POLY(2-OXAZOLINE)S: AN ALL-ROUND DRUG DELIVERY SYSTEM?

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Introduction

"Similia similibus solvuntur." Like dissolves like. This basic principle has become a major issue in the pharmacy. More than one third of the developed drugs are poorly water soluble and need an excipient in order to allow administration of the drug.[1] Development of such formulations with sufficient loading capacities and adequate final drug concentrations without additional side effects through the excipients continues to be a major challenge. Even more challenging is to discern a drug delivery system that can solubilize a variety of drugs efficiently. Recently, we presented doubly amphiphilic poly(2-oxazoline)s (POx) as high capacity carriers for poorly water–soluble drugs such as paclitaxel (PTX) and cyclosporine A (CsA).[2] Hydrophilic POx (e.g. poly(2-methyl-2-oxazoline)) exhibit good biodistribution[3], stealth properties[4], and when administered alone fast renal excretion.[5] In addition, POx are well tolerated *in vitro* and *in vivo*.[6,7] Here, we wanted to explore their limitations regarding their drug loading. We investigated a variety of other hydrophobic drugs, which were structurally diverse, in order to find a delimiter for POx based drug delivery systems.

Experimental

Materials. PTX, bexarotene (BXT), bortezomib (BTZ) and 17-allylamino-17-demethoxygeldanamycin (17-AAG) were purchased from LC Laboratories (Woburn, MA). Amphotericin B (AmB) was purchased at Riedel-de Haën (Seelze, Germany) and CsA at Alexis Corporation (San Diego, USA). Etoposide (ETO) and all other substances were obtained from Aldrich (München, Germany) and Acros (Geel, Belgium) and were used as received unless otherwise stated. Methyl trifluoromethylsulfonate (MeOTf), 2-methyl-2- oxazoline (MeOx), 2-butyl-2-oxazoline (BuOx), 2-nonyl-2-oxazoline (NonOx), chlorobenzene (ClBz) and acetonitrile (ACN) were refluxed over CaH_2, distilled and stored under nitrogen.

Instrumentation. NMR spectra were obtained with a Bruker DRX 500 P (^{1}H: 500.13 MHz) at room temperature (RT). The spectra were calibrated to the signals of residual solvent signals. Gel permeation chromatography (GPC) was performed on a Polymer Laboratories GPC-120 (1x PSS GRAM analytical 1000 and 1x PSS GRAM analytical 100) with N,N-dimethyl acetamide (5 mmol/L LiBr, 70 °C, 1 mL/min) as eluent and polymethylmethacrylate as standards. CEM Discover microwave was used for polymerization with a maximum power setting to 150 W. HPLC analysis was performed with an Agilent Technologies 1200 Series HPLC system (Nucleosil C18-5µ column, 250 mm x 4 mm). Dynamic light scattering (DLS) was performed with a Nano-ZS (Malvern Instruments Inc., UK). Pyrene fluorescence measurements were conducted with a HoribaJobinYvon Fluoro-Max4.

Synthesis of Methyl-P[MeOx$_{40}$-b-BuOx$_{21}$-b-MeOx$_{34}$]-piperazine. Under dry and inert conditions 0.29 g (1.76 mmol, 1eq) MeOTf and 5.24g (61.6 mmol, 35 eq) MeOx were dissolved in 30 mL dry ACN/ClBz (50/50, v/v) at RT. The mixture was irradiated for 60 min. After cooling to RT 4.37 g (34.4 mmol, 20 eq) BuOx were added and irradiated again. The procedure was repeated with 5.13 g (60.3 mmol, 34 eq) MeOx. Termination was carried out with 1.00 g (5.4 mmol,3 eq) 1-BOC-piperazine at RT and stirred over night. An excess of potassium carbonate was added and stirred for several hours. After filtration the mixture was concentrated, added into a mixture of chloroform/methanol (75/25, v/v) and thrice precipitated in cold diethylether. The residual was lyophilized and 13.2 g of a colorless powder were obtained. ^{1}H-NMR (ACN, 300 K): δ[ppm] = 3.38 (br, 379H, N-CH_2CH_2); 2.98/2.85 (m, 3H, N-CH_3^{Ini}); 2.33-2.18(m, 59H, CO-CH_2^{butyl}, CH_2^{Pip}); 2.04-1.08 (m, 222H, CO-CH_3); 1.52 (br, 42H, CH_2-CH_2-CH_2-); 1.42 (br, 7H, CH_3^{BOC}); 1.32 (br, 42H, CH_2-CH_3); 0.90 (br, 62H, CH_3^{butyl}).

5.1 g of the obtained polymer were dissolved in 32 mL of a mixture of trifluoroacetic acid (TFA), triisobutylsilane and water (95/2.5/2.5, v/v/v) and stirred for 3h at RT. The volatiles were removed under fine vacuum. The residual was dissolved in 25 mL DI water, transferred into a dialysis bag (MWCO 3500 g/mol) and dialyzed against 3 L DI water. The solution was recovered from the bag, lyophilized and 3.7 g of a colorless powder were obtained. GPC (DMAc): M_n= 11.6 kg/mol ($Đ_M$ =1.14).

Synthesis Methyl-P[MeOx$_{33}$-b-BuOx$_{26}$-b-MeOx$_{45}$]-piperazine. Synthesis was done accordingly using 0.30 g (1.82 mmol, 1 eq) MeOTf, 5.44 g (63.9 mmol, 35 eq) MeOx, 4.57 g (35.9 mol, 20 eq) BuOx, 5.34 g (62.7 mmol, 34 eq) MeOx and 1.03 g (5.6 mmol, 3 eq) 1-BOC-piperazine. 13.65 g of a colorless powder were obtained. ^{1}H-NMR (ACN, 300 K): δ[ppm] = 3.43 (br, 387H, N-CH_2CH_2); 2.98/2.85 (m, 3H, N-CH_3^{Ini}); 2.33-2.18(m, 48H, CO-CH_2^{butyl}, CH_2^{Pip}); 2.04-1.08 (m, 226H, CO-CH_3); 1.51 (br, 44H, CH_2-CH_2-CH_2-); 1.41 (br, 9H, CH_3^{BOC}); 1.31 (br, 45H, CH_2-CH_3); 0.89 (br, 65H, CH_3^{butyl}).
9.92 g of the polymer were deprotected with 60 mL TFA. 7.19 g colorless product were obtained. GPC (DMAc): M_n= 11.4 kg/mol ($Đ_M$ =1.14).

Synthesis Methyl-P[MeOx$_{34}$-b-NonOx$_{12}$-b-MeOx$_{34}$]-piperazine. Synthesis was done accordingly to [2] with 21.4 mg (0.13 mmol, 1 eq) MeOTf, 0.401 g (4.71 mmol, 36 eq) MeOx, 0.336 g (1.70 mmol, 13 eq) NonOx, 0.393 g (4.62 mmol, 36 eq) MeOx and 75.0 mg (4.03 mmol, 3 eq) 1-BOC-piperazine. 0.79 g of a colorless powder were obtained. ^{1}H-NMR (MeOD, 300 K): δ[ppm] = 3.53 (br, 320H, N-CH_2CH_2); 3.10/3.06/2.95 (m, 3H, N-CH_3^{Ini}); 2.58-2.33(m, 31H, CO-CH_2^{nonyl}, CH_2^{Pip}); 2.12-2.09 (m, 203H, CO-CH_3); 1.59 (br, 25H, CH_2-CH_2-CH_2-); 1.46 (br, 7H, CH_3^{BOC}); 1.31 (br, 151H,- CH_2-); 0.91 (br, 36H, CH_3^{nonyl}).
0.50 g of the polymer were deprotected with 3 mL TFA. 0.46 g colorless powder were obtained. GPC (DMAc): M_n= 11.8 kg/mol ($Đ_M$ =1.14).

Drug solubilization. Solubilzation was performed as described before.[2] Quantification of drug loading is defined via loading capacity LC (m_{drug}/m_{total}).

HPLC analysis of drug solubilization. PTX and 17-AAG were analyzed under isocratic conditions of ACN/ H_2O (55/45, v/v). BXT was determined using ACN/MeOH/H_2O (40/50/10, v/v/v) at 30 °C and 1 mL/min. HPLC analysis of ETO was performed with a step-wise gradient. First the analyte was eluted with ACN/MeOH/H_2O (5/5/90, v/v/v) for 10 min followed by ACN/ H_2O (60/40, v/v) for another 10 min. Column temperature was 40 °C. For BTZ analysis the mobile phase started for one minute with ACN/ H_2O (90/10, v/v) followed with ACN/MeOH/H_2O (35/35/30, v/v/v) for 10 min. The flow rate was 2.0 mL/min and column temperature was 55 °C. Detection was performed at 227 nm for PTX, ETO, 333 nm for 17-AAG and 270 nm for BTZ. HPLC analysis of CsA and AmB was described previously.[2]

Results and Discussion

(Physico)-chemical properties of amphiphilic POx. Living cationic ring-opening polymerization of 2-oxazolines allows the reproducible synthesis of precisely tailored, amphiphilic block copolymers. Three new batches of the triblock copolymer P[MeOx$_{37}$-b-BuOx$_{23}$-b-MeOx$_{37}$], which we used in our first studies are compared with a triblock copolymer containing a NonOx block (**Table 1**).

Table 1: Analytical Data and Composition of Triblock Copolymers Used in This Study.

Polymer composition[a]	M_n[a] [kg/mol]	M_n[b] [kg/mol]	Đ[b]
P[MeOx$_{37}$-b-BuOx$_{23}$-b-MeOx$_{37}$][c]	9.3	10.8	1.18
P[MeOx$_{33}$-b-BuOx$_{26}$-b-MeOx$_{45}$]	10.0	11.4	1.14
P[MeOx$_{40}$-b-BuOx$_{21}$-b-MeOx$_{34}$]	9.1	11.6	1.14
P[MeOx$_{34}$-b-NonOx$_{12}$-b-MeOx$_{34}$]	8.7	11.8	1.14

[a] As determined by end-group analysis from ^{1}H NMR spectroscopy.
[b] As determined by gel permeation chromatography.
[c] Data from Ref. 2.

Poly(2-butyl-2-oxazoline) is the first POx in the homologue series of poly(2-alkyl-2-oxazoline)s, which is non-watersoluble. However the repeating amide motif provides a polar group throughout the entire backbone. The resulting polarity within the hydrophobic core was determined with the solvatochromic fluorescence probe pyrene. As expected

the fluorescence intensity at 374 nm increased and a red shift in the excitation spectra of the (0,0) band from 333 to 339 nm was observed as is typical upon micelle formation due to the incorporation of pyrene into the micellar core.[8] However instead of a decrease in the ratio of the first and third band (I_1/I_3) of the vibrational fine structrure of pyrene, an increase was observed ($I_1/I_3 = 2.1$) in comparison to pyrene dissolved alone in water ($I_1/I_3 \sim 1.8$). Thus the microenvironment within the core appears more polar than water. In contrast the triblock copolymer containing a NonOx block features a decrease in ratio (Table 2), exhibiting a less polar microenvironment .

Table 2: Micellar Core Polarity and Loading Capacity (LC) of PTX of BuOx and NonOx Containing Triblock Copolymers in Comparison.

Polymer composition[a]	I_1/I_3	LC (PTX) [wt.%]
P[$MeOx_{37}$-b-$BuOx_{23}$-b-$MeOx_{37}$][a]	2.1	45.1
P[$MeOx_{34}$-b-$NonOx_{12}$-b-$MeOx_{34}$]	1.3	9.8

[a] Data from Ref. 2.

Interestingly, when comparing the solubilization capacity of PTX the polar BuOx core is more eligible with almost five times higher wt.% loading than the triblock copolymer with a NonOx core. Contemplating the molecular structure of PTX and many other drugs, a lot of polar groups are present. Thus not only the hydrophobicity of the drugs and their vehicles needs to be considered, but also the presence of polar motifs.

Solubilization of various hydrophobic drugs. Bearing the polarity in mind we selected a series of drugs, which display sufficient polar motifs. In addition, we chose another drug, bexarotene, which is mainly a hydrocarbon (**Figure 1**). The partition coefficients (logP) range from 0.8 up to 6.9. The best results were achieved with drugs similar in lipophilicity to PTX (logP = 3.5). 17 wt.% of CsA (logP = 4.3) formulations were easily prepared by thin film, as well as 17-AAG (logP = 4.7) with 26 wt.%. While AmB (logP = 0.8) also obtained an equal high drug content of 17 wt.%, the preparation is more complex due to the lack of a low boiling common solvent necessary for the thin film preparation. Thus solubilization of AmB was accomplished by solvent exchange via dialysis. ETO (and BTZ) were formulated by thin film preparation; however the resulting formulation precipitated after 2 days. In contrast, PTX or 17-AAG loaded micelles remained stable for at least two weeks. Thus drugs with logP ≤ 1 can be solubilized, but stability as well as preparation can become an issue. Nevertheless to some extend the solubilities of all those polar drugs were increased be several orders of magnitude. As for bexarotene, no siginifcant solubilization was observed. The drug seems to be too hydrophobic (logP = 6.9) to be solubilized by our BuOx containing triblock copolymer.

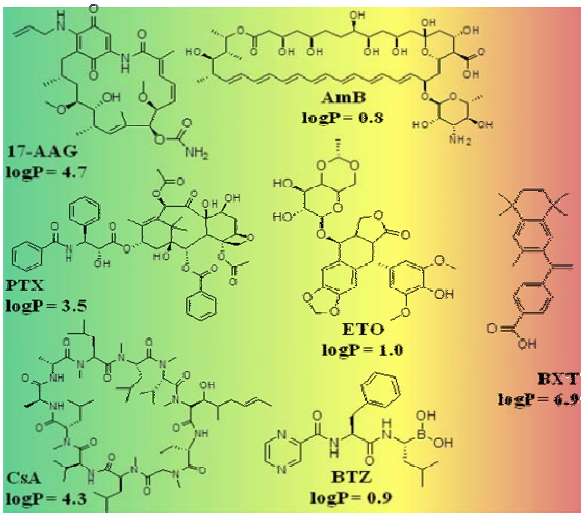

Figure 1: Selection of various poorly watersoluble drugs ordered by their solubilization behavior with BuOx containing triblock copolymers. From left to right: excellent (green), moderate (yellow), poorly (red).

Conclusion

In summary, the potential of POx as a drug delivery system has by far not been exploited to its fullest. Only considering BuOx comprised micelles, we were able to formulate CsA, an immunosuppressant drug, AmB, an antifungal compound and antineoplastic agents such as PTX, 17-AAG, ETO and BTZ, which address a variety of different types of cancer by diverse mechanism. In view of the scope of hydrophobic 2-oxazolines more active pharmaceutical ingredients are interesting targets for future studies. However, not only hydrophobicity but polarity as well needs to be considered when finding efficient matches. Like dissolves like is true in more than one aspect.

Acknowledgements. This study is financially supported by the National Cancer Institute Alliance for Nanotechnology in Cancer through the National Institutes of Health grant to the Cancer Nanotechnology Platform Partnership (U01 CA116591).

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