Biocompatibility of Polyglycidol with Human Peripheral Blood Mononuclear Cells

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ABSTRACT: Polyglycidol (PGly), a hyperbranched water-soluble polyether with numerous terminal hydroxyl groups, has structural similarities with polyethylene glycol. Our laboratory is exploring drug delivery using hyperbranched PGly-cytokines conjugates. Using human peripheral blood mononuclear cells and a tumor derived human B cell line, HBL-1; various PGly concentrations were tested for cytotoxicity. Our data indicated that hyperbranched PGly was similar in toxicity to PEG 8000, a commercial polymer. It was concluded that hyperbranched PGly did not exhibit significant cytotoxicity and showed promise for use as a biocompatible polymer. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 2275–2278, 2009

Key words: polyglycidol; PBMC; drug delivery; toxicity; polyethers

INTRODUCTION

There is an urgent need to identify novel delivery strategies for various candidate therapeutic molecules. The advantages of water-soluble and multivalent biomedical polymers have been widely studied, and these materials have been utilized as carriers in pharmaceutics and biomedical applications.[1,2] The field of "Polymer Therapeutics," although in its infancy, has caught increasing attention from many scientists as it yields potential vectors for drug delivery. Polyethylene glycol (PEG) is one of the most studied and widely used polymers in drug delivery.[3,4] Compared with linear polymers such as PEG and polyglutamic acid,[5] researchers suggest that branched (i.e., dendrimers and dendronized) polymers bring potential advantages in pharmaceutical applications due to their well-defined branched architecture, as reviewed extensively.[6–8] Hyperbranched polymers offer similar advantages to dendrimers, but are much more easily synthesized.

Besides the drug payload capacity of polymers, their safety in humans is an important issue in biomedical applications. Many polymers are cytotoxic, due to the chemistry of their core and surface structures.[2] Some studies have shown that more branched structures result in lower toxicity by increasing the surface coverage of a biopolymer[9,10]; that is, higher surface coverage may prevent cells from contacting the polymer core.[2]

Polyglycidol (PGly), a hyperbranched water-soluble polyether with numerous terminal hydroxyl groups, has structural similarities with PEG.[4] Because of these structural similarities, PGly is also expected to be biocompatible.[11] The properties of biomedical polymers depend not only on the chemistry of the core but also are strongly influenced by the nature of the polymer surface.[12] Thus, PGly is also expected to have the potential for drug delivery by conjugation with drugs or drug delivery candidates, as has been shown for PEG.[13,14] In addition, PGly can also be prepared in the linear form.[4] Hyperbranched PGly self-assembled monolayers were as protein resistant as PEG self-assembled monolayers, and were also thermally and oxidatively more stable,[15] suggesting that PGly is a promising biomaterial.

We are exploring the use of hyperbranched PGly in preparing cytokine conjugates as potential drug delivery systems. As a first step we report here the *in vitro* biocompatible properties of hyperbranched PGly prepared by cationic ring-opening polymerization.[16] Tests of hyperbranched PGly cytotoxicity were performed on human peripheral blood mononuclear cells (PBMC) and a tumor derived human B cell line, HBL-1, at various polymer concentrations.

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EXPERIMENTAL

Materials

Glycidol was purchased from the Sigma-Aldrich Co. (Milwaukee, WI). Boron trifluoride diethyl etherate ($BF_3 : OEt_2$) was obtained from Acros Organics (Geel, Belgium). Dichloromethane and methanol were obtained from Fisher Chemicals (Rockville, MD). All reaction materials were reagent grade; glycidol and dichloromethane, the reaction solvent, were stored over 4 Å molecular sieves, with glycidol being kept refrigerated.

Synthesis and analysis of hyperbranched PGly

Hyperbranched PGly (M_n = 1000 g/mol) was synthesized by $BF_3 : OEt_2$-catalyzed ring-opening polymerization in dichloromethane and analyzed as previously described.[16] Using YM-10 filters (Millipore Corp., Bedford, MA), the PGly was separated into two fractions, high- (>25,000 g/mol) and low-molecular weight (290 g/mol).[16]

Cell lines and culturing conditions

The tumor derived human B cell line, HBL-1, was a gift from Dr. Riccardo Dalla-Fevera, Columbia University, New York.[17] The cell line was cultured in complete RPMI-1640 media with 10 m*M* *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) buffer, 10% heat-inactivated fetal bovine serum (FBS), 2 m*M* L-glutamine, 10,000 IU/mL penicillin, and 10,000 μg/mL streptomycin (Cellgro®, Mediatech, Herndon, VA). Cultures were grown at 37°C with 5% CO_2 in a humidified incubator and subcultured every 3–4 days.

PBMC isolation

PBMC donated from Northwest Florida Blood Bank (Pensacola, FL) were recovered using Sepacell RS-2000 filters.[18] Twenty-five mL of room temperature stored phosphate buffered saline (PBS) were injected into the filter. The PBS pushed the blood into a 50-mL centrifuge tube. Twenty mL of Ficoll-Plaque® plus (Ficoll, Stemcell Technologies, British Columbia, Canada) phase separator were placed in a separate 50-mL centrifuge tube. Thirty mL of blood were gently layered on top of the Ficoll-Plaque to prevent mixing. The mixture was then spun in a centrifuge at 1000 × *g* for 45 min. After discarding the upper plasma layer without disturbing the plasma-Ficoll interface, the retained layer containing mononuclear cells was removed and transferred into a new tube. Mononuclear cells were washed twice with 15 mL PBS buffer and centrifuged at 2000 × *g* for 10 min. Pelleted PBMC was mixed with complete RPMI-1640 media at 10^6 cells per 1 mL and cultured for 24 h in 37°C with 5% CO_2 in a humidified incubator.

Biocompatibility testing

The PGly biocompatibility testing was carried out on 10^6 PBMC cells in 1 mL cell culture media seeded in COSTAR® 48-well flat-bottom cell culture plates (Corning Incorporated, Corning, NY). Five different PGly concentrations were tested at final concentrations of 100, 10, 1, 0.1, and 0.01 μg/mL. To the control cells only RPMI-1640 media was added. Cells were counted at 0, 2, 4, 6, 8, 24, 48, 72, and 96 h, using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay and also by Trypan Blue (Sigma) staining. MTT is a colorimetric assay to determine cytotoxicity of PGly by measuring cell viability. Briefly, yellow MTT is reduced to purple formazan in the mitochondria of living cells. A solubilization solution (10 μL of 0.1*N* HCl in isopropanol) is added to dissolve the insoluble purple formazan product into a colored solution. Because this reduction takes place only when mitochondrial reductase enzymes are active, the conversion can be directly related to the number of viable (living) cells. The amount of purple formazan produced by cells treated with PGly is compared with the amount of formazan produced by untreated control cells. The effectiveness of the PGly in causing death of cells was deduced through the production of a dose-response curve. Results were read using an MRX II Microplate Reader (Dynatech Laboratories) at 540 nm and automatically blanked to 690 nm to reduce background-reading errors.

We also tested the effect of PGly on the HBL-1 cell line using two additional PGly fractions, i.e., high- and low-molecular-weight PGly. Furthermore, we also tested the effect of PEG 8000, as a control, on these cells. PGly and PEG 8000 were tested at final concentrations of 1000, 100, and 10 μg/mL on 10^6 HBL-1 cells in 1 mL cell culture media seeded in a COSTAR 48-well flat-bottom cell culture plates. Cell numbers were counted as described earlier. A higher maximum concentration of PGly was used in tests on HBL-1 cells because this tumor-derived cell line was expected to be more robust than PMBCs.

RESULTS AND DISCUSSION

Chemical structure

The hyperbranched PGly used here was synthesized by a cationic ring-opening polymerization method.[16] This technique is known to yield PGly of molecular weight and polydispersity comparable with that prepared by anionic polymerization. However, the cationic polymerization is substantially simpler and

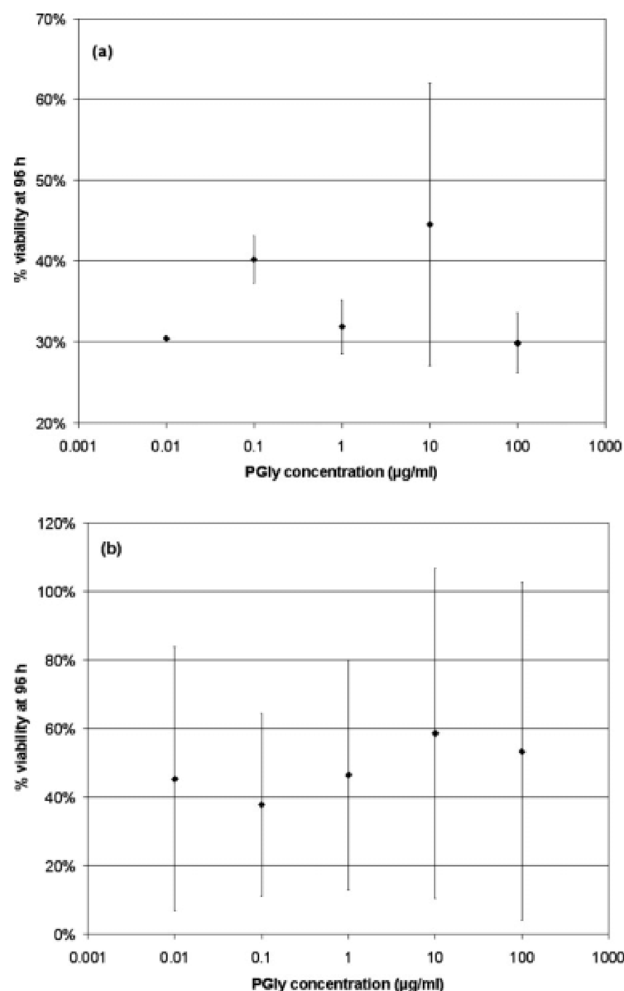


Figure 1 Average percent viability of PBMC at 96 h as a function of PGly concentration: (a) freshly-prepared PGly (error bars represent data range), (b) one-year-old PGly (error bars represent ±1 standard deviation). The average percent viability in the control sample at 96 h was 38%, with a standard deviation of 8%.

faster than the anionic polymerization, since the former technique can be carried out at room temperature and does not need an argon atmosphere. The degree of branching, polydispersity, core groups, and peripheral groups are different for PGlys synthesized by anionic versus cationic polymerization. The NMR spectra of these two PGlys were subtly different, indicating a slight chemical difference between these two substances.[16] The cationically polymerized PGly, for example, contained the dihydroxy end group $-OCH(CH_2OH)_2$, which is not present in anionically polymerized PGly (there is no route to this end group in the anionic polymerization mechanism). Because of these differences, it was necessary to test the cytotoxicity of cationically synthesized PGly, as has been done for its anionically polymerized analog.[4]

Statistical analysis

Student's *t*-tests were performed to differentiate between cell populations at 96 h in the presence of PGly at the highest concentrations used, and in its absence (controls). Cell populations in PGly-containing samples were assumed to have the same standard deviation as controls, so a pooled standard deviation was used in all *t*-tests. For PBMC and HBL-1 cell lines, the *t*-tests revealed that at the 95% confidence level, there was no difference in average cell population between controls and PGly-containing samples. This result was valid for all types of PGly tested, namely freshly prepared PGly, 1-year-old PGly and various fractions of PGly. Similarly, the 96-h population of HBL-1 cell line treated with the highest concentration of PEG 8000 was not statistically different from control, at the 95% confidence level. These findings lead us to conclude that the effects of hyperbranched PGly on cell mortality were not materially different from those of PEG 8000, for these cell types.

To evaluate whether there was a concentration-dependent toxicity effect, the percent viability of the PBMC population at 96 h was plotted as a function of PGly concentration. The viability of cells was calculated at any given time as

$$\%\ \text{viability} = \frac{\text{live cells remaining}}{\text{initial number of cells}} \times 100$$

These plots are shown in Figure 1 for freshly prepared PGly [Fig. 1(a)] and 1-year-old PGly [Fig. 1(b)]. No correlation between cell population and PGly concentration could be found [$R^2 = 0.16$ and 0.19 for the data in Fig. 1(a,b), respectively], suggesting that PGly did not exhibit concentration-dependent toxic effects on these cells.

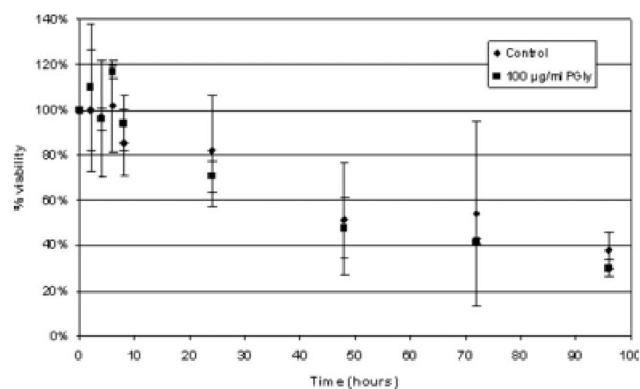


Figure 2 Viability of cells exposed to PGly at 100 μg/mL as a function of time. Viability was calculated as the percent of the initial cell population remaining at any time. Error bars are ±1 standard deviation for controls, and the entire data range for cells cultured with PGly.

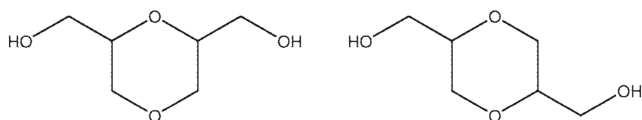
TABLE I
Average HBL-1 Cell Populations at 96 h in the Presence of Various Concentrations of Different PGly Fractions and PEG 8000

Polymer added	Concentration (μg/mL)	Avg. 96 h cell population (millions)
None	–	3.34
PEG 8000	10	2.68
PEG 8000	100	3.09
PEG 8000	1000	3.03
As-prepared PGly	10	3.08
As-prepared PGly	100	2.94
As-prepared PGly	1000	2.69
Heavy-fraction PGly	10	3.11
Heavy-fraction PGly	100	3.18
Heavy-fraction PGly	1000	2.72
Light-fraction PGly	10	2.98
Light-fraction PGly	100	2.80
Light-fraction PGly	1000	2.69

The viability of PBMC exposed to 100 μg/mL freshly prepared PGly is plotted in Figure 2 for a 96-h period, along with the viability of controls. The error bars were ±1 standard deviation for the controls. However, the entire data range was used for the cells exposed to PGly, that is, the error limits represent the maximum and minimum cell populations at the various times when counts were taken. It can easily be seen from this figure that the viabilities are the same for cells grown with PGly and the controls, within the limits of experimental error. Lower concentrations of PGly gave similar results.

Table I shows the average populations at 96 h for HBL-1 cells exposed to as-prepared, heavy-fraction, and light-fraction PGly, as well as to PEG 8000. Cells exposed to PGly showed a ~ 20% lower average 96 h population at 1000 μg/mL PGly compared with controls containing no added polymer. Lower concentrations of PGly and its fractions had no systematic effect on HBL-1 cells. As this behavior was also seen in the PEG 8000 samples (~ 10% lower 96 h population at 1000 μg/mL than controls), it was again concluded that PGly was similar in toxicity to PEG 8000.

There is some evidence that larger molecular weight polymers show increased cytotoxicity.[18] In contrast, there has been some concern about the toxicity of low-molecular-weight polyethers.[19] It is known that cationically polymerized PGly contains a certain amount of low-molecular-weight species including the dimers shown below



These and related species could be expected to form in our polymerization, and indeed, there was evidence for oligomer formation in the gel permeation chromatography scans of this polymer.[16] For this reason, the high- and low-molecular weight fractions of our PGly were separately tested for toxicity in the HBL-1 cell line. It was reassuring to find that high- (>25,000 g/mol) and low-molecular weight (290 g/mol) PGly were indistinguishable from controls at the 95% confidence level, as indicated by the Student's *t*-test.

As in the review of Sharma[20] on the current perspectives on cytokines for antiretroviral therapy in AIDS-relative B-cell lymphomas, our next goal is preparing and testing the stability of PGly-cytokine conjugates.

CONCLUSIONS

Hyperbranched PGly prepared by cationic ring-opening polymerization was tested for toxicity in PBMC and the HBL-1 cell line. Freshly prepared and 1-year-old PGly were tested to assess the shelf life of this polymer. The heavy and light fractions constituting PGly were also tested separately. All results indicated that PGly showed comparable toxicity to PEG 8000, that is, PGly showed promise for use as a biocompatible polymer.

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