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Use of lipid microspheres as a drug carrier for antitumour drugs

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9-Oxo-15-hydroxy-$\Delta^{7,10,13}$-prostatrienoic acid methyl ester (Δ^7-PGA_1), an antitumour drug was incorporated into lipid microspheres of 0·2 μm diameter (lipo-Δ^7-PGA_1). In in-vivo experiments, lipo-Δ^7-PGA_1 had a significantly greater antitumour activity than free Δ^7-PGA_1 against P388 leukaemia. Lipo-Δ^7-PGA_1 slightly, but significantly, prolonged the survival time of mice inoculated with L1210 leukaemia, whereas free Δ^7-PGA_1 did not. Against MM46 ascites tumour, the survival time after treatment with 10 mg kg^{-1} of lipo-Δ^7-PGA_1 was significantly greater than that after the same dose of free Δ^7-PGA_1. The results suggest that lipid microspheres can be used as drug delivery carriers for lipid soluble antitumour agents.

The use of liposomes as a drug delivery system in cancer chemotherapy is a promising approach (Fendler & Romero 1977; Kaye 1981). Lipid microspheres of 0·2 μm diameter have similar properties to liposomes in terms of tissue and cellular distribution (Hallberg 1965; Mizushima et al 1982) and lipophilic drugs are suitable candidates for encapsulation. We have incorporated dexamethasone palmitate (Mizushima et al 1982), indomethacin ethoxycarbonyl ester (Mizushima et al 1983a) and prostaglandin E_1 (Mizushima et al 1983b) in lipid microspheres to deliver the drugs to inflamed tissues and vascular lesions. A variety of antitumour drugs have been shown to exert more intensive antitumour activity, when integrated in liposomes (Fendler & Romero 1977; Kaye 1981; Brassinne et al 1983). We have entrapped a prostaglandin with antitumour activity in lipid microspheres and compared the anticancer activity with that of the free drug.

Materials and method

Lipid microspheres containing 9-oxo-15-hydroxy-$\Delta^{7,10,13}$-prostatrienoic acid methyl ester (Δ^7-PGA_1), synthesized as described by Sugiura et al (1984), were prepared by the method of Mizushima et al (1982, 1983a). Briefly, 1 g of Δ^7-PGA_1 was dissolved in 25 g of soya bean oil containing 3 g of yolk lecithin. The mixture was poured into 6·25 g of glycerol and was emulsified in distilled water with a Manton-Gaulin homogenizer, and the final volume was adjusted to 250 ml with water. The final concentration of Δ^7-PGA_1 in the microspheres (lipo-Δ^7-PGA_1) was 4 mg ml^{-1}. Δ^7-PGA_1 was extracted with ethyl acetate from lipid microspheres and quantitative analysis was carried out using HPLC (TSK-Gel OH-120: partition type; Hexane: EtOH = 95 : 5; UV detection at 250 nm) based on the standard curve of Δ^7-PGA_1. To determine the efficiency of entrapment and the ratio of leakage of Δ^7-PGA_1 from lipid microspheres in-vitro, lipo-Δ^7-PGA_1 (0·2 μm diameter) stored at 4 °C for 5 months was filtered through a 0·45 μm and then a 0·1 μm pore size millipore filter. The concentration of Δ^7-PGA_1 in each sample was measured. The results suggested that more than 90% of Δ^7-PGA_1 remained in lipid microspheres.

Antitumour activity. P388 mouse leukaemia cells, L1210 mouse leukaemia cells and the MM46 tumour were used. P388 cells at a dose of 10^6 were inoculated intraperitoneally into male BDF_1 mice aged 7 weeks. L1210 cells at 10^5 were injected intraperitoneally into male CDF_1 mice aged 6 weeks. MM46 tumour cells at 5×10^4 were inoculated intraperitoneally into male C3H/He mice aged 6 weeks.

Lipo-Δ^7-PGA_1 was diluted to a suitable concentration with 11% lipid microsphere suspension before the experiments. Free Δ^7-PGA_1 was dissolved in 100% ethanol at a concentration of 100 mg ml^{-1}, and was suspended in saline to prepare an appropriate concentration of Δ^7-PGA_1 suspension. The maximum concentration of ethanol was 3%. Saline containing 3% ethanol was used as the control solution. Lipid microsphere emulsion or saline alone was injected into the respective controls. The volume of cell suspension and drug solution injected was 0·1 ml per 10 g body weight. The drugs were injected intraperitoneally once a day from day 1 to 4 or 5 in the experiments with P388 and L1210 cells and on days 1, 3, 5, 7 in those with the MM46 tumour, and the survival times were noted.

Results

The mean survival times and percentage increases in life span (ILS) calculated from mean survival times of control and treated groups are shown in Tables 1–3. The antitumour effects of free Δ^7-PGA_1 and lipo-Δ^7-PGA_1 differed with different tumours. With P388 leukaemia cells (Table 1), Δ^7-PGA_1 at 20 mg kg^{-1} day^{-1} slightly increased the life span (%ILS: 18·9%), whereas lipo-Δ^7-PGA_1 at 5 mg kg^{-1} day^{-1} produced 33% ILS. Lipo-Δ^7-PGA_1 at 5 mg kg^{-1} had a significantly greater antitumour activity than free Δ^7-PGA_1 at 5 and 10 mg kg^{-1} against P388 leukaemia. With L1210 cells (Table 2), free Δ^7-PGA_1 did not prolong the survival time, but lipo-Δ^7-PGA_1 showed a small, but statistically significant ILS at doses of 3, 10 and 30 mg kg^{-1}. Against MM46 cells, free Δ^7-PGA_1 and lipo-Δ^7-PGA_1 at 10 and

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Table 1. Antitumour effect of Δ^7-PGA_1 incorporated into lipid microspheres (lipo-Δ^7-PGA_1) on P388 leukaemia in BDF_1 mice (n = 6).

Agents (mg kg^{-1} day^{-1})	Survival time ± s.d.[a] (days)	%[b] ILS
Δ^7-PGA_1		
30	10·8 ± 1·0*	13·7
20	11·3 ± 1·7*	18·9
10	10·3 ± 1·7	8·4
5	9·3 ± 0·7	−2·7
0	9·5 ± 0·9	0·0
Lipo-Δ^7-PGA_1		
30	11·0 ± 0·6**	22·2
20	11·0 ± 0·5**	28·9
10	10·4 ± 0·5**	15·6
5	12·0 ± 1·1**,⋆,⋆⋆	33·0
2·5	10·8 ± 0·7**	20·0
1	9·6 ± 0·5*	6·7
0	9·0 ± 0·0	0·0

BDF_1 mice were inoculated intraperitoneally with 10^6 P388 tumour cells on Day 0. Δ^7-PGA_1 or lipo-Δ^7-PGA_1 was given intraperitoneally on Days 1–5.

[a] Mean ± s.d. [b] percentage increase in life span over that of untreated controls.

*$P < 0.05$, **$P < 0.001$ (in relation to untreated control).

⋆$P < 0.1$, ⋆⋆$P < 0.001$ (between 5 mg kg^{-1} of lipo-Δ^7-PGA_1 and 10 or 5 mg kg^{-1} of Δ^7-PGA_1).

30 mg kg^{-1} markedly increased the survival days of mice. The survival time of 50 days after treatment with 10 mg kg^{-1} of lipo-Δ^7-PGA_1 was significantly greater than that after the same dose of free Δ^7-PGA_1.

Discussion

Fukushima et al (1984) and Fukushima & Kato (1984) demonstrated antitumour activity of Δ^7-PGA_1 in-vitro and in-vivo. We have studied its antitumour effect on mice bearing various tumours when incorporated into microspheres. Δ^7-PGA_1 in lipid microspheres (lipo-Δ^7-PGA_1) exerted a significantly greater antitumour activity than did the free Δ^7-PGA_1 in some tumour systems.

Table 2. Antitumour effect of Δ^7-PGA_1 incorporated into lipid microspheres (lipo-Δ^7-PGA_1) on L1210 leukaemia in CDF_1 mice.

Agents (mg kg^{-1} day^{-1})	Number of mice	Survival time ± s.d.[a] (days)	%[b] ILS
Δ^7-PGA_1			
30	6	8·0 ± 0·6	1·3
10	12	7·9 ± 0·7	0·0
3	6	7·5 ± 0·5	−5·1
0	12	7·9 ± 0·5	0·0
Lipo-Δ^7-PGA_1			
30	11	8·5 ± 0·7**	9·0
10	12	8·6 ± 1·3*	10·3
3	12	9·1 ± 2·4*	16·7
0	12	7·8 ± 0·4	0·0

CDF_1 mice were inoculated intraperitoneally with 10^5 L1210 tumour cells on Day 0. Δ^7-PGA_1 or lipo-Δ^7-PGA_1 was given intraperitoneally on Day 1–4.

[a] Mean ± s.d. [b] Percentage increase in life span over that of untreated controls.

*$P < 0.1$, **$P < 0.01$ (in relation to untreated controls).

Table 3. Antitumour effect of Δ^7-PGA_1 incorporated into lipid microspheres (lipo-Δ^7-PGA_1) on MM46 tumour in C3H/He mice.

Agents (mg kg^{-1} day^{-1})	Number of mice	Survival days[a] (mean ± s.d.)	Survival ratio on[b] 50 days
Δ^7-PGA_1			
30	6	38·0	5/6*
10	18	26·2 ± 12·6	8/18*
3	17	19·3 ± 7·6	0/17
0	24	16·9 ± 3·6	0/24
Lipo-Δ^7-PGA_1			
30	6		6/6*
10	24	29·0 ± 7·3	14/18*,⋆
3	17	19·8 ± 2·1	0/17
0	23	17·7 ± 1·9	0/23

C3H/He mice were inoculated intraperitoneally with 5 × 10^4 MM46 tumour cells on Day 0. Δ^7-PGA_1 or lipo-Δ^7-PGA_1 was given intraperitoneally on Days 1, 3, 5, 7.

[a] Mean survival days for mice which died before 50 days.

[b] Number of survival mice more than 50 days/total animals.

*$P < 0.001$ (in relation to untreated controls).

⋆$P < 0.05$ (in relation to 10 mg kg^{-1} of Δ^7-PGA_1).

Electron microscope studies have indicated that liposomes (Kosloski et al 1978; Todd et al 1980) and lipid microspheres (unpublished observation) are taken up by some tumour cells through fusion and endocytosis, which may partly explain the enhanced antitumour activity of lipo-Δ^7-PGA_1. Another reason for the increased antitumour activity of lipo-Δ^7-PGA_1 may be that lipid microspheres are retained in the abdominal cavity. Moreover, several studies have shown that liposomes (Gregoriadis et al 1974; Richardson et al 1978; Kaye et al 1981) and lipids (Konno et al 1983) accumulate in and around some tumours. Unlike liposomes, lipid microspheres can be produced on a large scale and are stable on storage for 2 years at room temperature. These findings indicate that lipid microspheres have clinical potential as drug delivery carriers of antineoplastic prostaglandins and other lipid soluble antitumour agents.

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Re-evaluation of the L-dopa loading effect on dopamine metabolism in rat striatum

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Investigation by HPLC with electrochemical detection of dopamine (DA) metabolism in rat striatum after L-dopa + benserazide treatment allowed quantification of the time course evolution of DA, 3,4-dihydroxyphenylacetic acid and homovanillic acid levels. Furthermore, four peaks which did not appear in controls, were detected in treated striatum. One was identified as 3-methoxytyrosine, the level of which was still high 9 h after treatment. 3-Methoxytyrosine, has been detected previously in plasma of parkinsonian patients treated with L-dopa, and the disturbance in DA metabolism could explain some of the side-effects induced by that treatment.

L-Dopa with benserazide has been widely used in the treatment of Parkinson's disease (Bartholini et al 1968). L-Dopa loading has been examined in rats for its effects on conditioned avoidance response (Seiden & Martin 1971), circling behaviour (Melamed et al 1984) and 'bizarre social behaviour' (Lammers & van Rossum 1968).

The evaluation of the central biochemical effects of this treatment up to date has been essentially with fluorimetric methods. However, these did not permit the detection of unexpected metabolic modifications. Since 1980, methods that associate a high performance liquid chromatographic separation with an electrochemical detection (LC-ECD) have become available. The detection limit, the specificity, and the wide range of analytical possibilities of these LC-ECD methods allow the simultaneous determination of a large number of catechols and indoles in the same sample. On the chromatograms, in addition to the known peaks, other compounds can be co-eluted and investigated. With these methods, several authors have studied the L-dopa loading effect on the dopamine (DA) metabolism in various tissues (Hefti et al 1981; Beers et al 1984; Ehrenstrom & Johansson 1985).

By using this method, we aimed to investigate the effects of L-dopa + benserazide treatment, and focused our attention on the levels of DA and its metabolites in rat striatum.

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Materials and methods

Male, Sprague Dawley rats (Charles River, France), 225–250 g, maintained under standard conditions were used. The schedule of the treatment was according to a 'balanced lattice design' (Cochran & Cox 1957). Animals were injected with L-dopa (125 mg kg^{-1} i.p.), 30 min after pretreatment with benserazide hydrochloride (50 mg kg^{-1} i.p.). Treatment began every day at 0930h. Control rats received i.p. 0·9% NaCl (saline) injections. Decapitation was 0·5, 1, 1·5, 3, 4·5, 6, 7·5 and 9 h after L-dopa administration. The brain was rapidly removed and striatum isolated on a plate chilled to −15 °C. The samples were stored at −80 °C until assayed. Each time, 5 treated and 5 control rats were used, to take into account eventual circadian changes on the levels of DA and its metabolites. Sample preparation and chromatographic procedure were according to a LC-ECD method previously described (Orosco et al 1985). All chemicals used were of nanograde purity. L-Dopa and benserazide were generously supplied by Roche laboratories; catechol compounds used in the external standard mixture (Fig. 1A) were from Sigma.

Results

The qualitative and quantitative study took into consideration the dopaminergic compounds. In Fig. 1(B), the chromatogram from a control striatum showed the dopaminergic peaks usually measured (3,4-dihydroxyphenylacetic acid (DOPAC). DA and homovanillic acid (HVA). In Fig. 1(C), the chromatogram from a treated rat exhibited the same dopaminergic peaks plus four unexpected peaks. One of these has been identified as 3-methoxytyrosine (3-*O*-Me-dopa), which in the control striatum was barely detectable. In order to verify the identity of this peak, retention times of 3-*O*-Me-dopa