

Efficacy of Sustained-Release Radioprotective Drugs *in vivo*

Jashovam Shani, Ph.D., Shimon Benita, Ph.D., Muhamed Abdulrazik, M.D., and Aharon Yerushalmi, Ph.D.*

Departments of Pharmacology and Pharmacy, The Hebrew University School of Pharmacy, Jerusalem, and *Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel

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In previous publications from this laboratory we suggested the use of radioprotective drugs *in a sustained-release form* as a practical way to cope with their high toxicity and quick metabolism and excretion. Cysteine and cysteamine, well-established radioprotectants, were used as model drugs and compressed at various concentrations (0–65%) into an insoluble tablet matrix, composed of ethylcellulose and stearic acid at various ratios and compression pressures. We demonstrated *in vitro* that when the release rate of the radioprotectants was measured under nitrogen, the kinetic data conformed with the Higuchi square root of time equation, indicating that the release of both drugs correlates with Higuchi's diffusional mechanism. In the present *in vivo* study, tablets containing cysteine or cysteamine in a slow-release matrix were implanted, into the stomachs of female rats. The rats were irradiated at various time intervals up to 12 h after implantation and their survival recorded daily. Utilizing a 1:3 ratio of ethylcellulose:stearic acid as a matrix, the protective effect of the drugs was remarkable eight hours after tablet implantation. The results reported indicate that slow-release tablet formulation is a possible method for delivering of radioprotective drugs over an extended period of time.

Introduction

The search for new radioprotective agents during the past two decades joined efforts on syntheses of new compounds, hoping to get chemicals with dose-modifying effects more profound than those of the classic radioprotectants cysteine and cysteamine. Such radioprotectants have to be administered shortly before exposure to radiation, are toxic to mammals, and are rapidly excreted and degraded [1]. Some of them produce a state of shock when injected at radioprotective dose levels, as they are hypotensive, vasodilative and cause enhancement in vascular permeability [2]. One way to keep a high serum level of these drugs without actually exposing the subject to high doses at any particular time, is to use a phosphorylated drug, which is then dephosphorylated to its active form *in vivo*. Another approach, developed in this laboratory, is to compress the radioprotective drug in a slow-releasing matrix [3, 4].

Previous *in vitro* studies from our laboratory presented kinetic data on the release of cysteine and

cysteamine from an insoluble matrix. The data conformed with the Higuchi square root of time equation [4] and allowed us to select the best matrix for *in vivo* studies as far as composition of the tablet and tablettation pressure were concerned. Our preliminary *in vivo* studies demonstrated that survival of rats irradiated 10 Gy at various time intervals after implantation of such sustained-release tablets containing 50% cysteine, was the highest when the tablets were implanted into the rats' stomachs 8 h before the irradiation [3]. Due to the increasing need for sustained-release radioprotectants and the lack of new, less toxic ones, we undertook to investigate the radioprotective effect of two well-established radioprotectants, cysteine and cysteamine, embedded in controlled-released matrix, after their implantation into stomachs of rats, and subsequently exposed to whole-body gamma irradiation.

Materials and Methods

Sustained-release tablets containing the radioprotective drugs cysteine or cysteamine were surgically implanted under ether anesthesia and careful electrocoagulation into stomachs of female albino Lewis rats weighing 220–250 g each. In order to assure tablet uniformity, cysteine HCl and cysteamine HCl

Reprint requests to Prof. Dr. Jashovam Shani, Department of Pharmacology, Hebrew University School of Pharmacy, P.O. Box 12065, Jerusalem 91120, Israel.

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(Sigma, St. Louis, Missouri, USA), of the same particle size distribution, were used during the entire investigation. Just prior to pressing the tablets, both powders were dried and suspended under continuous stirring in melted stearic acid USP XVI, in a Pyrex glass container. The mixture was continuously stirred even after heat removal, until complete solidification, then granulated by a 20-mesh screen and mixed with various proportions of ethylcellulose (N type, having ethoxy content of 47.5–49.0%) in a mortar. The ratio of ethylcellulose (Hercules N-100) to stearic acid (Merck, Darmstadt, Germany) was 1:3. Various concentrations of cysteine (0–65%) and cysteamine (0–25%) were mixed with this matrix and were compressed into cylindrical tablets of 13.1 mm in diameter and a mean surface area of 400 mm², weighing 500 mg each. A laboratory press having a vacuum KBr die was used, and the compacting pressure was fixed at 4 tons throughout the study.

Tablet implantations were performed between 8–10 am, into 18-hour-starved rats. The rats and the tablets of each specific concentration were selected at random, and one tablet was implanted per rat. Under anesthesia an incision was made in the mid-line of the abdominal body wall, the stomach exposed and a 2 cm long cut was performed along its outer curve. The selected tablet was then pushed through the cut with the aid of forceps, and the cut sutured using cat gut or silk threads. Electrocoagulation was done carefully whenever necessary, to minimize bleeding, the body wall and the skin sutured and the anesthetic removed. The moment each rat turned on its abdomen was recorded, and the irradiations were done at fixed time intervals after this time point.

For the whole-body irradiation the rats were placed into a circular case mounted around a radioactive source, with 86 cm animal-to-source distance. They were exposed at 0.75 Gy/minute, to a total body irradiation of 10 Gy (⁶⁰Co unit, model 150 A, Atomic Energy of Canada). The radiation dose was monitored and confirmed by thermoluminescent dosimeters. Eight to ten rats were pooled in each experimental group. After irradiation the rats were divided into cages, two per cage, received Purina pellets and water ad libitum, and were kept in a well-ventilated room at 20 °C. Daily survival was monitored for fourteen days post-irradiation, and expressed graphically. Survival rates were obtained from those curves.

Results

The first factor determined in this study was the radiation dose, aimed at defining a dose which will kill the unprotected rats in less than 14 days, so that any differences between the matrix-treated and the drug-treated rats will be noticeable on day 14 after irradiation. Eight, 10 and 15 Gy were used, but while 8 Gy was too low a dose (50% of the untreated rats survived over 14 d), and 15 Gy killed all the rats within 8 days, 10 Gy was chosen for subsequent experiments (Fig. 1).

The second factor studied was the length of the radioprotective effect. This was performed by implanting into the rats' stomachs tablets with 50% cysteine HCl, and irradiating them 2, 6, 8 or 18 h after implantation. Fig. 2 demonstrates that out of the four time-intervals (between implantation and irradiation) tested, 8 h is the optimal one. Apparently 2 and 6 h were too short for a protective dose to be released, and 18 h was a too-long time lapse to retain radioprotective activity. Therefore, 8 h was chosen as the time interval for all other subsequent experiments with cysteine protection.

The dose of the cysteine tablets implanted 8 h before irradiation was also monitored, and even though only two concentrations were actually used, it was clear that the higher concentration (65%) was already less effective than the 50%, as it forced reduc-

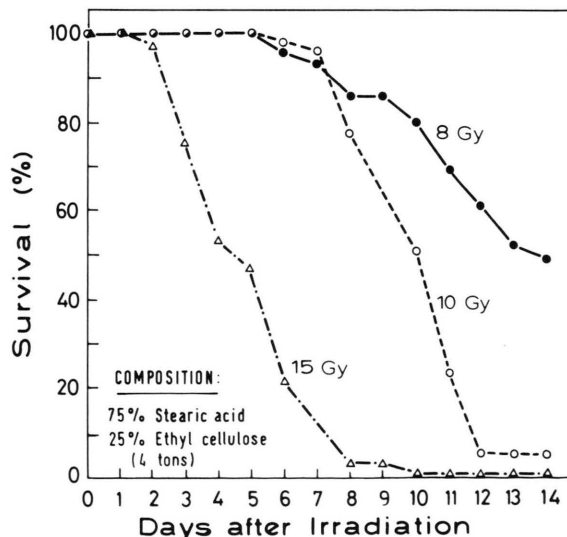


Fig. 1. Survival of rats irradiated 8, 10 or 15 Gy, two hours after implantation of matrix tablets without radioprotective drug into their stomachs ($n = 8-11$).

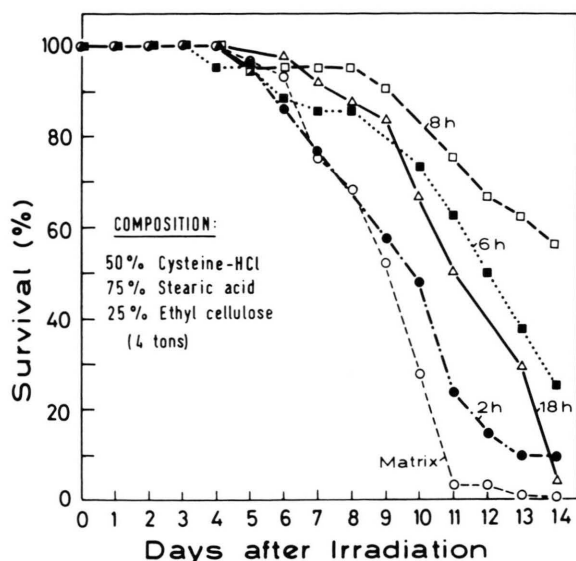


Fig. 2. Survival of rats irradiated 10 Gy, at various time intervals after implantation of matrix or sustained-release cysteine (50%) tablets into their stomachs ($n = 8-10$).

tion in weight of the slow-release matrix, causing a quicker release and consumption of the drug. 50% seems to be a practical concentration of cysteine to be used in such tablets (Fig. 3).

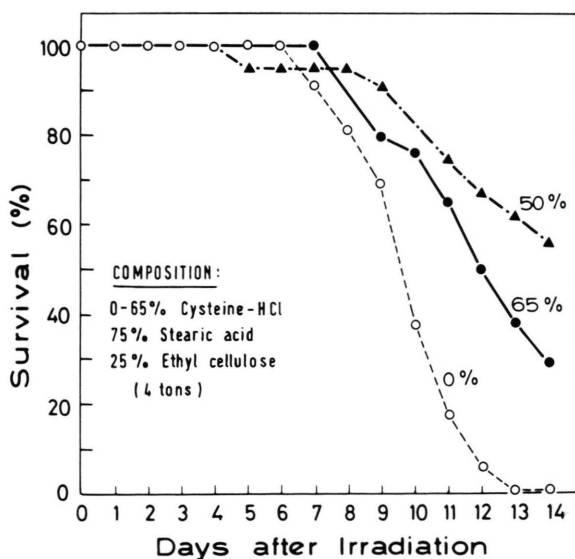


Fig. 3. Survival of rats irradiated 10 Gy, eight hours after implantation of matrix or sustained-release cysteine tablets at various concentrations (0, 50 and 65%) into their stomachs ($n = 9-10$).

Due to the high toxicity of cysteamine, only concentrations below 25% of it were used in our study. While concentrations below 12% did not increase the survival rate, as compared to controls, concentrations above 15% were already toxic. We concluded that under our experimental conditions (10 Grays and 8 h interval), 15% cysteamine was the optimal concentration for its experimental use as a radioprotectant (Fig. 4).

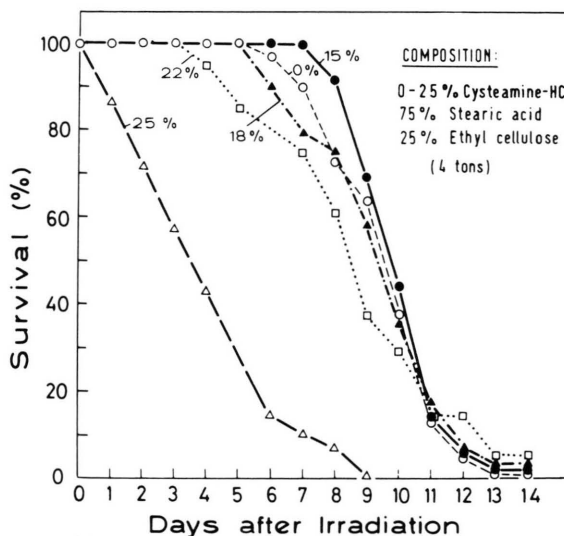


Fig. 4. Survival of rats irradiated 10 Gy, eight hours after implantation of matrix or sustained-release cysteine tablets at various concentrations (15, 18, 22 and 25%) into their stomachs ($n = 8-11$).

Discussion

Although it has been established that cysteine and cysteamine protect against gamma-irradiation, these drugs are toxic to all mammals investigated [5]. The Dose Reduction Factor (DRF) for both cysteine and cysteamine in mice is 1.7, their LD_{50} values in rats are 700 and 130 mg/kg and their protective doses in rats are 1200 and 600 mg/kg, respectively [6, 7]. For comparison, the LD_{50} of WR-2721, a thiophosphate derivative of cysteine, is 550 mg/kg, while its protective dose is 400 mg/kg, 80% of its LD_{50} . At that dose it has a whole body DRF of 2.6 [8].

To the best of our knowledge, no previous results on radioprotection of animals given sustained-release radioprotective tablets have been published so

far. The goal of our study was, therefore, not only to achieve an effective prolonged supply of two well-established radioprotectants, but also to supply the drugs in a less toxic modality. Cumulative toxicity of repeated iv doses of such drugs introduce some difficulty in studying protection against radiation. Hence, repeated injections of split doses of cysteine and cysteamine were shown to be better tolerated than one large dose of the same drugs, provided that an interval of a few hours is maintained between administrations. Prolonged supply was shown to have very low toxicity: when cysteamine was supplied to rats in the food in concentration of up to 0.5% for 27 days, no toxic reactions were noticed [9], but its protective effect against radiation was low.

When preparing the tablets we noticed that the efficiency of the two radioprotectors correlated with their toxicity. While cysteine could be compressed and used in concentrations of up to 65%, cysteamine could not be given to rats in concentrations exceeding 20%, due to its high toxicity. We actually limited further experiments to 20% of the latter drug, when noticing that in higher concentrations its adverse reactions were even more profound when pressed in tablets containing over 75% of stearic acid. As frequent administration of cysteine and cysteamine to humans is impractical — we suggest this slow released modality which lowers the toxicity and retains part of its radiation protection ability as compared to conventional drug administration.

We chose rats for stomach implantation because in this model the tablet matrix does not pass from the stomach to the intestine for a few days, allowing controlled dissolution of its active ingredients in a narrow range of pH values, thus isolating the dissolution rate factor from other factors involved in the stomach-to-intestine transfer of food. Another justification of this model is its simplicity: hardly any of the negative control rats (operated but not irradiated) died, and complications due to the operation were minimal. Out of a random selection of 27 rats, none developed any visible ulceration [9].

Even though in our preliminary studies we followed-up the rats for 30 days post irradiation, we found out that a 14-day follow-up after irradiation is enough for our *comparative* studies as this was the critical period of time for acute radiation damage.

For that reason, the result of the studies reported here were not presented beyond this time period. In order to further justify this decision, the radiation dose per rat was increased to 10 Gy, as compared to 6–8 Gy which might have been sufficient in similar experiments with rats followed up for 24–30 days after irradiation [1].

The best hematopoietic radioprotective effect was obtained in this study using tablets containing 50% cysteine and transplanted 8 h before irradiation. Neither a higher (65%) concentration of the drug nor shortening the time interval between implantation and irradiation exhibited any superior protection. Cysteamine showed a very poor protection, if at all, as the standard deviation for the different tablet concentrations from the control tablets was very small. The major reasons for cysteine's better protection as compared to cysteamine are that under identical experimental conditions cysteine dissolves slower and is more stable in solution, consequently enhancing bioavailability to the irradiated cells. As noticed in our previous publications [3, 4] the kinetic data of the drugs' dissolution conformed with the Higuchi's square root equation and first order release. Because both plots were linearly acceptable, a statistical method for release mechanism identification was used, showing that aminothiols release from the matrix tablets follows the Higuchi square root equation, with solvent penetration (which also follows the square root of this relationship) being the rate-limiting factor in the release process. This relationship between the cumulative amount of the drug released and the square root of time is linear until 80% of the active components are released from the matrix.

This study suggests that simple pharmaceutical modifications of the drug dosage form can be used for prolonged hematopoietic protection of human subjects against internal contamination with radionuclides or against external irradiation during radiation therapy and at various emergency conditions.

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