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Der Pharmacia Lettre, 2012, 4 (3):979-982
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Dendritic architechture for the delivery of anticancer bioactive against myelogenous (AML-193) leukemia

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ABSTRACT

The antileukemic activity of the Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer of Tyrosin kinase inhibitor antitumor bioactive was studied. The ascetic form of myelogenous leukemia AML-193 (transplantation dose 1x10^5 tumor cells/mouse, i. p.), in hybrid mice BDF1, was used as tumor model. An antileukemic activity of the studied Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer was found. The criterion "increase of life span" (ILS %) reached maximally 278.7 % for the drug loaded dendrimer. The studied dendrimer with Imatinib showed lower toxicity with improved antileukemic activity in comparison with free Imatinib. The further experiments in this field are in progress, aiming to design better dendritic formulations, with potential clinical use.

Keywords: Polypropyleneimine dendrimers, PEGylation, Imatinib, antileukemic activity

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INTRODUCTION

Dendrimer represents a novel type of polymeric material. It is also known as starburst [1] or cascade [2] or molecular trees [3] or arborols, or polymers. They attract the increasing attention of all because of their unique structure, high degree of control over molecular weight and the shape that has led to the synthesis of unimolecular micelles [4-7]. Considering the use of dendrimers for drug delivery, it is necessary that they are nontoxic and biocompatible. However, it has been demonstrated that widely used dendrimers, such as PAMAM and poly(propylene imine) (PPI) dendrimers bearing primary amino group termini, are quite cytotoxic, and also these dendrimers were cleared rapidly from the circulation when administered intravenously [8-12]. Poly ethylene glycol (PEG) is typically a clear, colorless odorless substance that is soluble in water, stable to heat, inert to many chemical agents, that does not hydrolyze or deteriorate, and is generally non-toxic, PEG is considered to be biocompatible, which is to say that PEG is capable of coexistence with living tissue or organisms without causing harm, as reviewed earlier [13,14]. It has been shown that covalent attachment of poly(ethylene glycol) to proteins decreases their immunogenicity and increases their circulation time (15,16). Moreover, a number of studies have demonstrated that poly (ethylene glycol) chains grafted to surface of polymer micelles and liposomes suppress their interaction with plasma proteins and cells and prolong their blood elimination half-life [17-22]. On the basis of these findings, it seems that dendrimers covered with poly(ethylene glycol) grafts are attractive compounds as drug carriers in in vivo. The purpose of the present experimental investigations was to assess the antileukemic activity of an Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer in comparison with free Imatinib.

MATERIALS AND METHODS

Materials

PEG4000, Reney Nickel (Sigma, Germany), Raney Nickel (Merck, India), triethylamine, ethylenediamine, acrylonitrile (CDH, India), N, N dicyclohexyl carbodiimide (DCC), Cellulose dialysis bag (MWCO 12-14 Kda, Himedia, India), Imatinib was a benevolent gift from Shasun Pharmaceuticals, Chennai, India.

Methods

Synthesis of 5.0G PPI dendrimer

5.0G PPI dendrimer was synthesized by following the procedure reported by (De Brabender-Van Den Berg and Meijer) [23] using EDA as initiator core [24]. Briefly, ethylenediamine (EDA) was used as initiator core and acrylonitrile was added to it in a double Michael addition-reaction method to produce half generation (-CN terminated), followed by heterogeneous hydrogenation using Reney Nickel as catalyst to produce full generation (-NH_2) dendrimers. The reaction sequence was repeated cyclically to produce PPI dendrimers up to fifth generation (PPI-5.0G) as shown in Figure 1.

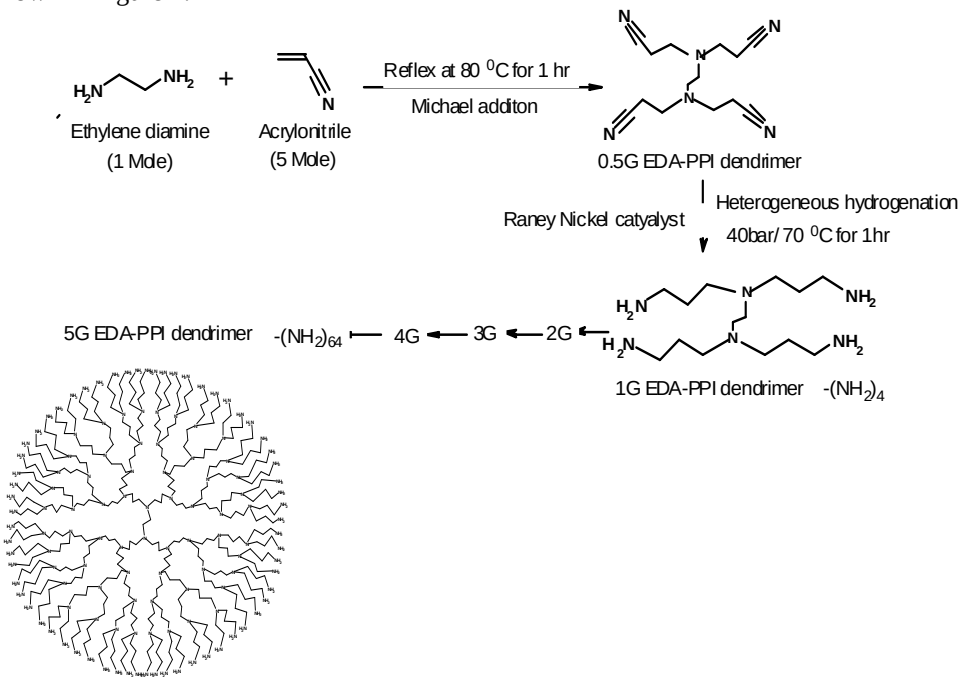
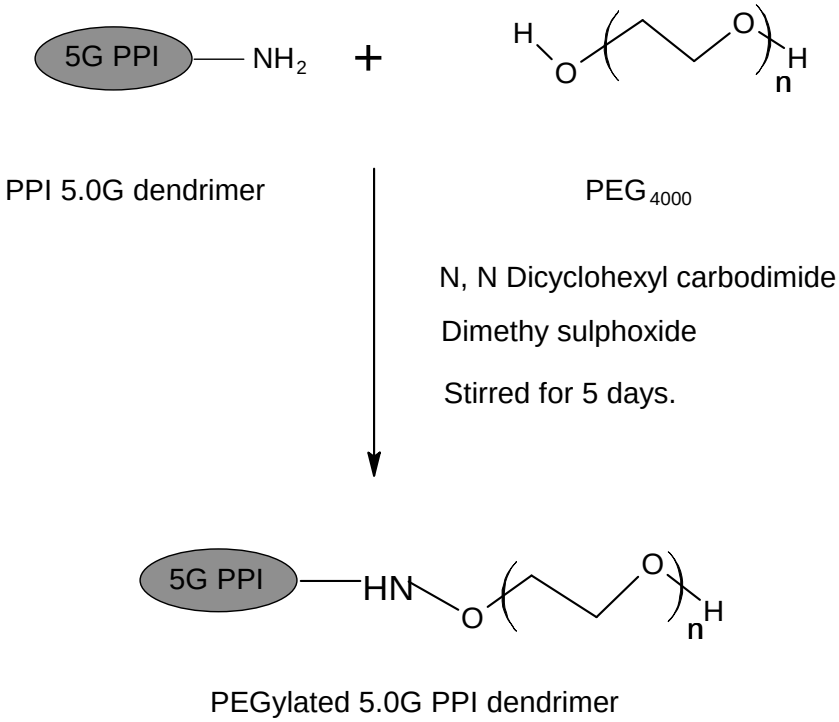


Figure1. Schematic diagram for synthesis of PPI-5G dendrimer



Synthesis of PEGylated 5.0G PPI Dendrimers

To a solution of 5G EDA-PPI dendrimer (0.01 mmol) in dimethyl sulfoxide (DMSO) (10 ml), PEG 2000 (0.32 mmol) in DMSO (10 ml) and N, N dicyclohexyl carbodiimide (DCC) (0.32 mmol) in DMSO (10 ml) were added and the solution was stirred for 5 days at room temperature. The product was precipitated by addition of water,

filtered and dialyzed (MWCO 12-14 Kda, Himedia, India) against double distilled water for 24 h to remove free PEG 2000, DCC and partially PEGylated dendrimers followed by lyophilization (Heto drywinner, Germany).The preparation of PEGylated 5.0G PPI dendrimers was shown in Figure 2.

Drug Loading in Formulation

The known molar concentrations of EDA-PPI dendrimer and PEGylated 5.0G dendrimers were dissolved separately in methanol and mixed with methanolic solution of Imatinib (100 mol). The mixed solutions were incubated with slow magnetic stirring (50 rpm) using Teflon beads for 24 h. These solutions were twice dialyzed in cellulose dialysis bag (MWCO 1000 Da Sigma, Germany) against double distilled water under sink conditions for 10 min to remove free drug from the formulations, which was then estimated spectrophotometrically (λ_{max} 254 nm) (UV-1601, Shimadzu, Japan) to determine indirectly the amount of drug loaded within the system. The dialyzed formulations were lyophilized and used for further characterization.

Antileukemic activity

The in vivo studies were performed in male hybrid BDF1 mice. The antileukemic activity was studied on ascitic form of myelogenous AML-193 leukemia, with transplantation dose of $1x10^5$ tumor cells/mouse, on day 0, intraperitoneally (i. p.). Imatinib and Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer were introduced intraperitoneally, once a day, on day 1, day 4 and day 8 after the tumor transplant. The antileukemic activity was assessed by use of the criterion T/C %, where T was the mean survival time (MST, days) of the drug treated mice, bearing AML-193 myelogenous leukemia and C – the mean survival time (MST, days) of untreated control animals, bearing the same leukemia[25].

Statistical analysis

The activity was assessed by use of the criterion T/C %, where T was the mean survival time (MST, days) of the drug treated mice, bearing AML-193 myelogenous leukemia and C – the mean survival time (MST, days) of untreated control animals, bearing the same leukaemia. T/C %>125% is considered as significant.

RESULT AND DISCUSSION

The polypropyleneimine dendrimer was synthesized by using ethylenediamine as a core. The synthesized dendrimer were further PEGylated with PEG_{4000}. The PEGylated dendrimer is used as carrier system, in which Imatinib was loaded and drug entrapment efficiency was calculated as 61.2±0.03. The antileukemic activity was assessed by use of the criterion T/C %. The results obtained from this study on the effect of Imatinib and its Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer on BDF_1 hybrid mice-bearing AML-193 leukemia are shown on the Table 1. According to these results, the free Imatinib exhibited a pronounced and dose-related antileukemic activity on mice-bearing AML-193 leukemia. An increase of the free Imatinib dose over 0.25 mg/kg x 3, i. p., caused an increase in its acute toxicity. This fact was registered by the progressive decrease in the ratio T/C (treated/control). The dose of the free Imatinib of 2 mg/kg x 3, i. p., was toxic (T/C% < 125%). The Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer exhibited an antileukemic activity against ascitic myelogenous leukemia AML-193 in BDF_1 mice, in four of the used doses – from 0.5mg/kg x 3 to 8.0 mg/kg x 3, i. p., with T/C% varying between 197.2% and 278.7%. The experimental results on activity of the Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer showed that an increase in dose levels of equivalent to the free drug led to an increase in the ratio T/C, indicating lower toxicity. The dose of 8.0 mg/kg x 3, i. p., was not toxic (T/C% = 278.7%). The antileukemic activity of the Imatinib loaded PEGylated Polypropyleneimine (PPI) dendrimer was also higher than the activity of free Imatinib, that was favorable by clinical point of view. The chemical and pharmacological investigations in this field are in progress, aiming to analyse the results and trying to design better formulation of selected antitumor drugs with dendrimers, for potential clinical use.

Table 1.Antileukemic activity of free Imatinib and its Imatinib loaded PEGylated olypropyleneimine (PPI) dendrimer on BDF1 hybrid mice-bearing AML-193 leukemia

Agent	Dose (mg/kg) x 3, i.p	. MST (in days)	T/C (%)
Imatinib	0.25	27.7	256.4
	0.5	27.4	253..7
	1.0	22.5	208.3
	1.5	13.8	127.7
	2.0*	8.3*	76.8*
Imatinib loaded PEGylated (PPI) dendrimer	0.5	21.3	197.2
	1.0	23.7	219.4
	2.0	25.9	239.8
	4.0	27.3	252.7
	8.0	30.1	278.7
Untreated control	0	10.8	-

MST – mean survival time (days); T – survival time of treated mice (days);C – survival time of control mice (days); Significant antileukemic effect at T/C% > 125% was accepted.
** Toxic dose at T/C% < 125%.*

Acknowledgement
The authors are thankful to Prof (Dr). J.Anbu, Department of Pharmacology, Vels University, Chennai. For providing facility as well as guidance to do Antileukemic activity of prepared system.

REFERENCES

[1] DA Tomalia, AM Naylo, WA Goddar , *Angew. Chem*, **1990**, 102-119.
[2] D Tomalia, HD Durst **,** *Top. Curr. Chem*, **1993,** 165-193.
[3] JMJ Fréchet **,** *Science*,**1994**, 263, 1710.
[4] BI Voit **,** *Acta Polym*, **1995**,46-87.
[5] N Ardoin, D Astr**,** *Bull. Soc. Chim. Fr* ,**1995**, 132, 875.
[6] FZeng ,ST Zimmerman, *Chem. Rev*, **1997**, 97, 1681.
[7] FG Johan, A Jansen, MM Elien, DE Brabander- van den Berg, EW Meijer, *Science*, **1994,** 266, 1226.
[8] JC Roberts. MK Bhalbat RT Zera, *J. Biomedical. Mater. Res.,* **1996,** 30, 53.
[9] N Malik, R Wiwattanapapee, R Klopsch, K Lorenz , H Frey, JW Weener, EW Meijer, W Paulus, R Duncan, *J. Control. Release*, **2000,** 65, 133.
[10] C Kojima, K Kono, K Mayuyama, T Takagishi, *Bioconjugate Chem*., **2000,**11, 910.
[11] MF Neerman, HT Chen, AR Parrish, EE Simanek, *Mol. Pharm.* **2004,** 390.
[12] HT Chen, MF Neerman, AR Parrish, EE Simanek, *J. Am. Chem. Soc.*, **2004,** 126, 10044.
[13] D Bhadra, S Bhadra, P Jain, NK Jain, *Pharmazie*, **2002,** 57, 5.
[14] D Bhadra, S Bhadra, NK Jain, *Drug Del. Sci. Tech*., **2005,** 15. 65.
[15] A Abuchowski, NC Palczuk, T Van Es, FF Davis, *J. Biol. Chem*., **1977,** 252, 3578.
[16] A Abuchowski, JR McCoy, NC Palczuk, T van Es, FF Devis, *J. Bio. Chem*., **1977**,252, 3582.
[17] M Yokoyama, T Okano, YSakurai, H Ekimoto, C Shibazaki, K Kataoka, *Cancer Res*., **1991,**51, 3229.
[18] A Klibanov, K Maruyama, VP Torchilin, L Huang, *FEBS Lett*, **1990,** 268, 235.
[19] B Blume, G Cevc, *Biochim. Biophys. Acta*, **1990,** 1029, 91.
[20] MC Woodle, DD Lasic, *BioChim. Biophys. Acta, **1992,*** 1113,171.
[21] DD Lasic, D Needham, *Chem. Rev*., **1995,** 95, 2601.
[22] LD Margerum, BK Campion, M Koo, N Shargill, JJ Lai, A Marumoto, PC Sontum, *J. Alloys Compd*., **1997,** 249, 185.
[23] De Brabender-Van Den Berg and EM Meijer, *Angew Chem Int Ed Engl*. **1993,** 32(a):1665-1688.
[24] D Bhadra, AK Yadav, S Bhadra and NK Jain *Int J Pharm* , **2005,** 295, 221-233.
[25] N Todorova, K Maneva, M Ilarionova *Biotechnol. Biotechnol*. **2003,** Eq., **17** (2), 51-153.