THE EFFECTS OF REPEATED CHLOROQUINE ADMINISTRATION IN GROWING FEMALE RATS.

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ABSTRACT

The effects of repeated administration of 40mg kg^{-1} chloroquine diphosphate, given by daily intraperitoneal injections for 21 days have been examined in growing female rats. Some factors contributing to the observed effects were investigated. Chloroquine depressed the body weight of the rats which retarded their growth and caused stunting, as assessed from femur length and circumferential growth. Chloroquine reduced the dry mass of organs examined without an appreciable effect on their water content. There were some behavioural disturbances observed after chloroquine administration.

INTRODUCTION

Chloroquine was introduced as an antimalarial drug, and in the recommended dose of 1.5—2. 0g of the base over a 3— day period (11) the toxicity and side effects are generally minimal (1, 3). Large doses of chloroquine given over long periods of time have been used for the treatment of various connective tissue diseases (2, 11). The doses (0.25 — lg daily for many months to years) are much larger than those required for the treatment of malaria with higher incidences of toxicity to the patient.

Following administration, chloroquine is concentrated by most tissues and organs such as the liver, spleen, kidney, lung, melanin containing tissues and some endocrine cells (4, 10). It is thus possible that repeated administration of chloroquine in malarious areas due to reinfection or resistant strains could result in sufficient amounts of the drug being accumulated to produce toxicity to the host.

Several toxicity studies of chloroquine have been undertaken and the findings summarised (11, 12). The main toxic effect of chronic chloroquine administration was loss of body weight reported in man (1, 3), rats (7), rhesus monkeys & dogs (11) and chicks (9). The reasons advanced for the reduction in body weight by these workers include reduction in food intake, water intake, cell size and cell number.

Most of the reported toxicity studies of chloroquine in man and experimental animals have been carried out using male subjects or animals. The aim of the present study was therefore to investigate the toxicity of repeated chloroquine administration in female rats to see if these would be similar to those reported for male animals. Some of the factors contributing to the toxicity were investigated.

METHODS

Female rats (Wistar strain) aged 28 days were weight matched into two groups (n = 8 in each group) and placed in two separate cages. One group received 40mg kg^{-1} chloroquine diphosphate daily by intraperitoneal injections while the second group received the equivalent volume of saline to serve as the control.

The rats were fed commercial rat cubes and were kept in a room maintained at a temperature of 26 ± 1°C. Water was provided through a plastic water tube.

Acute effects of chloroquine: The rats were observed for up to 3 h every 3 days for any signs of acute toxicity.

Effects on body weight. The rats were weighed daily between 08.00 — 08.30 h, before drugs were administered, for up to 21 days. Thereafter, the rats were killed by stunning and bleeding for other investigations as described subsequently.

Effects on Femur bone development. The femur bones from chloroquine treated or control rats were removed, cleaned and their lengths measured. The circumference of the middle part of each bone was determined.

Effect on selected organs. Various organs from the rats were removed, cleaned, blotted dry and weighed. They were then dried to constant weight in a constant temperature drying oven (Gallen Kamp) at 100°C.

The heart (together with 0.5cm of adjoining vessels) was cut open to remove any blood clots.

The other organs studied were the liver, together with the gall bladder; the spleen; both lungs; the kidneys; the uterus and both adrenal glands.

Drugs. Drug solutions were always freshly prepared in such concentrations that not more than 0.2 ml was injected. The drug used was chloroquine diphosphate (Sigma).

Statistical analysis. Results were analysed by means of Student's t-test.

RESULT

Acute Effects of Chloroquine Rats injected with Chloroquine were less physically active than the control for up to 2 h; this inactivity appeared to decrease in severity and duration as drug administration progressed. During the period of inactivity the rats did not eat or drink as much as the control; they were insensitive to the stimulus of touch and leg pricking and they were ataxic.

Effects of chloroquine on body weight. The body weight of chloroquine and saline treated rats did not differ from each other ($p > 0.05$) at the beginning of the experiment. The body weight of saline treated (control) rats

continued to increase daily throughout the experimental period. The body weight of rats given chloroquine fell below ($0.05 > p > 0.02$) pretreatment level and the control ($0.05 > p > 0.02$) 1 day after administration. The body weight of the chloroquine group continued to fall gradually throughout the experimental period such that it was always less ($p < 0.001$) than the control and the pretreatment value (Fig. 1). The difference became more pronounced as treatment progressed.

Effects of chloroquine on femur bone development. The length or circumference of femur bones taken from the left legs of saline or chloroquine treated rats did not differ ($p > 0.05$) from those of their right legs. This indicates that growth of female rats in the presence of chloroquine is not disproportionate.

During growth, the femur bone increases in length and circumference. Chloroquine reduced ($p < 0.001$) the lengthening and circumferential growth in the female rats. The reduction was more pronounced on the femur circumference (Fig. 2).

Effects of chloroquine on various organs. Chloroquine administration reduced ($p < 0.001$) the wet weights of the spleen, kidneys, lungs, uterus and adrenal glands. These reductions were due to reductions ($p < 0.001$) of the mass of such organs since their water content was unaffected ($p > 0.05$). The reduction ($p < 0.01$) of the wet weight of the liver was largely due to a reduction ($p < 0.001$) in its tissue mass but was also associated with a slight reduction ($0.05 > p > 0.02$) in its water content. The heart was the organ least affected; the reduction ($0.02 > p > 0.01$) in its wet weight being due to a reduction ($0.05 > p > 0.02$) in its mass.

DISCUSSION.

Among the various toxic effects of chloroquine reported for man and experimental animals are reduction in body weight and stunting of growth (1, 7, 9, 11). Most of these observations have been on male subjects or animals such that observations reported for female rats in the present study provide a useful information on possible influences of sex variation in response to chloroquine toxicity. It is thus interesting that chloroquine reduced the body weight of the rats compared to the control. However, results from the present study showed that the body weight of the rats continued to fall progressively below the pretreatment level throughout the period of administration. This response of growth retardation varies from the inhibition of body weight increase in chicks. (9). The dose of chloroquine employed in the present study which retarded growth was less than, that administered to growing chicks. This is further supported by the fact that drug administration began at an earlier age for chicks since they do not require reonal care, yet they were more to-lerant to the drug.

It has been shown that reduction in body weight produced by chronic chloroquine administration to rats and chicks is dose – dependent (7, 9). However, only one dose of chloroquine was employed in the present study.

Chronic chloroquine administration has been shown to produce a dose – dependent stunting of growth as assessed from femur length and circumferential growth in chicks (9). The administration of a single dose of chloroquine in the present study also produced stunting of growth which was more pronounced with femur circumference than the length. Stunting of growth has also been reported for rats (7) which is confirmed by results from the present study. Like the chronic administration of chloroquine to chicks (9), there was no evidence of disproportionate growth in the present study as reported for a child from a mother treated with chloroquine for lupus erythematous during pregnancy (8).

Among the various factors contributing to the reduction in body weight induced by chloroquine are reductions in food and water intakes (1, 8). Similar investigations were not carried out for female rats in the study. However, the observation that the rats were depressed for up to 3h after drug administration and that during such periods they did not eat or drink well would tend to suggest a similar effect on the female rats.

The main findings from examination of most organs from control and chloroquine treated female rats was a reduction in their wet and dry weights. The heart appeared to be the organ least affected in this regard. Since the water content of most organs was unaffected, the effect of chloroquine was primarily a reduction in the muscle mass. The mechanism of this effect was not investigated but might be due to reductions in cell size and number as reported for chicks (9), consonant with the ability of chloroquine to inhibit cell division. Thus, the toxicity of chloroquine to female rats appear similar to

those of their male counterparts although there are indications of variations to the extent of severity of such effects. The female rats appear to be more sensitive to chloroquine than chicks possibly due to species variation.

REFERENCES

1. Alving, A.S. Eichelberger, L., Craige, B. Jr, Jones, R. Jr, Whorton, C.M. and Pullman, T.N. (1948). J Clin. Invest. 27: 60-65.
2. Bagnall, A.W. (1957). Can. Med. Assoc. J. 77: 182-194.
3. Berliner, R.W. Earle, D.P. Jr., Taggart, J.V., Zubrod, C.G., Welch, W.J., Conan, N.J., Bauman, E., Scudder, S.T. and Shannon, J.A. (1948) J. Clin. Invest. 27: 98-107.
4. Dencker, L. and Lindquist, N.G. (1975) Arch Otolaryngol. 101: 185-188.
5. Dencker, L., Lindquist, N.G. and Tjalve, H. (1976). Med. Biol. 54: 62-68.
6. Ekelund, M., Ahren, B., Hakanson, R., Lindquist, I. and Sundlur, F. (1980). Histochemistry 66: 1-9.
7. Fitzhugh, O.G., Nelson, A.A. and Holland, O.L. (1948). J. Pharmacol. Exp. Ther. 93: 147-152.
8. Hart, C.W. and Naunton, R.F. (1964) Arch Otolaryngol. 80: 407-412.
9. Lot, T.Y. and Bennett, T. (1982). Med. Biol. 60: 210- 216.
10. Rubin, M., Bernstein, H.N. and Zvaifler, N.J. (1963). Arch. Opthalmol. 7: 474-481.
11. Thompson. P.E. and Werbel, L.M. (1972). Antimalarial agents: Chemistry and Pharmacology. Academic Press, New York.

12. Wiselogle, F.Y. (1946). A survey of antimalarial drugs, 1941-1945 Vol. 1 pp. 398-399. Edwards. MI.

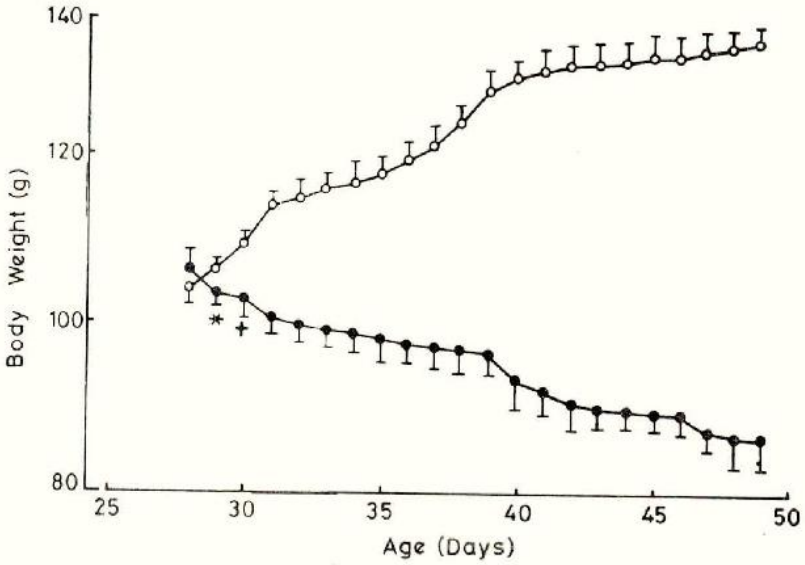

FIGURE 1

Mean (± SEM) body weight of rats (n = 8 in each group) treated by daily intraperitoneal injections of saline (control, open circles) or 40mg kg^{-1} chloroquine (closed circles). * $0.05 > p > 0.02$ + $p < 0.001$ by Student's unpaired t-test (compared to control).

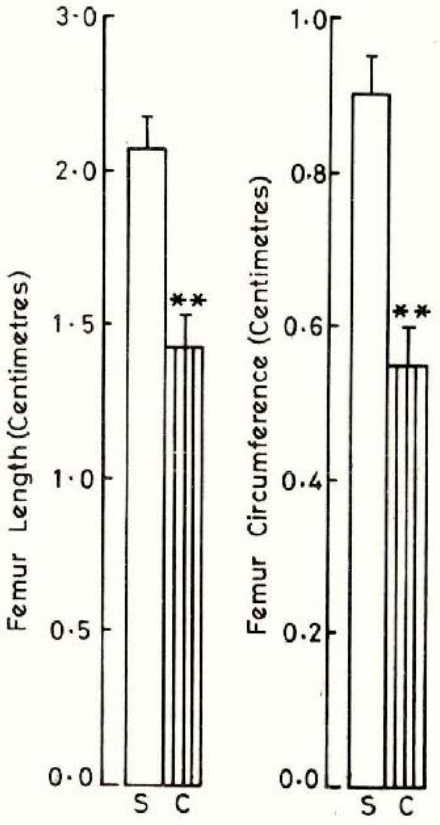

FIGURE 2

Mean (± SEM, n = 8 in each group) femur length or femur circumference of control (Saline – treated) rats (S, Δ) or rats given 40mg kg^{-1} chloroquine (C, Δ)

* * $p < 0.001$ by Student's unpaired t-test (compared to the control).