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Preparation and evaluation of polyglutaraldehyde nanoparticles containing 5-fluorouracil

G. Mukherji [1], R.S.R. Murthy [2] and B.D. Miglani [3]

[1] Hamdard College of Pharmacy, Hamdard Nagar New Delhi (India), [2] Department of Pharmacy, Faculty of Tech. and Engg., M.S. University, Baroda (India) and [3] College of Pharmacy, Pushp Vihar, New Delhi (India)

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Summary

Polyglutaraldehyde (PGL) nanoparticles have been formed by aldol condensation under alkaline conditions. The particles are spherical, with an average diameter of 270 nm. In-vivo deposition of the drug in some tissues, 2 h after injection, has been measured using high-pressure liquid chromatography.

Introduction

The problem of directing a cytostatic agent to specific sites is a current topic of research. Use of drug carriers may improve the drug's anti-cancer activity at a lower dose and reduce toxicity. The use of endocytizable and lysosomotropic carriers such as liposomes (Arrowsmith, 1983) and DNA complexes (Trouet, 1972) appears promising. Microcapsules (Bakan and Anderson, 1976), nanocapsules (Speiser, 1976), macromolecular complexes (Uchman and Grezeskowiak, 1981), erythrocyte carriers (Updike and Wakamiya, 1983) and emulsions (Katsuo et al., 1980) are other examples of colloidal drug delivery systems used either for their sustained release or specific-release properties. Another group of micron-, or even, submicron-sized delivery systems are solid particles, termed microparticles or nanoparticles (NPs).

Polyglutaraldehyde (PGL) NPs. ranging in size from 50 to 1500 nm could be obtained by aldol polycondensation of monomeric glutaraldehyde at alkaline pH (Margel et al. 1979). In the presence of surfactants, such as Aerosol 604, or a mixture of 1% Guar C-13 and 5% polyethylene oxide with a molecular weight of 10,000 and in basic aqueous solutions, Margel et al. (1979) obtained spherical colloidal particles due to precipitation of PGL. Highly fluorescent or magnetic PGL NPs were produced by addition of fluorescent substances or ferric oxide to the initial reaction mixture.

PGL microspheres were prepared according to the procedures of Margel et al. and after washing and sizing, Tokes et al. (1982) coupled the drug, Adriamycin, to them. Sensitivity studies of murine leukemic cell lines to free and PGL-coupled adriamycin showed the latter to be much more toxic.

Correspondence: G. Mukherji, Hamdard College of Pharmacy, Hamdard Nagar, New Delhi, India.

Margel has briefly described the development and properties of a novel adsorbent system consisting of PGL microspheres encapsulated in agarose matrix. Since the microspheres contain aldehyde groups, various amino ligands can be covalently bound in a single step under physiological pH (Davis et al., 1984).

PGL spheres have shown to possess strong adsorbing and entrapping properties. In the present study, PGL NPs have been prepared containing the anticancer drug, fluorouracil (5-FU) and assessed for the distribution of the drug in a rat model.

Materials and Methods

Chemicals and apparatus

5-FU was obtained from Hoffman La Roche, Switzerland. Glutaraldehyde (25% solution in water) of Riedel-De Haenag, Hannover, Sodium carboxy methyl cellulose (High Purity Chem., N. Delhi), Tween 80 (G.S. Chemicals Testing Lab & Allied Ind., N. Delhi), hydrochloric acid, concentrated (Glaxo Lab. (India) Ltd., Bombay) and sodium hydroxide pellets (Sarabhai M. Chemicals, India) were used. Ethyl acetate, A.R. (I.D.P.L., India), sodium phosphate, monobasic (Reechem Pvt. Ltd., India), sodium acetate (Glaxo Labs. (India) Ltd., Bombay) and acetic acid (I.D.P.L., India), and acetonitrile and methanol, both of HPLC grade (LKB Biochrom Ltd., U.K.) were used for in-vivo study.

Philips scanning electron microscope (PSEM 501 B), refrigerated centrifuge (Caltan, India) UV double-beam spectrophotometer (Model CE-594; Cecil Instruments, U.K.) and a tissue homogenizer (Grover Enterprises, India) were used. An LKB Bromma HPLC system with Ultropac column packed with Lichrosorb RP 18 (10 μm) and measuring 25 cm × 4.0 mm were used for in-vivo estimations at 280 nm.

Formula: 5-Fluorouracil, 1.0 g; Sodium CMC, 0.1%; Tween 80, 5.0%; Glutaraldehyde (5% soln.), to make 100 ml.

Preparation of PGL NPs

The pH of the initial reaction mixture was adjusted to 8.2. The resulting solution was deaerated using nitrogen, the container was tightly closed and stirred for 24 h. After 0.5 and 4 h of stirring, the pH was readjusted to 8.2 and after 4 h, the mixture was again deaerated with nitrogen. After 24 h, the dispersion was passed through Cellulose nitrate filter (0.2 μm in pore size). The NPs retained over the filter were repeatedly washed with water and finally resuspended in water. The dispersion was reduced to dryness by evaporation of the solvent (water) at 45 ° C under vacuum. The yield of PGL NPs was obtained by dry weight analysis of the sediment obtained after repeated centrifugation at 20,000 rpm.

Detection of PGL NPs by scanning electron microscopy (SEM)

An aqueous dispersion of the nanoparticles was finely spread over a stub and was dried by keeping in a desiccator. The dried film of the NPs was given a 25 nm thick gold layer and was observed by SEM. The size of 100 NPs was estimated to determine the overall size and range of the particles.

Determination of 5-FU entrapped in PGL NPs

The filtrate obtained during the purification of PGL NPs was adjusted to volume. The above solution (10 ml) was further diluted to 100 ml with water and 1 ml of the diluted solution was mixed with 0.1 ml of 0.5 M NaH_2PO_4 solution and extracted with 8.0 ml of ethyl acetate by vigorous shaking for 10 min. Measurement of the optical density at 280 nm of the clear, organic layer obtained after centrifugation at 5000 rpm for 5 min. indicated the quantity of free 5-FU in the filtrate.

The payload of 5-FU in the NPs was determined by calculating the difference between that added initially and the amount that is unincorporated in the NP.

Tissue distribution study in albino rats

Albino rats (Speiser, 1976) of either sex (200–300 g) were starved for 18 h prior to drug dosing. The rats were anaesthetised with ether. PGL NPs (8.0 mg) were dispersed in 10 ml of distilled water. A dose of 1.0 ml, containing 114.61 μg of 5-FU was injected through the femoral vein of an albino rat. After 2 h the rats were sacrificed and liver, lung and intestine were excised. They were homogenized in 10 ml of isotonic phosphate buffer (pH 8) (Yoshioka et al., 1981). The supernatant obtained after centrifugation at 10,000 rpm for 10 min was used to analyse for 5-FU.

Analysis of 5-FU in tissue samples

The method followed for preparing samples for HPLC analysis has been reported by Sadee and Beelen (1980). 1.0 ml of the supernatant obtained from liver, lung and intestinal tissue homogenates was mixed with 0.1 ml of 0.5 M NaH_2PO_4 buffer and extracted with 8.0 ml of ethyl acetate. After centrifugation at 5000 rpm for 5 min, 5 ml of the organic layer was evaporated under nitrogen at 55°C. The residue was redissolved in 500 μl methanol and 50 μl was injected into the HPLC. The mobile phase was 0.01 M sodium acetate buffer (pH 4) in acetonitrile (95 : 5 v/v) at a flow rate of 0.5 ml/min.

Result and Discussion

The SEM of the PGL NPs shows discrete spheres, with an average diameter of 270 nm (Fig. 1). The size ranged between 120 nm and 1.8 μm (Fig. 2). The PGL NPs prepared by Margel et al. (1979) had a mean diameter of 450 nm and involved extensive dialysis and prolonged centrifugation to purify and isolate. The filtration method, as adopted in this instance, simplified both the steps.

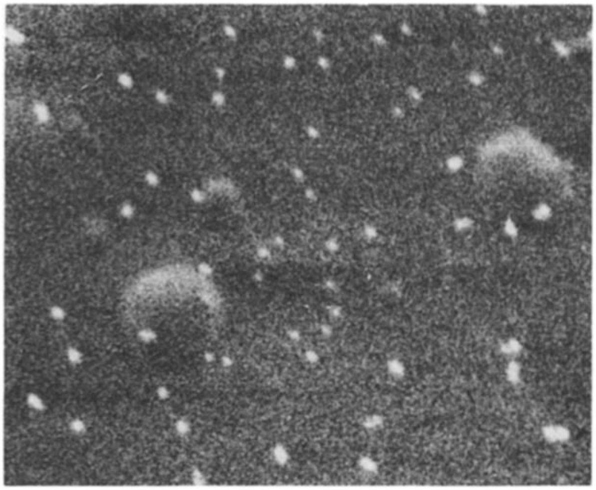

Fig. 1. SEM of PGL NPs. Thickness of the gold layer was 25 nm. ×5300.

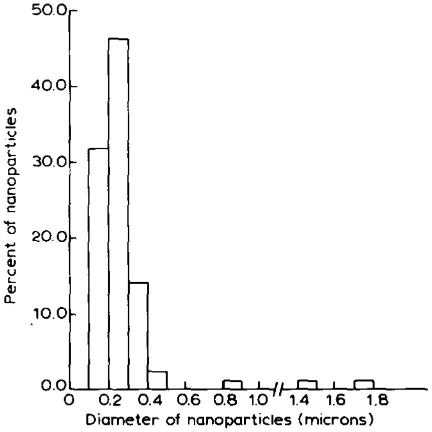

Fig. 2. Particle size distribution pattern of PGL NPs containing 5-FU.

Glutaraldehyde undergoes slow aldol condensation in the absence of surfactants, at pH 7. In the presence of surfactants, in basic solution, PGL precipitates out in the form of colloidal, spherical particles. The rate of polymerization increases at greater pH values (pH greater than 7.0) and the average diameter of PGL NPs was found to reduce from 450 nm to 200 nm on increasing the pH from 9.5 to 12.0 (Margel et al. 1979).

In the present study, one important problem encountered was the incompatibility of alkaline pH with 5-FU. 5-FU is stable in solutions which are not strongly basic (pH less than 9). When subjected to strongly basic conditions, 5-FU is hydrolyzed to urea, fluoride, and an aldehyde. This hydrolysis is enhanced by increased pH and temperature. Some of the urea formed on hydrolysis reacts further giving ammonia and CO_2 (Florey, 1973). Thus, the preparation of NPs of still smaller size was not feasible, which would, otherwise, have formed above pH 12. Thus, an optimum pH value had to be fixed to avoid the degradation of 5-FU, while obtaining convincingly minute particles at the same time. Tween 80, as a surfactant, in combination with sodium CMC which increased the viscosity of the dispersion and stabilized it, was used to produce the NPs.

Removal of unreacted glutaraldehyde was effected by membrane filtration, instead of the more complicated dialytic step.

PGL NPs were found to be insoluble in most of the normally employed solvents. Measurement of free 5-FU in the filtrate, an indirect method to measure the amount of drug entrapped in PGL NPs has been adopted.

In this technique, the initial reaction mixture has been divided into two equal volumes. The PGL NPs were recovered from the first portion by filtration as the final product. The filtrate so obtained was used to estimate for free 5-FU after proper dilution. The second fraction was used to determine the amount of PGL NPs formed. The PGL NPs were collected in ethanol after sedimentation in a high-speed centrifuge. Centrifugation was repeated to ensure that all the particles were collected. Dry-weight analysis gave an estimation of the amount of PGL NPs formed. Thus, it was found that 1 mg of 5-FU was entrapped in 6.98 mg of PGL NPs.

5-FU distribution in rat tissues

Fig. 3 depicts the isolation of 5-FU (Rt = 4.3 min) by HPLC.

Distribution of 5-FU in rat tissues following the injection of PGL nanoparticles is given in Table 1.

The highest quantity of the drug was found in liver (35.87 μg) which was 31.3 ± 3.02% of the administered dose of the drug. However, calculations based on the distribution of the drug per gram of the tissue show maximum concentration of the drug in lung tissue and minimum concentration in the intestine.

The in-vivo distribution pattern of 5-FU as reported after extensive studies revealed that the free drug does not remain in plasma or in any tissue long enough for being detected by any of the modern, accurate, analytical techniques. (Florey, 1973). The activity of 5-FU which continues over a prolonged time, possibly several days following a single injection, is due to the persistence of the active metabolite FdUMP in tumour, as well as in normal tissues. Based on this consideration, a comparative evaluation using the free drug was not regarded as necessary. Thus, the amounts of free 5-FU detected in liver and lung in the present study reflect an excess distribution of the free drug from what would, otherwise, have been obtained by an injection of 5-FU in solution. The result not only shows a gradual release of the drug and hence its gradual conversion to 5-fluoro-2′-deoxyuridine-5′-phosphate (FdUMP), but also a higher localisation of 5-FU in liver, lung and intestine, which account for 65% of the initially incorporated drug.

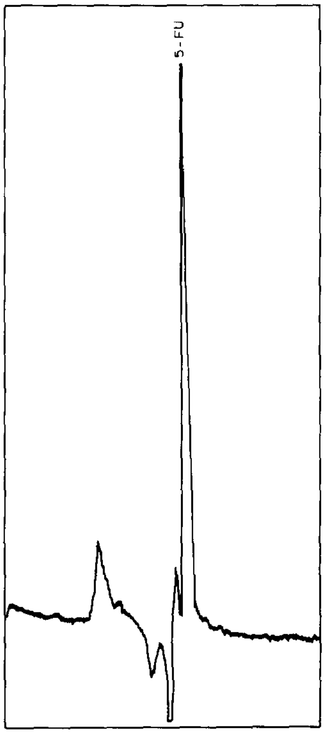

Fig. 3. Diagram showing the peak due to 5-FU in HPLC analysis.

TABLE 1

Tissue accumulation of 5-FU 2 h after the injection of PGL NPs

Tissue	Amount of 5-FU		
	μg/whole tissue	% injected drug per whole tissue	μg/g of tissue
Liver	35.87 ± 3.46	31.03 ± 3.02	5.57 ± 0.59
Lung	20.80 ± 0.47	18.15 ± 0.41	9.56 ± 2.32
Intestine	19.03 ± 1.53	16.60 ± 1.34	3.96 ± 0.71

Conclusion

The study has shown that 5-FU can be entrapped in PGL NPs in high concentration. The presence of 5-FU in the tissues indicates that the drug is readily released from the spheres, considering that they are non-biodegradable, unlike gelatin.

References

Arrowsmith, M., Hadgraft, J. and Kellaway, I.W., *Int. J. Pharm.*, 17 (1983) 91.

Bakan, J.A. and Anderson, J.L., in Lachman, L., Lieberman, H.A. and Kanig, J.L. (Eds.) *The Theory and Practice of Industrial Pharmacy*, 2nd edn., Lea and Febiger, Philadelphia, 1976, p. 420.

Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E., (Eds.), *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, p. 441.

Florey, K., (Ed.), *Analytical Profiles of Drug Substances (Vol. 2)* Academic, 1973, 234.

Katsuo, U., Goto, A., Kagaya, S., Murota, H., Echigo, M. and Takahashi, T., *Byoin Yakugaku*, 6 (1980) 14.

Margel, S., Zisblatt, S., and Rembaum, A., *J. Immunol. Meth.*, 28, (1979) 341.

Sadee, W., and Beelen, G.C.M. (Eds.), *Drug Level Monitoring-Analytical Techniques, Metabolism and Pharmacokinetics*, Wiley, 1980, 246.

Speiser, P., *Prog. Colloid. Polym. Sci.*, 59 (1976) 48.

Sugibayashi, K., Morimoto, Y., Nadai, T.,Kato, Y., Hasegawa, A. and Arita, T., *Chem. Pharm. Bull.*, 27 (1979) 204.

Tokes, Z.A., Rogers, K.E. and Rembaum, A., *Proc. Natl. Acad. Sci. U.S.A.* 79, 2026–2030 (1982).

Trouet, A., Deprez-Decampenere, D. and De Duve, C., *Nature (Lond.)*, 239 (1972) 110.

Uchman, G. and Grezeskowiak, E., *Farm. Pol.* 37 (1981) 275.

Updike, S.J. and Wakamiya, R.T., *J. Lab. Clin. Med.*, 101 (1983) 679.

Yoshioka, T., Hashida, M., Muranishi, S. and Shozo, H., *Int. J. Pharm.*, 8 (1981) 131.