obvious species differences exist with respect to the presence of functional α_2-adrenoceptors in the venous vascular bed.

We acknowledge the generous donation of drugs by Bayer, Burroughs Wellcome, Delagrange and Pfizer.

REFERENCES

Andén, N. E., Golembiowska-Nikitin, K., Thornström, U. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 321: 100–104

De Jonge, A., Knape, J. Th. A., Van Meel, J. C. A., Kalkman, H. O., Wilffert, B., Thoolen, M. J. M. C., Timmermans, P. B. M. W. M., Van Zwieten, P. A. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 321: 309–312

Fegler, G. (1954) Quart. J. Exp. Physiol. Cog. Med. Sci. 39: 153–158

Gerold, M., Haeusler, G. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322: 29–33

Guyton, A. C. (1976) Textbook of Medical Physiology, W. B. Saunders Company Ltd, London, Philadelphia, Toronto, pp 168–175

Kalkman, H. O. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322: R 42

Schümann, H. J. (1980) TPS 1: 195–201

Shepherd, J. T., Vanhoutte, P. M. (1975) Veins and Their Control, W. B. Saunders Company Ltd, London, Philadelphia, Toronto, pp 38–39, 205

Van Meel, J. C. A., De Jonge, A., Timmermans, P. B. M. W. M., Van Zwieten, P. A. (1981) J. Pharmacol. Exp. Ther. 219: 760–766

Van Meel, J. C. A., Timmermans, P. B. M. W. M., Van Zwieten, P. A. (1982) J. Pharmacol. (Paris) 13: 367–379

Wilffert, B., De Jonge, A., Thoolen, M. J. M. C., Smit, G., Timmermans, P. B. M. W. M., Van Zwieten, P. A. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. in the press

Zandberg, P., Timmermans, P. B. M. W. M., Van Zwieten, P. A. (1984) J. Cardiovasc. Pharmacol. in the press

J. Pharm. Pharmacol. 1984, 36: 268–269
Communicated October 3, 1983

Effect of chloroquine and some other antimalarials on the immune mechanism in experimental animals

S. K. BHATTACHARYA, C. R. PILLAI†, M. MATHUR, P. SEN*, *Department of Pharmacology, University College of Medical Sciences, Ring Road, New Delhi-110029, †Biochemistry Division, N.I.C.D., Delhi-110054, India*

The effects of chloroquine and some other antimalarials on the immune responses in experimental animals have been examined. Chloroquine and quinine caused significant decrease of serum anti-SRBC haemagglutination titre. Chloroquine lowered the serum IgM level and also reduced plaque-forming cells in the spleen of mice. The delayed-type hypersensitivity responses to SRBC and the passive cutaneous anaphylaxis were also diminished in rats treated with chloroquine. Thus, the immunosuppressant activity of chloroquine may explain its efficacy in various types of immune disorders.

It is well documented that prostaglandins are involved in various forms of experimental arthritis and rheumatoid arthritis and that chloroquine antagonizes these effects (Tietz & Chrisman 1975). Prostaglandins are also known to play a major part in the function of T- and B-lymphocytes (Pelus et al 1977) which are involved in the immune mechanism and inhibit lymphocyte transformation, possibly by stimulation of adenylate cyclase activity (Smith et al 1971). That the beneficial effect of antimalarials on various immune disorders could be due to their effects on the immune mechanism, remains to be explored. Hence, we have examined the effect of antimalarials on both humoral and cellular immune processes in experimental animals.

* Correspondence.

Method

Groups of albino mice of either sex were immunized by intravenous injections of 1×10^9 sheep red blood cells (SRBC) on 0 day either alone or with different doses of chloroquine ($20\ mg\ kg^{-1}$), quinine ($20\ mg\ kg^{-1}$), mepacrine ($10\ mg\ kg^{-1}$) or primaquine ($15\ mg\ kg^{-1}$) given from 0–5th day of immunization; similar doses were also used by Ayitey Smith (1980). All mice were bled on the 6th day and the anti-SRBC haemagglutination titres were estimated (Ferrante et al 1979). Estimation of total serum protein (Varley et al 1980) was also made in each group to examine if the antimalarials interfered with protein synthesis. Estimation of serum haemagglutination titre in presence of 2-mercaptoethanol was made to determine the type of antibody synthesis affected by the drugs (Carpenter 1975). Determination of antibody forming cells in the spleen of mice immunized by SRBC (plaque forming cells) was by the method based on Jerne's plaque technique (1963) with modifications (Janah et al 1970).

Delayed-type hypersensitivity responses which are mediated through the T-lymphocytes were studied by the footpad thickness method (Liew 1977); here two groups of mice were primed with 1×10^8 SRBC subcutaneously (s.c.) in the back (day 0) and subsequently treated with either 0·9% NaCl (saline) or with chloroquine. Both these groups were challenged with SRBC (s.c.) in the hind footpad on day 5. The increase

in footpad thickness was measured 24 h after challenge by applying the fluid displacement method as well as by caliper, and the degree of delayed-type hypersensitivity was expressed as per cent increase in footpad thickness.

Effects on passive cutaneous anaphylaxis (PCA) was studied in groups of rats sensitized with egg albumin (12.5 mg) with Freund's complete adjuvant (0·5 ml) s.c. The test group received chloroquine for 6 days while the control group was treated with saline. The sera were collected after 6th day, serial dilutions were made and 0·25 ml of different dilutions were injected into the shaven dorsal skin of normal rats. After 72 h, intravenous challenge was given with egg albumin and Evans blue (1%) and readings were taken after 1 h.

Determination of organ-body weight indices (Sen et al 1969), estimation of adrenal ascorbic acid content (Roe & Kuether 1943) and total and differential leucocyte counts in mice were done routinely to evaluate the role of pituitary-adrenal axis in the beneficial effect of chloroquine. Statistical analysis was with Student's *t*-test.

Results and discussion

In the initial experiment, it was observed that the anti-SRBC haemagglutination titre was only 44·47 ± 13·59 (n = 17) in the chloroquine-treated group (control value being 223·07 ± 35·48; n = 28) followed by quinine (63·52 ± 29·51; n = 17) and mepacrine (184·53 ± 42·90; n = 15), while in the primaquine-treated group, the titre was 223 ± 67·26; n = 16). Thus it was evident that of the four antimalarials, only chloroquine and quinine affected the humoral immune mechanism significantly. Quinine has limited therapeutic use, chloroquine, on the other hand, is still extensively used. Further studies, therefore, were mainly confined to the effects of chloroquine on the immune mechanism. Suppression of humoral antibody formation by chloroquine could be a specific effect since there was no significant change in the total serum protein level between the control group (7·1 ± 0·36; n = 35) and the test group (6·0 ± 0·70; n = 21) which was also observed with lower doses of chloroquine (1–20 mg kg^{-1} s.c.). Knox & Owens (1966) reported that the beneficial effect of chloroquine in the treatment of collagen disorders might involve serum IgM level; hence, the next group of experiments conducted were to determine whether chloroquine has any specific effect on serum IgM level and the plaque forming cells in the spleen of mice which synthesize IgM type of antibodies. It was observed that the number of plaque-forming cells was significantly reduced from 104·87 ± 14·6 in control group to 24·43 ± 10·4 in the chloroquine treated group ($P < 0·01$); there was also a simultaneous reduction of serum IgM levels in these mice.

The results of delayed-type hypersensitivity responses clearly indicate that chloroquine has a definite suppressive effect on the cell-mediated immune responses, as it significantly suppressed the footpad thickness (0·064 ± 0·009) when compared with the control group (0·226 ± 0·06) (n = 7; $P < 0·025$).

The observations made on the effect of chloroquine on the PCA in rats indicate that in the control group, there was a definite swelling and intense blueing at the site of injection up to a dilution of 1/1024 while it was not detectable in any of the animals treated with chloroquine.

Involvement of pituitary-adrenal axis in the mediation of these actions of chloroquine was ruled out since it failed to modify significantly any of the parameters, viz. organ-body weight indices, adrenal ascorbic acid content or haematological picture.

It has been reported that chloroquine could inhibit the antigen-antibody reaction (Stollar & Levine 1963; Holtz & Fischer 1973) although it did not inhibit antibody production in response to antigen (Thompson & Bartholomew 1964). Our results clearly indicate that chloroquine and quinine suppress both humoral and cellular immune responses, as evidenced by the suppression of serum anti SRBC haemagglutination titre, inhibition of serum IgM level and plaque forming cells of spleen and reduction of cell mediated immune responses, indicating towards an immunological basis for the mechanism of action of these drugs.

Chloroquine sulphate (Nivaquine) was a generous gift from May & Baker (India) Ltd.

REFERENCES

Ayitey Smith, E. (1980) Arch. Int. Pharmacodyn. 243: 192–196

Carpenter, P. L. (1975) in: Immunology and Serology, W. B. Saunders Company, Philadelphia. pp 1061–1076

Ferrante, A., Rowan-Kelly, B., Thong, Y. H. (1979) Clin. Exp. Immunol. 38: 70–76

Holtz, G., Fischer, M. (1973) Z. Immunitaetsforch. 146(2): 145–147

Janah, S., Hussain, Q. Z., Chaudhuri, S. N. (1970) Ind. J. Med. Res. 58: 1206–1216

Jerne, N. K., Nordin, A. A., Henry, C. (1963) Cell-bound antibodies. Wister Institute Press, Philadelphia, pp 109

Knox, J. M., Owens, D. W. (1966) Arch. Dermatol. 94: 205–214

Liew, F. Y. (1977) Eur. J. Pharmacol. 7: 714–718

Pelus, L. M., Strausser, H. R. (1977) Life. Sci. 20: 903

Roe, J. H., Kuether, C. A. (1943) J. Biol. Chem. 147: 399

Sen, P., Arora, S., Lahiri, P. K. (1969) Ind. J. Chest. Dis. 11: 26–30

Smith, J. W., Steiner, A. L., Parker, C. W. (1971) J. Clin. Invest. 50: 442

Stollar, D., Levine, L. (1963) Arch. Biochem. 101: 335

Thompson, G. R., Bartholomew, L. (1964) University of Michigan Med. Centre. J. 30: 227–230

Tietz, C. C., Chrisman, O. D. (1975) Clin. Orthop. 108: 264

Varley, H., Gowenlock, A. H., Bell, M. (1980) Practical Clinical Biochemistry, Vol 1. Heinemann, London, pp 545–546