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INTERACTION OF CHLOROQUINE AND CARRAGEENAN*

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Abstract—Amounts of chloroquine from 0·25 to 5·0 mg, when mixed with a carrageenan suspension and injected into the rat's paw, inhibit the swelling normally induced by the injection of carrageenan alone. This effect appears to be explainable in terms of a physicochemical interaction between chloroquine and carrageenan. The interaction can be demonstrated *in vitro* by determining the extinction of chloroquine–carrageenan mixtures at 400 nm. The reaction is pH dependent. The chloroquine–carrageenan complexes are soluble in saline solutions. When amounts of chloroquine and carrageenan are reacted which do not result in a detectable precipitate, the u.v. absorbancy curve of chloroquine is quenched. The amount of chloroquine recovered from the chloroquine–carrageenan complexes is linearly related to the amount of chloroquine added until the excess (by weight) of chloroquine to carrageenan exceeds about 3. It is suggested that the interaction between mucopolysaccharides and chloroquine may be of importance for the accumulation of antirheumatic concentrations of chloroquine in connective tissues.

CARRAGEENAN-induced edema of the rat's paw is a method widely used to demonstrate the anti-inflammatory activity of drugs. It is know that the edema is subject to "nonspecific" inhibition by the systemic administration of irritants[1–5] or toxic agents.[6] These types of agents are effective inhibitors even in adrenalectomized rats. Shanahan[7] has suggested that "anti-inflammatory" substances which exert their effect through a counter-irritant mechanism can be distinguished from "true" anti-inflammatory compounds by administering them locally. An irritant, when mixed with carrageenan and injected into the rat's paw, will cause an amount of swelling greater than that produced by the injection of carrageenan alone, while conventional anti-inflammatory compounds are still effective in reducing the amount of edema when administered in this manner.

Chloroquine has been claimed to produce anti-inflammatory–antirheumatic effects in humans.[8] In general, it has not been particularly effective after systemic administration in experimental models of inflammation,[9] and these include carrageenan-induced edema. The present report deals with the demonstration of marked activity for chloroquine, when it is administered locally in the carrageenan assay, and the suggestion that a physicochemical interaction between the acidic mucopolysaccharide, carrageenan, and the basic amine, chloroquine, is the basis for the demonstrated activity. A partial characterization of the interaction *in vitro* between these two substances has been accomplished.

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EXPERIMENTAL

Materials. λ-Carrageenan (Seakem 402) was purchased from Marine Colloids, Inc., Springfield, N.J. Chloroquine diphosphate was purchased from Sigma Chemical Co.

Carrageenan-induced edema of the rat's paw. Male rats (Simonsen Laboratories, Sprague–Dawley derived), 130–170 g, were used in these studies. The animals were injected in the plantar tissues of one hind paw with 0·1 ml of a 0·5% suspension of carrageenan in saline or with the same volume of the 0·5% carrageenan suspension to which quantities of chloroquine had been added. The other hind paw was injected with 0·1 ml of a saline solution (0·9%). Three hr later, the volumes of both hind paws were determined by mercury displacement using a Plethysmographe ΔV-3 (Ugo Basile). The difference in volume between the carrageenan (± chloroquine)-injected paw and the saline-injected paw was recorded. Per cent inhibitions of the difference which occurred in the control group (carrageenan without chloroquine added) were determined for each experimental group.

Assays in vitro. Aliquots of solutions of carrageenan and chloroquine were mixed together, allowed to react for 30 min, and the turbidity which occurred was quantified by measuring the extinction of the mixture at 400 nm[10] with either a Bausch & Lomb Spectronic 20 or a Beckman Acta II spectrophotometer. The volume of the reaction mixture was always 3·0 ml, except in the experiments in which the amount of chloroquine precipitated was determined, in which case the volume was 12·0 ml. The ionic strength and pH of the mixture varied in certain experiments and these are indicated in the appropriate figures. Analysis of the precipitate formed after the reaction between carrageenan and chloroquine was accomplished by centrifugation, two cold water washes, and dissolution of the precipitate in 0·9% saline. The extinction of the saline extract was determined at 330 nm for estimation of chloroquine content.

RESULTS

The effect of the local administration of chloroquine on carrageenan-induced edema of the rat's paw is shown in Fig. 1. Amounts of chloroquine as low as 0·25 mg

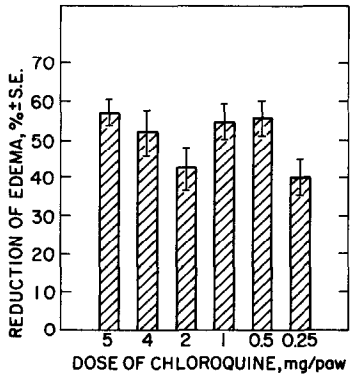

FIG. 1. Effect of local administration of chloroquine on carrageenan-induced edema of the rat's paw. Chloroquine in the indicated amounts was added to a 0·5% suspension of carrageenan and 0·1 ml of this mixture was injected into the hind paw of rats. The degree of swelling of the paw was assessed 3 hr later. Per cent inhibitions of the swelling which occurred in rats injected with carrageenan without added drug were determined. Ten rats were used to establish each mean value.

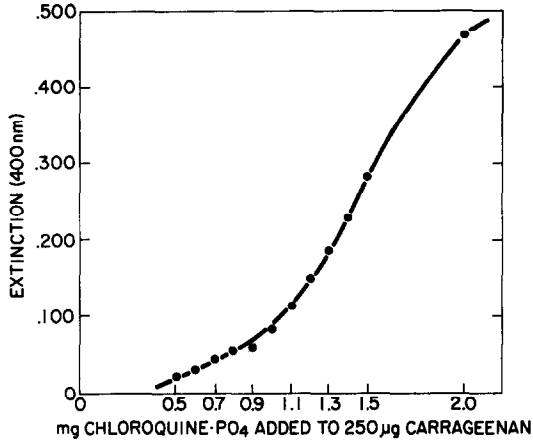

FIG. 2. Turbidometric reaction between chloroquine and carrageenan *in vitro*. Aqueous solutions of carrageenan and chloroquine phosphate were mixed together, allowed to react for 30 min, and the extinction of the mixture at 400 nm was determined. Final volume of the mixture was 3·0 ml, pH *ca.* 5.

significantly decreased the amount of edema. It was apparent during the preparation of the carrageenan–chloroquine mixture that an interaction between the two compounds was occurring.

The reaction between the two substances can be quantified by measuring the extinction of the mixture at 400 nm (Fig. 2). This is essentially a measurement of turbidity, since the reaction *in vitro* results in an opalescence from the precipitate formed. The curve appears to be sigmoid and larger quantities of chloroquine (added to 250 μg carrageenan) do not result in further increases in the extinction of the mixture. The chloroquine–carrageenan complexes are soluble in saline solutions and the degree of solubility is dependent on the concentration of saline (Fig. 3). The solubility of the complexes in saline solutions is not due simply to ionic strength *per se*,

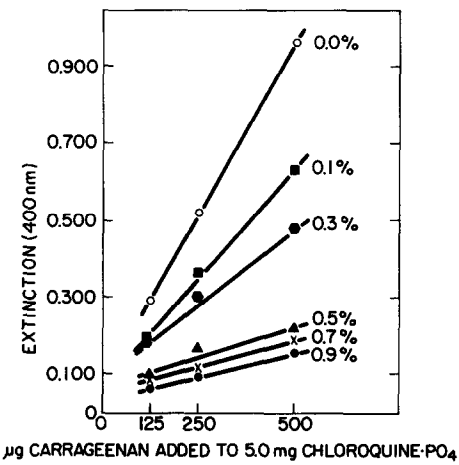

FIG. 3. Effect of the concentration of saline on the turbidometric reaction between chloroquine and carrageenan *in vitro*. Carrageenan and chloroquine phosphate were dissolved in the indicated saline solutions, mixed together, allowed to react for 30 min, and the extinction was determined at 400 nm. Final volume of the mixture was 3·0 ml, pH *ca.* 5.

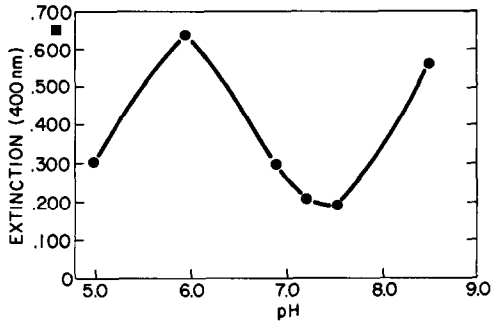

FIG. 4. Effect of pH on the turbidometric reaction between chloroquine and carrageenan *in vitro*. Carrageenan and chloroquine phosphate were dissolved in appropriate buffers (0·15 N) of the indicated pH, mixed together, allowed to react for 30 min, and the extinction of the mixture at 400 nm was determined. Final volume of the mixture was 3·0 ml.

since such complexes are much less soluble in 0·15 N acetate buffer of the same pH. [Compare the extinction of the mixture in acetate buffer at pH 5 in Fig. 5 with that in 0·9% (*ca.* 0·15 N) saline in Fig. 3.]

The reaction is pH dependent with an apparent optimal pH of around six (Fig. 4). In the absence of a visible precipitate, an interaction between the two substances can be demonstrated by determining the u.v. absorbancy spectrum of the mixture. Addition of carrageenan results in a quenching of the absorbancy of chloroquine at all wavelengths (Fig. 5). The amount of chloroquine recovered from the carrageenan–chloroquine complexes is linearly related to the amount of chloroquine added until the excess (by weight) of chloroquine to carrageenan exceeds about 3 (Fig. 6).

DISCUSSION

Sulfated mucopolysaccharides can be considered as cation exchangers[11] and chloroquine is an organic cation. Greiling[10] demonstrated the reaction between chloroquine and certain mammalian sulfomucopolysaccharides *in vitro*. The reaction

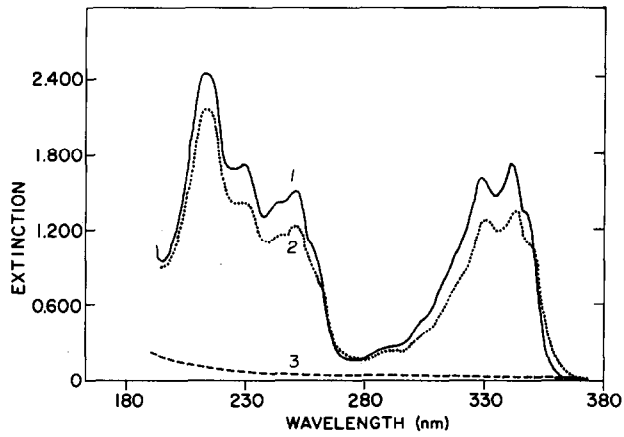

FIG. 5. Ultraviolet absorbancy spectra for solutions of chloroquine (curve 1), chloroquine plus carrageenan (curve 2), and carrageenan (curve 3). Final concentration of chloroquine was 0·2 mg/ml and of carrageenan 0·02 mg/ml. Aqueous solutions, pH *ca.* 5.

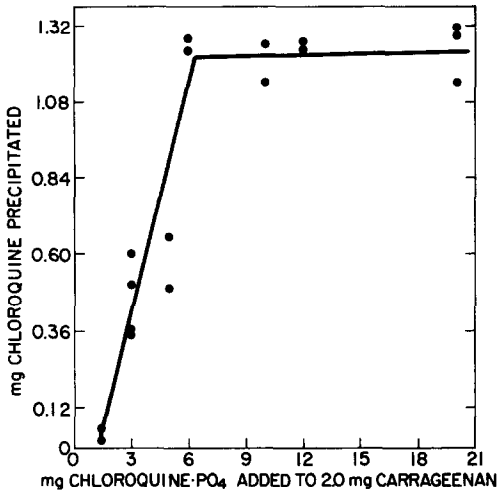

FIG. 6. Recovery of chloroquine from carrageenan–chloroquine complexes. Aqueous solutions of chloroquine and carrageenan were mixed together, allowed to react for 30 min, centrifuged, the precipitate was washed with cold water and dissolved in 0·9% saline solution, and the extinction of the resultant solution was determined at 330 nm. Estimation of chloroquine content of the extract was made by comparison to the absorbancy curve of standard solutions of chloroquine. Each point represents a single determination.

was specific enough for that investigator to suggest that chloroquine might be used as a reagent to distinguish sulfated from nonsulfated mucopolysaccharides. λ-Carrageenan is highly sulfated[12] and these groups may be the site of interaction of chloroquine. Stone[13] has pointed out the relationship between carrageenan and certain mammalian polysaccharides like the chondroitin sulfates.

In patients with rheumatoid arthritis, the maximal therapeutic response with chloroquine is not achieved until several months after initiation of therapy.[8] The trapping of chloroquine in joint tissues by virtue of their relatively high content of sulfomucopolysaccharides might be important for the accumulation of effective concentrations of chloroquine. Cowey and Whitehouse[14] concluded that chloroquine did not bind sufficiently strongly to acidic mucopolysaccharides to prevent their degradation by mucopolysaccharases. These investigators felt that chloroquine might protect connective tissue from degradation by inhibiting autolytic proteases. They pointed out that, although the concentrations of chloroquine required *in vitro* to inhibit such enzymes appeared high, the ability of connective tissues to "fix" chloroquine would make possible the accumulation of effective concentrations of drug there.

Vinegar *et al.*[15] could prevent the development of the second phase of carrageenan-induced edema in rats by prior heat denaturation of the carrageenan. Only when esterified sulfate was released from the carrageenan by this treatment was its edema-provoking activity lost. The observed "neutralization" of the inflammagenic activity of carrageenan by chloroquine may have to do with the sulfate groups of the polysaccharide. In support of this hypothesis, it has been shown (K. F. Swingle, unpublished observations) in one experiment that the local administration of chloroquine has no effect on the swelling of the rat's paw during the 1st hr after injection

of carrageenan, but markedly inhibits the swelling (e.g. 83 per cent at 1·0 mg/paw) occurring between the 2nd and 3rd hr (the "second phase" of Vinegar *et al.*[15]).

Certain other organic bases (e.g. chloropromazine) are capable of reacting with carrageenan *in vitro* at molar concentrations comparable to those of chloroquine, but not all of these will neutralize the edemagenic activity of the polysaccharide (K. F. Swingle, unpublished observations). If this is true, there may be a basis for the anti-rheumatic activity of chloroquine. It is conceivable that there occurs some natural counterpart of carrageenan at certain sites of inflammation. It is known that certain mammalian mucopolysaccharides (e.g. chondroitin sulfate) are capable of producing inflammation when injected locally.[16]

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