Experimental chloroquine myopathy¹

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In 1948 Nelson and Fitzhugh fed small doses of chloroquine to rats over a period of two years and showed that they developed skeletal muscle lesions. This appears to have been forgotten for 15 years until Whisnant, Espinosa, Kierland, and Lambert (1963) published their biopsy findings on four patients who had developed muscle weakness while taking 250-1,000 mg. of chloroquine a day for several months to seven years. These showed muscle damage with vacuolar myopathy. Muscle necrosis is not a commonly reported side effect of drugs, and in view of the fact that chloroquine is now being used as a malaria prophylactic, it was decided to try and investigate the mode of production of chloroquine myopathy.

MATERIAL AND METHODS

Ten rabbits of mixed breed weighing 1-2 kg. were given 25 mg. of chloroquine phosphate per kilogram of body weight daily. In six animals this was given in tablet form by mouth and in four by intramuscular injection. The number of doses given to each animal ranged from seven to 42.

The cerebrum, cerebellum, spinal cord, posterior root ganglia, peripheral nerve, heart, kidney, liver, lung, and skeletal muscle were examined in paraffin sections. The muscles examined were the erector spinae, quadriceps femoris, gluteus maximus, tibialis anterior, soleus and triceps brachii. Peripheral nerves were stained with osmium tetroxide, teased and examined under a low-power microscope. Silver impregnations were done on the terminal innervation in the muscle by Schofield's method. The skeletal muscle was also examined histochemically using techniques for phosphorylase (Takeuchi and Kuriaki, 1955), succinic dehydrogenase with menadione (Wattenberg and Leong, 1960), and acid phosphatase (Holt, 1959).

RESULTS

All the animals remained healthy throughout their treatment and steadily put on weight. There was no clinical evidence of any muscular weakness or wasting.

The animal given only seven doses showed no pathological abnormality, the other nine animals given 12 to 42 doses all showed changes, the most

severe abnormalities occurring in the animals receiving the largest amount. Histological examination of the tissues showed changes only in the heart and skeletal muscle. In particular the central nervous system and the peripheral nerves, including the terminal arborization within the muscle, were normal. The cardiac muscle showed vacuolation of muscle fibres (Fig. 1) and in some cases necrosis with

FIG. 1. Cardiac muscle from a rabbit which had received a total of 700 mg. of chloroquine per kilogram of body weight. It shows vacuolation of muscle fibres. Haematoxylin and eosin $\times 400$.

FIG. 2. Cardiac muscle from a rabbit which had received 600 mg. of chloroquine per kilogram of body weight. It shows loss of muscle fibres. Haematoxylin and eosin $\times 125$.

¹This work was assisted by a grant from the Muscular Dystrophy Group of Great Britain.

FIG. 3.

FIG. 6.

FIG. 4.

FIG. 7.

FIG. 5.

FIG. 3. Skeletal muscle from a rabbit which had received 800 mg. of chloroquine per kilogram of body weight. It shows a single necrotic muscle fibre full of histiocytes. The surrounding muscle is normal. Haematoxylin and eosin $\times 115$.

FIG. 4. Another muscle from the same animal as in Fig. 3 showing two necrotic fibres. Haematoxylin and eosin $\times 135$.

FIG. 5. Skeletal muscle from a rabbit given 1,050 mg. of chloroquine per kilogram of body weight. It shows a vacuolar myopathy. Haematoxylin and eosin $\times 300$.

FIG. 6. Skeletal muscle from a rabbit given 800 mg. of chloroquine per kilogram of body weight. It is a succinate dehydrogenase preparation with a neutral red, nuclear counterstain. The central fibre which is 'red' is shrunken and contains a central nucleus. The surrounding 'white' fibres are normal. $\times 500$.

FIG. 7. A similar preparation from the same animal. The central 'red' fibres show central nuclei and blurring of the mitochondria so that the cytoplasm appears stained. $\times 500$.

FIG. 8. A similar preparation from a rabbit given 600 mg. of chloroquine per kilogram of body weight. It shows a normal red fibre lying next to a spindle which is badly damaged and contains histiocytic nuclei. The rather blurred remnant of the dark intrafusal fibre can be seen. $\times 500$.

histiocytic invasion (Fig. 2). The changes in the cardiac muscle appeared earlier and were on the whole more severe than in the skeletal muscle. They were most marked in the region of the inter-ventricular septum. The changes in the skeletal muscle consisted mainly of scanty single fibre necrosis with replacement of the sarcoplasm by histiocytes (Figs. 3 and 4). The rest of the muscle was normal except for a few central nuclei. There was no perivascular or interstitial inflammatory reaction and no fibre regeneration was seen. In the two animals receiving the largest dose there was more extensive myopathy with vacuolation (Fig. 5).

Histochemically there was no significant alteration of the basic distribution of the two types of fibre. These consist of small 'red' fibres with large numbers of mitochondria as demonstrated by succinic dehydrogenase, and large 'white' fibres dependent on anaerobic glycolysis and having very few mitochondria. The latter are demonstrated by their high phosphorylase content. The necrotic fibres contained neither succinic dehydrogenase nor phosphorylase but their lysosomes were positive for acid phosphatase. Many of the 'red' fibres were shrunken with dark, swollen mitochondria and large sarcolemmal nuclei which were migrating inwards

(Figs. 6 and 7). The cytoplasm in between the organelles was purplish as if some of the enzyme had spilt into the cytoplasm as a result of mitochondria damage. The spindles showed an occasional swollen fibre and slight increase in cellularity (Fig. 8). It is possible that damage to these structures was more severe than appeared. Once the intrafusal fibres are destroyed and replaced by histiocytes they are difficult to identify. A number of such collections were seen in the neighbourhood of the neurovascular bundle where one might expect to see a spindle. Normal intrafusal fibres are 'red' and contain closely packed mitochondria.

DISCUSSION

Evidence of chloroquine toxicity either ocular or muscular does not appear until the patient has been taking the drug for a very long time, usually more than a year. This is probably related to the fact that more than 50% of a given dose is retained in the body (Rubin, Zvaifler, Bernstein, and Mansour, 1965). Rubin and his colleagues report cases where patients were still excreting measurable quantities of chloroquine up to five years after the last dose. Consequently patients taking the drug in small doses for a long time eventually retain a large and potentially toxic dose. By giving large doses to rabbits we have produced lesions in a comparatively short time, suggesting that myopathy depends on the total dose and not on some other complex effect resulting from administration over a prolonged period.

The histological lesion in chloroquine myopathy is necrosis of muscle fibres in cardiac and skeletal muscle rather more extensive in the former than the latter. The histochemical pattern, apart from the dead fibres, is unaltered. This is in marked contrast to corticosteroid myopathy in which phosphorylase activity is reduced some days before there is any histological abnormality (Smith, 1964). By the time any dead fibres can be seen, the phosphorylase activity is almost lost and there is an increase in mitochondria in 'white' fibres. The histochemical pattern is thus uniform, all fibres appearing 'red'. Loss of phosphorylase appears to be an early indicator of fibre damage (Smith, 1965) and its preservation in most of the fibres in chloroquine myopathy suggests that these really are normal. Once a fibre is dead and has lost its normal enzyme activity, it is impossible to say whether it was originally 'red' or 'white'. However, a number of 'red' fibres can be found in chloroquine-treated animals which are shrunken, with swollen mitochondria and with migrating nuclei. It is possible that these are in an early stage of necrosis and suggests that the fully necrotic fibres seen were 'red'. Cardiac muscle

contains very little phosphorylase and from a histochemical standpoint is all red. It is also unaffected in corticosteroid myopathy. It seems therefore that chloroquine may be selectively attacking 'red' fibres, which are dependent on mitochondrial sources of energy, in contrast to corticosteroids which affect glycolytic, 'white', fibres primarily.

Dialysis experiments with chloroquine and protein (Rubin *et al.*, 1965) have shown evidence of drug binding by albumin and globulin, and particularly by haemoglobin. Chloroquine can be extracted from red blood corpuscles in patients taking the drug and also from those who have taken it in the last two or three years (Rubin *et al.*, 1965). This affinity for haemoglobin and red cells is probably the basis for its therapeutic value in malaria. 'Red' muscle fibres originally received their name, not because of their high content of mitochondria, but because of their high content of myohaemoglobin which gives them their colour. In view of the molecular similarity of haemoglobin and myohaemoglobin it is possible that the drug is bound by myohaemoglobin and this could be the basis of the fibre necrosis.

No histological damage could be found in the peripheral nervous system but there was probably damage to spindle muscle fibres which might account for the diminished tendon reflexes reported by authors describing chloroquine toxicity as a 'neuromyopathy' (Whisnant *et al.*, 1963).

SUMMARY

Skeletal and cardiac muscle fibre necrosis has been produced by the administration of chloroquine. The predominant damage is to 'red' fibres, and it is possible that it is due to binding of the drug by myohaemoglobin.

We are grateful to Dr. L. Goldman and Bayer Products for gifts of chloroquine phosphate and to Mr. P. Crocker for the photographs.

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