The efficiency of a PAMAM dendrimer toward the encapsulation of the antileukemic drug 6-mercaptopurine

Michael F. Neerman

Anti-Cancer Drugs 2007, **18**:839–842

Keywords: antileukemic, dendrimers, drug delivery, encapsulation, 6-mercaptopurine

Department of Pathology, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA

Correspondence to Dr Michael F. Neerman, PhD, Department of Pathology, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75390-9073, USA
Tel: +1214 590 8443; fax: +1214 590 5003;
e-mail: mneerm@parknet.pmh.org

Received 20 November 2006 **Accepted** 12 January 2007

Introduction

Although chemotherapy proves to be an invaluable method for treating leukemia, there are a number of shortcomings that are associated with conventional chemotherapy. One of the most prevalent flaws with many anticancer drugs is that they possess poor aqueous solubility and are thus delivered simultaneously with cosolvents or undergo chemical modification into prodrugs: all of which have the possibility to alter the drug's pharmacokinetics, attenuate the efficacy of the drug or even produce detrimental side effects [1,2]. Therefore, increasing water solubility while not altering the efficacy of anticancer drugs is of great concern.

Formulations facilitating the solubilization of poorly soluble anticancer agents can be designed to reduce the peak plasma concentration and thus extend exposure to an effective drug concentration potentially leading to both attenuation in nonspecific organ toxicity along with increased efficacy [3–6]. An attractive architecture for such an application which has gained much attention in recent years is a class of hyperbranched, macromolecular polymers known as dendrimers [7,8]. Of particular interest are the Starburst polyamidoamine (PAMAM, Dendritech, Inc., Midland, Michigan, USA) dendrimers bearing surface group functionalities ($-NH_2$, $-COOH$ or $-OH$) that can serve as attachment sites for the conjugation of drugs, targeting groups, etc. [9]. Furthermore, the hydrophobic internal environment of the dendrimer facilitates the entrapment of molecules as well as increasing the aqueous solubility of hydrophobic therapeutics [10,11]. The fact that dendrimers are water soluble and can enhance the solubility of poorly water-soluble drugs makes them attractive architectures for potential vehicles for controlled drug release.

A chemotherapeutic drug of interest most commonly used to treat certain leukemias is the antipurine 6-mercaptopurine (6-MP) (Puri-Nethol, DSM Pharmaceuticals, Inc., Greenville, North Carolina, USA). The drug 6-MP is a 6-thiopurine analogue of naturally occurring purine bases: hypoxanthine and guanine, but is a prodrug requiring intracellular activation to thiopurine nucleotides to exert an antileukemic effect [12]. The drug inhibits *de novo* purine synthesis via the inhibition of phosphoribosylpyrophosphate amidotransferase leading to purine deprivation [13–16]. This effect will subsequently initiate the inhibition of DNA synthesis and will decrease cell proliferation [16].

Herein, we report the efficiency of a generation 4, PAMAM dendrimer bearing hydroxyl terminal groups toward encapsulation of the hydrophobic antileukemic drug 6-MP (Fig. 1) and to see what effect pH has on this efficiency.

Materials and methods

Materials

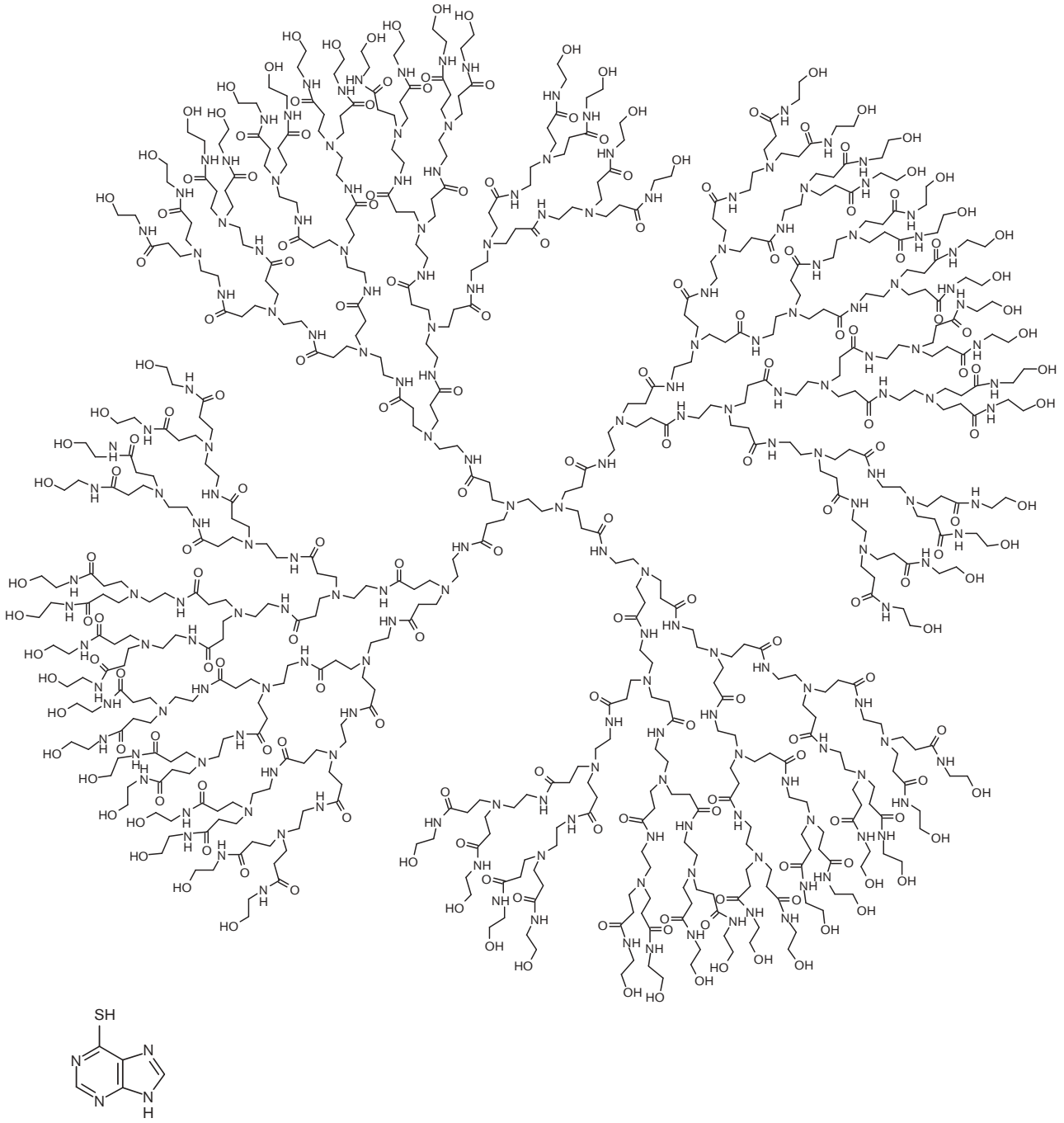
Generation 4, PAMAM-OH dendrimer (molecular weight 14.2 kDa, 64 terminal hydroxyl groups) and 6-MP were purchased from Sigma (St Louis, Missouri, USA). All of the other reagents were of analytical grade.

Solubility studies

As the PAMAM-OH came as a 10% w/v stock solution in methanol, an aliquot was placed in a CentriVap (Labconco Corporation, Kansas City, Missouri, USA) to concentrate the dendrimer. Dendrimer solutions were then prepared (10 mg/ml) in distilled water at differing pH values (3, 5 and 7) and 1 ml of each test solution was added to 5-ml glass vials. To each sample was added an excess of 6-MP (15 mg) and sonicated for 4 h in the dark. After equilibration, the solutions were then subjected to syringe filtration employing a 0.2-μm filter membrane to remove any unbound drug [17]. The resultant solution was then assayed spectrophotometrically ($\lambda = 327$ nm) for the quantitation of encapsulated drug. The approximate number of drug molecules incorporated was determined as follows:

$$\text{Number of drug molecules per molecule of dendrimer} = \frac{\text{moles of drug incorporated/solubilized}}{\text{moles of dendrimer}}.$$

Fig. 1



The generation 4, PAMAM-OH dendrimer and the antileukemic drug 6-mercaptopurine.

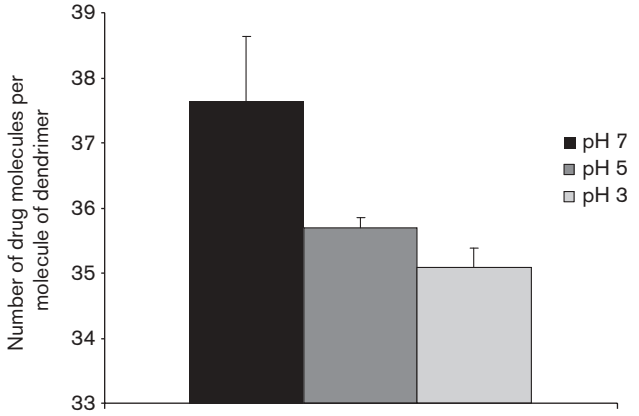
The encapsulation efficiency of drug molecules incorporated was determined as follows:

$$\text{Encapsulation efficiency}\,(\%) = \frac{\text{encapsulated drug}}{\text{total drug added}\,(15\,\text{mg})} \times 100$$

Results

Quantifying the number of 6-MP drug molecules per molecule of a generation 4, PAMAM-OH dendrimer at differing pH values was explored (Fig. 2). It was found that at neutral pH 7, the number of drug molecules incorporated into the dendrimer was the highest at around 38 drug molecules. As the pH dropped to 5 and 3, however, so did the number of drug molecules per dendrimer to around 36 and 35, respectively. Although there was a decrease in the average number of drug molecules incorporated, it is imperative to emphasize that this decrease was not significant.

Fig. 2



The average number of 6-mercaptopurine drug molecules incorporated within each PAMAM-OH dendrimer. Results were done in groups of $n=3$ and expressed as the mean ± SD.

Table 1 The encapsulation efficiency of PAMAM-OH as a function of pH

pH	Encapsulation efficiency (%)
7	26.7 ± 0.67
5	25.5 ± 0.21
3	24.9 ± 0.21

Results were done in groups of $n=3$ and expressed as the mean ± SD.

The percent encapsulation efficiency was also determined by comparing the average amount of drug encapsulated with the total drug added (Table 1). Mirroring the trend in drug molecule incorporation per dendrimer, the percent encapsulation efficiency was also modestly affected by pH. Again, at the highest pH value of 7, roughly 28% of the added drug was incorporated using a 10 mg/ml dendrimer solution. At the lower pH values of both 5 and 3, the percent efficiency was only slightly lowered to around 25%.

Discussion

Previous studies have shown that acidic conditions tend to hinder the encapsulation efficiency of drugs within dendrimers [18,19]. This is presumably owing to the protonation of the dendrimer's internal tertiary amines, thereby decreasing the amount of drug that can be incorporated within the macromolecular system [10]. These results, however, exhibit only a modest decrease in encapsulation efficiency under acidic conditions, therefore it is questionable as to whether this same principle applies. A plausible rationalization is that the hydrophobic interactions between the drug and the dendrimer overcome the immediate effects of protonation of the dendrimer's internal amines.

Shortcomings of this study exist, however, that could also further explain why there is no significant change in drug encapsulation. The first problem resides in the process by which removal of free drug takes place. Many previously described methods rely on syringe filtration to remove any unbound drug. This presents a major limitation in that if a drug possesses minor water solubility, some of the drug could still freely pass through the syringe filter, thereby producing a falsely elevated encapsulated drug level. Moreover, characterization of the dendrimer–drug complex is needed to substantiate as to whether encapsulation is actually taking place and to elucidate the exact mechanism of encapsulation. Finally, subsequent studies are warranted to investigate the long-term effects of pH on the stability of these complexes along with examining the drug release profiles under physiological conditions.

Acknowledgements

The present scientific work was supported by funds from Dr Frank Wians (Department of Pathology, The University of Texas Southwestern Medical Center at Dallas).

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