21.8(2.9)	22.6(1.0)	..	20.2(1.0)	..	23.3(1.4)	28.0(1.5)
4. 0.1	a	99.7(4.71)	90.0(5.80)	60.0(6.3)	56.6(5.1)	..	63.3(5.1)	..	..	98.3(4.05)
	b	20.0(4.65)	9.3(1.14)	22.5(1.0)	24.1(1.6)	..	27.3(1.02)	..	..	28.3(1.00)
5. 0.2	a	..	98.4(3.7)	95.0(5.4)	96.6(5.1)	36.6(5.1)	95.0(5.5)	36.6(5.7)	..	..
	b	..	15.0(1.61)	23.3(2.6)	24.5(1.7)	27.5(1.1)	28.8(1.7)	24.3(1.0)	..	..
6. 0.3	a	..	..	..	..	56.7(5.1)	..	58.3(4.1)	..	..
	b	..	..	..	..	29.0(2.3)	..	24.8(1.0)	..	..
7. 0.4	a	..	..	..	..	96.7(5.1)	..	93.3(8.1)	..	..
	b	..	..	..	..	30.9(2.6)	..	24.7(1.8)	..	..

a—Mean percentage of failure of response. b—Mean duration of anaesthesia in minutes.
The values in parentheses represent standard deviation.

TABLE II

'Median effective dose' for surface anaesthesia with the lignocaine analogues and the reference drugs

Drugs	Cocaine	Lignocaine	Comp G.	Comp H.	Comp I.	Comp J.	Comp K.	Comp L.	Comp M.
Median effective dose (per 100 *ml.*)	0.07	0.06	0.08	0.08	0.25	0.07	0.25	0.02	0.03
True fiducial limits (P=0.95)	0.022 —0.095	0.027 —0.161	0.043 —0.189	0.043 —0.189	0.217 —0.389	0.043 —0.189	0.211 —0.389	0.011 —0.047	0.022 —0.095

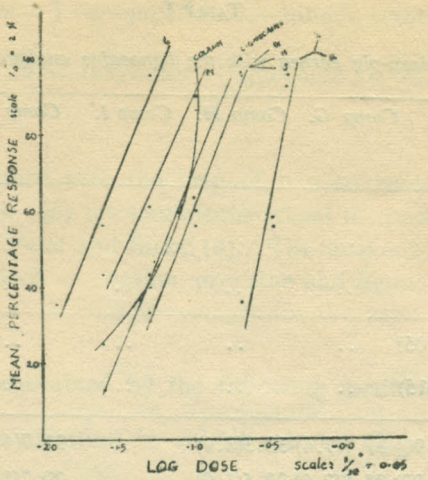

Fig. 1. *Surface anaesthesia in guinea-pig cornea.*
The figure shows the relationship between the log dose and the mean percentage failure of response with lignocaine analogues, cocaine and ilignocame—Each point represents the means of 20 observation..

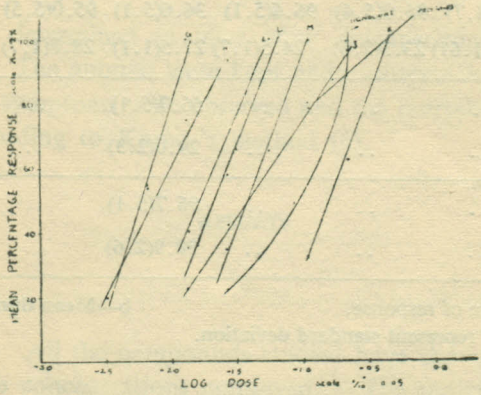

Fig. 2. *Intradermal anaeshtesia in guinea-pig :*
The figure shows the relationship between the log dose and the mean percentage of failure of response will lignocanine analogues, procine and lignocaine; Each point represents the mean of 20 observations.

Intradermal Anaesthesia : Graded concentrations ranging from 0.0031 to 0.4% of the drugs under investigation were injected intradermally in guinea-pigs. The results varied with different compounds. The individual compounds generally showed a uniform relationship between the effect and the drug concentration (Table III). A dose response curve was obtained with all the compounds and is presented graphically (Fig. 2).

It is evident from the results that compound G is most potent as a local anaesthetic for intradermal anaesthesia in guinea-pigs and has been found to be 32 and 16 times more potent than procaine and lignocaine respectively, while compound H, I, J, K, L, M and 16,16,2,1,16,4, times respectively as potent as procaine and 8,8,1,1/2, 8, 2 as potent as lignocaine. The duration of anaesthesia was approximately double than the reference drugs (Table III).

TABLE III

Intradermal anaesthesia on guinea-pig with the lignocaine analogues and the reference drugs

% Con. of drug Solu		Procaine	Lignocaine	Comp G.	Comp H.	Comp I.	Comp J.	Comp H.	Comp L.	Comp M.
0.0031	a	..	..	21.6(4.0)	21.6(4.0)	..	..	..	..	..
	b	..	..	62.5(2.7)	64.0(3.7)	..	..	..	..	..
0.0062	a	..	..	55.3(5.0)	56.6(5.0)	..	..	..	..	..
	b	..	..	74.2(3.6)	65.8(3.3)	..	..	..	..	..
0.0125	a	..	..	100.0(0.0)	75.0(22.9)	41.6(4.4)	23.3(4.8)	..	36.6(5.0)	..
	b	..	..	92.5(7.5)	77.4(5.2)	80.8(4.9)	51.7(2.5)	..	70.0(2.2)	..
0.025	a	..	23.6(14.35)	..	93.8(5.0)	80.0(0.0)	43.3(4.8)	..	58.3(4.2)	36.6(5.0)
	b	..	19.1(5.38)	..	81.0(2.0)	99.0(3.7)	66.3(2.5)	..	75.0(3.2)	70.8(3.8)
0.05	a	..	36.1(5.51)	..	100.0(0.0)	100.0(0.0)	66.6(8.0)	..	100.0(0.0)	75.0(5.0)
	b	..	21.6(4.69)	..	95.0(0.0)	103.3(6.2)	74.0(3.7)	..	88.3(3.8)	75.0(3.2)
0.1	a	78.5(7.24)	78.5(10.50)	..	..	..	80.0(8.2)	33.3(5.9)	..	100.0(0.0)
	b	31.7(2.44)	31.7(2.44)	..	..	..	77.5(2.7)	86.6(4.7)	..	76.0(3.6)
0.2	a	88.7(3.30)	97.2(3.92)	..	..	..	96.6(5.9)	63.3(7.7)	..	..
	b	38.3(2.41)	44.2(6.08)	..	..	..	78.6(3.1)	97.5(1.7)	..	..
0.4	a	100.0(0.0)	..	..	..	..	..	100.0(0.0)	..	..
	b	48.3(7.46)	..	..	..	..	..	101.0(5.2)	..	..

a—Mean percentage of failure of response b—Mean duration of anaesthesia in minutes.
The values in the parentheses represent standard deviation.

Local Irritancy :

None of the compounds have shown local irritation or tissue injury in any of the experiments performed. No systemic reactions were observed with any of the compounds in concentrations ranging from 0.0031 to 0.4% which were also employed for testing their local anaesthetic activity.

Acute Toxicity :

In small doses all the compounds studied showed symptoms of depression on the central nervous system such as somnolence, diminished movements, and hypnosis. The animals could, however, be aroused by external stimuli. Higher doses showed powerful stimulation of the central nervous system as demonstrated by tonic and clonic convulsions, ultimately resulting in death due to respiratory paralysis. The LD_{50} of these compounds as calculated by Karber's method is given in Table IV. The results show that none of the compounds is as toxic as lignocaine.

TABLE IV

Results of acute toxicity experiments after intraperitoneal injections of lignocaine analogues in mice using Karber's method

Compound	*LD_{50} in mg/kg*
G	732
H	690
I	480
J	436
K	430
L	712
M	680
Lignocaine	153

DISCUSSION

The results obtained in the present study have shown that some of the tested compounds were more potent than the reference drugs. As a surface anaesthetic compound L has been found to be 4 and 2 times more potent than lignocaine and cocaine respectively ; < as a block anaesthetic, 8 and 4 times more potent than procaine and lignocaine respectively. However, compound G was found to be the most potent in this respect which was nearly 32 and 16 times as potent as procaine and lignocaine respectively (Table III) . The local anaesthesia produced by both the compounds under test was reversible and normal sensitivity could be restored within a reasonable period of time. However, the period of duration of intradermal anaesthesia with compounds G and L was nearly double than the reference drugs. None of the compounds showed any sign of toxicity such as irritation, smarting, inflammation, necrosis, etc. at the site of application or injection, which indicates greater therapeutic value for parenteral administration and mucous membrane application. It is usually seen that potency and toxicity run parallel with local anaesthetics. Compounds G and L were, however, exceptions to this phenomena. The studies on acute toxicity in mice showed that the potent local anaesthetic compounds G and L were less toxic than other compounds tested including lignocaine. The present study has thus shown the superiority of compounds 'G and L' over the existing local anaesthetics, on account of high potency, longer duration of action in low concentration and relatively low toxicity.

SUMMARY

Seven new lignocaine analogues were studied for their local anaesthetic activity, irritancy and toxicity. All the compounds exhibited significant degree and duration of local anaesthesia but compound 'G' [N (p-) 4' - chlorobenzyloxy phenyl]—α—diethenolamino propionamide hydrochloride and compound 'L' [N (p-benzyloxy phenyl)—α—diethyl amino propionamide

hydrochloride] were found to be the most potent as surface and block anaesthetic. All the compounds were found to be atoxic in concentrations used for producing local anaesthesia. Acute toxicity studies have shown that compounds G & L though most potent local anaesthetic, are less toxic than other tested compounds including reference drugs. Compounds G and L thus hold promise as future most potent, non-irritant and less toxic local anaesthetic compounds.

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