

TOXICOLOGICAL STUDIES ON CERTAIN AMINOALKYL ETHERS AS POTENTIAL LONG-ACTING LOCAL ANAESTHETICS.

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ABSTRACT

The acute intravenous toxicities of a selection of three potential long-acting local anaesthetics belonging to the series of 2- phenoxyethyl dialkylamine were determined in mice. Two of the compounds were tertiary amines and the third a quaternary ammonium derivative. The I.V. median lethal dose, (LD₅₀) of one of the tertiary compounds, 21A [N, N- diethyl-2- (2,6- diisopropyl) ethylamine hydrochloride] was over 2.5 times that of lignocaine, while the other, 30A [N, N- diethyl-2- (4-sec-butyl) ethylamine hydrochloride] had an LD₅₀ of 1.5 times. On the other hand, the quaternary ammonium derivative FA [N- benzyl-N-N- dimethyl -2- (2- tert- butyl) ethylamine chloride] was more toxic, with an LD₅₀ value of one quarter of the standard. The test compounds, particularly compound 21A, seem to have good properties that would warrant further investigation as potential long-acting local anaesthetic agents.

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INTRODUCTION

Generally, toxicity could result from a single dose administration, from continuous parenteral infusion or from repeated doses. It is normally established by the appearance of certain symptoms and/or phenomena such as ataxia, convulsions, loss of consciousness and death or could be morphological with functional changes or other anatomical alterations in tissues and organs. These changes may be reversible or may ultimately be lethal. The toxic symptoms could be either localized or generalised or both. The local tissue toxicity, e.g. irritation, swelling, edema, induration, necrosis and ulceration, of the local anaesthetic agent is an important problem which must be studied carefully.

This is because there are a number of agents that have tolerable and acceptable systemic toxicity, but are certainly undesirable clinically due to high and unacceptable degrees of tissue damage when applied topically, parenterally or adjacent to the nerve trunks. Both local and systemic toxicities must therefore be investigated and evaluated. Local tissue toxicity may be evaluated by the rabbit or guinea pig intradermal wheal test, with further confirmation by histological examinations. Systemic toxicity due to acute, chronic or repeated administration of a substance could be investigated by observing certain symptoms as end points, e.g. ataxia, convulsions or death. The dose that statistically causes convulsions (CD₅₀) or death (LD₅₀) in 50% of the animals is then calculated. The values of CD₅₀ or LD₅₀ are not constants as other physical constants such as a pK_a value. They may show considerable variability since they are based on a biological end point which is influenced by numerous factors such as: genetic, physiological and pathophysiological status of the animal, external environments,

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experimental conditions, factors inherent to the administration of a compound, and route and rate of administration of the test agent¹⁻¹² (see Table I).

Acute toxicity signifies the toxic effects produced by a single dose of a compound. It is investigated in experimental animals in the course of the safety evaluation of chemicals. It is assessed in rodents such as: mice, Syrian hamsters, Chinese hamsters, rats, guinea pigs and rabbits and further examined in non-rodents such as dogs and cats. The scope of the measurement of acute toxicity depends on the over-all plan of the safety evaluation of a chemical.

Generally, toxicity is greatest by the route that carries the compound to the blood stream most rapidly. The I.V. toxic dose is greatly affected by the rate of administration. A slow rate of infusion might equilibrate with the rate of detoxification of the compound and not induce a severe toxic response. This problem should be kept in mind, especially when the estimation of the I.V. lethal dose is attempted in dogs or in cats using only a few animals. The site of injection also has a bearing on the toxic response of a compound.

During our pharmacological screening¹³ of certain novel derivatives of 2-phenoxyalkyldialkylamine, the results were promising. These compounds showed good profiles of local anaesthetic properties. They were as good as or better than both of the standard agents, lignocaine and bupivacaine, in terms of onset of action, potency, and duration of full local anaesthesia using the guinea pig intradermal wheal test. Moreover, the test compounds showed neither obvious signs of local tissue toxicity, e.g. irritation, swelling, edema, induration, necrosis or ulceration nor systemic toxicity at the dosage levels applied (0.5-2% W/V). To confirm whether these compounds are comparable or superior to the standard, further toxicological studies will be needed before clinical trials.

Three compounds of the afore mentioned series were selected and their acute intravenous toxicities (LD_{50}) were determined on male mice. The preliminary findings are reported. Two tertiary amines and one quaternary ammonium derivatives of 2-phenoxyalkyldialkylamine were studied.

EXPERIMENTAL

Materials

Male NMRI mice (18-23 g) were used. Solutions (0.05-0.2% W/V, Ph 5.9-6.1) of the test compounds and the lignocaine standard were prepared in distilled water. Lignocaine (Xylocaine, Astra Läkemedel AB, Södertälje, Sweden) was used as the standard. The assayed compounds were primarily coded as: 21A, 30A and FA. Later on they were recoded by Astra Pharmaceutical Company as: RAP 456, 37902; RAP 457, 37903 and RAP 458, 37904, respectively.

Methods

The animals were kept for a week in the laboratory before use. This is to ensure their proper health and to allow for their adaptation to the new external environment. Animals of similar body weight (not more than 10% difference), selected one day before, were chosen and assigned randomly to the various groups. Ten animals were selected for each dose level, and a similar number, treated with the vehicle only, serving as control. Lignocaine was used as the standard. Each animal was then caged individually. A freshly prepared solution of each compound was used. It was kept at room temperature. The volume injected was in the range of 0.1-0.7 ml for each animal. The intravenous administration was kept at a constant rate for each dose level.

Various acute intravenous doses of the test and standard compounds in the tail vein of 3-6 groups of ten animals. The LD_{50} was established by observing the following symptoms: ataxia and/or convulsions. It was then calculated on probit graph paper.¹⁴

RESULTS

The results and some of the pertinent physicochemical and biological data of the compounds are presented in Table I.

DISCUSSION

Because of the availability of uniform strains of mice, ease of housing, size, relatively low cost and abundance of published toxicologic data on this species, they were chosen for the measurement of acute toxicity. However, the drugs should also be evaluated in other species.

The biological response to a chemical substance is usually related to its concentration at the receptor sites. It is affected by the dose administered and the pharmacokinetic properties of the substance. The differences in these pharmacokinetic properties between different species, individuals and various environmental conditions contribute to the variations in toxicological responses. Accordingly, recognition of the factors that influence its variability permit a standardization of measurement and therefore, a greater precision. The important factors affecting laboratory animals and their reactions are listed in Table II.

A close look at the results, shown in Table I, indicates that the acute intravenous toxicity of compound 21A is more favourable compared to lignocaine, i.e. the intravenous median dose (LD_{50}) of lignocaine was about 20 mg/kg, while that of compound 21A was 54 mg/kg. In contrast, the toxicity of the standard was

Table I. Results of acute intravenous toxicity test and some of the pertinent physicochemical and biological data of the assayed compounds.

Compounds	Dose (mg/kg)	Mortality	End point	Approximate LD ₅₀ (mg/kg) (Base)	PKa	Log P	WT
21A	20	0/10	ataxia		8.15	0.71	6
(RAP 456, 37902)	30	0/10	Ataxia				
	40	0/10	Convulsions	54			
	40	0/10	Convulsions	(average value)			
	50	1/10	Convulsions				
	60	4/10	Convulsions				
	70	7/10					
30A	20	0/10	Convulsions	29	8.56	1.40	>6
(RAP 457, 37903)	30	4/10	Convulsions	(average value)			
	40	10/10	Convulsions				
FA	4	0/10	ataxia	5	§	§	§
(RAP 458, 37904)	5	4/10	ataxia	(average value)			
	6.25	10/10	Coinvulsions				
Lignocaine				≈20	7.9	1.13	1-2

§ = data not available.

PKa = ionization constant.¹³Log P = partition coefficient in octanol/Ph 7.4 phosphate buffer.¹³

WT = the duration of action of the anaesthetic agent tested as 0.5% W/V solution in the guinea pig intradermal wheal test (h).

lower than that of compounds 30A and FA. It seems that there is a correlation between the degree of toxicity and its lipophilicity or partition coefficient (Log P). This is only applicable within the same series of compounds. The higher the Log P, the greater the degree of penetration through blood-brain barrier leading to more systemic untoward effects. Compound 21A has a lower Log P than that of compound 30A, and hence a lower penetration through the blood-brain barrier. This, in turn results in a lower incidence of toxic effects. These compounds were previously shown¹³ to have comparable rates of recovery from full local anesthesia (*in vivo* rate sciatic nerve test and the guinea pig intradermal wheal test). This phenomenon was initially ascribed to the fact that they may have comparable rates of tissue clearance or systemic absorption. But the degree of the toxicities does not seem to be due to this factor, otherwise we should have comparable toxic effects. The high level of LD₅₀ of compound 30A could be attributed to the fact that it may have lower rates of metabolism and excretion. Since the biotransformation of local anaesthetics appears only after their resorption into the blood stream. It seems that metabolism is quite important for their toxicity and excretion, while it has scarcely any direct effect on their activity.¹⁵ Similar findings were found by Kisch, et al.¹⁶ who expressed the opinion that the apparent contrast between the long duration of local anaesthetic activity in the esters investigated by them and their rapid

Table II. Important factors affecting the results of simple acute toxicity experiments on laboratory animals.

- 1- Genetic status of the animal.....e.g. species, strain and individual variations, etc.
- 2- Physiological status of the animal.....e.g. sex, age, maturity, estrous cycle, pregnancy, lactation, weight, etc.
- 3- Pathophysiological status of the animal.....e.g. health, deficiencies, spontaneous and acquired immunity, clinical and latent infections, physical activity, fever, stress, obesity, hormonal state gastro-intestinal diseases, renal and hepatic functions, pH of the body fluids, etc.
- 4- External environment of the animal
 - a. Microclimate.....e.g. season, etc.
 - b. Macroclimate.....e.g. temperature, relative humidity, barometric pressure, atmospheric constituents, air circulation, electric charges, light intensity, light spectrum light cycles, noise, etc.
 - c. Feed and feeding (Diet)... constituents, quantity, mode of application, etc.
 - d. Drinking.....e.g. water quality, water quantity, mode of application, etc.
 - e. Caging.....e.g. size, material, shape
 - f. Bedding.....e.g. source, quantity, frequency of changes, etc.
 - g. Handling.....e.g. physical contacts, noise, commotion, personnel qualities such as: training, temperament, replacement, human-animal interactions, animal-animal interactions, handler attitude, etc.
- 5- Experimental conditions.....e.g. transfer, regrouping, altered surroundings adaptation, recovery, etc
- 6- Factors inherent to the administration of a compound.....e.g. volume, type of the solution. concentration of the solution, solubility, pH, osmolarity, etc.
- 7- Route and rate of administration.....e.g. orally, parenterally, site of injection, slow, moderate or rapid rate of administration, etc.

hydrolysis by serum cholinesterase could be explained by the fact that the hydrolysis begins only after passage from the site of action into the blood stream and the liver. Therefore, the improved activity of the more stable esters is rather to be ascribed to their more satisfactory physicochemical properties and higher reactivity with the reactive sites of the receptors. It is well known that cinchocaine is a highly toxic local anaesthetic, and is attributed to its high chemical stability and minimal degrees of metabolism and excretion, while hostacaine, which is degraded more rapidly, is significantly less toxic¹⁰

Generally, ether derivatives are chemically more stable than both esters and amides. Thus, it is not expected that they suffer a significant biochemical degradation at the site of injection or during their transport to the site of action in the biophase and after

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their passage into the blood stream. They are most probably excreted unchanged or after metabolism in the liver. Accordingly, it seems that the rational development of a new safe local anaesthetic must include the search for easily metabolizable derivatives since they have the advantage of low toxicity. Moreover, they should have satisfactory physicochemical properties and higher reactivity with the reactive groups (receptors) of the site of action.

The high toxicity of the quaternary ammonium derivative could be attributed to the high degree of interaction of its cationic group with the ionic oxygen of the phosphatide structure of the receptor site. This is further enhanced by the interaction of the ether functional group of the drug and other lipophilic groups with certain cationic and lipid moieties of the site of action. This is termed specific interaction. It could also react with the binding sites of other phosphatides and protein molecules, called non-specific interaction. The specific interaction leads to structural and functional alterations in the nerve membrane resulting in interruption of nerve conduction.¹⁰ The non-specific binding, on the other hand, is responsible for the production of various untoward and toxic effects.

Currently available local anaesthetics differ from each other in potency, duration of action, toxicity, irritancy and their ability to produce topical anaesthesia,¹⁷ but all share the tendency to produce untoward reactions in adose-related manner. Likewise, the toxic reactions of the test agents appear to be related to the dose administered, the problem most likely encountered in clinical practice is excitation of the central nervous system, possibly resulting in ataxia and convulsions, particularly after an overdose or an inadvertant intravascular injection.¹⁸ Similarly, our findings seem to be in agreement with the aforementioned conclusions.

Finally, one should be cautious in drawing general conclusions solely from the data of acute toxicity tests. Though these tests reveal toxic effects that interfere with the vital functions within the period of observation, other toxic effects may be expected to remain undetected because of limitations of examinations and numerous other factors that influence the results. Thus, the measurement of acute toxicity represents only a small portion of the safety evaluation conclu-

sion. Further toxicological studies must be performed, paying special attention to all the factors that could modify the response pattern of the animal, thus affecting the outcome.

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