SUSTAINED-RELEASE OF RADIOPROTECTIVE AGENTS IN VITRO

Jashovam Shani, Shimon Benita, Amram Samuni and Max Donbrow

Departments of Pharmacology, Pharmacy and Molecular Biology, Hebrew University Medical School and School of Pharmacy, Jerusalem, Israel

The major functional group of radioprotective agents contains a sulfur atom, either as a thiol (-SH) or in the oxidized state (-S-S-). These compounds protect against radiation as a result of their ability to trap primary free radicals formed via degradation radiolysis of water (1). The practical use of those radioprotectants is limited by their cumulative toxicity and rapid excretion and degradation. Moreover, for provision of adequate protection against irradiation effects, the concentration of the drug requires precise regulation, a difficult goal to achieve due to the rapidity of depletion on the one hand, and the low protective index on the other. Some protective doses and LD_{50} values for a highly effective radioprotective agent, cysteamine, in various mammalian species, are given in the following table (2):

Species	Route	Protective Dose (mg/kg)	LD_{50} (mg/kg)
Mouse	ip	75-150	260
Rat	ip	75-150	140
Dog	iv	75-110	
Rabbit	iv		150
Man	iv	150-400	

The radioprotective drugs chosen for the first phase of this study were cysteamine (β-mercaptoethylamine, $H_2N-CH_2-CH_2-SH$) and cysteine (β-mercaptoalanine, $H_2N-CH(COOH)-CH_2-SH$), the most effective agents explored in mammals. The objective of this research is to improve the efficacy of these radioprotectants by development of new pharmaceutical formulations rather than by synthesising of new agents. With the growing use of nuclear enrgy throughout the world, and the risk of accidental irradiation becoming a dangerous hazard, we considered it desirable to develop a sustained-release radioprotec= tive formulation. Prolonged release of the agent in the body at effective concentrations is aimed at avoiding increase in toxicity, and will provide a model for a novel approach to human protection against accidental irradiation. The ultimate goal of this study is then better control of the bioavailabilityof the drug in the serum, with increase in its protective index.

MATERIALS AND METHODS

Cysteamine and cysteine (Sigma) were dried, pulverised and mixed

with stearic acid BP 1973 (sigma) and ethylcellulose (Hercules), in various proportions, in a mortar. Various concentrations of the active material, not exceeding 50%, were prepared in defined ratios of the matrix mixture. The mixture was than compressed into cylindrical tablets of 13.1 mm in diameter and a mean of $4.0cm^2$ surface area (13.6 mm height) in a vacuum KBr die, with a laboratory press, at various pressure values (3), as given previosly (4). Release of the drug was measured spectrophotometrically (Unicam, model SP-1805) at 240 nm, using a rotating basket dissolution apparatus as described in USP XIX. The dissolution medium (phosphate beffer pH 7.4) was kept under nitrogen. Exactly one liter of the buffer, previously heated and maintained at $37\pm0.3^{o}C$, was used for each experiment. The basket was immersed in the buffer and rotated at 100 rpm. Experiments were carried out for 8-16 hours, and the concentration of the unoxidized drug was monitored continuously, using a 10 mm flow-cell fed by a peristaltic pump (Buchler, model 2-6100), at flow rate of 60 $ml.min^{-1}$. The tablets were wrapped in aluminium foil immediately after their compression, and kept at room temperature pending assay. Experimental results obtained spectrophotometrically were computerized, using a programme to fit Higuchi equation treatment described as follows: 10-20 points from each spectrophotometric run were fed into a PDP-15 computer and plotted against the square root of t. The parabolic UV absorption curve, which demonstrates the accumulated released drug when plotted on a linear scale of t, is converted into a linear correlation when plotted against the square root of t, following the Higuchi law. For these calculation the molar extinction coefficients for cysteine (427 $M^{-1}.cm^{-1}$) and cysteamine (563 $M^{-1}.cm^{-1}$) were determined experimentally under nitrogen.

The mechanisms for the sustained-release of drugs dispersed uniformly in hydrophobic matrices were proposed by Higuchi in 1963 (5). He developed equation suitable both for drugs partially soluble in the matrix, and for drugs insoluble in the matrix but soluble in the external medium. In the latter type of system the drug particles are released by leaching action of the penetrating solvent. The Higuchi square-root law, which has been validated in numerous cases (6-8), takes the following form:

$$Q = \sqrt{\frac{D.\varepsilon}{\tau} (2A - \varepsilon . C_s) C_s . t }$$

where Q = the amount of drug released at time t per unit exposed area
ε = the porosity of the matrix
τ = the tortuosity of the matrix
D = the dffusibility of the drug in the dissolution medium
A = the total amount of drug present in the matrix / unit volume
C_s= the solubility of the drug in the dissolution medium

With all other parameters fixed, the release rate is linearily related to the square root of t. Release rate from spherical pellets by this mechanism does not follow a first-order relationship, as the time required to release 50% of the drug from a matrix is expected to be approximately 10% of the time required to dissolve the last trace of the solid drug phase in the center of the pellet. It was shown (9) that dissolution of a soluble drug at high concentration from an insoluble matrix follows the Higuchi square-root equation except during an initial lag phase and a terminal diffusion phase. The main

assumptions made are that the two-dimentional cross-sectional porosity
has the same mean as the volumetric porosity, and that the dissolving
substance is sufficiently dilute not to affect the porosity. A graphic
demonstration of a tablet before (left) and during (right) the drug-
dissolution process is shown in the following figure, where the lined
areas represent the solvent liquid, and the bold lines - the drug (9):

porosity ε porosity $\varepsilon + A$ $(1-\varepsilon)$
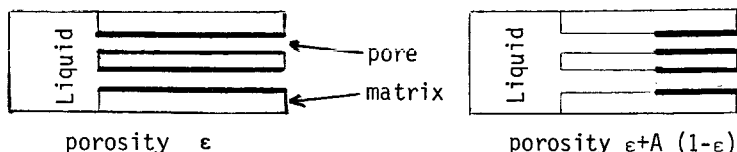
RESULTS AND DISCUSSION

A very distinct correlation was obtained in this study between
the stearic acid / ethylcellulose ratios and the release rate of 20%
cysteine , pressed at 4 tons, from the tablets. The mean release rate
at five increasing ratios (expressed as percentage of stearic acid in
the matrix mixture) is shown in the following table:

Stearic acid (% in matrix mixture)	Release rate of 20% cysteine
25	4.90
33	4.52
50	3.77
56	3.02
75	2.86

Tablets of 100% stearic acid as matrix liquified during their
preparation and could not be manufactured. The significant decrease
in rate of cysteine release with increasing the concentration of
stearic acid was calculated, and obtained graphically by the computer.
In general, the release rate of 20% cysteamine from similar matrix
mixtures were much higher than those of cysteine, with 17.20% for 0%
stearic acid (i.e. pure ethylcellulose) and 7.10% for 50% stearic acid
(i.e. 1:1 ratio). The release rate of cysteamine also decreases with
increasing stearic acid concentration in the matrix, and experiments
with 90-100% of stearic acid are being carried out currently.
The relation between the concentration of the active drug and
its rate of release was also investigated. Preliminary results suggest
that the rate increases with increasing percentage of stearic acid.
Further studies are needed in order to validate this preliminary
finding.
One other factor that has been investigated in this study was
the effect on the release rate of the pressure applied to the tablet
during its formation. Here, preliminary findings suggest that with 20%
cysteamine as the active material, a higher formation pressure results
in quicker drug release, while with 20% cysteine no such irregularity
was noticed. Although both components of the matrix are hydrophobic,
stearic acid is much more hydrophobic than ethylcellulose, due to its
waxy character. This character is probably the reason for the decrease
in the porosity of the matrix.

Studies of the release of the radioprotectants from their matrices in simulated gastro-intestinal juices are currently being carried out. The results of this work indicate that a correlation might be anticipated between the in vitro profile of release and the in vivo efficacy of the radioprotective agent, due to the sustained-release of the drug.

REFERENCES

1. Bacq, Z.M. (1975): Sulfur-containing Radioprotective Agents. Perganon Press.
2. Doull, J., Plzak, V. & Brois, S.J. (1962): A survey of Compounds for Radiation Protection. USAF Aerospace Medical Division, Texas.
3. Parrott, E.L. & Sharma, V.K. (1967): J. Pharm. Sci. 56,1341.
4. Donbrow, M. & Friedman, M. (1975): J. Pharm. Pharmacol. 27,633.
5. Higuchi, T. (1963): J. Pharm. Sci. 52, 1145.
6. Desai, S.J., Singh, P., Simonelli, A.P. & Higuchi, W.I. (1966): J. Pharm. Sci. 55, 1224.
7. Desai, S.J., Singh, P., Simonelli, A.P. & Higuchi, W.I. (1966): J. Pharm. Sci. 55, 1230.
8. Desai, S.J., Singh, P, Simonelli, A.P. & Higuchi, W.I. (1966): J. Pharm. Sci.,55, 1235.
9. Fessi, H., Marty, J.P., Puisieux, F. & Carstensen, J.T. (1978): Intern. J. Pharmac., 1, 265.