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Sustained release of a dual antimalarial system

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The sustained release of a dual drug system in a biodegradable carrier was evaluated in rhesus monkeys and mice. The two drugs were sulphadiazine (WR-7557) (^{3}H-labelled) and 2,4-diamino-6-(2-naphthylsulphonyl)quinazoline (WR-158122) (^{14}C-labelled). The carrier was a polymer of L-lactide (90 parts by weight) and glycolide (10 parts), with a molecular weight of 46 000. The dual system injected was a blend of two preparations, each 50% (w/w) of the appropriate drug in the polymer, in a weight ratio of ten parts of the sulphadiazine system to one part of the WR-158122 system. This blend, as cryogenically ground particles, was injected intramuscularly into three monkeys; a fourth monkey received an equivalent dose of a 10:1 mixture of the pure drugs. Excretion of radioactivity in urine and faeces was measured over 13 weeks. Similar studies were done with mice. In monkeys, ^{14}C-labelled material derived from WR-158122 was excreted at a nearly uniform rate; expressed as WR-158122, the rate of excretion from the pure drug mix was approximately 50 μg day^{-1} from week three to week 13, while the drug/polymer matrix released the drug at approximately 26 μg day^{-1} during this period. Recovery of tritiated materials derived from sulphadiazine was approximately 82% of the injected dose in the first three weeks and only a slight difference in release characteristics of the pure drug mix and the mixed drug/polymer matrices was observed. Results of studies with both mice and monkeys were generally consistent.

The unreliability of self-administration of prophylactic antimalarial drugs is well recognized (Canfield 1972); an implanted sustained release drug delivery system would relieve the individual of responsibility for self-medication. Recently, results were reported on the sustained release of the antimalarial drugs 2,4-diamino-6-(naphthylsulphonyl)-quinazoline (WR-158122) and sulphadiazine (WR-7557). Implants of a finely divided ($<125\,\mu$m particle size) spray-dried matrix of DL-lactide/glycolide copolymer containing WR-158122 had antimalarial activity in mice over 14 weeks (Wise et al 1976). For sulphadiazine, an array of polymers and physical forms demonstrated a wide range of release rates in mice (Wise et al 1978). With this background of extended drug release, the studies reported here were toward achievement of the simultaneous administration of more than one drug.

MATERIALS AND METHODS

Glycolide and L-lactide, prepared from glycolic and L(+)-lactic acids, were polymerized following procedures reported previously (Wise et al 1976; 1978). The copolymer contained 90 parts by weight L-lactide and 10 parts glycolide, with a molecular weight of 46 000.

WR-158122, [^{14}C] WR-158122 (labelled at the 2-position of the quinazoline ring) and sulphadiazine were supplied by the Division of Experimental Therapeutics, Walter Reed Army Institute of Research. Tritiated sulphadiazine was obtained from New England Nuclear. The particle size of both drugs was approximately 10 μm.

The polymer was solvent-blended in benzene with each drug in amounts to produce systems containing 50% drug by weight. Films were cast, benzene was removed and the two drug/polymer preparations were extruded separately into 0·8 mm diameter cylinders. The cylinders were independently ground cryogenically, sieved, and particles of 90–180 μm size were retained. The two matrices were mixed in a conical rotating blender in a weight ratio of ten parts of the sulphadiazine matrix to one part of the WR-158122 matrix; this mixture was used in the experimental injections. A mixture of the two drugs (no polymer) was similarly prepared. Before injection into animals, specific activities of both systems (mixed drug/polymer and mixed drugs) were determined by dissolving samples in dioxane and assaying radioactivity by liquid scintillation techniques.

Four monkeys (*Macaca mulatta*)* of either sex, 3–5 kg, were placed in individual cages equipped with

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* In conducting the research described in this report, the investigators adhered to the principles set forth in the Guide for Care and Use of Laboratory Animals as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute for Laboratory Animal Resources, National Research Council—National Academy of Sciences.

automatic watering systems and centre drain waste collectors. The animals were fed once daily and had free access to water. Urine was collected daily for one week before injection and for two weeks after drug administration. The collecting pan was rinsed with tap water and the rinse was combined with the urine and the total volume recorded. About 60 ml of each daily collection was frozen for analysis. After two weeks, urine was pooled on a weekly basis by combining 10% of each day's output; 30 ml of this pool was frozen. Faeces were collected daily for one week before and two weeks after drug administration and then pooled weekly. Collection was continued for three months after injection; collections were homogenized. All samples for analysis were frozen over dry ice. Combustion analysis of faeces and scintillation analysis of urine were done following conventional techniques described previously (Wise et al 1976; 1978).

Three monkeys received the dual drug/polymer matrix and one received the mixture of drugs without polymer. Three vials containing 800 mg of the drug/polymer formulation and one vial containing 400 mg of the mixed drugs, without polymer, were prepared. Before injection, each sample was suspended in 1% (w/w) methocel to a total volume of 4 ml by agitation with a Vortex laboratory mixer. The volume of injection was 0·5 ml kg^{-1}, equivalent to 50 mg kg^{-1} as the drugs in the mixtures (WR-158122 4·5 mg kg^{-1} and sulphadiazine 45·5 mg kg^{-1}). Injection was made through an 18 gauge needle into the hamstring muscle of the thigh. After injection, the syringe, needle, vial, and leakage from the injection site (recovered with cotton swabs) were rinsed with scintillation grade dioxane to a final measured volume of approximately 100 ml. An aliquot of each rinse was analysed for radioactivity.

Heparinized blood was withdrawn at intervals following injection. The plasmas were separated and single 1, 2 or 3 ml samples were air-dried overnight. Non-volatile ^{3}H and ^{14}C activity was assayed by combustion analysis.

Procedures employed in studies with mice were essentially those described above. A group of five CD-1 mice (Charles River Breeding Laboratories) each ~20 g, was given subcutaneous injections of the mixed drug polymer formulation (5·0 mg/mouse) and a second group of five mice received the mixture of drugs without polymer (2·5 mg/mouse). These doses corresponded to sulphadiazine 113·6 mg kg^{-1} and WR-158122 11·4 mg kg^{-1}. Urine and faeces were collected over 13-weeks and analysed for radioactivity.

RESULTS

Excretion by mice of tritium-labelled materials derived from sulphadiazine in the drug/polymer matrix and the pure drug mixture is presented in Fig. 1. Both preparations released considerable amounts in the first week, 39% of the initial dose of the pure drug mixture and 29% from the mixed matrix. These correspond to 92% and 72%, respectively, of the total recovered in the 13-week study. Tritium was present in diminishing quantities until

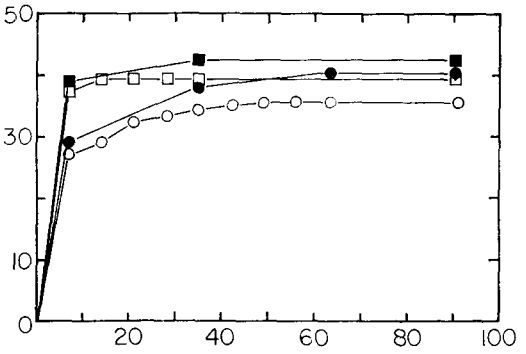

FIG. 1. Recovery of ^{3}H-labelled material from sulphadiazine implanted in mice. Preparations containing 10 parts sulphadiazine to one part WR-158122 as a mixture of the pure drugs and as a 50% mixture of the drugs in matrices of a copolymer of L-lactide and glycolide were implanted in mice. The excretion of sulphadiazine in urine and faeces was quantitated by assay of ^{3}H-activity derived from ^{3}H-labelled sulphadiazine in the implanted material. Circles represent data from the drug mixture and squares the drug/polymer preparation. The figure is a plot of the cumulative recovery, calculated as percentage of the implanted material, on the ordinate, vs time in days, on the abscissa. ● urine and faeces; ○ urine; ■ urine and faeces; □ urine.

week seven in urine of mice with the drug/polymer matrix, whereas none was detectable after week three in urine of mice with the drugs alone. Total recoveries in the study were 42·3% (drug mixture) and 40·2% (drug/polymer) of the originally implanted quantities.

Recovery of ^{14}C-labelled material from WR-158122 in preparations implanted in mice is shown in Fig. 2. Urinary excretion patterns were similar and the 13-week recovery was approximately 26% of the originally implanted material in both cases. The effect of the polymer may be observed in the total (urinary plus faecal) excretion. After one week, recovery from the drug mixture was 21% compared with 10·5% from the drug/polymer matrix. In 13 weeks the recovery from the drug mixture was 80%, but that from the drug/polymer matrix was only 50%. During weeks 9–13, the rate of release from the drug mixture was 0·8% per week whereas that from the drug/polymer matrix was 1·4% per week.

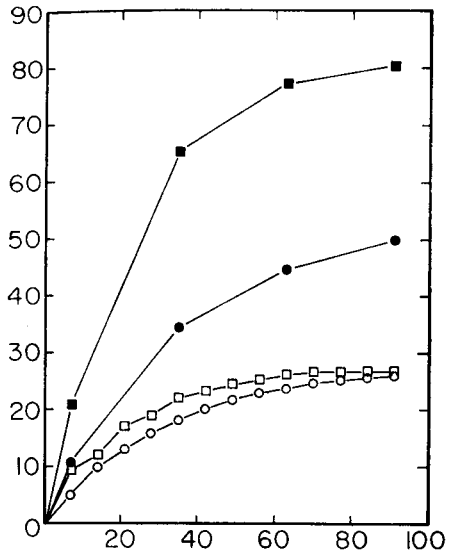

FIG. 2. Recovery of ^{14}C-labelled material from WR-158122 implanted in mice. Studies were done as described in Fig. 1. The excretion of WR-158122 was quantitated by assay of ^{14}C-activity derived from ^{14}C-labelled WR-158122 in the implanted material. Symbols as in Fig. 1.

Quantities injected into monkeys were determined by the differences between the initial radioactivity and that remaining in the vials and syringes and recovered from the injection, as determined by liquid scintillation. Monkeys I, II, and III received the drug/polymer matrix. The average dose delivered was 85·8 mg of matrix kg^{-1} whereas the target dose was 100 mg kg^{-1}. Monkey IV received the pure drug mixture at the dose of 52·2 mg drug kg^{-1}, which was close to the target dose of 50·0 mg kg^{-1}.

Tritium excretion by all monkeys was virtually complete after three weeks and all monkeys had excreted between 74·0 and 93·5% of the injected tritium at the completion of the 13-week study. As illustrated in Fig. 3, the polymer did not significantly retard the release of tritiated materials derived from sulphadiazine.

Fig. 4 shows plasma concentrations of sulphadiazine (determined as tritium activity). Maximum values were obtained at the first sampling, 6 h after injection. The values were very nearly the same for both the drug/polymer matrix and the drug mixture

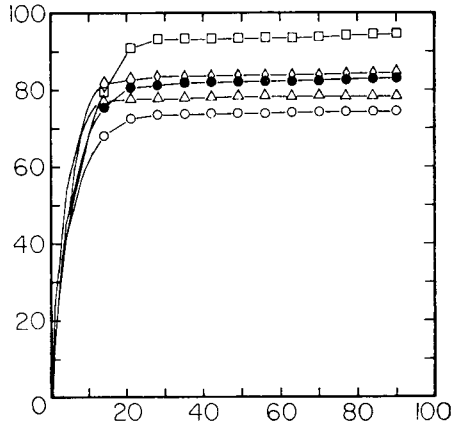

FIG. 3. Recovery of ^{3}H-labelled material from sulphadiazine implanted in monkeys. A preparation containing 10 parts sulphadiazine to one part WR-158122 was implanted in a monkey (IV △) and a preparation with the same proportions as 50% mixtures of the drugs in matrices of a copolymer of L-lactide and glycolide was implanted in three monkeys (I ◇, II □, III ○). The excretion of WR-7557 in urine and faeces was quantitated by assay of ^{3}H-activity derived from ^{3}H-labelled sulphadiazine in the implanted material. The figure is a plot of the cumulative recovery, calculated as percentage of the implanted material, on the ordinate vs time in days on the abscissa. ● Average of I, II, III.

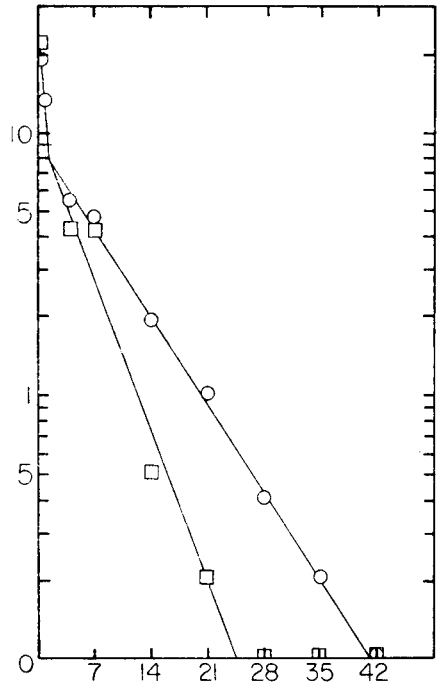

FIG. 4. Plasma concentrations of sulphadiazine in monkeys. Implantations were described in Fig. 3. Blood was withdrawn, plasma was separated and the concentration of sulphadiazine in plasma was quantitated by assay of ^{3}H-activity derived from ^{3}H-labelled sulphadiazine in the implanted material. Squares represent data from the monkey implanted with drugs alone and circles represent the mean values from the three monkeys implanted with the matrix of drug/polymer. The figure is a plot of plasma values of sulphadiazine, calculated as $\mu g\ ml^{-1}$ by means of the specific activity of [^{3}H] sulphadiazine, on the ordinate, vs time in days, on the absicssa.

(18·9 and 21·8 μg ml^{-1}, respectively), indicating similar rates of exit of drug from the injection sites. The plasma values fell more rapidly with the pure drug mixture and the half-lives were determined to be 3·6 and 6·3 days for the two preparations. The matrix system yielded plasma values several times greater than those of the drug mixture in the period one to six weeks after injection.

As shown in Fig. 5, after 13 weeks the total excretion of ^{14}C-labelled materials by monkeys injected with the drug/polymer matrix varied from

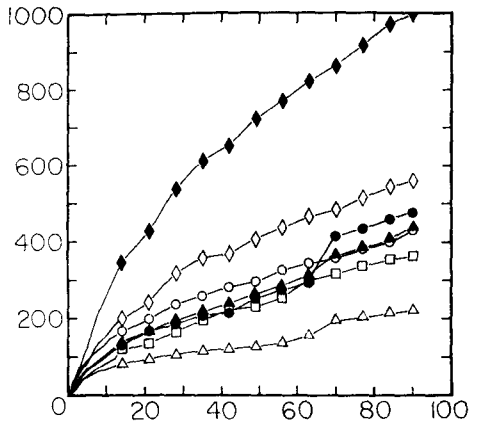

FIG. 5. Excretion of ^{14}C-labelled material from WR-158122 implanted in monkeys. Studies were done as described in Fig. 3. The excretion of WR-158122 was quantitated by assay of ^{14}C-activity derived from ^{14}C-labelled WR-158122 in the implanted material. The figure is a plot of the cumulative excretion of ^{14}C-activity, calculated as microcuries, on the ordinate vs time in days on the abscissa. ○ Monkey III urine and faeces; □ monkey II urine and faeces; ● monkey I urine and faeces; ◇ monkey IV urine; ◆ monkey IV urine and faeces; △ average of I, II, III urine; ▲ average of I, II, III urine and faeces.

0·36 to 0·47 μCi, of which 0·19 to 0·29 μCi had been excreted in urine. The total excretions were approximately 30% of the initial dose. The recipient of the pure drug mixture excreted substantially more radioactivity in 13 weeks: 0·56 μCi in urine and 1·0 μCi total (47% of the total radioactivity injected). Rates of release were computed from the slopes of the plots of excretion vs time between days 21 and 90. For monkey IV this was 53·5 μg day^{-1} and an average of 25·8 μg day^{-1} for the other three animals. Plasma values for WR-158122, as ^{14}C-activity, were below the limit of reliable measurement.

DISCUSSION

The purpose of having a system with two drugs rather than a single drug is reduction of the bulk of implanted material required for effective antimalarial activity. These two drugs have demonstrated synergism of activity against malaria (Schmidt 1973) and, thus, the dosage of the combination should be much less than that of either drug singly. The ratio of 10:1 of sulphadiazine to WR-158122 was selected as an approximation of that which may be expected to be the optimal combination of the two drugs.

The results showed that the drug/polymer matrix provided longer term release than did the pure drug mixture. However, it is apparent that the polymer matrix was not optimal for both drugs. Release of WR-158122 was approximately as desired but that of sulphadiazine was much too rapid. This indicates that each drug must have a specifically developed individual polymer matrix to control the release of that drug in the mixture of two drug/polymer preparations.

Acknowledgements

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REFERENCES

Canfield, C. J. (1972) Proc. Helminthol. Soc. Wash. 39: 15–18

Schmidt, L. H. (1973) Trans. R. Soc. Trop. Med. Hyg. 67: 446–474

Wise, D. L., McCormick, G. J., Willet, G. P., Anderson, L. C. (1976) Life Sci. 19: 867–874

Wise, D. L., McCormick, G. J., Willet, G. P., Anderson, L. C., Howes, J. F. (1978) J. Pharm. Pharmacol. 30: 686–689