Medical Journal of the
Islamic Republic of Iran

Volume 6
Number 1
Bahar 1371
Spring 1992

A NEW BIOLOGICAL SCREENING SYSTEM FOR LOCAL ANAESTHETICS BY INHIBITION MOBILITY OF *TETRAHYMENA PYRIFORMIS*

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ABSTRACT

An alternative *in vitro* approach to drug screening has been the use of human cell cultures for antiviral agents and microbial cell cultures for the assessment of the carcinogenic potential of selected compounds. A number of protozoan species have been also used as drug screens for anti-protozoal agents. The ciliated protozoan *Tetrahymena pyriformis* species has been widely utilised as a drug screen for a variety of pharmacologically active agents.

Accordingly, it was decided to investigate whether *T. pyriformis* could be used as a preliminary drug screen for evaluation of the local anaesthetic activity and duration of action of certain commercially available local anaesthetics. In this communication, the results of this new *in vitro* biological drug screen are reported. It is based on the complete protozoan cell immobilisation by the anaesthetic solution. A positive inverse correlation was observed between the lowest concentration (minimum inhibitory concentration=MIC) that wholly inhibits the mobility of all cells of *T. pyriformis* and the duration of action of the test compounds. Generally, MIC was high for the short-acting anaesthetics and low for the long-acting ones. The results suggest the suitability of this new microbiological assay system for the evaluation of local anaesthetic activity and duration of action and possibly irritancy and toxicity of other local anaesthetics as well as potentially active therapeutic agents which possess surface activity.

MJIRI, Vol. 6, No. 1, 55-58, 1992

INTRODUCTION

The ciliated protozoan *T. pyriformis* species has been used as an alternative *in vitro* drug screen to evaluate a number of biologically active substances such as antiprotozoals[1], antitumor agents,[2-11] antihormones,[12,13] antimicrobials,[14] antimalarials,[15] antihistamines,[16] antipsychotics,[17,18] antivitamins,[19] hypocholesterimics,[20,21] inhalational anaesthetics,[22] and others.[23-30] Nandini-Kishore, et al[31] and Thompson, et al[32,33] have suggested the usefulness of *T. pyriformis* cell as a model system for membrane studies. There is a pronounced difference in lipid composition among the cell's various functionally distinct membrane systems which gives a significant lipid specificity to the membrane sites. In an effort to develop new *in vitro* biological assay method to evaluate local anaesthetics, this investigation has been initiated. *T. pyriformis* species was used as a preliminary microbiological system for screening the local anaesthetic activity and duration of action of five commonly clinically em-

* Presented at the Ist Seminar on Iranian Self-Sufficiency of Pharmaceutical Industries, Tehran, Iran, 1988.

ployed local anaesthetic agents. It is based on the examination of the complete immobilisation of whole *T.pyriformis* cells in the sample by the assayed compound. The results of this study are reported in this communication.

METHODS

Three-day old *T. pyriformis* cells (GL Strain) maintained in an unshaken axenic culture at room temperature in neopeptone broth (Difco), were washed twice in phosphate buffer (6.7 mM, pH 7.2) by centrifuging (80 g for 4 min) and left overnight in fresh buffer. In the flat bottomed wells of a micro-titre plate, 0.2 ml volumes of buffered cell suspension (5×10^4 cells/ml) were mixed with 0.2 ml buffered anaesthetic solutions of various dilutions. The lowest concentration (MIC) which completely inhibited motility after 60 min was determined by examining the wells microscopically (160× magnification).[34]

RESULTS

The results of inhibition of *T.pyriformis* mobility test by the test agents and some of their pertinent properties[34-38] are presented in Table I. MIC and clinical anaesthetic concentration (CAC) are expressed in % W/V. The onset (OA) and duration (DA) of actions are expressed in minutes and the serum half-life (SHL or T ½) in hours, while pKa is the ionisation constant of the test compounds.

Table I. Results of inhibition of T.pyriformis mobility test by the assayed compounds and some of their properties

Compounds	MIC	CAC	OA	DA	t½	pka
Lidocaine.HCl	1.50-2.00	0.5-4.0	5-10	30-90	1.6	7.86
Prilocaine.HCl	1.25-1.50	1.0-3.0	3-10	60-120	--	7.86
Etidocaine.HCl	1.00-1.25	0.5-1.5	5-15	60-150	2.7	7.74
Amylocaine.HCl	0.75-1.00	--	5-15	90-150	--	--
Tetracaine.HCl	0.175-0.350	0.2-1	7-15	120-180	--	8.39

* Figures of MIC are average of three experiments performed in duplicate.

DISCUSSION

A close scrutiny of the results indicates that there is an apparent inverse relationship between the MIC of the test agents on *T. pyriformis* and their duration of actions. MIC was high for the short-acting anaesthetics and low for the long-acting ones while the duration of action generally increases in the opposite aforementioned order. On the other hand, a positive direct correlation was observed between MIC and CAC of the test compounds, i.e. MIC decreases in the following order; lidocaine > prilocaine > amylocaine > tetracaine.

Affinity for the nerve membrane has been related to DA.[39-43] In an isolated nerve preparation, the major factors influencing DA are the specific interaction at the active receptor site and nonspecific binding of adjacent tissues.[40,44] Moreover, the rate of diffusion of a test agent through capillary endothelial cells increases with increasing lipid solubility or partition coefficient (Log P) of the agent. It is also influenced by the volume of the injected anaesthetic solution.[45,46] It has been found with several long-acting local anaesthetics that an increase in hydrophobicity or Log P is generally associated with increased DA,[34-36] and it seems that such anaesthetics resist systemic absorption by adsorption to nonspecific acceptor, i.e. membranes or proteins, near the site of action. Furthermore, it is well known that compounds with high Log P exhibit strong affinity to cellular membranes which are lipoid or hydrophobic in nature. Thus, a positive correlation between MIC and DA and, in turn, with Log P could indicate that the anaesthetic compounds are presumably dissolving in the lipid portion of the protozoal cell membrane through hydrophobic interactions resulting in membrane fluidization and, subsequently, loss of the ciliary function.

The transport of local anaesthetics to the site of action in the nerve membrane is influenced by their physicochemical properties, e.g. Log P, pKa and surface activity, and the nature of the protein structures of the tissue at the site of application and surrounding the nerve bath, i.e. specific and nonspecific protein-binding.[47,48] Apparently, a correlation was not observed neither between MIC and pKa nor between MIC and OA. However, it would be worth while to conduct further investigations to find out whether such a correlation exists between MIC and other factors or not.

Finally, this simple, quick and inexpensive microbiological assay appears to be able to discriminate between local anaesthetics of different duration of actions and, therefore, could provide an appropriate basis for screening other potential series of local anaesthetics as well as other clinically useful therapeutic agents, preferably those that possess surface activity. Moreover, additional investigations on a large series of local anaesthetics are required to draw ultimately a statistically valid and concrete general conclusion.

ACKNOWLEDGEMENT

We would like to express our thanks to Dr. A.J. Bailliel, School of Pharmacy and Pharmacology, University of Strathclyde, for his cooperation in this project.

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