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Dendrimers: Relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of ^{125}I-labelled polyamidoamine dendrimers in vivo

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Abstract

Dendrimers are highly branched macromolecules of low polydispersity that provide many exciting opportunities for design of novel drug-carriers, gene delivery systems and imaging agents. They hold promise in tissue targeting applications, controlled drug release and moreover, their interesting nanoscopic architecture might allow easier passage across biological barriers by transcytosis. However, from the vast array of structures currently emerging from synthetic chemistry it is essential to design molecules that have real potential for in vivo biological use. Here, polyamidoamine (PAMAM, Starburst™), poly(propyleneimine) with either diaminobutane or diaminoethane as core, and poly(ethylene oxide) (PEO) grafted carbosilane (CSi–PEO) dendrimers were used to study systematically the effect of dendrimer generation and surface functionality on biological properties in vitro. Generally, dendrimers bearing -NH_2 termini displayed concentration- and in the case of PAMAM dendrimers generation-dependent haemolysis, and changes in red cell morphology were observed after 1 h even at low concentrations (10 μg/ml). At concentrations below 1 mg/ml CSi–PEO dendrimers and those dendrimers with carboxylate (COONa) terminal groups were neither haemolytic nor cytotoxic towards a panel of cell lines in vitro. In general, cationic dendrimers were cytotoxic (72 h incubation), displaying IC_{50} values=50–300 μg/ml dependent on dendrimer-type, cell-type and generation. Preliminary studies with polyether dendrimers prepared by the convergent route showed that dendrimers with carboxylate and malonate surfaces were not haemolytic at 1 h, but after 24 h, unlike anionic PAMAM dendrimers they were lytic. Cationic ^{125}I-labelled PAMAM dendrimers (gen 3 and 4) administered intravenously (i.v.) to Wistar rats (~10 μg/ml) were cleared rapidly from the circulation (<2% recovered dose in blood at 1 h). Anionic PAMAM dendrimers (gen 2.5, 3.5 and 5.5) showed longer circulation times (~20–40% recovered dose in blood at 1 h) with

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generation-dependent clearance rates; lower generations circulated longer. For both anionic and cationic species blood levels at 1 h correlated with the extent of liver capture observed (30–90% recovered dose at 1 h). ^{125}I-Labelled PAMAM dendrimers injected intraperitoneally were transferred to the bloodstream within an hour and their subsequent biodistribution mirrored that seen following i.v. injection. Inherent toxicity would suggest it unlikely that higher generation cationic dendrimers will be suitable for parenteral administration, especially if they are to be used at a high dose. In addition it is clear that dendrimer structure must also be carefully tailored to avoid rapid hepatic uptake if targeting elsewhere (e.g. tumour targeting) is a primary objective.

Keywords: Dendrimers; Biocompatibility; Drug delivery; Gene delivery

1. Introduction

The growing body of clinical data arising from the development of polymer therapeutics suggests that polymer–protein and polymer–drug conjugates constitute an important new class of anticancer agents (reviewed in Refs. [1–6]). This has aroused considerable interest in the design of second generation polymer-based anticancer treatments, and also in the potential use of polymer therapeutics for management of other diseases. Traditionally, polymer–drug conjugates comprise a linear hydrophilic polymer backbone covalently bound to a potent antitumour drug via a biodegradable spacer [3–5]. However, the novel highly branched macromolecules arising from innovative synthetic chemistry commonly called dendrimers, arborols or cascade polymers offer particular advantages compared with linear polymers. Dendrimers display narrow polydispersity, the possibility to tailor-make their surface chemistry, and the reduced structural density in the intramolecular core is amenable to host–molecule entrapment with opportunities for subsequent controlled release. These interesting nanometre sized architectures were first introduced by the pioneering chemistry of Tomalia et al. [7], Newkome et al. [8], Buhleier et al. [9] and Hawker and Frechet [10], and the last decade has seen an explosion in the number of related structures (reviewed in Ref. [11]).

The vast majority of dendrimers so far described [11] were not intended for pharmaceutical use. They are not soluble in aqueous solutions and their structure would predict general toxicity. However, materials are now being selected more specifically for application as drug-carriers [12,13], gene delivery systems [14–17] and as imaging agents [18]. Opportunities provided by synthetic chemistry continue to expand exponentially. For example, dendrimers have been prepared with surfaces modified by carbohydrate residues [19,20], with multiple arrays of peptidyl epitopes for use as vaccines [21], as dendritic boxes that encapsulate guest molecules [22] and in the form of dendrimer–protein and –antibody conjugates [23,24]. We have recently become interested in the development of dendrimers as carriers for anticancer agents [25] and as drug delivery systems for oral delivery [26]. Unfortunately, there are still very few studies that have systematically investigated the basic biological properties of these novel macromolecules.

For a polymeric carrier to be suitable for in vivo application it is essential that the carrier is nontoxic and nonimmunogenic, and it should preferably be biodegradable. It must display an inherent body distribution that will allow appropriate tissue targeting (reviewed in Refs. [3–5]). Here we have examined the haematotoxicity and in vitro cytotoxicity of five classes of dendrimer (Tables 1 and 2 and Fig. 1). The dendrimers used include three closely related families prepared by the divergent synthesis: polyamidoamine (PAMAM, Starburst™), and poly(propyleneimine) dendrimers with either a diaminobutane (DAB) or a diaminoethane (DAE) core. These molecules were synthesised and characterised as described in the literature [27,28]. The oligoethyleneoxide-terminated carbosilane dendrimers (CSi–poly(ethylene) oxide; CSi–PEO) were prepared by addition of the respective thiols catalysed by AIBN [29,30]. Additionally, preliminary experiments were conducted to determine the haemolytic properties of polyether dendrimers prepared by the convergent route [31] after the methodology first described by Hawker and Frechet [10].

As biocompatibility (general toxicity) of a poly-

Table 1
Characteristics of the dendrimers arising from divergent synthesis

Dendrimer	No. of surface groups	MW range (Da)	Termini
PAMAM dendrimers			
gen 1	8	1,430	$-NH_2$
gen 2	16	3,256	$-NH_2$
gen 3	32	6,909	$-NH_2$
gen 4	64	14,215	$-NH_2$
gen 1.5	16	2,935	$COO^- Na^+$
gen 2.5	32	6,267	$COO^- Na^+$
gen 3.5	64	12,931	$COO^- Na^+$
gen 5.5	256	52,913	$COO^- Na^+$
gen 7.5	1024	212,841	$COO^- Na^+$
gen 9.5	4096	852,555	$COO^- Na^+$
DAB dendrimers			
gen 2	16	1,687	$-NH_2$
gen 3	32	3,514	$-NH_2$
gen 4	64	6,910	$-NH_2$
gen 1.5	16	2151	COOH
gen 2.5	32	4,442	COOH
gen 3.5	64	8,766	COOH
PEA dendrimers			
gen 1	8	745	$-NH_2$
gen 2	16	1,659	$-NH_2$
gen 3	32	3,486	$-NH_2$
CSi–PEO dendrimers			
gen 1	12	4,022	PEO
gen 2	36	12,289	PEO

mer is profoundly influenced by its biodistribution in vivo, the body distribution (1 h) of cationic ^{125}I-labelled PAMAM dendrimers (gen 3 and 4) and anionic ^{125}I-labelled PAMAM dendrimers (gen 2.5, 3.5 and 5.5) was investigated after intraperitoneal (i.p.) or intravenous (i.v.) administration to rats.

2. Materials and methods

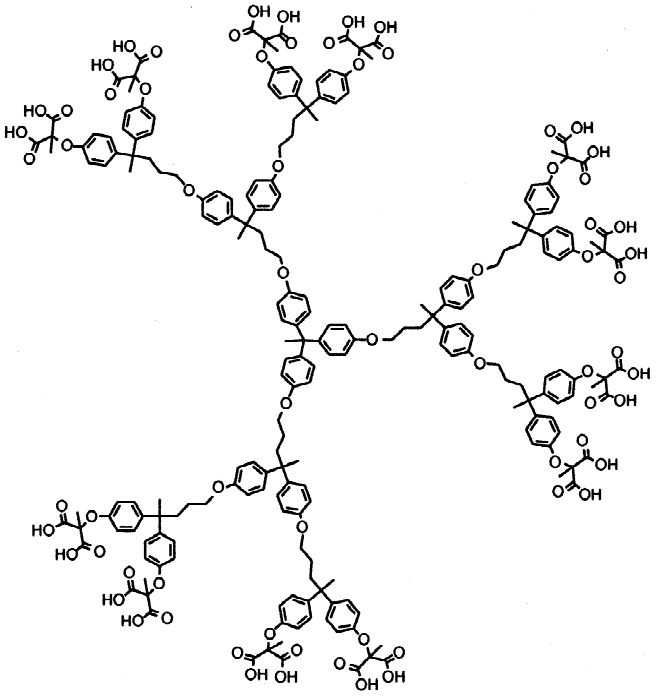
2.1. Materials

Dendrimers prepared by divergent (Tables 1 and Fig. 1) or convergent (Table 2) synthesis were either purchased or obtained via collaboration. PAMAM dendrimers were obtained from Aldrich (UK) or Dendritech (US). DAB dendrimers and DAE dendrimers were prepared by the well known Michael addition–hydrogenation sequence [11] and the CSi–PEO dendrimers were prepared as previously described [29,30]. Preparation and characterisation of the polyether dendrimers is also described in full elsewhere [31]. Full generations (1, 2 etc.) describe amine functionalised surfaces, while the half generations (1.5, 2.5 etc.) describe carboxylic acid or sodium carboxylate end groups at the surface.

All general reagents, 3-[4,5-methylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), ethylenediamine, optical grade dimethyl sulphoxide (DMSO), Triton-X-100, dextran (MW=74 000 Da), poly(L-lysine) (PLL) (MW=56 000 Da), and polyethyleneimine (PEI) (MW=70 000 Da) were from Sigma (UK). 1-Ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was from Pierce Warriner (UK), and ^{125}I-labelled Bolton and Hunter reagent was from Amersham (UK).

Wistar rats were from Banton and Kingman (UK) and all animal experiments were conducted in accordance with the UK Home Office Guidelines. Cells

Table 2
Characteristics of polyether dendrimers prepared by convergent synthesis



gen 2 malonate as a typical structure

Dendrimer	No. of surface groups	MW range (Da)	Termini
Poly ether dendrimers			
gen 0	3	481	Carboxylate
gen 0	6	655	Malonate (carboxylate)
gen 2	12	3292	Carboxylate
gen 2	24	3988	Malonate (carboxylate)

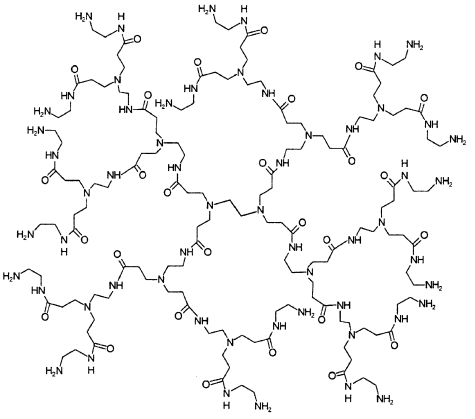
were from ECACC, UK and cell culture media from Gibco (UK). Oxygen (medical grade) and isofluorothane was supplied by Abbott (UK).

2.2. Haemolysis assay

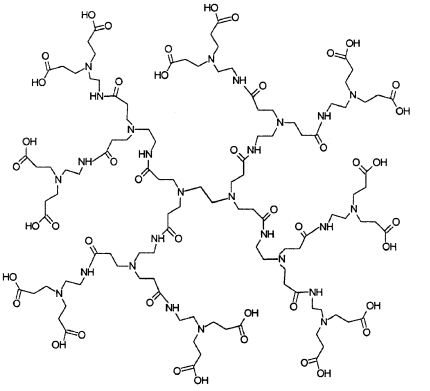
Dendrimers and the reference polymers dextran, PLL or PEI (0–5 mg/ml) were added to a 2% w/v solution of freshly prepared rat red blood cells (RBC) in phosphate-buffered saline (PBS) and incubated for 1 h or 24 h at 37°C in a shaking water bath [32]. At each sample time the solution was centrifuged (1500 *g* for 10 min) and the supernatant assayed spectrophotometrically for the presence of free haemoglobin (optical density=550 nm). Haemolysis is expressed as a percentage of the haemoglobin release induced by Triton-X-100 (1% v/v).

2.3. In vitro cytotoxicity

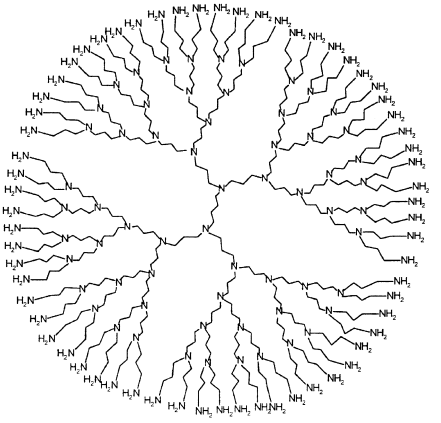
Dendrimers and the reference polymers dextran, PLL or PEI were incubated with three cell lines; B16F10, CCRF or HepG2. Cells were seeded at a



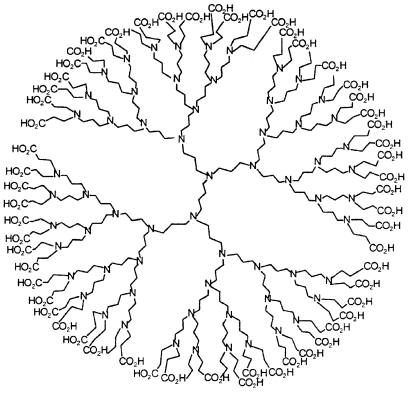
PAMAM
(Amine terminated)



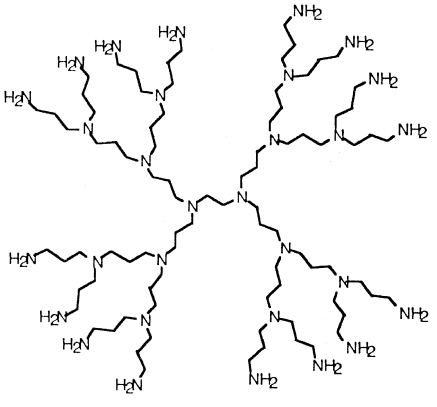
PAMAM
(Carboxylic acid terminated)



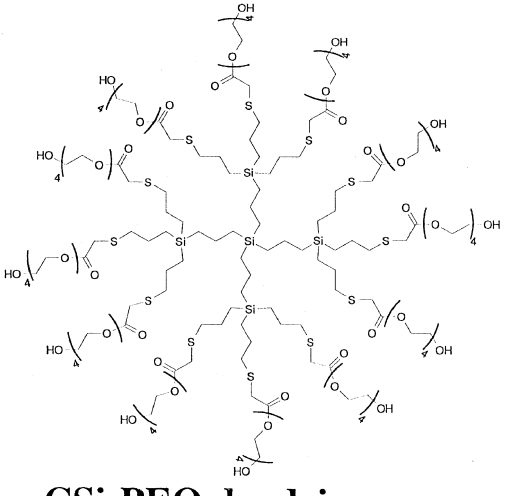
DAB-dendrimers
(Amine terminated)



DAB-dendrimers
(Carboxylic acid terminated)



DAE-dendrimers
(Amine terminated)



CSi-PEO dendrimers

Fig. 1. Structures of the PAMAM (gen 1.5 and 2), DAB (gen 3.5), DAE (gen 2), and CSi–PEO (gen 1) dendrimers.

density of $1 \cdot 10^5$ cells per ml (except CCRF cells which were seeded at $5 \cdot 10^4$ cells per ml) into 96 well microtitre plates using serum-containing media. Cells were left to recover for 24 h before addition of fresh serum-containing medium containing polymer (0–5 mg/ml) and then they were incubated for 72 h. After 67 h MTT (5 mg/ml, 20 μl) was added and the cells left for the final 5 h. At 72 h the medium was removed and DMSO (100 μl) was added to dissolve the MTT crystals and the optical density at 550 nm was measured using a Titerteck plate reader [33]. The viability of cells exposed to dendrimer was expressed as a percentage of the viability of cells grown in the absence of polymer.

2.4. Scanning electron microscopy (SEM)

To obtain samples for SEM, RBC were incubated with dendrimer as above (1 h) or B16F10 cells, the cells were grown at a cell density of $1 \cdot 10^5$ per ml on coverslips in a six well tissue culture plate in the presence of dendrimer (concentrations shown) for 1 h. In both cases cells were then harvested, washed twice with PBS, and then placed in 1 ml of 2.5% SEM grade glutaraldehyde for 24 h. After washing with PBS cells were placed in 1% osmium tetroxide and left for 1 h before step-wise dehydration in increasing concentrations of ethanol solutions and a final wash with 100% ethanol. Cells were resuspended in HMDS and placed onto glass coverslips. After the evaporation of HMDS, gold deposition was performed using a K550 coater set for 5 min at 50 μA.

2.5. Introduction of an amine into PAMAM dendrimers to allow radioiodination by the Bolton and Hunter reagent

Anionic dendrimers (gen 2.5, 3.5 and 5.5) were supplied in methanol (10% w/v) as a sodium salt. Samples (10 mg) were first dried under a stream of nitrogen to a solid residue and then redissolved in double distilled water to give a final concentration of 10 mg/ml. The pH was monitored and adjusted to 6.5 with dilute HCl. EDC (a molar ratio sufficient to modify one carboxylate residue per dendrimer) was added and the reaction left stirring for 30 min at ambient temperature. Ethylenediamine (a molar equivalent to EDC) was then added slowly to prevent crosslinking. The reaction was left for 4 h and unreacted EDC removed by dialysis. The ninhydrin assay was used to verify the number of amino groups on the surface of the cationic- and anionic-modified dendrimers [34].

2.6. ^{125}I-Radiolabelling of PAMAM dendrimers

The cationic PAMAM dendrimers generations 3 and 4 (10 mg), and the anionic dendrimers generations 2.5, 3.5 and 5.5 modified with ethylenediamine (20 mg) were dissolved in borate buffer (pH 8.5, 0.1 M). ^{125}I-Labelled Bolton and Hunter reagent (0.5 mCi; 100 μl in benzene) was carefully dried under a stream of nitrogen. The dendrimer solution was then added and allowed to react for 15 min on ice, mixing periodically. A sample (5 μl) of the reaction mixture was removed for analysis and the remaining solution was carefully purified by dialysis against NaCl (1%). The ^{125}I-labelled dendrimer preparations were then stored at 4°C until use. The labelling efficiency and percentage of free [^{125}I]iodine in each sample was determined by paper electrophoresis.

2.7. Body distribution of ^{125}I-labelled dendrimers

Rats (Wistar, male, 250 g) were injected either i.p. or i.v. with ^{125}I-labelled dendrimer at doses of ~50 000 cpm; specific activity 1–12 μCi/mg. Animals were left in metabolic cages to allow collection of faeces and urine over 1 h at which time they were then humanely killed and the principle organs (liver, heart, lung, spleen and kidney) were removed, the bladder was emptied and all organs washed in PBS, and finally weighed. For i.p. injections an aspirate wash of the peritoneal cavity was also taken. Organs were then placed in 5 ml of water and homogenised using a blade homogeniser. The radioactivity was assessed using a gamma counter and the results expressed as percentage of dose administered accumulating in each organ or the percentage of recovered dose in each organ. Only the results for blood and liver are shown here.

3. Results and discussion

3.1. Haemolytic and cytotoxicity testing in vitro

Observation that the cationic dendrimers (with amine end groups) studied here induced haemolysis (Fig. 2), and were cytotoxic (Fig. 3 and Table 3) was not surprising as the toxicity of polycations such as PLL, PEI and chitosans is well documented [32,35]. Interestingly, subtle differences in haemolytic behaviour were seen according to the precise structure of the dendrimer under study. All cationic dendrimers (except PAMAM gen 1) were lytic above a concentration of 1 mg/ml, but the DAB and DAE dendrimers showed no generation-dependency in activity (Fig. 2a). Both families were equally lytic and this is predictable owing to the similarity of their repeat branch structure. In contrast, PAMAM-induced haemolysis was clearly generation-dependent over the range generations 1 to 4. Although the

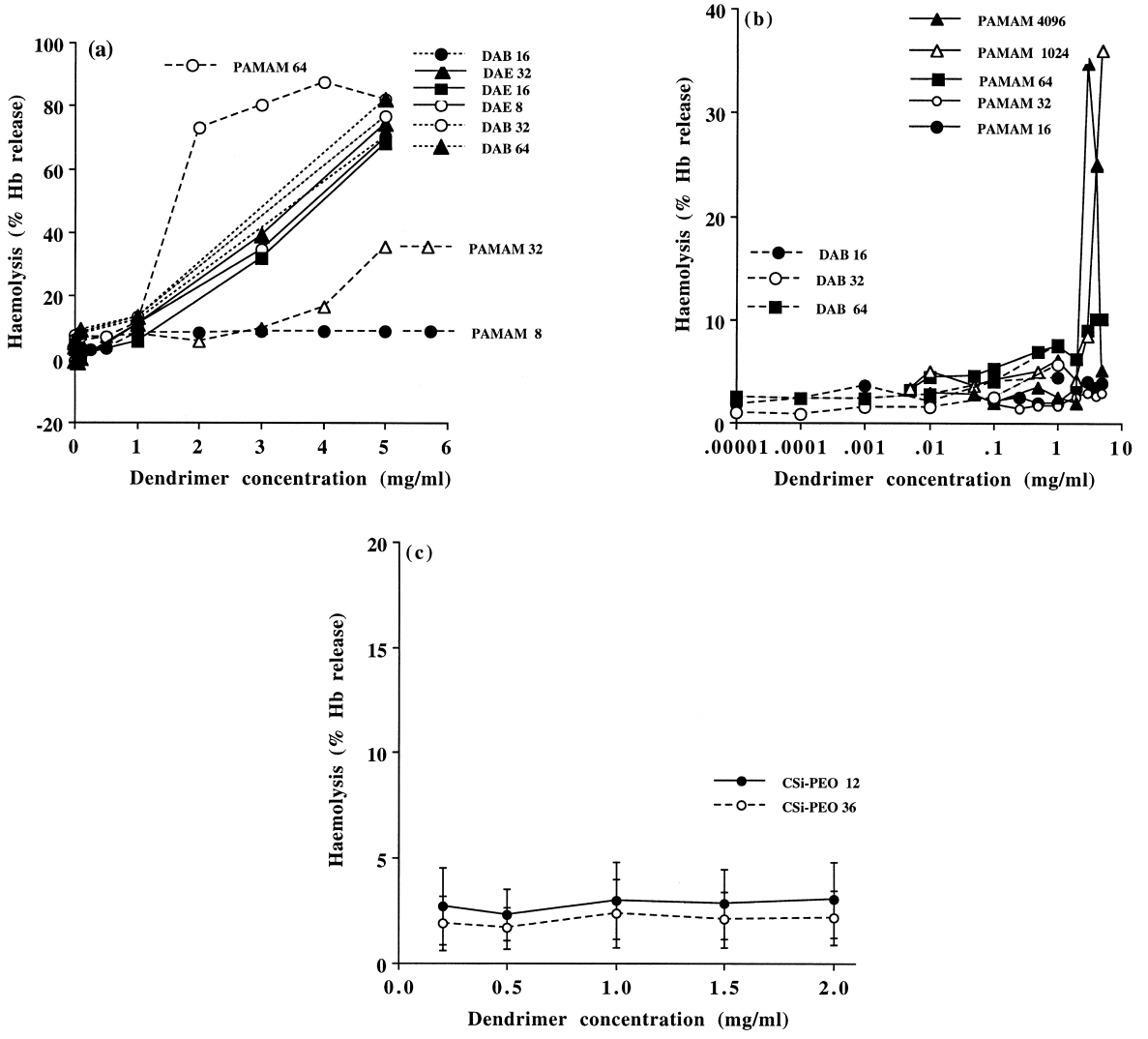


Fig. 2. Dendrimer-induced haemolysis. Panel (a) shows the lysis caused by cationic PAMAM, DAB and DAE dendrimers, panel (b) the lysis caused by anionic PAMAM and DAB dendrimers and panel (c) lysis caused by CSi–PEO dendrimers. To avoid confusion, the key shows the number of surface groups rather than the generation number in each case.

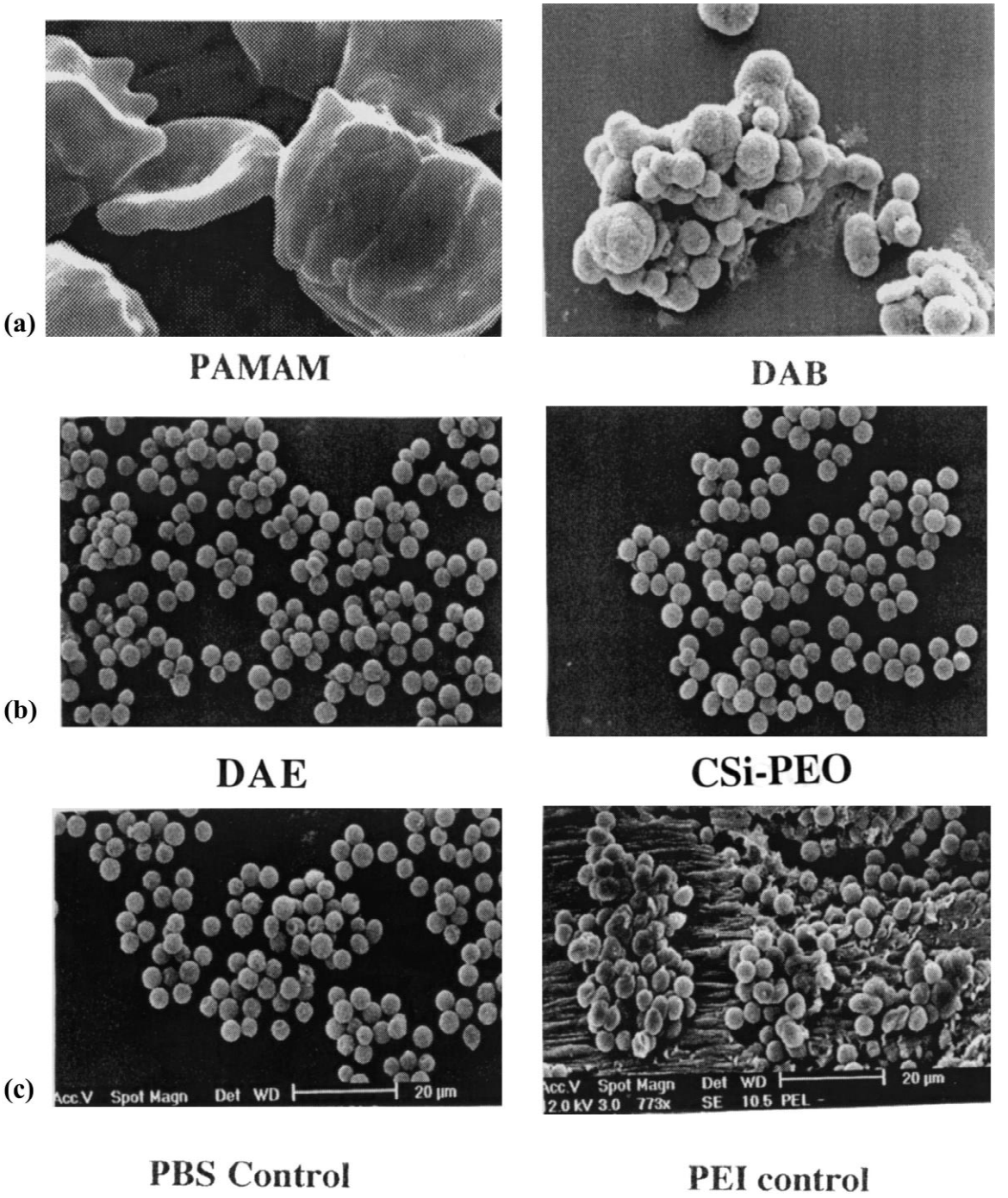


Fig. 3. Cytotoxicity of dendrimers against B16F10 cells. Panel (a) shows the cytotoxicity of cationic PAMAM, DAB and DAE dendrimers, panel (b) the cytotoxicity of anionic PAMAM and DAB dendrimers and panel (c) cytotoxicity of CSi–PEO dendrimers. To avoid confusion, the key shows the number of surface groups rather than the generation number in each case.

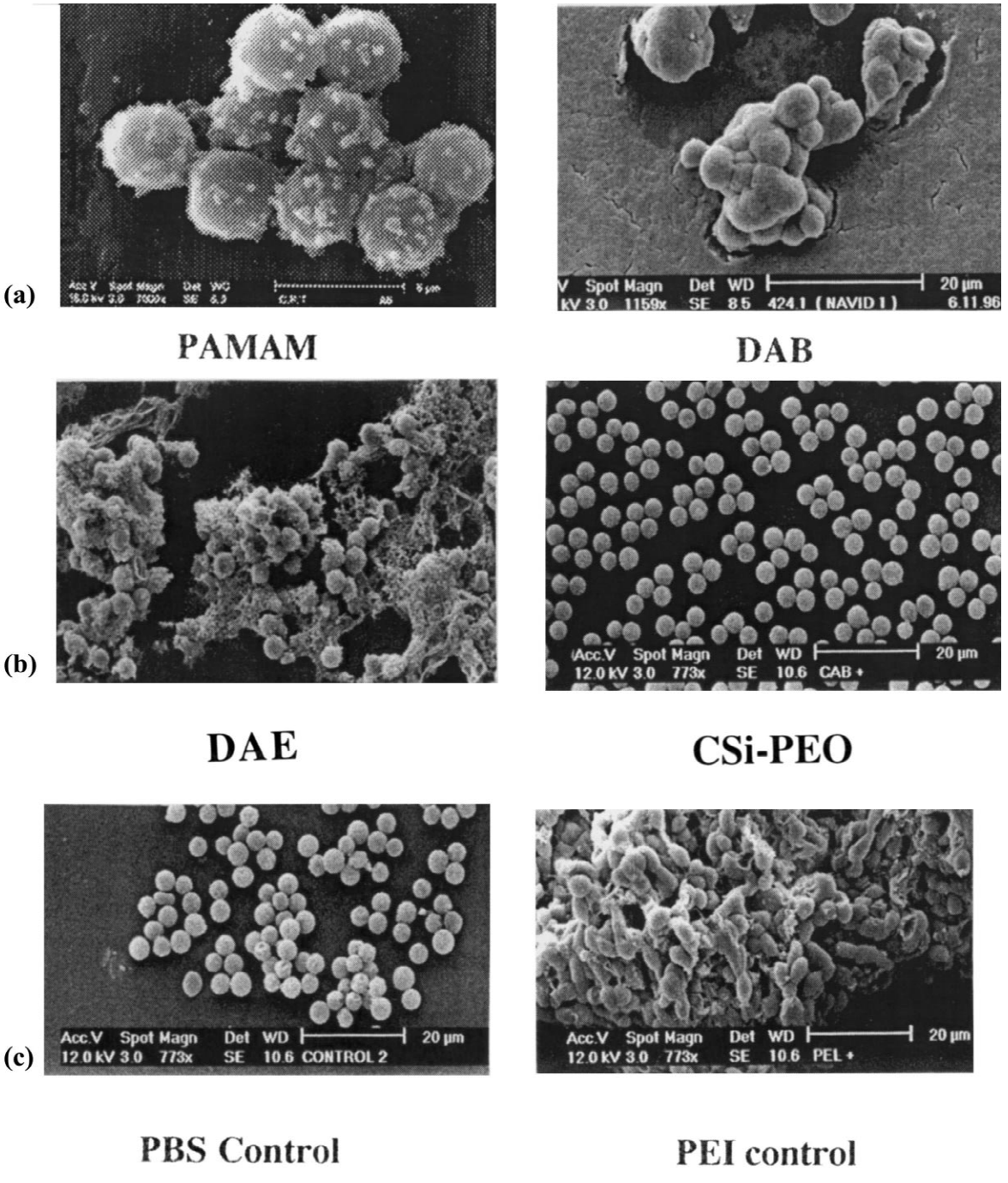


Fig. 3. (*continued*)

Table 3
Cytotoxicity of PAMAM, DAB, PEA and CSi–PEO dendrimer against B16F10 cells

Test substance	Cytotoxicity[a]							
	gen 1.5	gen 2.5	gen 3.5	gen 1	gen 2	gen 3	gen 4	No dendrimer
Dendrimers						0.1	0.1	
PAMAM	>2	>2	>2	>2	>2	0.05	0.05	–
DAB	>5	>5	>5	–	0.3	0.05	0.05	–
PEA	–	–	–	>0.1	>0.1	0.05	–	–
CSi–PEO	–	–	–	>2	>2	–	–	–
Reference controls								
Poly-L-lysine	–	–	–	–	–	–	–	0.01
Dextran	–	–	–	–	–	–	–	>2

[a] Cytotoxicity measured as an IC_{50} value (mg/ml) after 72 h incubation (MTT assay).

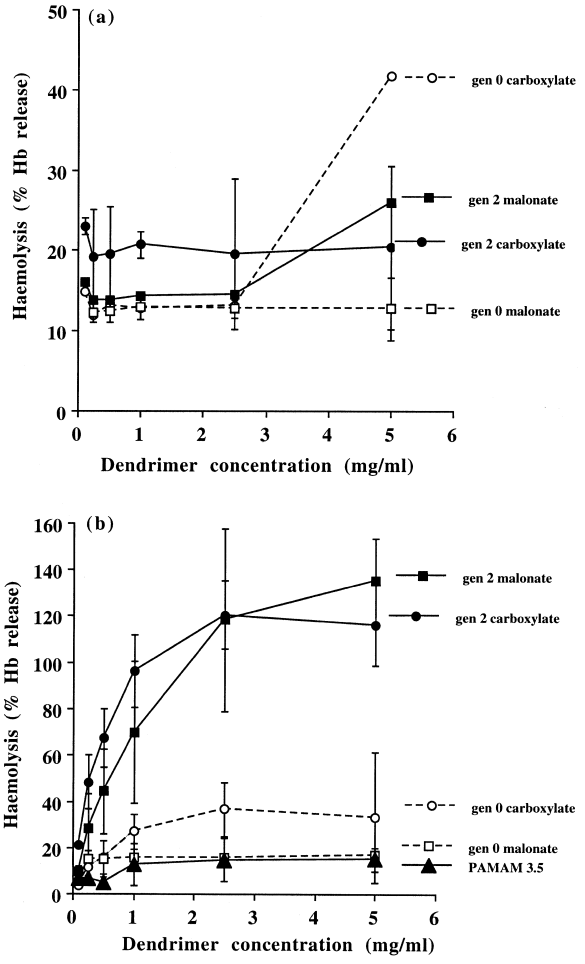


Fig. 4. Haemolysis induced by polyether dendrimers bearing either carboxylate or malonate surface groups. Panel (a) 1 h and panel (b) 24 h.

cationic PAMAM dendrimers, like DAB and DAE dendrimers, have primary amino groups as termini, their interior structure is markedly different with both amide and tertiary amino groups present in the repeat branch unit. This may contribute to the reduced haemolysis observed, but the molecular weight of each dendrimer type for a given number of surface groups is so different that this may also be a contributory factor.

The anionic PAMAM and DAB dendrimers, and also the PEO-modified CSi–PEO dendrimers were not haemolytic up to concentration of 2 mg/ml (Fig. 2a,c), but the higher generation carboxylate PAMAMs were. As the polyether dendrimers contain a large number of aromatic groups one might anticipate haemolytic behaviour as a result of hydrophobic membrane interaction. However, after 1 h, as for the anionic and CSi–PEO dendrimers, no haemolysis was observed, except in the case of the carboxylate core (gen 0) at high concentration (Fig. 4a). Extension of incubation time to 24 h led to a marked increase in haemolysis that was not observed in the case of anionic PAMAMs (Fig. 4b). Further experimentation is required to document in more detail the toxicology and immunogenicity of this type of convergent dendrimer, but it is clear that carboxylate species can be prepared that are much less haemolytic than cationic dendrimers.

Even at a nonhaemolytic concentration (10 μg/ml), cationic PAMAM and DAB dendrimers caused substantial changes in RBC morphology after only 1 h (Fig. 5a). RBCs typically showed a rounded appearance and cells were obviously brought into close contact, probably by dendrimer crosslinking. Exposure to higher dendrimer concentrations (1 mg/

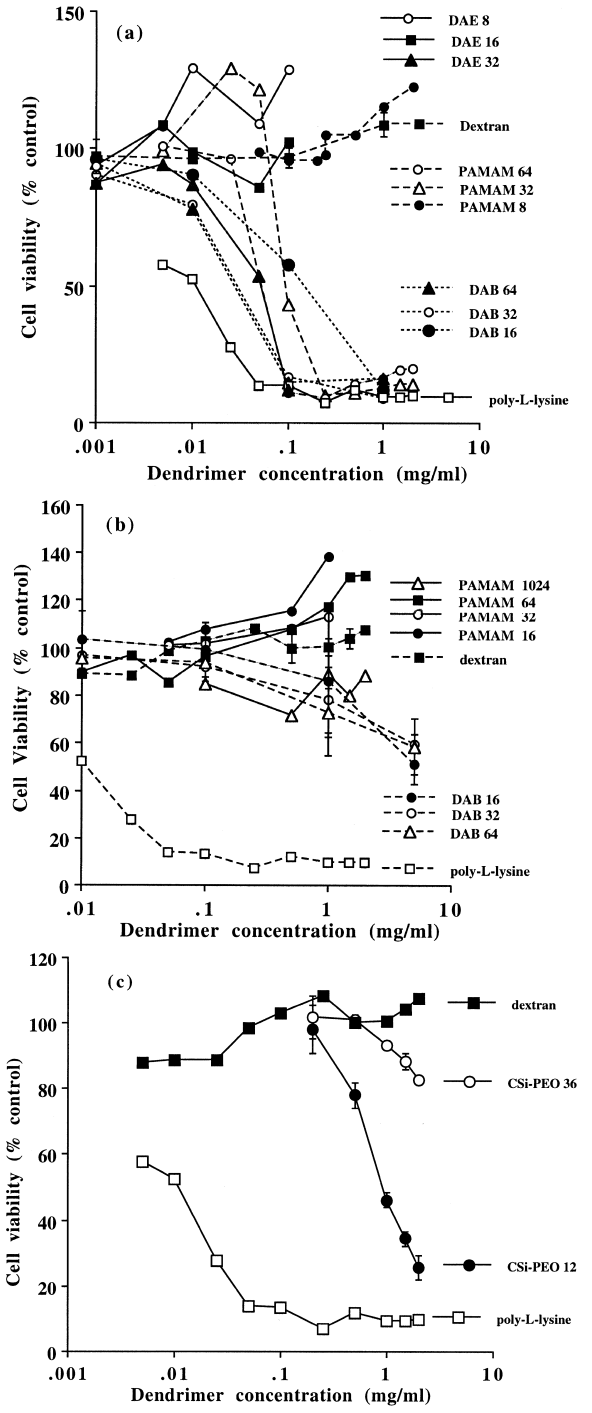


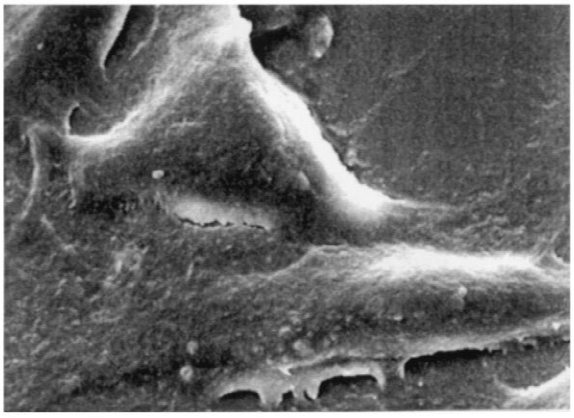
Fig. 5. SEM of RBC incubated exposed to dendrimers for 1 h. Panel (a) shows RBC morphology after incubation with dendrimer or PEI for 1 h at a polymer concentration on 10 μg/ml. Panel (b) shows RBC morphology after exposure to polymers for 1 h at 1 mg/ml.

ml) exaggerated this ''clumping'' behaviour. PEI, the reference polymer, caused substantial membrane damage at a concentration 1 mg/ml (Fig. 5b). RBCs exposed to the anionic PAMAM dendrimers of generation 3.5 to 9.5 showed no morphological changes up to a concentration of 2 mg/ml (results not shown). These experiments illustrate the value of using SEM to detect cellular changes not overtly obvious from the biochemical assay.

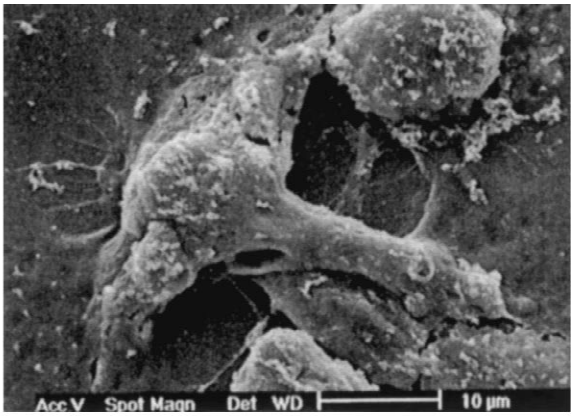
Consistent with the RBC haemolysis study, the anionic dendrimers were not cytotoxic towards B16F10 cells up to concentrations of 1 mg/ml (72 h). Cell viability was never significantly different from the dextran control expect at the highest DAB concentration used (Fig. 3b). SEM of the B16F10 cells confirmed absence of morphological changes in the presence of anionic dendrimers (results not shown). Although the cationic DAE dendrimers generations 1, 2 and 3 and PAMAM generation 1 were not toxic towards B16F10 cells up to the concentration of 100 μg/ml tested, the cationic PAMAM and DAB dendrimers, and DAE generation 4 were all markedly cytotoxic (Fig. 3), displaying IC_{50} values similar to those seen for PLL (Table 3). Again the PAMAM dendrimers showed generation-dependant toxicity. PAMAM dendrimers of equivalent surface functionality were slightly less toxic than DAB dendrimers with the same number of surface groups. Substantial changes in cell morphology were seen after exposure of B16F10 cells to DAB and DAE dendrimers at 1 mg/ml (Fig. 6). No morphological change was apparent 1 h after exposure to PAMAM dendrimers (1 mg/ml), but damage began to appear after 5 h (results not shown).

The cytotoxicity observed is supportive of the observations of Roberts et al. [36] who described concentration- and generation-dependent cytotoxicity of cationic PAMAM dendrimers (gen 3, gen 5 and gen 7) when incubated with V79 Chinese hamster lung fibroblasts for 4 h and 24 h. Cell viability fell to <10% after exposure to PAMAM generations 3 (1 nM), 5 (10 μM) and 7 (100 nM) for 24 h.

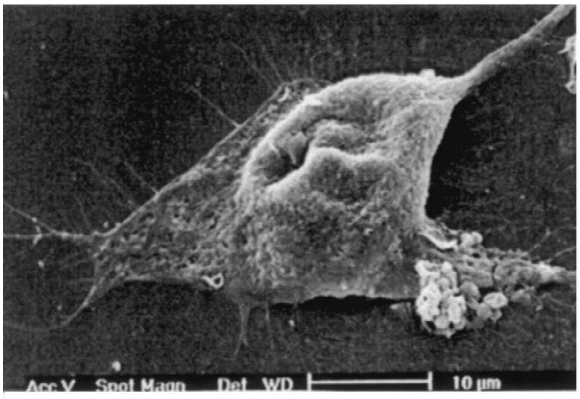
Although the PEO-modified CSi dendrimers were not toxic when incubated (up to 2 mg/ml) with CCRF and HepG2 cells (results not shown), the lower generation CSi–PEO dendrimer was surprisingly cytotoxic towards B16F10 cells at higher concentrations (Fig. 3c). This toxicity diminished as the branching increased. It is interesting to note that



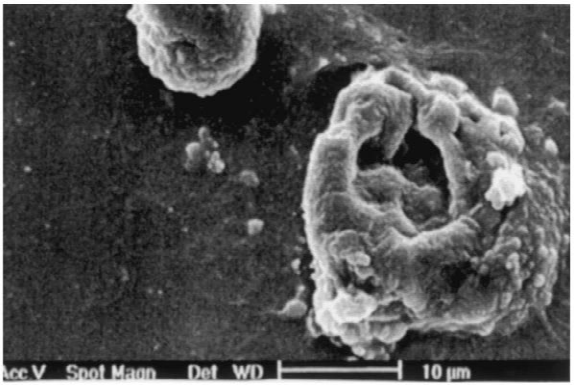
DAB (10μg/ml)



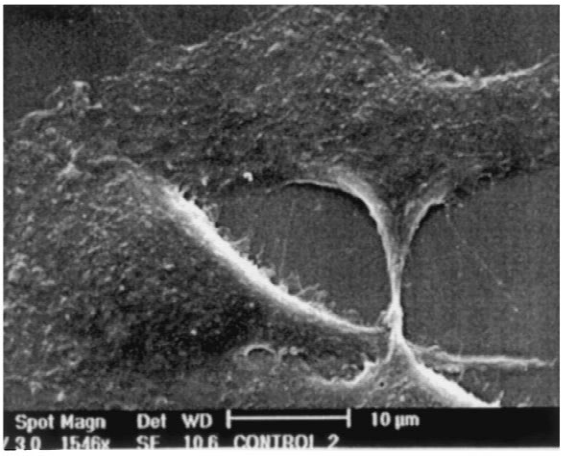
DAB (1mg/ml)



DAE (10μg/ml)



DAE (1 mg/ml)



PBS Control

Fig. 6. SEM of B16F10 cells exposed to dendrimers for 1 h at different concentrations.

a potentially toxic dendrimer core will be more accessible to the cell when presented as a low generation, with a more open molecular structure. Probably the increased branching and a greater surface coverage with biocompatible terminal groups (like PEO) can be more widely used to generate biocompatible dendrimers.

3.2. Body distribution

In vitro biocompatibility assays can only give a relative index of potential toxicity of a new polymeric carrier. Fig. 7 summaries IC_{50} values obtained using standardised conditions and the B16F10 cell line. It is interesting to note that cationic dendrimers are considerably more toxic than most chitosans [35] and linear polyamidoamines [37]. However, the ultimate toxicological profile of any polymeric carrier will depend on its biodistribution in vivo, and the rate, location and mechanism of metabolism. After i.v. and i.p. administration cationic ^{125}I-labelled PAMAM dendrimers were readily cleared from the circulation. Only 0.1–1.0% of the recovered dose was detected in blood at 1 h (Fig. 8). Liver showed by far the highest levels of radioactivity at this time; 60–90% of the recovered dose. Although the anionic ^{125}I-labelled PAMAMs displayed longer circulation times (15–40% of the recovered dose in blood at 1 h) they also showed significant liver accumulation (25–70% of the recovered dose) (Fig. 7). In the i.v. experiments the dose recovered was 70–100% for the cationic PAMAM dendrimers and lower (43–50%) for the cationic PAMAM dendrimers.

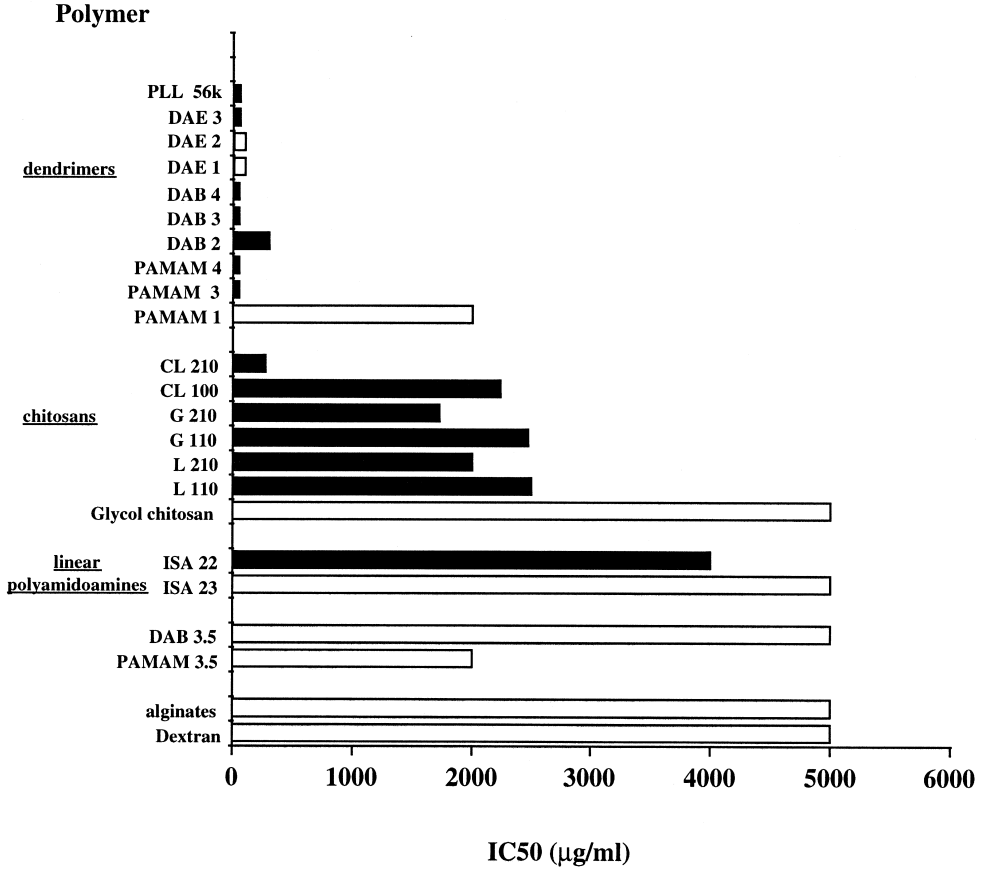


Fig. 7. Comparison of the cytotoxicity of cationic polymers incubated with B16F10 cells (72 h). The empty bars show the highest concentration of polymer used.

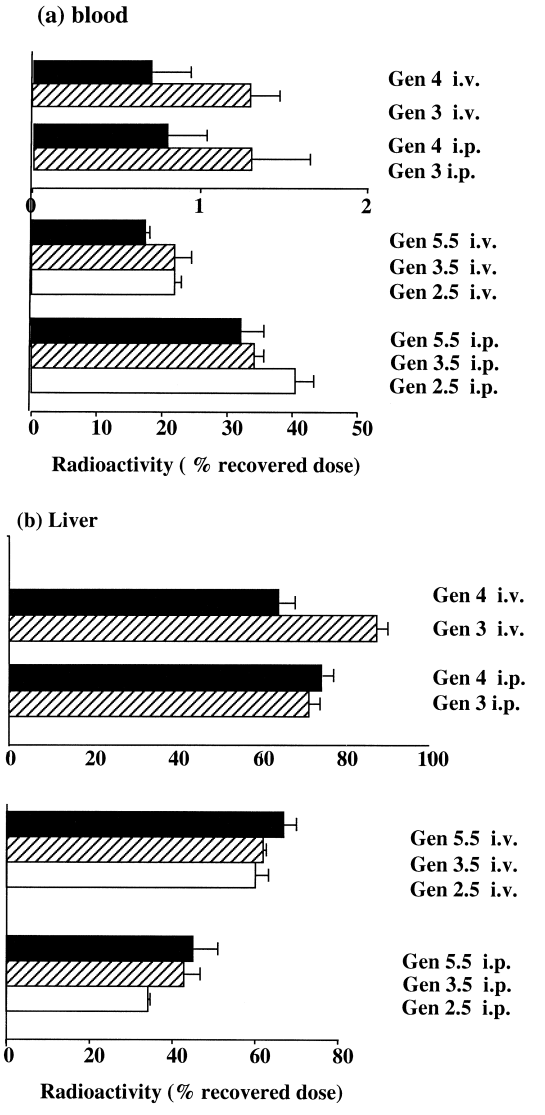


Fig. 8. Blood and liver distribution (1 h) of ^{125}I-labelled PAMAM dendrimers after i.v. and i.p. administration.

Wilbur et al. [38] also showed that ^{125}I-labelled iodobenzoate-biotinylated-PAMAM dendrimers (gen 0,1, 2, 3 and 4) were cleared quickly with low blood levels (0.13–0.2% dose/g) and higher kidney and liver levels at 4 h after i.v. administration. In this case the highest concentration of radioactivity was found in kidney (8–48% dose/g). Similarly, using magnetic resonance imaging (MRI) to follow generation 3 PAMAM dendrimer–gadolinium chelates in rats, Margerum et al. [39] noticed high liver levels still present at 7 days (1–40%). The degree of liver localisation and blood clearance of these probes was related in some fashion to conjugate molecular weight (18.4 kDa and 61.8 kDa probes were used) and also whether or not poly(ethylene) gyycol (PEG) was grafted to the dendrimer surface. With addition of PEG, the blood half-life increased significantly and liver accumulation fell to 1–8% at 7 days. However, no precise correlation of molecular weight with biodistribution was seen suggesting that dendrimer molecular weight, architecture and surface functionality act in concert to determine biodistribution.

There is increasing interest in the potential use of dendrimers as components of vectors for tumour targeting. We have already shown that after i.v. injection, a PAMAM generation 3.5–platinate is able to selectively increase the platinum content of palpable B16F10 subcutaneous tumours approximately 50-fold compared to that seen after i.v. administration of cisplatin at its maximum tolerated dose [13]. This is due to passive localisation of the dendrimer–platinate in tumour tissue by the enhanced permeability and retention effect [13]. Moreover, the dendrimer–platinate displayed antitumour activity in the B16F10 model which is refractory to treatment with cisplatin. Interestingly the generation 3.5 PAMAM–platinate showed lower levels of liver localisation (platinum levels measured by atomic absorption spectroscopy, AAS) than the parent ^{125}I-labelled dendrimer, implying a relationship between the surface carboxylate groups, which are consumed during formation of the platinate ligand, and liver tropism (unpublished data).

Tumour targeting might also be promoted using receptor-mediated localisation of dendrimers and PAMAM dendrimers bearing epidermal growth factor (EGF) are under investigation for this purpose [41]. However, using Fischer rats bearing a C6-EGF transfected glioma it was found that i.v. injection of ^{131}I-labelled boronated-PAMAM generation 4 containing EGF resulted in relatively low levels of tumour localisation; 0.01 and 0.006% dose/g at 24 h and 48 h respectively. Concomitantly 5–12% dose/g of the radioactivity was localised in liver and spleen [41]. EGF receptors in liver or inherent propensity of dendrimer to localise there could be responsible.

The suitability of dendrimers for parenteral administration in the clinical setting will ultimately be

determined by their toxicity in vivo and also the toxicological profile of the drug payload that the dendrimer is designed to carry. Few in vivo toxicological studies involving dendrimers have been reported. Certainly PAMAM dendrimers bearing a carboxylate surface are less toxic than the cationic derivatives. Three daily doses of PAMAM generation 3.5 i.p. at a dose of 95 mg/kg caused no adverse weight change in C57 mice bearing B16F10 tumours [40]. In studies with cationic PAMAM dendrimers, Roberts et al. [36] administered generations 3, 5 and 7 to mice at maximum doses of 2.6 mg/kg, 10 mg/kg and 45 mg/kg respectively. The dendrimers were given either as single dose or repeatedly once a week for 10 weeks. Although no behavioural changes or weight loss was reported, administration of generation 7 did seem to ''have the potential to induce problems'' and 1/5 animals died. In the multiple dose study a degree of liver vacuolarisation was also observed during histopathology. These cationic PAMAMs were not immunogenic as measured by an immunoprecipitation assay and the Ouchterlony double diffusion assay [36]. Plank et al. [42], found that PAMAM dendrimers, high molecular weight PLL and PEI were all strong activators of complement. As optimised formulations of the respective polymer–DNA complexes showed decreased complement activation, it was suggested that problems might be avoided with preparation of appropriate complexes.

4. Conclusions

Regardless of internal repeat unit structure, cationic dendrimers were generally haemolytic and cytotoxic dependent on molecular weight (generation) and the number of surface groups. This has implications for their future use as parenteral drug carriers. Conversely, anionic dendrimers were neither lytic nor cytotoxic over a broad concentration range, and previous studies have shown no evidence of toxicity in vivo after repeated i.p. (95 mg/kg) injection to mice. Dendrimer surface modification using PEO, as illustrated here in the case of CSi–PEO dendrimers, or in the future other hydrophilic coatings may usefully render a potentially unsuitable dendrimer core nontoxic. However, before in vivo use, consideration must be given to the ultimate metabolic fate and likely degradation products that could themselves be harmful. Absolute chemical characterisation of dendrimers is notoriously difficult and often debated. It is noteworthy, that the dendrimers used here (obtained from several different sources) essentially behaved very similarly according to their size and surface characteristics.

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