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Antitumour Activity of Mitoxantrone-loaded Chitosan Microspheres Against Ehrlich Ascites Carcinoma

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Abstract

Glutaraldehyde cross-linked chitosan microspheres containing the antineoplastic agent mitoxantrone were prepared and the antitumour activity was evaluated against Ehrlich ascites carcinoma in mice by intraperitoneal injections. The tumour inhibitory effect was followed by monitoring animal survival time and change in body weight for a period of 60 days.

While the mean survival time of animals which received 2 mg and 1 mg of free mitoxantrone intraperitoneally was 2·1 and 4·6 days, respectively, animals which received 2 mg mitoxantrone via microspheres showed a mean survival time of 50 days. Five out of 8 animals treated using microspheres lived beyond 60 days. The percentage ratio mean survival time of the treated group divided by the mean survival time of the untreated group for animals treated using mitoxantrone-loaded chitosan microspheres containing 2 mg of the drug was 290 compared with 12·2 for those which received 2 mg of the free drug.

The antitumour effect of mitoxantrone-loaded microspheres against Ehrlich ascites carcinoma was much higher than that of doxorubicin-loaded microspheres reported by previous workers. Our data demonstrate the potential of mitoxantrone-loaded chitosan microspheres for sustained drug delivery to minimize drug toxicity and maximize therapeutic efficacy.

Anticancer drugs are often limited in their use by high systemic toxicity, poor stability and short biological half-life (Balis et al 1983), Hence, selective efficient and targeted delivery of drugs to the desired site of action assumes importance in cancer chemotherapy (McLaughlin & Goldberg 1983). Among various drug delivery systems investigated to achieve this goal, biodegradable polymeric microspheres have received much attention (Davis et al 1984). Thus, many protein and polysaccharide based microspheres have been evaluated for sustained and targeted delivery of cytotoxic drugs (Guiot & Couvreur 1986; Puisieux et al 1994).

Chitosan is a deacetylated derivative of chitin, a biodegradable natural polysaccharide second only in abundance to cellulose in nature. It is reported to be suitable for a number of biomedical applications (Allan et al 1984; Muzzarelli et al 1986). In recent years, there has been considerable interest in chitosan as a drug carrier (Zikakis 1984; Ouchi et al 1989; Thanoo et al 1992; Ohya et al 1993; Shiraishi et al 1993). The polysaccharide per se and some of its derivatives have been reported to possess antitumour properties. In recent studies, we have shown that the biodegradability of the chitosan matrix and the release profiles of many drugs incorporated therein could be effectively controlled by glutaraldehyde crosslinking of the polysaccharide (Thanoo et al 1992; Jameela et al 1994; Jameela & Jayakrishnan 1995). Thus, sustained release of the antineoplastic agent mitoxantrone, and a model protein such as bovine serum albumin were demonstrated in-vitro for several weeks from glutaraldehyde crosslinked chitosan microspheres.

Since our in-vitro studies have already shown that glutaraldehyde cross-linked chitosan microspheres could function as a long-acting drug-delivery vehicle for the anticancer drug mitoxantrone, this study was undertaken to evaluate the antitumour activity of mitoxantrone-loaded chitosan microspheres in a tumour model such as Ehrlich ascites carcinoma in mice.

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Materials and Methods

Materials

Chitosan having 74% deacetylation and a molecular weight of $3{\cdot}15 \times 10^5$ Da was from a Central Institute of Fisheries and Technology, Cochin, India and was used without further purification. Glutaraldehyde, (25% solution) and sorbitan sesquioleate were from Sigma Chemical Co., USA. Mitoxantrone was from American Cyanamid Co., New York. Liquid paraffin having a viscosity of 18 cPs at 30°C, petroleum ether and other chemicals were from SD Fine Chemicals, Bombay, India.

Preparation and characterization of mltoxantrone-loaded chitosan microspheres

Mitoxantrone-loaded chitosan microspheres were prepared essentially as reported before except that microspheres of smaller size were prepared using sonication (Jameela & Jayakrishnan 1995). Briefly, 24 mg of the drug was mixed with 6 g of a 4% solution of chitosan in 5% acetic acid containing 2% NaCl and dispersed in a mixture of 35 mL liquid paraffin and 25 mL petroleum ether containing 0·85% sorbitan sesquioleate as stabilizer in a beaker using a probe-type sonicator (Cole-Parmer 4710, USA) for three min. The mixture was then transferred into a 100-mL round-bottomed flask and stirred using a paddle stirrer at 2000 rev min^{-1} for 5 min. Toluene saturated with glutaraldehyde (10 mL) was then added followed by 1 mL 25% aqueous glutaraldehyde after 15 min. After stirring for 2 h, the hardened spheres were centrifuged, washed several times with petroleum ether, twice with 5%

sodium bisulphite, followed by water and finally with acetone and dried. Particle-size analysis of the microspheres was carried out by laser diffraction using a particle size analyser (Galai, CIS-1. Israel). The distribution was plotted using a computer program supplied by the manufacturer. Scanning electron microscopy (SEM) of the microspheres was performed using a Hitachi instrument (Model S 2400, Japan). Mitoxantrone content in the microspheres was determined as reported previously (Jameela & Jayakrishnan 1995).

Antitumour activity in mice

Male Swiss albino mice, 25 to 35 g, were inoculated with 2×10^6 Ehrlich ascites carcinoma cells. On the second day after inoculation, one group of animals (n = 8) was administered mitoxantrone-loaded microspheres equivalent to 2 mg of drug dispersed in 2 mL phosphate-buffered saline containing 0·05% Tween 80 using a 23 G needle intraperitoneally. A second group (n = 5) was injected with an equivalent amount of placebo chitosan microspheres in the same vehicle, and the third and the fourth groups (n = 8) were given 2 and 1 mg of mitoxantrone in the same vehicle; a control group (n = 8) was left untreated. Therapeutic efficacy was determined by recording the change in body weight and survival time of each animal for 60 days.

Results and Discussion

Mitoxantrone, an anthracenedione is a novel anticancer agent with a wide spectrum of antitumour activity. Anticancer activity of mitoxantrone is comparable with that of doxorubicin, methotrexate, cyclophosphamide and cytosine arabinoside against P388 and L1210 leukaemias and B16 melanoma and colon tumour in mice. Compared with doxorubicin., mitoxantrone has much reduced cardiotoxicity (Cheng et al 1979). The pharmacokinetics of mitoxantrone in man and laboratory animals has been reviewed by Batra et al (1986).

Microspheres prepared were smooth and spherical in shape as evidenced by SEM (Fig. 1). Particle-size analysis showed a volume average particle size of 37 ± 13 μm and mitoxantrone analysis showed a drug loading of approximately 4%. In-vitro release studies in 0·1 M phosphate buffer (pH 7·4) at 37°C showed a slow diffusion profile for the entrapped drug molecule reaching only approximately 30% in 2 weeks. The antitumour effect of the mitoxantrone-loaded microspheres was evaluated by following the animal survival data. The percentage mice survived is plotted against time in Fig. 2. Animals

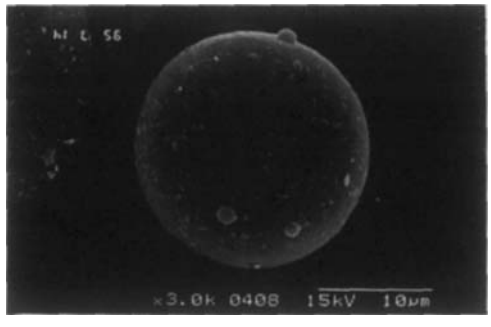

FIG. 1. Scanning electron micrograph of 4% mitoxantrone-loaded chitosan microsphere.

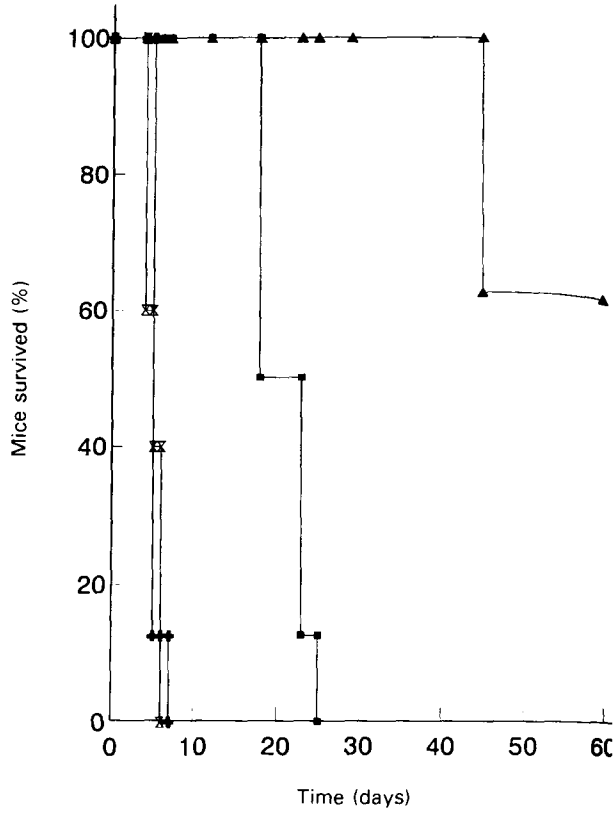

FIG. 2. Percentage survival of mice receiving mitoxantrone therapy plotted against time. (▲) Mice receiving 2 mg, mitoxantrone encapsulated in chitosan microspheres, (■) mice receiving no therapy, (+) mice receiving 2 mg mitoxantrone as free drug, (×) mice receiving 1 mg mitoxantrone as free drug.

which received Ehrlich ascites carcinoma cells but no therapy showed a survival time of $17·2 \pm 1·13$ (mean ± s.e.) days. All animals died within 25 days. The mean survival time of animals (n = 5) which received placebo chitosan spheres was $19·5 \pm 1·1$ days (data not shown) which was not significantly different from the value for the untreated group. All animals in this group died within 22 days. The mean survival time of animals which received therapy via mitoxantrone-loaded chitosan microspheres was $50 \pm 4·6$ days which was significantly different from the value of $2·1 \pm 0·67$ days for those which received 2 mg of the free drug ($P < 0·001$) or $4·6 \pm 0·67$ days for those which received 1 mg of the free drug ($P < 0·001$). Five out of eight animals which received mitoxantrone-loaded chitosan microspheres were still alive at 60 days. In the case of animals which received 2 mg free drug, seven out of eight died within 5 days and the other died on the 7th day. In the case of animals which were given 1 mg free drug, all animals died within 6 days. Drug toxicity was apparent in the groups which received free mitoxantrone. The percent T/C ratio (mean survival time of treated group divided by the mean survival time of untreated control) for the group treated using microspheres as the vehicle was 290 compared with 27 for those which received 1 mg free drug or 12·2 for those which received 2 mg free drug. The LD50 of mitoxantrone administered intraperitoneally in mice and rats ranges from 8·0 to 19·7 mg kg^{-1} (American Cyanamid Co., 1991) It is remarkable that even at a dose of 4 to 8 times the LD50 of the drug, the therapeutic efficacy of the microsphere prepara-

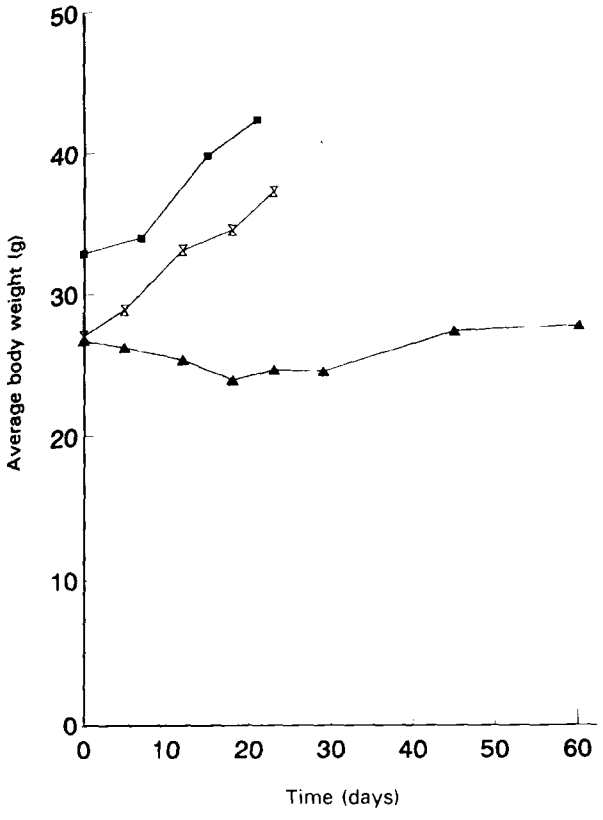

FIG. 3. Average body weight of animals bearing Ehrlich ascites carcinoma and receiving mitoxantrone therapy. (■) Mice receiving placebo chitosan microspheres, (⊠) mice receiving no therapy, (▲) mice receiving chitosan microspheres containing 2 mg mitoxantrone.

tion is very significant. Thus, as seen in the in-vitro studies, the very slow diffusion of the drug from the matrix is believed to be responsible for the excellent therapeutic effect seen in-vivo.

The antitumour effect was also evaluated by following the change in body weight of animals with time (Fig. 3). In the case of animals untreated as well as those which received placebo microspheres, progressive growth of tumour is observed as evidenced by the increase in the body weight. Animals which received mitoxantrone therapy via microspheres showed a more or less constant body weight with respect to time. Therefore, it was evident that mitoxantrone-loaded microspheres were not imparting any toxicity during the period of study. Drug toxicity was apparent in the case of animals which received 1 or 2 mg of the free drug.

There are very few reports in the literature on the antitumour effect of sustained polymeric formulations of antineoplastic agents against Ehrlich ascites carcinoma. Miyazaki et al (1985, 1986) investigated the antitumour effect of 5-fluorouracil-loaded ethylene-vinyl alcohol (EVA) copolymer discs and doxorubicin-loaded fibrinogen microspheres against Ehrlich ascites carcinoma intraperitoneally in mice. Given at a dose roughly corresponding to the LD50 of 5-fluorouracil, the percent T/C ratio observed with EVA discs was approximately twice that of the free drug. With fibrinogen microspheres, however, the percent T/C ratio observed was approximately three times that of free doxorubicin administered at a dose corresponding to 2–5 times the LD50 of the drug. Although 5-fluorouracil is reported to be active against Ehrlich ascites carcinoma, it is not very effective due to rapid elimination. The antitumour activity of doxorubicin and mitoxantrone on the other hand are comparable against many neoplastic diseases. In the present study, it can be seen that the percent T/C ratio of chitosan microspheres containing 2 mg mitoxantrone is approximately 23 times that of the free drug at the same dose. Also noteworthy is the fact that the mean survival time of mice treated via microspheres was 50 days and survival at 60 days was 62·5%. On the other hand, with doxorubicin-containing fibrinogen microspheres administered intraperitoneally against Ehrlich ascites carcinoma, Miyazaki et al (1986) found a mean survival time of 40 days with only one out of six mice surviving at the end of 60 days. It is possible that the much reduced cardiotoxicity of mitoxantrone coupled with its slow sustained release is responsible for the higher survival rate seen in the present investigation.

The data obtained in this preliminary study conclusively demonstrate that intraperitoneal administration of mitoxantrone-loaded chitosan microspheres is an effective means of therapy against Ehrlich ascites carcinoma. The formulation minimizes drug toxicity and enhances the therapeutic efficacy. The biocompatible and biodegradable nature of glutaraldehyde cross-linked chitosan microspheres have already been demonstrated (Jameela et al 1994; Jameela & Jayakrishnan 1995). Therefore, glutaraldehyde cross-linked chitosan microspheres loaded with antineoplastic agents appear to have good potential as a sustained drug delivery system in the treatment of cancer.

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