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Liposome Mediated Delivery of 2-Mercaptopropionyl Glycine: Entrapment of MPG in Liposome.

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Introduction

2-mercaptopropionyl glycine (MPG), an amionothiol, has been widely used in experimental radioprotection in mice because the effective dose (20 mg/kg. body weight) is far below the toxic dose (2100 mg/kg. body weight) (1-2). The radio-protective effect of MPG was also reported *in vitro* for gamma induced radiolysis of catalase (3). However, gamma induced DNA strand breaks in human lymphocytes *in vitro* showed that depending on the dose of radiation, the MPG concentration must be adjusted for optimal radioprotection (4). Furthermore, DNA strand break analysis in human lymphocytes *in vitro* revealed that adjustment of optimal concentration of MPG in relation to the dose of radiation was a must otherwise MPG acted as a rediosensitizer instead of radioprotector (5). Therefore, in order to exploit the radioprotective potential of MPG in radiotherapy, it is highly desirable to deliver a calculated amount of MPG for the computed dose of radiation to the target tissue.

The widely used protocol of MPG administration for radiotherapy is intravenous injection of MPG 15 to 30 min prior to irradiation (6). Obviously, distribution of MPG in various tissues of the experimental animals or patients in directly controlled by the physiological conditions. In this situation, it is assumed that MPG concentration available in the tissue of interest is optimal for radioprotection. However, if this assumption is not correct, then, in light of our previous results (4-5), the desired radioprotective effect of MPG is undesirable and is likely to undermine the radioprotective potential of MPG.

In order to overcome this problem of delivery of calculated amount of MPG in specific target tissues, we have taken the approach of liposome mediated delivery of MPG. In principle, liposomes can encapsulate drugs in its aqueous content. The liposomes then can be targeted to a particular tissue by linking specific antibodies on to the surface of the liposome. Upon administration, the modified liposomes specifically migrate to the desired tissue and deliver the drug (7).

Because MPG may act as radioprotector as well as sensitizer, liposome mediated MPG delivery system may offer several advantages over the conventional mode of administration. These may include (1) delivery of MPG to specific target tissues, (2) amount of MPG delivered in the tissue can be controlled, and (3) MPG delivered in the tissue can escape possible metabolism enroute the target tissue due to encapsulation.

The present investigation was aimed to enhance the radiomodulatory potential of MPG. This report brings out the preliminary results of attempts to encapsulate MPG in liposomes.

Methods and Materials

Chemical: MPG (tiopronine) was a gift from Prof. T. Sugahara and Santen Pharmaceutical Co., Japan. Dipalmitoyl phosphatidyl choline (DPPC), dicetylphosphate (DCP), cholesterol and dithiobis 2-nitro benzoic acid (DTNB) were obtained from Sigma Chemical Co., USA, Sepharose CL-4B from Pharmacia Fine Chemicals, Sweden and triton X-100 from Glaxo Labs., India. All other reagents used were of anlytical grade.

Preparation of liposomes:

A. Wet method: Liposomes were prepared as described earlier (8). In short, DPPC: Cholesterol:DCP were taken in test tube in molar ratio of 1.0:0.9:0.25 and dissolved in 0.2 ml chloroform by vortexing. 1.0 ml aqueous solution, phosphate buffered saline (PBS), pH 7.4 or 10 mM MPG in 10 mM PBS, pH 7.4 was added in 0.2 ml aliqotes followed by vortexing. The mixture was transferred in a round bottom flask and chloroform was removed in a rotary evaporator at 30-35°C. The remaining trace of chloroform was removed during gel filtrating chromatography.

B. Dry film method: Liposomes were made as described by Bangham et al (9). In short, DPPC: Cholesterol: DCP mix in the same molar ratio, as above, were dissolved in 0.2 ml chloroform by vortexing. The chloroform was removed by nitrogen flushing at 40°C while cohtinuously rotating the tube. This resulted in a thin and uniform film on the walls of the tube. The dried film was dispersed in 1 ml of PBS or 10 mM MPG solution by vortexing.

Separation of liposomes from the free MPG: The liposomal suspensions were passed through a Sepharose CL-4B column (30x15 cm) equilibrated with 10 mM PBS, pH 7.4 containing 0.02 % sodium azide. The column was developed with the same buffer. Fractions were collected and read at 280 nm.

Determination of MPG concentration: The MPG was quantified by the assay of -SH group using the method of Ellman (10) with minor modifications. In short, 2.9 ml of reagent (flushed with nitrogen) consisting of 10 mM DTNB in 100 mM phosphate buffered saline (pH 7.9) containing 0.1 mM EDTA was added to 0.1 ml of sample and absorption monitored at 412 nm immediately after mixing. Cystein was used as a standard.

Results and Discussion

Lipid composition and lipid/MPG ratio used in liposome preparation is shown in Table 1. 10 mM MPG solution in PBS (10 mM, pH 7.4)was employed for encapsulation. Liposomes containing 10 mM MPG or PBS (blank liposomes), prepared as discussed in Methods and Materials section, were separated from the unentrapped MPG by gel filteration chromatography on Sepharose CL-4B column. Liposomes eluted in the void volume were assayed for the content of -SH group to quantitate encapsulated MPG into the liposomes. The percent entrapment of MPG in liposomes prepared by both the method are shown in Fig. 1. 56 % entrapment

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was obtained by the wet method, whereas 30 % by dry film method. Poor entrapment efficiency has been reported by dry film method earlier (9). We have reported 75% entrapment efficiency by the above wet method for a plant protein, gelonin, of molecular weight 30 kd (8). The decrease in entrapment efficiency of MPG, as reported here, may be because of its smaller molecular weight. However, the entrapment value of MPG reported by wet method is very close to the high entrapment efficiency methods for proteins and drugs described in literature (11-14). Our procedure is not only easy but also reproducible compared to any other high entrapment techniques described so far (11). Each experiment was repeated 3 to 4 times and the value of liposome encapsulation of MPG are meant.

The results of our studies open the possibility to develop a more effective MPG delivery system. It must be emphasized here, however, that these findings are still of a preliminary nature and further investigations are continuing.

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Table 1 Lipid Composition and Lipid/MPG Ratio of Liposomes.

Composition	Amount of lipid(mg)	Molar ratio	Lipid/MPG ratio
DPPC:Cholesterol: DCP	5:2.5:1	1:0.9:0.25	5.21

Fig. 1 MPG Entrapment Efficiency in Liposomes