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PATTERN OF CHANGES IN LOCOMOTOR BEHAVIOUR IN RATS AND MICE FOLLOWING ADMINISTRATION OF CHLOROQUINE

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ABSTRACT

Chloroquine (CQ) is a known neurotoxic drug. Its possible effect on locomotor behaviour was studied using rats and mice. The experiments were divided into two groups. In group one, 18 Albino Wistar rats (210 – 300g body weight) were divided into 3 subgroups of 6 rats each. One subgroup served as control and was administered 0.2ml physiological (normal) saline i.p. The other subgroups received 0.57mg/kg and 3mg/kg of CQ i.p. respectively. The animals were allowed to explore the open field maze for 5 minutes each and their locomotor behaviours scored. In another group, 35 mice (15 – 32g body weight) were used. The animals were subdivided into 7 subgroups of 5 mice each. The control subgroup also received normal physiological saline (0.2ml) while the test subgroups received graded doses of CQ (5, 10, 20, 40, 80mg/kg i.p. respectively). The mice were observed for 30 minutes (broken into 5 minutes per session) in an observation cage and various locomotor behaviours scored. Flumazenil (2mg/kg), a GABA blocker, was administered i.p. to a group of test animals and five minutes later, CQ (80mg/kg) was administered and the animals were observed. Results obtained showed significant decrease in locomotor and exploratory activities (Line crossing, rearing and walling) in test groups compared to control ($p< 0.001$) in the rats. There was also a dose dependent decrease in Novelty induced total locomotor activity (NITLA) in the test groups compared to their control ($p< 0.001$). The NITLA observed with flumazenil (2mg/kg) + 80mg/kg CQ (46.8 ± 4.35/30mins) was not significantly different, when compared with 80mg/kg CQ alone (50.0 ± 8.37). Therefore, flumazenil did not block the effect of chloroquine. In conclusion, chloroquine reduces locomotor activity in a dose dependent manner and therefore has a sedative effect in mice and rats. The effect is however not mediated by the GABAergic mechanism. If these results are applicable to man, chloroquine may not be beneficial in depressed individuals.

KEYWORDS: Chloroquine, neurobehaviour, rats, mice.

INTRODUCTION

With the ever increasing incidence of malaria in Tropical Africa, the major cause for concern has been finding a more convenient drug which has minimal side effects. One of the drugs readily available over the counter for the treatment of malaria has been chloroquine. Chloroquine, a 4-aminoquinoline derivative (Clark *et al*, 1992; Webster, 1992) is commonly used in the chemotherapy of malaria fever, and also as an anti inflammatory disease modifying agent in patients with rheumatoid arthritis or systemic lupus erythematosus (Onigbogi *et al*, 2000). Chloroquine is however, not without side effects. Among these side effects are gastrointestinal upset, and pruritus which are common in black Africans (Ajayi, 1998). Others are mild and transient headache, precipitation of acute intermittent pophyria in susceptible individuals, mental disturbances, interference with cardiac rhythms (especially when given intravenously) and visual disturbances. Most of these side effects are infrequent or may be mild and tolerable at normal doses of the drug used for treatment and prophylaxis of malaria and they are reversible on withdrawal of the drug. For instance, corneal deposits of chloroquine may be asymptomatic or cause mild photophobia (Laurence *et al*, 1999). However, these side effects can be threatening on treatment with high doses and or prolonged administration of the drug.

Retinal toxicity of chloroquine which in most cases is irreversible has been reported by several researchers (Ekanem and Caxton-Martins, 2000; Keller *et al*, 2003; Mahon *et al*, 2004). Chloroquine also causes blurred vision as one of its side effects, probably due to its effect on the lateral geniculate body of the thalamus (Ekanem *et al*, 2000).

The other neurologic side effects of chloroquine include depression, psychosis and delirium. The basis for these was demonstrated in using the whole cell patch clamp method, which showed that chloroquine could depress *in vitro* neuronal activity perhaps through inhibition of calcium channels (O'shaughnessy *et al*, 2003).

In spite of all these neurologic and neurotoxic effects of chloroquine, no report has been found on its effect on locomotor behaviour. It was therefore the aim of this study to ascertain locomotor behavioural effects of chloroquine using the rats and mice.

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MATERIALS AND METHODS

The experiments were divided into two groups. Group one was made up of 18 Albino Wistar rats (weighing between 210 – 300g), which were divided into 3 subgroups (1a, 1b and 1c) of 6 rats each. All animals were raised under standard laboratory conditions and given free access to normal rat chow and clean tap water. Subgroup 1a animals (control) were administered 0.2ml normal saline intraperitoneally (i.p.). Subgroup 1b and 1c were test groups and received 0.57mg/kg and 3mg/kg of CQ (Glaxo, Nigeria) i.p. respectively. 0.57mg/kg of chloroquine served as the human therapeutic dose, while 3mg/kg was the pharmacological dose.

Study of locomotor behaviour

The animals were allowed to explore the open field maze for 5 minutes each and their locomotor behaviours scored in accordance with the method of Brown *et al* (1999). The horizontal locomotor activity was assessed by the number of lines crossed in the open field maze per 5 minutes exposure. while the vertical locomotor and exploratory activities were assessed by the frequency of rearing and walling. Other behaviours scored include the frequency and duration of grooming and centre square activity.

Group two experiments consisted of 35 mice (weighing between 15g – 32g) body weight. The animals were divided into 7 subgroups (2a, b, c, d, e, f and g) of 5 mice each. The animals in subgroup 2a served as control and were administered normal saline (0.2ml) i.p. The animals in subgroups 2b to 2f were test groups and were administered graded doses of CQ (5, 10, 20, 40, 80mg/kg i.p. respectively).

Drug administration and observation

Flumazenil (2mg/kg – Sigma chemicals, UK), a GABA blocker, was administered i.p. to animals in subgroup 2g and five minutes later, CQ (80mg/kg) was administered i.p. The mice were observed for 30 minutes (broken into 5 minutes per session) in an observation cage and various locomotor behaviours, such as rearing, walling, grooming, scratching and face wash were scored.

Statistical Analysis

Statistical analysis was done using ANOVA and post hoc student-Newman-Keuls test. Results were expressed as means ± standard error of the mean and were regarded as statistically significant at $p < 0.05$.

RESULTS

Locomotor activity

The number of lines crossed by animals in the two test subgroups given graded doses of chloroquine (0.57 and 3.0mg/kg respectively) was significantly lower ($p < 0.001$) when compared to control (saline treated) group, (Table 1).

Table 1: Patterns of locomotor activity following administration of chloroquine in rats

Groups / Behaviour (activity)	Control, n = 6	Concentration of chloroquine (CQ)	
		0.57mg/kg body weight, n = 6	3mg/kg body weight, n = 6
Locomotor activity (Line crossing)	78.2 ± 3.9	46.7 ± 3.5***	45.2 ± 4.4***
Rearing (/5mins)	14.5 ± 2.4	7.8 ± 1.8**	3.1 ± 1.3***
Walling (/5mins)	14.0 ± 2.6	6.3 ± 0.8**	9.3 ± 1.2**
Frequency of grooming (/5mins)	5.0 ± 1.4	3.0 ± 0.7^{NS}	3.7 ± 1.1^{NS}
Frequency of centre square entry (/5mins)	8.3 ± 2.4	7.4 ± 2.1^{NS}	1.7 ± 1.3*
Duration in centre square (sec)	6.7 ± 3.5	24.5 ± 7.7*	40.8 ± 6.0**

n = Number of animals
NS = Not significant compared to control
* = Significant at P< 0.05, compared to control
** = Significant at P< 0.01, compared to control
*** = Significant at P< 0.001, compared to control

Exploratory behaviour in the open field maze: Frequency of rearing and walling.

Rearing and walling are forms of exploratory behaviours. The frequency of rearing in the animals in subgroup 1b, 7.8 ± 1.8/5 minutes and that in subgroup 1c, 3.1 ± 1.3/5 minutes were significantly lower ($p< 0.01$, $p< 0.001$ respectively) than in the control subgroup Similarly, walling in the test subgroups 1b and 1c respectively were significantly lower than their control subgroup (14.0 ± 2.6/5minutes $p< 0.01$, Table 1).

Grooming

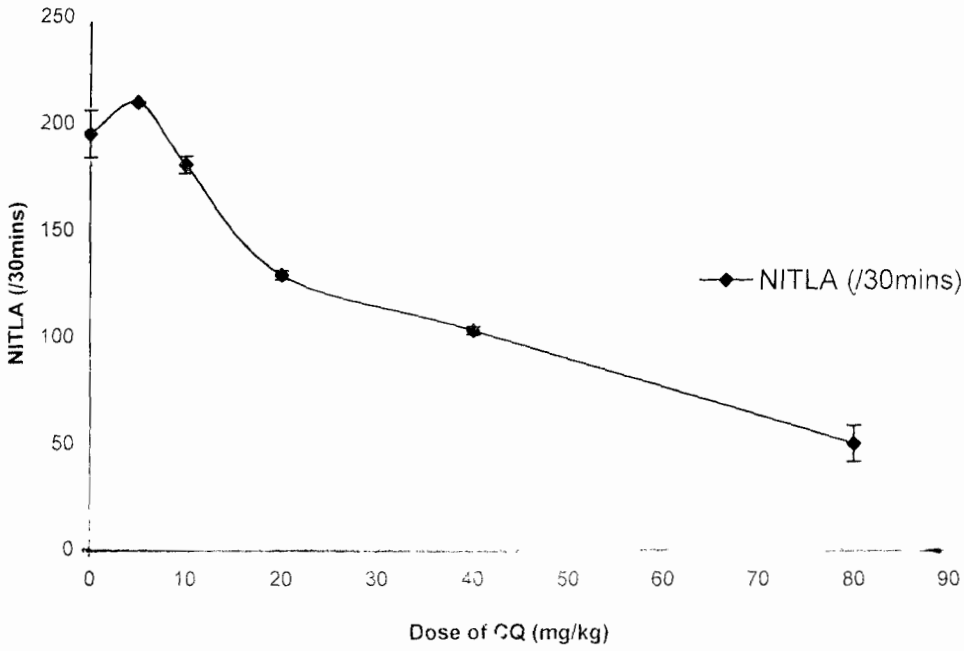
Although the frequency of grooming did not statistically differ, the duration of grooming was significantly higher in subgroups 1b and 1c compared to the control group. Grooming in subgroup 1c animals was for 40.8 ± 6.0 seconds; group 1b animals groomed for 24.5 ± 7.7 seconds while animals in subgroup 1a (control) groomed for 6.7 ± 3.5 seconds Grooming duration in subgroups b and c were higher than in control group ($p< 0.05$ and $p< 0.001$ respectively). See Table 1.

Centre square activity

The frequency of entry into the centre square was not significantly different from control. However, there was a significant difference in the time spent (duration) in Centre Square The animals in group1c spent the least time in the centre square when compared to the control (1a) and group 1b ($p< 0.05$). Control animals (group 1a) entered the centre square 5.0 ± 1.4 times/5minutes, spent 8.3 ± 2.4 seconds while groups b and c animals entered 3.0 ± 0.7; 3.7 ± 1.1 times and spent 7.4 ± 2.1, 1.7 ± 1.3 seconds respectively (Table 1).

Dose response relationship showing the novelty induced locomotor activity following administration of graded doses of chloroquine in mice.

Novelty induce locomotor activity (NITLA) refers to the sum total of all locomotor activities observed when the mice were exposed to a new environment. When graded doses of chloroquine (5, 10, 20 40, 80mg/kg) were administered to mice in subgroups 2b to 2f, NITLA decreased progressively with increasing dose of CQ (Fig. 1). When the dose was raised to 160mg/kg, convulsions occurred about 10 minutes after administration. NITLA was significantly different for the different subgroups ($p< 0.001$).

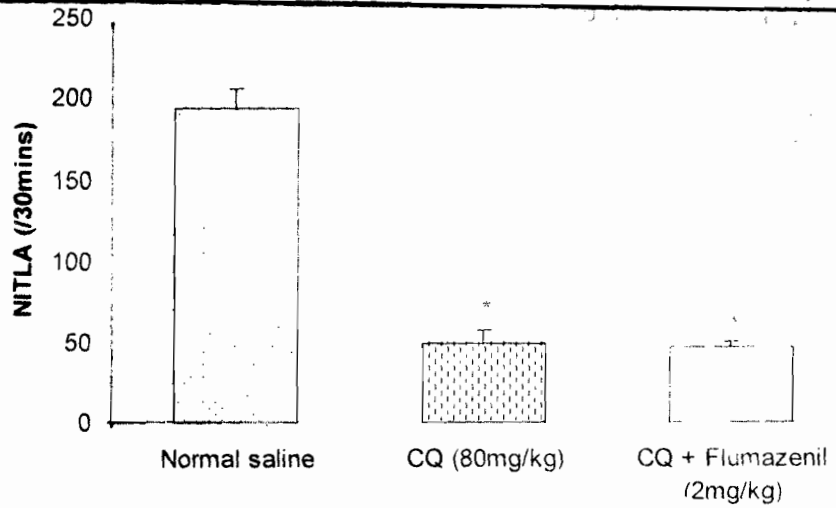


$F(4,24) = 110.5$; $p< 0.001$

Fig. 1: Dose response relationship showing the novelty induced total locomotor activity following administration of graded doses of chloroquine in mice.

Comparison between NITLA in mice following administration of normal saline, CQ (80mg/kg) and CQ + Flumazenil (2mg/kg).

When Flumazenil (2mg/kg), a GABA blocker was administered (i.p) five minutes before CQ (80mg/kg i.p.) to mice in subgroups 2g, the NITLA was 46.8 ± 4.35/30mins. This value was however, not statistically different from the value obtained when CQ (80mg/kg) was administered alone, which was 50.0 ± 8.37 The NITLA for the normal saline administered group (control) was 195.0 ± 11.0 and it was higher compared to CQ alone and CQ + flumazenil (Fig. 2).



* - Significant at p< 0.05 in comparison with control.

Fig. 2: Comparison between NITLA in mice following administration of normal saline, CQ (80mg/kg) and CQ + Flumazenil (2mg/kg).

DISCUSSION

In this study the open field maze of Weiss and Greenberg (1996) was employed to assess locomotor and exploratory behaviour in albino Wistar rats and mice following the administration of chloroquine. The open field apparatus is used to assess the emotionality of an animal in a novel environment as well as assess locomotion and exploration using behaviours such as line crossing, rearing and defaecation.

The number of lines crossed (i.e. frequency of line crossing) in the two test groups with graded doses of chloroquine was significantly lower than the control group. This means that the animals in the test groups showed decreased locomotor activity (Walsh and Cummins, 1976).

The exploratory activity of the animals administered chloroquine was also significantly decreased. This was shown in the decreased frequency of rearing and walling. The decreased locomotor and exploratory behaviour observed in the chloroquine-administered groups is probably due to the depressed neuronal activity via the inhibition of the calcium ion channel (O'shaughnessy et al 2003).

The duration of grooming was longer in the two test groups (chloroquine administered) groups than in control. The test animals therefore, scratched their bodies more than their control. This most probably was as a result of the effect of chloroquine in inducing pruritus (Ajayi, et al, 1998).

The duration of time spent in the centre square was only significantly reduced in the test group of animals with a higher (pharmacological) dose of chloroquine. The animals dared less into the centre square. According to Walsh and Cummins (Walsh and Cummins, 1976), it could be concluded that the animals in this group were more fearful and thus made less entries. This could only be speculative since it is also likely that the generalised depression of neuronal activity could be responsible for the decreased centre square activity.

The dose response relationship showed that higher doses of chloroquine produced greater lowering effects on novelty induced total locomotor activity. At a much higher dose, however, the drug was injurious as it caused convulsion and death. This is consistent with earlier studies, which reported death due to chloroquine poisoning (Kemmenoe, 1990; Marquadt and Albertson, 2001).

Administration of flumazenil (a GABA blocker) was an attempt to elucidate the mechanism by which chloroquine decreases activity. When flumazenil was administered 5 minutes before chloroquine, it did not change the NITLA. Therefore, flumazenil did not decrease NITLA caused by chloroquine, inferring that CQ may not be acting via the GABAergic mechanism.

In conclusion, chloroquine reduces locomotor activity in a dose dependent manner and therefore has a sedative effect. This effect is however, not mediated by the GABAergic mechanism. If these results are applicable to man, chloroquine may not be beneficial in depressed individuals or those individuals with related neurological problem.

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