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Sustained-Release Characteristics of a New Implantable Formulation of Disulfiram

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Abstract □ The object of this study was to evaluate the sustained-release characteristics of a new formulation of disulfiram. Solid rods (500 mg) made of a composite of 80% poly(glycolic-co-L-lactic acid) and 20% ^{14}C-labeled disulfiram were implanted subcutaneously in five Wistar CD-1 rats; a control group received 100 mg of ^{14}C-labeled disulfiram subcutaneously. Excretion of radiolabeled material in the urine and feces was monitored for 88 d. Sustained mobilization of drug was observed in the copolymer–disulfiram implant group, reaching a peak value 30 d after implantation. The control group exhibited first-order kinetics of drug mobilization. At necropsy, there was no encapsulation of the residual rods. The copolymer–disulfiram composite performed as a true sustained-release system, and improved formulations may have clinical applications in the treatment of alcoholic humans.

Keyphrases □ Disulfiram—sustained-release implantable formulation, rats, poly(glycolic-co-L-lactic acid) □ Sustained-release formulations—implantable disulfiram, poly(glycolic-co-L-lactic acid), rats

Disulfiram is widely prescribed to discourage alcoholics from drinking alcohol, since the two drugs interact to produce a subjectively unpleasant experience characterized by facial flushing, nausea, tachycardia, and hypotension (1–4). The effectiveness of disulfiram as a treatment for alcoholism is severely limited by the willingness of patients to take the drug every day; many stop taking their tablets so that they might resume drinking alcohol as soon as the effects have worn off (5). Frequent failures of treatment with the orally administered drug have stimulated interest in parenteral therapy with subcutaneously implanted disulfiram tablets, but numerous studies during the past 25 years have demonstrated that these implants have miniscule pharmacological effects, possibly due to their poor bioavailability (6–8).

However, animal studies have demonstrated that disulfiram can be rapidly mobilized from a subcutaneous site, provided that the drug is injected in an appropriate vehicle, *e.g.*, suspended in arachis oil (9) or dissolved in polyethylene glycol (10). These findings suggest that it might be possible to prepare a sustained-release disulfiram implant with a true pharmacological effect. Ideally, such a formulation would combine disulfiram with a vehicle which would deliver the drug into the circulatory system at a steady rate for several weeks or months at a time and be free of any significant local or systemic toxicity. A vehicle which appears to offer these features is a new biodegradable polymer, poly(glycolic-co-L-lactic acid) (PLGA). When implanted subcutaneously, the copolymer appears to degrade slowly into its parent monomers, lactic acid and glycolic acid, while continuously releasing any bound drug at a steady rate. *In vivo* studies of PLGA combined with contraceptives, narcotic antagonists, and antimalarials have shown that these implants can deliver the drug continuously into the circulatory system for several months at a time (11). We describe a study of the sustained-release characteristics of a new formulation of disulfiram combined with the copolymer, which was undertaken to investigate the feasibility of using such a preparation in the treatment of alcoholic humans.

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EXPERIMENTAL SECTION

Polymer Synthesis—The general principles of poly(glycolic-co-L-lactic acid) synthesis have been described (11). It was synthesized in bulk in an evacuated glass vessel from a mixture of 90 g of L-lactide and 10 g of glycolide with 1 g of *p*-toluenesulfonic acid monohydrate as a catalyst. After deacrating the mixture to <0.05 mm Hg for 45 min, the vessel was sealed, rotated to mix the ingredients, heated at 118°C for 2 h, mixed again, then heated for 10 d at 118 ± 2°C. The resulting polymer was dissolved in tetrahydrofuran, filtered through glass wool, precipitated in distilled water, and vacuum dried. The mean molecular weight of the copolymer (34,428 ± 877) was determined by dissolving the polymer in tetrahydrofuran (5 mg/mL) and assaying by gel-permeation chromatography using an HPLC system[1] equipped with μ-Styragel columns and a differential refractive index detector.

Dilution of Radiolabeled Disulfiram—Radiolabeled (5.0 mCi) disulfiram, [1-^{14}C]bis(diethylthiocarbamoyl)disulfide[2], was diluted with 14.9991 g of unlabeled disulfiram[3] by stirring for 2 h at room temperature in 650 mL of reagent-grade ethanol[4]; 14.9860 g of diluted disulfiram was recovered after vacuum drying at 60°C for 10 d. Two samples of the diluted radiolabeled drug were assayed in triplicate for specific activity on a liquid scintillation counter[5]; the mean specific activity was 336 μCi/g.

Composite Preparation—A cosolution of ^{14}C-labeled disulfiram and PLGA (1:4, w/w) was prepared in dichlormethane and cast as a thin film on a clean glass surface. The film was adjusted to 0.06 cm (0.025 in.) in thickness with a film spreader[6], air dried, then vacuum dried at 45°C. The final dosage form was prepared by extruding rods of this composite through a 0.3175-cm die, at a temperature between 70–80°C and pressures up to 140 psi. These disulfiram–copolymer composite rods (20:80) had a mean specific activity of 62.92 ± 8.11 μCi/g.

Implantation—Two groups of animals were studied: a test group receiving the subcutaneous ^{14}C-labeled disulfiram–copolymer implant, and a control group receiving subcutaneous uncompounded ^{14}C-labeled disulfiram. Each group was comprised of five Wistar CD-1 male rats (100–200 g). After anesthesia[7] was administered a slit was cut in the skin of the interscapular region and a small subcutaneous pocket was dissected (large enough for the introduction of the 0.3175-cm diameter rods). Each rat received 500 mg of the compounded 20% rods (containing 100 mg of ^{14}C-labeled disulfiram and 400 mg of the copolymer) and the wound was closed with clips. The second group of rats served as controls and similarly received 100 mg of uncompounded powdered ^{14}C-labeled disulfiram. No attempt was made to preserve strict asepsis during these procedures.

Sample Collection and Analysis—All rats were individually housed in metabolism cages to facilitate the separate collection of urine and feces. Prior to implantation, three fecal and urine samples were collected to determine background counts. Subsequent samples of all excreted urine and feces were collected 1, 3, 7, 9, 11, 15, 18, 22, 25, 29, 32, 39, 46, 53, 60, 67, 74, 81, and 88 d after implantation. Urine samples were assayed for ^{14}C-labeled material in a liquid scintillation counter[8], and the extraction was calculated as the mass of implanted disulfiram which contained an equivalent amount of radiolabel. Feces were combusted to $^{14}CO_2$ with an oxidizer[9] and adsorbed in oxyfluor-CO_2[10] for counting. At the conclusion of the study, the rats were sacrificed and the excised rods from the experimental animals were assayed for residual drug. Pathological studies were performed on the injection sites of two animals from each group: the sites were inspected for gross changes and tissue sections were prepared (thickness, 7–10 μm), stained with hematoxylin and eosin, and examined microscopically.

Data Analysis—All data were stored on diskettes using a microcomputer[11] and software[12] to calculate the mean and standard deviation of total disulfiram excretion during each collection interval in the test and control groups. The line of best fit for the control group was calculated by the least-squares method.

RESULTS

Drug Excretion—The average daily excretion of radiolabeled material is shown for the test and control groups in Figs. 1 and 2. The combined excretion

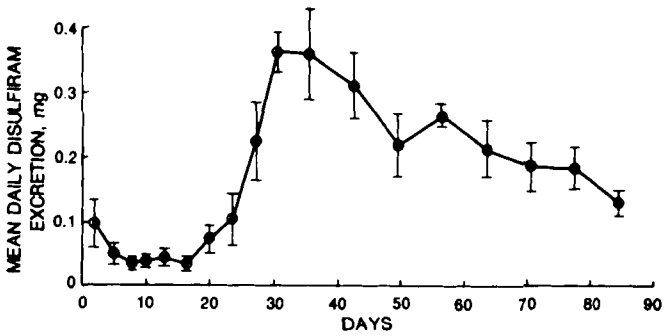

Figure 1—*Disulfiram–copolymer composite group mean daily excretion of radiolabeled metabolites in urine and feces following subcutaneous implantation. Bars indicate 1 SD.*

in urine and feces is expressed as the mass of implanted disulfiram which originally contained an equivalent amount of radiolabel. In all collection periods, the quantity of radiolabeled material in the urine was approximately 10 times greater than the amount detected in the feces. This was true for both groups of animals throughout the study. The cumulative recovery of radiolabeled material in urine and feces (expressed as a percentage of the total administered dose ±1 *SD*) was 17.41 ± 2.27% for the group receiving the disulfiram–copolymer composite and 80.54 ± 9.56% for the control group.

Residual Drug in Implanted Composite—The residual radiolabeled material in the excised composite rods accounted for an additional 55.53% (*SD* = 14.83%) of the administered dose of disulfiram. Hence, the total recovery of radiolabel from the rats receiving the disulfiram–copolymer composite accounted for 72.94% (*SD* = 14.49%) of the implanted dose. The mean weight of the rods recovered from the implantation sites was 55.53 mg (*SD* = 12.84). No residual drug was observed at the injection site in the control group.

Pathological Findings—One rat in the control group died on day 63 of the study; the cause of death was unknown. Excretion data from this animal did not deviate statistically from the others in the group and were included in the study. In the remaining animals, tissues from the injections sites were grossly and microscopically normal, with no evidence of residual drug, necrosis, or inflammatory reaction.

One rat in the test group excreted aberrantly large quantities of radiolabel during the early days of the study; at autopsy, a large abscess was found at the implantation site with no remaining rods. Data from this animal was not included in the findings reported above. The implantation sites of the remaining animals were grossly and microscopically normal, with no evidence of necrosis, inflammatory reaction, or encapsulation of the rods.

DISCUSSION

All excretion of radiolabeled material in this study was expressed in terms of the equivalent quantity of implanted disulfiram. This was not meant to imply that unchanged disulfiram was measured in the urine and feces; to the contrary, there is strong evidence that disulfiram is very rapidly metabolized in the blood and liver, and the breakdown products are promptly excreted (10, 12, 13). At least one of the metabolites, carbon disulfide, is a volatile compound excreted in the breath, which might account for some part of the discrepancy between the implanted dose and the total amount of drug recovered. The real utility of expressing excretion of radiolabel in terms of quantity of the parent drug lies in the insight it provides into the rate of mobilization of the disulfiram from the injection site.

The mobilization of drug from the disulfiram–copolymer composite (Fig. 1) demonstrates that the major objective of the study was achieved: sustained systemic delivery of disulfiram for a 3-month period. During that time, the drug was delivered continuously, although fluctuations were observed in the rate of delivery. An ideal implantable drug delivery system would achieve zero-order kinetics, *i.e.*, the rate of delivery would rapidly reach its maximal value and remain there until the supply of drug was exhausted. However, in clinical practice, even a nonideal delivery system may achieve a near-ideal pharmacological effect, provided that the rate of delivery oscillates within a defined "therapeutic window" (with upper and lower limits defined as the rates of delivery at which either toxic effects or inadequate clinical responses are observed). Consequently, even though fluctuations were observed in the rate of delivery of disulfiram from the composite implant, it could provide an acceptable therapeutic effect for a period of 3 months or longer.

The mobilization of injected powdered disulfiram in the control group provided an unexpected finding (Fig. 2), in view of the known poor bioavailability of disulfiram in subcutaneously implanted tablets. The first-order

[1] Waters Associates.
[2] Amersham.
[3] USP grade; Ayerst Laboratories.
[4] Fisher.
[5] Model LS-100C; Beckman Instruments.
[6] Boston-Bradley.
[7] Penthrane; Abbott Laboratories.
[8] Model LS-230; Beckman.
[9] Harvey Biological.
[10] New England Nuclear.
[11] TRS-80 Model 1; Radio Shack, Tandy Corporation, Ft. Worth, Tex.
[12] VisiCalc; Personal Software Inc., Sunnyvale, Calif.

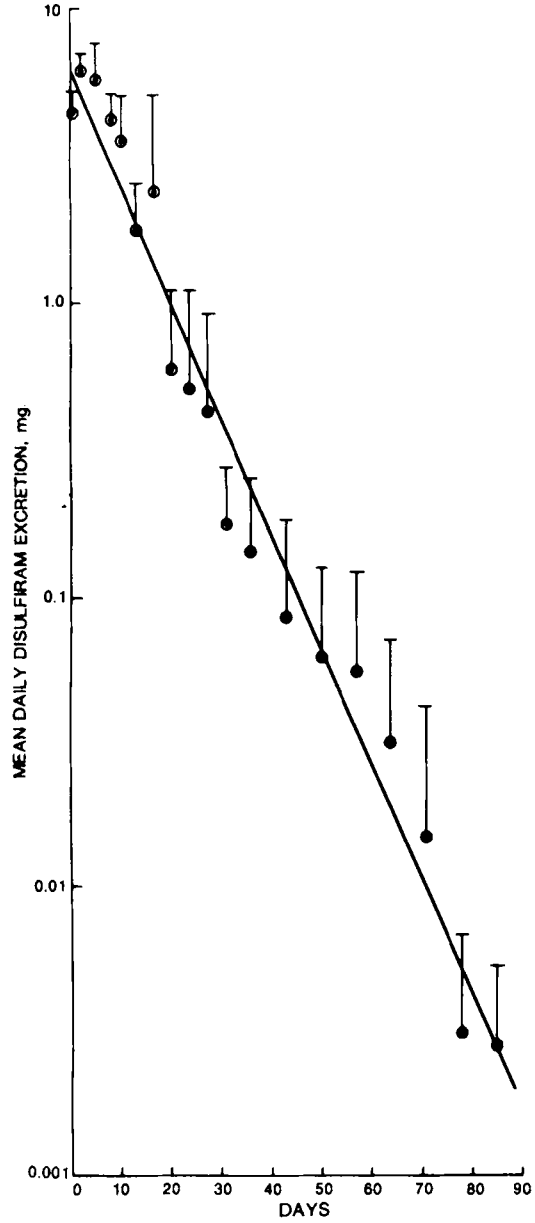

Figure 2—*Control group mean daily excretion of radiolabeled metabolites in urine and feces following subcutaneous injection of powdered disulfiram* $t_{1/2}$ = *7.53 d. Bars indicate 1* SD. (*Log* y = −0.04 x + 0.80; r^2 = 0.97; $p < 0.0001$.)

mobilization kinetics are consistent with the commonly observed kinetics of other parenterally administered drugs (14). It is possible that the bioavailability of the disulfiram was enhanced by administering it in powder form, thus greatly increasing the surface area available for the dissolution of the drug in the interstitial fluid. The long half-life of the powdered disulfiram (7.53 d) may be a function of its poor solubility in water, and raises the possibility that this preparation may also have potential clinical application.

These data demonstrate that the disulfiram–copolymer composite has the properties of a sustained-release formulation when implanted subcutaneously in rats, with no evidence of local or systemic toxicity. However, a number of factors must be taken into account before any trial of a similar material is contemplated in human subjects. First, the ratio of disulfiram to the copolymer is low, so that prohibitively large volumes of composite might be required for a prolonged period of treatment. For use in human subjects, an improved composite with a higher ratio of drug to vehicle would probably be desirable. Second, the daily dosage requirement for parenteral disulfiram is not yet known. Although patients generally require 250–500 mg of the oral drug each day to achieve a clinical effect, more research is required to determine whether or not parenteral disulfiram needs to be administered in similar quantities. Pending these advances in technology and basic knowledge, we suggest that sustained-release disulfiram implants may eventually achieve a clinical application in the treatment of alcoholic humans.

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