Chloroquine Drug Interactions
Part I: Interaction with drugs acting at the neuromuscular junction

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Chloroquine is extensively used in the management of malaria in Kenya. It is widely available for self medication. Often it is used concurrently with other drugs. In the present paper, possible drug interactions with Chloroquine have been investigated.

Isolated rat phrenic nerve diaphragm preparation was used to study the effect of Chloroquine alone and in combination with several drugs on neuromuscular impulse transmission. Chloroquine in the dose range 0.025 - 0.3 μg/ml organ bath concentration induced a dose-dependent neuromuscular junction (NMJ) transmission blockade.

The drug significantly potentiated the NMJ transmissionblockade induced by commonly used agents gallamine, succinylcholine and lignocaine. It antagonised the NMJ facilitatory action of physostigmine, calcium chloride and barium chloride.

Chloroquine could be interfering with ion conductance processes. It is suggested that Chloroquine should be used with caution in conditions characterised by muscle contractile disorders or during treatment with drugs that cause decreased skeletal muscle activity.

Key Words: Chloroquine, interactions, neuromuscular junction.

INTRODUCTION

Short term therapy with conventional doses of chloroquine has been associated with mild side effects. However, in long term therapy with high doses as in the treatment of amoebiasis, rheumatoid arthritis and systemic/discoid lupus erythematosus (S.L.E.) a number of serious toxic effects are manifested. These include skin eruptions, photosensitivity, alopecia, bleaching of the hair, leucopenia, retinopathy [1], generalised muscle weakness [2], peripheral neuropathy [3] and cardiovascular disturbances [4, 5].

Neuromuscular junction (NMJ) transmission blockade can occur due to interference with pre or post synaptic mechanisms. Vartanian and Chinyanga [6] found that therapeutic doses of chloroquine induced muscle paralysis characterised by decreased excitability of electrically excitable membranes of the axon and muscle fibre, decreased transmitter release at end-plate plus decreased firing index and decreased amplitude of the action potential. However, there was no change in the resting membrane potential [7]. This effect is similar to that induced by local anaesthetics. Chloroquine possesses local anaesthetic effect [8], calcium antagonistic effect [6], and kaliuretic effects [9].

Although there is documented evidence on the NMJ transmission blockade induced by chloroquine [2,7,10,11] the likely drug interactions during combination therapy particularly with drugs acting at the NMJ have not been fully assessed hence, the need for the current study.

MATERIALS AND METHODS

Materials

White Wistar rats weighing 200 - 300 g and bred by the National Public Health Laboratory (NPHL) Kenya, were used. Lignocaine HCl was obtained from the Pharmaceutical Manufacturing Company (Kenya); physostigmine, acetylcholine, barium chloride from BDH, Poole (UK), gallamine, calcium chloride from May & Baker Ltd. Dagenham (Eng.) succinylcholine from Asta-Werke (Germany) and chloroquine from E.T. Monks (Nairobi, Kenya).

Phrenic nerve-diaphragm preparation

The isolated rat phrenic nerve diaphragm muscle preparation was dissected and set up as per the method described in literature [12]. The tissue was mounted in a 50 ml double walled organ bath containing modified Kreb's Henselleit physiological solution [13] of the following composition; NaCl 6.87; KCl 0.4; $NaHCO_3$ 2.1 and glucose 2.0 grammes per litre). The tissue was adequately aerated by gassing with Carbogen (95% Oxygen and 5% Carbon dioxide) and also

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thermostatically maintained at 37°C.

The tissue was allowed to stabilise for 20 minutes after which the nerve was electrically stimulated at a frequency of one pulse/second and at a voltage of 5 volts.

Although the recommended tissue drug contact time is normally 2 minutes, preliminary results had shown that after chloroquine treatment, the tissue took 30 - 40 minutes to recover with 4-5 rinsings using fresh physiological solution before the next test could be done. Changes in contractile activity of the nerve-diaphragm muscle were recorded via a kymograph (Bioscience, USA 400). Concentrations of chloroquine ranging from 0.025 to 0.37 µg/ml were tested allowing one minute tissue drug contact time before indirectly stimulating the diaphragm muscle via the nerve.

Modification of chloroquine effect by Gallamine, Succinylcholine and Lignocaine

Gallamine 0.2mg/ml bath concentration was tested on the phrenic nerve diaphragm preparation with and without pre-treatment with a standard dose of chloroquine. The drug mixture was left in contact with the tissue for a maximum of two minutes during which changes in the electrically induced contractile response were recorded.

Succinylcholine at a dose of 0.125 mg/ml bath concentration was tested singly and in combination with Chloroquine 0.125 µg/ml bath concentration. Similarly, lignocaine at a dose of 0.125 µg/ml was tested. The tissue-drug contact time was similar to that of gallamine.

Modification of chloroquine effect by Physostigmine, Barium Chloride and Calcium Chloride

Physostigmine (0.075 and 0.15 µg/ml) was left in contact with the phrenic-nerve diaphragm preparation for 1 minute while recording the effect. Without washing, a known concentration of chloroquine was added into the bath and change in contractile activity noted for one to two minutes.

Alternatively, physostigmine was tested on the tissue after pretreatment with a known concentration of Chloroquine.

Similarly, barium chloride and calcium chloride were tested at doses 0.125-0.25 mg/ml and 0.05 mg/ml respectively.

RESULTS

Chloroquine caused a dose dependent neuro-muscular junction transmission blockade. The drug significantly potentiated the NMJ transmission blockade induced by gallamine, succinyicholine and lignocaine (Table 1).

TABLE 1 : Influence of chloroquine on the response of skeletal muscles on drugs n=8 means (SEM)

Drug and Drug Combination	Final Bath Conc.	Mean % Contractile Response (SEM)
Chloroquine	0.025 µg	-12.4 (6.5)
	0.025 µg	-15.0(3.1)
	0.125 µg	-45.5 (2.1)
	0.25 µg	-50.3 (0.7)
	O.375 µg	-64.5 (1.6)
Succinylcholine	0.15 mg	-33.3(1.3)
Lignocaine	0. 125 mg	-56.2 (1.9)
Barium Chloride	0.25 mg	+30.6 (2.3)
Calcium Chloride	0.O5 mg	+16.6 (2.1)
Physostigmine	0.075 mg	+33.3 (1.5)
Gallamine	0.2 mg	-48.5 (1.7)
Gallamine + Chloroquine	0.2 mg + 0.125 µg	-66.0 (1.4)
Succinylcholine + Chloroquine	0.125 mg + 0.0125 µg	-56.1(2.4)
Lignocaine + Chloroquine	0.125 mg + 0.125 µg	-61.1(2.1)
Barium Chloride + Chloroquine	0.125 mg + 0.25 µg	-39.3 (1.8)
Barium Chloride + Chloroquine	0.25m g + 0.25 µg	-51.9 to +33µ3*
Barium Chloride + Chloroquine	0.25 mg + 0.37 µg	-78.6 to +23.8*
Calcium Chloride + Chloroquine	0.O5 mg + 0.125 µg	-20.0 (1.9)
Physostigmine + Chloroquine	0.075 µg + 0.125 µg	-45.5(1.8)
Acetylcholine + Chloroquine	0.25 µg + 0.125 µg	-54.5 (2.7)
Calcium Chloride + Gallamine	0.O5 mg+0.2 mg	-50.0 (2.4)
Succinylcholine + Gallamine	0.125 mg + 0.2 mg	-62.5 (3.6)
Succinylcholine + Lignocaine	0.125 mg + 0.125 mg	-69.6 (1.3)

\- Means contractile inhibition + Means contractile stimulation

* The neuromuscular junction blockade induced by chloroquine was effectively antagonised and reversed by increased doses of barium chloride

The stimulatory actions of calcium, physostigmine and barium on the skeletal muscle were antagonised by chloroquine. The anti-calcium effect was less pronounced than the anti-physostigmine and anti-barium effects.

Physostigmine at a dose of 0.075 µg/ml bath concentration, caused 33.3 ± 1.5% increase in contractile activity, but 45.5 ± 1.8% contractile inhibition when combined with chloroquine, 0.125ug/ml bath concentration. At this dose level, chloroquine completely antagonised the muscular stimulatory effects of physostigmine.

The antagonism between chloroquine and barium chloride seemed to be competitive in nature since an increase in the dose of barium chloride could reverse the effect of chloroquine.

DISCUSSION

The NMJ transmission blocking effects of chloroquine have previously been documented [2]. The effect has been attributed to the local anaesthetic effect [8] and possible calcium antagonistic effect [6].

The present study highlights the drug interactions with chloroquine that are related to its NMJ transmission blockade. The results presented show that chloroquine caused a dose dependent NMJ transmission blockade of rat phrenic-nerve diaphragm preparation.

It potentiated the NMJ transmission blockade induced by non-depolarising NMJ blocker, gallamine, depolarising NMJ blocker, succinylcholine, and a local anaesthetic, lignocaine. This implies that chloroquine affects or alters a step in the overall transmission mechanism that is common to all three pharmacological agents. It could be exerting a direct non-specific NMJ transmission blockade by affecting the sodium conductance mechanisms [7] or by inhibiting the sodium/potassium pump through physiological depletion of either sodium or potassium [9].

Abnormalities of NMJ transmission ranging from minimal muscular weakness to complete paralysis have been associated with hypokalaemia [14]. Potassium is the principal cation of the intracellular fluid, but it is also a very important constituent of the extracellular fluid where it plays a role in the sodium/potassium pump to regulate muscle activity. *In vivo* animal experiments have shown that chloroquine enhances urinary potassium loss and potentiates the kaliuretic effect of diuretics [9]. It is therefore possible that neuromuscular blocking property of chloroquine may be related to its ability to cause potassium depletion, hence making it unavailable for the contractile mechanisms.

Present findings also show that chloroquine has dose-related calcium antagonistic effect at the neuromuscular junction. It probably acts by blocking the calcium mediated excitation - release coupling mechanism hence inhibiting transmitter release [15, 16]. This anti-calcium effect of chloroquine contributes to its neuromuscular junction transmission blocking effects.

Due to the synergistic effect of Chloroquine with succinylcholine and gallamine concurrent administration of this drug with any of the two muscle relaxants may prolong the recovery of muscular activity after general anaesthesia and increase the risk of death of patients from respiratory paralysis.

Myasthenic patients are another high risk group since the neuromuscular blocking effect of chloroquine may precipitate myasthenic crisis in this type of patients [10, 11]. This is more so when physostigmine, an anticholinestrase agent used in the management of myasthenia gravis, does not show any appreciable antagonism of the chloroquine induced NMJ transmission blockade.

A dose of physostigmine (0.075 µg/ml) that induced increased muscular contraction of the indirectly stimulated phrenic-nerve diaphragm muscle (33. ± 1.5%) reversed to inhibition of muscular contraction (-45.4 t 1.8%) when combined with chloroquine 0.125 µg/ml bath concentration. This observation indicates that the NMJ transmission stimulatory effects of physostigmine were completely masked in presence of chloroquine (Table I).

The possibility and risk of interaction between chloroquine and other drugs acting at the NMJ is increased by the fact that chloroquine has a long biological half-life, greater than 48 hours [17]. In addition, the drug is taken up into tissues in the body [18] and therefore its effects (other than antimalarial) may be felt for quite sometime even after stopping therapy.

Although the results presented were obtained from animal experiments, they are pointers to the interactions likely to occur in human. Supportive evidence is provided by the findings that chloroquine induces myopathy [2, 19] degeneration of muscle fibres [19] and myasthenia [11] when used for long term therapy as in arthritic conditions. However, even in normal therapeutic antimalarial dose, one of the commonly reported side effect of chloroquine is generalised muscular weakness and malaise which can scientifically be attributed to the ability of the drug to induce NMJ blockade.

It is therefore suggested that chloroquine should be contraindicated or used with caution in patients with skeletal-muscular disorders or those on treatment with drugs that may cause decreased skeletal muscle activity.

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