J. Pharm. Pharmac., 1978, **30**, 686–689 *Received May* 5, 1978

Sustained release of sulphadiazine

DONALD L. WISE†, GERALD J. MCCORMICK*, GLORIA P. WILLET*, LYNNE C. ANDERSON AND JOHN F. HOWES**

*Dynatech R/D Company, Cambridge, MA 02139, *Walter Reed Army Institute of Research, Washington, DC and **SISA, Inc., Cambridge, MA* 02138, *U.S.A.*

An implantable system was developed which released sulphadiazine in mice over an extended period of time efficacious against infective challenges by *Plasmodium berghei*. The most successful preparation was a copolymer of L(+)-lactic acid + (±)-lactic acid (90 and 10% by weight, respectively) with a molecular weight of 150 000, with which sulphadiazine was mixed at 33.3% of the total weight, in a formulation as beads of 1.5 mm diameter. This preparation released sulphadiazine at a nearly constant rate over three months as measured by the appearance in urine of mice of radioactivity from [^{35}S] sulphadiazine in transplanted material. When implanted in mice, the beads gave effective protection against repetitive (weekly) infective challenges with *P. berghei* by implanted beads at dosages equivalent to 57 mg kg^{-1} sulphadiazine and greater over 21 weeks.

Successful sustained release of the antimalarial drug 2,4-diamino - 6 - (2-naphthylsulphonyl) - quinazoline (WR-158122) was achieved in a delivery system with a copolymer of (±)-lactic acid and glycolic acid as the implantable vehicle (Wise, McCormick & others, 1976). In mice, release took place over a 14-week study and protection against infection by the rodent malaria *Plasmodium berghei* was observed through the same period. The object of the present study was the development of a similar delivery system for another effective antimalarial drug, sulphadiazine. Preparations in various combinations of chemical composition and physical form were evaluated for duration of release and effectiveness against *P. berghei*.

MATERIALS AND METHODS

Glycolide and lactide, synthesized from glycolic acid, (±)-lactic acid, and L(+)-lactic acid, were polymerized by procedures reported previously (Schwope, Wise & Howes, 1975; Wise & others, 1976). Three preparations were studied, a copolymer of 50% (by weight) L(+)-lactic acid and 50% (±)-lactic acid, a copolymer of 90% L(+)-lactic acid and 10% glycolic acid, and a polymer of L(+)-lactic acid (100%).

Sulphadiazine (WR-7557) was supplied by the Division of Medicinal Chemistry, Walter Reed Army Institute of Research. [^{35}S]Sulphadiazine was obtained from Amersham/Searle Corporation, Arlington Heights, Illinois.

Preparations of drug in polymers were produced following procedures reported previously (Schwope & others, 1975; Anderson, Wise & Howes, 1976; Wise & others, 1976) in three physical forms: molded spherical beads (1·5 mm diameter), rods (0·75 mm diameter), and cryogenically ground powder (90–180 μm particle size).

Release *in vitro* was examined in duplicate experiments in which rods, beads, or powder (approximately 50 mg) were placed in a Whatman extraction thimble suspended by a thin wire into a test tube containing 65 ml of phosphate buffer solution at pH 7. The assembly was held in an oil bath at 37° with gentle agitation. Samples were removed for analysis when the buffer solution was changed, daily during the initial week(s) and at approximately weekly intervals thereafter. The amount of sulphadiazine was measured by spectrophotometric (Bratton & Marshall, 1939) or liquid scintillation techniques.

In studies of release *in vivo*, rods, beads or powder were implanted subcutaneously into the scapular region of male albino CD-1 mice 20 g. The rods and beads were introduced using a trochar; the powder was suspended in 1·0% methylcellulose and injected. Each mouse was placed in a metabolic cage and urine was collected in semiweekly periods during the first four weeks and weekly thereafter. Faeces were not regularly collected because essentially 100% of the excretion of drug (or metabolites) was found to be in urine. The amount of sulphadizine excreted was determined by spectrophotometric analysis, initially, and in later experiments by assay of ^{35}S activity using liquid scintillation techniques. At termination of the experiments the mice were killed, the appearance of residual implanted material noted and the site examined for signs of encapsulation or tissue irritation. In experiments with [^{35}S]sulphadi-

† Correspondence.

azine in rods and beads, residual drug at the site was determined; the implants were removed, dissolved in scintillation grade dioxane, and the radioactivity measured by liquid scintillation technique. Residues of matrices in powder form could not be isolated for analysis.

Efficacy *in vivo* against *P. berghei* was tested following procedures described previously (Wise & others, 1976). Preparations in bead form were introduced beneath the skin in the scapular region through a 10-gauge needle which had been inserted through a remote incision and manipulated into position. Implantations of 1, 2, 4, and 8 beads were made. The incisions were closed by suture. The preparation in powder form was suspended in 0·7% carboxymethylcellulose and injected subcutaneously through an 18-gauge needle into the scapular region to yield dosages of 10, 20, 40, 80, and 160 mg sulphadiazine kg^{-1} body weight. Immediately after administration of the delivery system, each animal received an intraperitoneal injection of infected mouse blood (2×10^7 parasitized cells, *P. berghei* NYU-2 strain, maintained by serial passages in mice). Observation of appearance and survival was done daily except at weekends. At seven-day intervals, blood smears were taken and examined for parasites. Each animal with a negative smear received an injection of *P. berghei*-infected blood, as above, on that same day. Control groups of five animals were injected similarly each time.

In conducting the research described in this report, the investigators adhered to the principles set fourth 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.

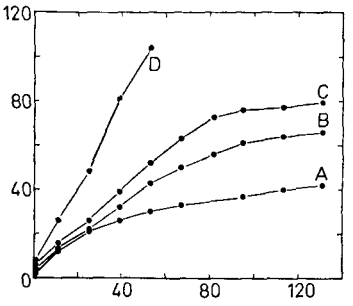

FIG. 1. Effect of drug concentration on the rate of release *in vitro*. Preparations of L(+)-lactic acid polymer containing A 10, B 16·7, C 20 and D 33·3% sulphadiazine by weight, in powder form (90–180 μm diameter), were extracted with pH 7 phosphate buffer. The amount of drug released into the buffer was quantitated by assay of ^{35}S activity. The figure is a plot of the cumulative recovery, calculated as percentage of the original sample, on the ordinate *vs* time in days on the abscissa.

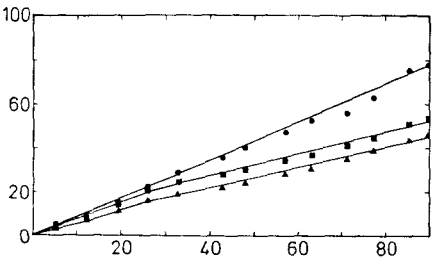

FIG. 2. Effect of copolymer molecular weight on the rate of release *in vitro*. Preparations of copolymers of L(+)-lactic and (±)-lactic acids (50% each by weight) with molecular weights of 150 000 (●) 210 000 (■) and 450 000 (▲), containing 33·3% sulphadiazine by weight, in bead form (1·5 mm diameter), were extracted with pH7 phosphate buffer. The amount of drug released into the buffer was quantitated by assay of ^{35}S activity. The figure is a plot of the cumulative recovery, calculated as percentage of the original sample, on the ordinate *vs* time in days on the abscissa.

RESULTS

The effect on release rate *in vitro* of the relative concentrations of sulphadiazine in the drug/polymer preparation is illustrated in Fig 1. In this experiment the matrix was the polymer of L(+)-lactic acid. Preparations with sulphadiazine contents of 10, 16·7, 20, and 33·3% of the total weighed were cryogenically ground to 90–180 μm size powders. It is evident that the greater the drug concentration, the more rapid was the release. The preparation containing 20% sulphadizine released the drug over a three-month period, with depletion of the drug reservoir shortly thereafter. The 33·3% preparation released

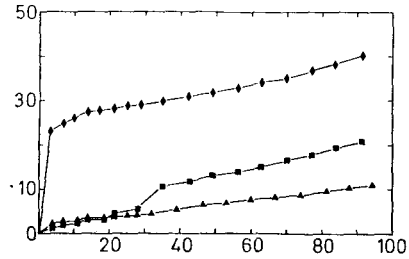

FIG. 3. The effect of physical form of implanted material on the rate of release *in vivo*. Preparations of the copolymer of L(+)-lactic acid and (±)-lactic acid (50% each, by weight) with 150 000 molecular weight and containing 33·3% sulphadiazine by weight were implanted in mice as powder (90–180 μm diameter ◆), rods (0·75 mm diameter ▲), and beads (1·5 mm diameter ■). The amount of drug excreted in urine was quantitated by assay of ^{35}S activity. 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.

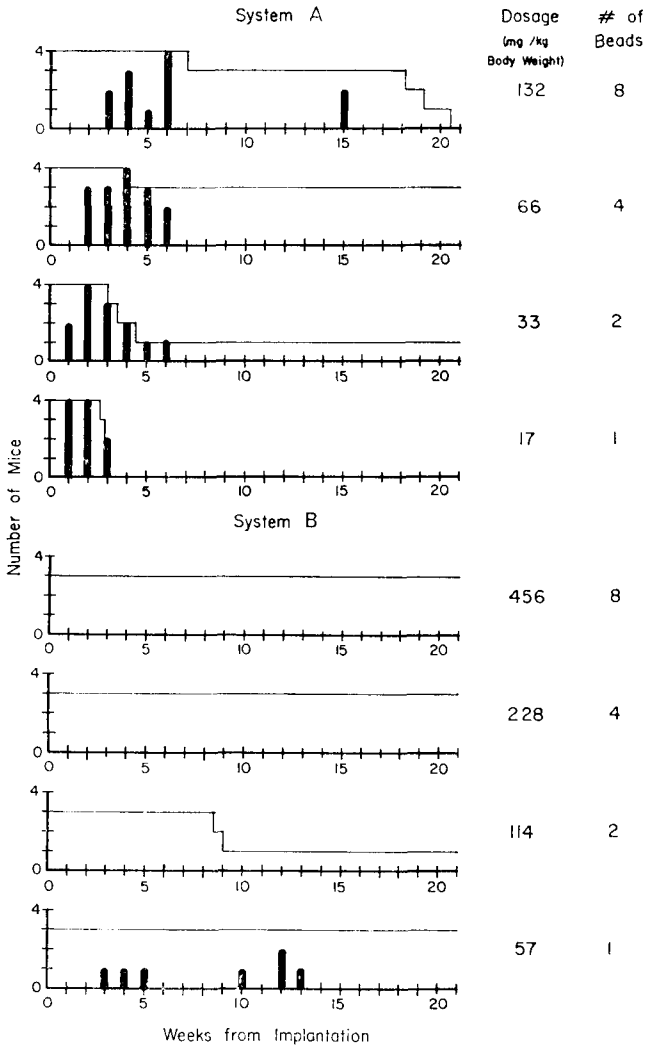

FIG. 4. Efficacy of implanted drug-release systems against *Plasmodium berghei* in mice. Part A: Preparation of copolymer of L(+)-lactic acid and (±)-lactic acid containing 33·3% sulphadiazine, in bead form (1·5 mm diameter). Part B: Preparation of copolymer of L(+)-lactic acid and glycolic acid (90 and 10%) by weight, respectively) containing 10% sulphadiazine, in bead form (1·5 mm diameter). Preparations were implanted subcutaneously in mice as 1, 2, 4 or 8 beads per mouse and weekly challenges with *P. berghei*-infected mouse blood were administered to all mice with blood smears negative for parasitaemia. The figure is a plot of numbers of surviving mice (—) and mice with positive blood smears (■) on the ordinate *vs* time in weeks on the abscissa.

material very rapidly and the preparations with less than 20% drug released material much more slowly.

The effect of polymer molecular weight on the release rate *in vitro* is illustrated in Fig. 2. Copolymers of L(+)-lactic and (±)-lactic acids were the matrices. Preparations containing sulphadiazine at 33·3% of the total weight were formulated at 1·5 mm diameter beads. The copolymer of 150000 molecular weight released the drug most rapidly and most closely approximated a three-month release system. As the molecular weight increased, the release rate decreased.

A study of the effect of physical form on release rate is illustrated in Fig. 3. This is a study *in vivo* in which the most appropriate molecular weight (150 000) copolymer of L(+)-lactic and (±)-lactic acid with 33·3% sulphadiazine, described above, was formulated as beads, rods, and powder. The plot shows the cumulative recovery as percentage of the drug implanted. After the first four days, the release

was similar in rate, approximately 1–2% per week, from each preparation. After three months, residual sulphadiazine in rods and beads was approximately 50% of the originally implanted amount. The rapid release from the implant in powder form is typical of that observed in studies with powder forms of other polymer matrices. This is attributed to the greater relative surface area of the particles in the powder. The ratios of percentages released in the first four days from powder, rods, and beads (15·7: 1·7: 1) are in close agreement with the calculated ratios of surface of these forms (16: 1·5: 1).

In the studies *in vivo* of drug efficacy there was no external evidence of rejection (site-localized ulceration, loss of hair, and/or sloughing of skin) during the period of study with any preparation.

Part A of Fig. 4 shows efficacy data for the L(+)-lactic acid, (±)-lactic acid copolymer with 33·3% sulphadiazine in 1·5 mm diameter beads. The appearance of parasitaemia was delayed to three weeks at the lowest dose (one bead, equivalent to 57 mg kg^{-1}) and there was complete protection at the higher doses. Two anomalous deaths occurred in the two-bead group. At autopsies of three mice at 18, 23, and 24 weeks, respectively, the implanted beads were localized subcutaneously in sacs which also contained blood (by appearance). At autopsies of seven mice from 27 to 52 weeks, the implanted beads were intact in sacs without blood or evidence of local reaction.

Part B of Fig. 4 shows efficacy data for a preparation in bead form in which the matrix was a copolymer of L(+)-lactic acid (90%) and glycolic acid (10%) with sulphadiazine at 10% of the total weight. The appearance of parasitaemia was delayed to two and three weeks by implants of four and eight beads (equivalent to 66 and 132 mg kg^{-1}). Some antimalarial activity was present through 20 weeks of infective challenging.

The preparation in powder form which was tested for efficacy (L(+)-lactic acid polymer with 20% sulphadiazine) had minimum activity. Patency was delayed to two weeks only at 80–160 mg kg^{-1}, all mice were malarious by the second week, and only two mice of 23 in the study survived beyond five weeks.

All control animals in all studies were malarious at one week after infection and subsequently died.

DISCUSSION

It is clear that subdermal implantation of the drug/polymer preparations was a successful procedure for delivery of sulphadizine. The systems were efficacious against *P. berghei*; with the preparation of the copolymer of L-(+)lactic acid (90%) and (±)-lactic acid (10%) with 33·3% sulphadiazine formulated as beads of 1·5 mm diameter, there was complete protection at doses of 114 mg kg^{-1} and greater. Other systems had less success, but this may be attributable in part to the generally lower ranges of dosage. The studies of release *in vivo* and *in vitro* generally correlated well with the efficacy studies. Bio-incompatibility was negligible.

Based on the results of this and the previous study (Wise & others, 1976) prospects appear to be good for eventual development of a system for human application. The benefits to be derived are convenience and control. There would be a single administration without repetitive requirements and (when the implant is of an intact configuration) an easy removal if desired in case of adverse reaction or when the requirement for protection against malaria ends. The proper dose would be present for a predetermined period with no subsequently scheduled drug administrations with problems such as avoidance by the individual, schedule conflicts, or shortages in supply of the drug. Such a system is of potential prophylactic value in malarious areas.

Acknowledgement

This work was carried out under Contract DAMD-17-74-C-4120 of the U.S. Army Medical Research and Development Command. This is Contribution No. 1465 from the U.S. Army Research Program in Malaria.

REFERENCES

ANDERSON, L. C., WISE, D. L. & HOWES, J. F. (1976). *Contraception*, **13**, 375–384.
BRATTON, A. C. & MARSHALL, E. K., JNR (1939). *J. biol. Chem.*, **128**, 537–550.
SCHWOPE, A. D., WISE, D. L. & HOWES, J. F. (1975). *Life Sci.*, **17**, 1877–1885.
WISE, D. L., MCCORMICK, G. J., WILLET, G. P. & ANDERSON, L. C. (1976). *Ibid.*, **19**, 867–874.