SYNTHESIS OF A POLYMERIC PRECURSOR BY ATRP FOR CONVERSION TO POLYMER-DRUG CONJUGATES

A Godwin[1], M Hartenstein[2], AHE Müller[2] and S Brocchini[1]

[1]Centre for Polymer Therapeutics, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK. [2]Universität Bayreuth, Makromolekulare Chemie II, Universitätsstr. 30 D-95440 Bayreuth, Germany

Introduction

Covalent conjugation of a cytotoxic drug to a soluble, biocompatible polymer can improve the efficacy of the drug. The three main parts of a polymer-drug conjugate are (1) polymer, (2) pendent chain linker and (3) conjugated drug (e.g. **5**, Scheme 1). Taken together these components produce a distinct profile of properties typical of polymer-drug conjugates. The polymer is not a mere carrier for the pharmacologically active drug since its properties are directly responsible for altering the biodistribution of the pharmacologically active molecule (e.g. doxorubicin **4**). Unlike most low molecular weight drugs, polymer-drug conjugates exhibit prolonged blood circulation. This can alter the biodistribution and the conjugate can preferentially permeate into diseased tissue (e.g. solid tumours [1,2]) which tend to be more permeable and able to retain large molecules [1] than healthy tissue.

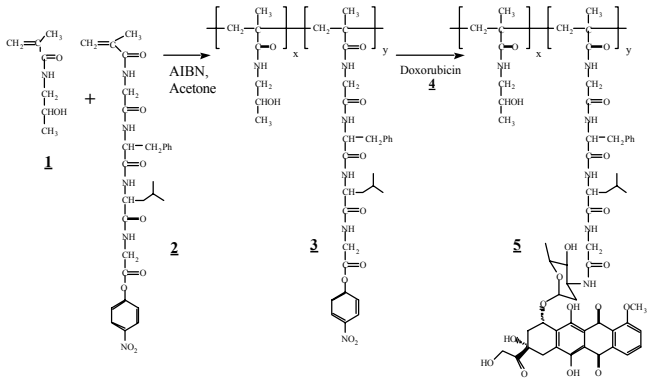
Altered biodistribution is further augmented because cellular entry of large molecules proceeds almost exclusively by pinocytosis [3]. Appropriately designed polymer-drug conjugates use drug conjugating pendent chains (i.e. linkers) that are stable in blood circulation but degrade in the lysosome resulting in the intracellular release of the drug.[4-6] Copolymers of N-(2-hydroxypropyl) methacrylamide (HPMA) in the molecular weight range of about 25,000-30,000 g/mol which is below the renal threshold have been extensively studied for the conjugation of cytotoxic drugs for cancer chemotherapy. The HPMA copolymer doxorubicin conjugate **5** is currently undergoing Phase II evaluation in the UK.[7]

While the concept entailing the appropriate conjugation of a drug to a polymer for treatment of cancer is tangible and been proven viable in a clinical environment, the future widespread use of polymer conjugates will depend on these therapeutic agents fulfilling stringent requirements considered by regulatory authorities for any new drug entity. Since polymer-drug conjugates tend to be structurally non-uniform with respect to molecular weight distribution, obtaining knowledge of all chemical species required during regulatory approval is difficult. Polymers with narrow molecular weight distribution are needed for study. Also, for a given therapeutic candidate during preclinical development, it is necessary to study many analogues to optimise properties. Future development of polymer-drug conjugates will thus depend on practical synthetic routes using appropriate common polymer precursors. The current study describes the synthesis of polymer **7** to be used as a polymeric precursor to prepare polymer-drug conjugates (e.g. **5** as shown in Scheme 2).

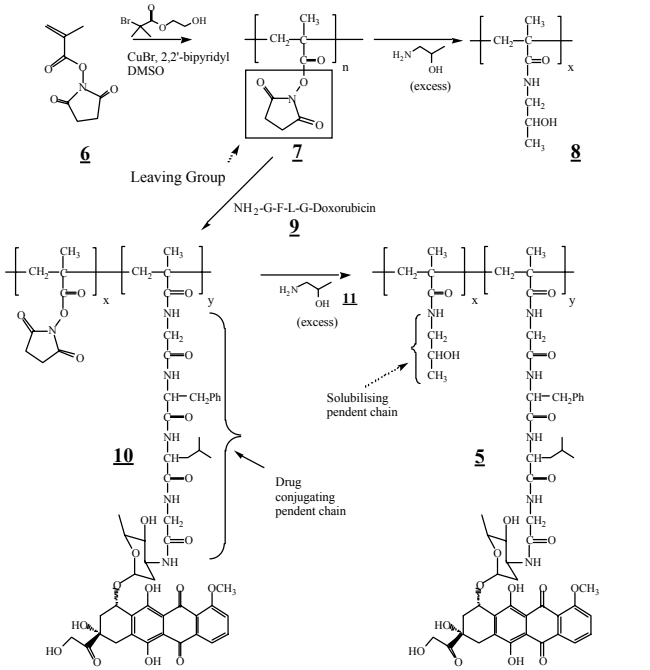
Results and Discussion

Currently HPMA copolymer-drug conjugates (e.g. **5**) are prepared in a polymer analogous reaction with the active ester precursor **3** and the drug to be conjugated (e.g. doxorubicin **4**) (Scheme 1).[8] Variation of the amount of doxorubicin to be conjugated requires the use of precursors **3** prepared from different molar amounts of **2**. If the molar amount of doxorubicin conjugated with precursor **3** is less than a particular *y* repeat, the remaining active ester pendent chains must then be quenched with an amine. Moreover, since the structure of the drug conjugating pendent chain must be optimised during the development of a polymer-drug conjugate, a precursor analogous to **3** must be prepared for each drug conjugating pendent chain to be studied. Synthesis of a large number of precursors **3** by conventional free radical polymerisation leads to precursors with a wide range of molecular weight distributions. In principle, laborious fractionation and/or optimisation of the co-polymerisation chemistry is required for each conjugate that is to be studied.

In an effort to minimise the number of polymeric precursors required during the preclinical development of polymer-drug conjugates and to prepare water soluble copolyacrylamides that have narrow molecular weight distributions, we have examined the use of atom transfer radical polymerisation (ATRP) [9] to give a common polymeric precursor **7** (Scheme 2). ATRP is an example of a controlled radical polymerisation (CRP) process and has been used with a variety of vinyl monomers to give polymers with narrow molecular distributions (>1.3). One exception has been the preparation of a wide range of narrow molecular weight poly(meth)acrylamides by ATRP. Precursor **7** has been prepared (Scheme 2) so (1) CRP processes can be used to make polyacryl- and poly(meth)acrylamides with narrow molecular weight distributions and (2) families of polymer-drug conjugates for study (e.g. **5**) with the same molecular weight characteristics can be prepared.



Scheme 1. Synthesis of HPMA copolymer precursor **3** followed by a polymer analogous reaction with doxorubicin **4** to give the polymer-drug conjugate **5**.).[8]



Scheme 2. Synthesis of an active ester polymeric precursor **7** by ATRP followed by conjugation with 1-amino-2-propanol to give water soluble HPMA homopolymer **8**. The route to polymer-drug conjugates such as **5** to be followed using the polymeric precursor **7** is also shown.

Although other active ester acrylates were considered [10], the methacryloxy succinimide monomer **6** [11,12] was used because of the relative hydrolytic stability of N-hydroxysuccinimide (NHS) esters compared to other active esters.[13] The comparatively small and hydrophilic N-hydroxysuccinimide moiety was also preferred over moieties such as N-hydroxyphthalimide because there would presumably be greater monomer and polymer solubility. Initial polymerisations (0.5 g scale in monomer **6**) were attempted in solvents such as ethyl acetate and THF which have been used successfully with ATRP. The monomer **6** was not readily soluble in ethyl acetate and precipitation of the polymer **7** during polymerisation occurred in THF. It had been hoped that only high molecular weight polymer would be insoluble in THF.[11] These initial reactions did give polymer **7** possessing a narrow molecular weight distribution, but isolated yields were less than 40% (Table 1). This implied that premature polymer precipitation may have terminated the reaction. Since it appeared that monomer **6** could be

polymerised with quite narrow molecular weight distribution and since reproducibly prepared polymeric precursors **7** at different target molecular weights (M_{cal}) would eventually be required for study, it was necessary to find polymerisation conditions that would ensure the polymerisation went to completion to give the active ester polymer **7**.

Solvent	Wt % **6**	**6**:initiator: CuBr: PMDETA	M_{cal}	M_n	M_w/M_n
THF	25	100:1:1:1.2	18,300	14,800	1.10
THF	22	200:1:1:1.2	36,600	1,800	1.12
THF	22	100:1:0.3:1.2	18,300	13,100	1.09
DMF	33	100:1:1:1.2	18,300	3,200	1.10

Table 1. Reactions were conducted on 0.5 g scale of monomer 6 for 16 h at 70 °C. The yield of the polymer was in the range of 10-40%.

When larger scale polymerisation reactions (1.5 g in monomer **6**) were attempted in THF, it appeared that the monomer and possibly some copper species were insoluble. CuBr (0.01 g) and each of the two ligands under study, N, N, N', N'', N''-pentamethyldiethylenetriamine (PMDETA) and 2,2'-bipyridine (Bpy) (0.03 g) were observed to give heterogeneous solutions in THF and ethyl acetate. While we observed that a solution of CuBr alone in DMF became homogeneous after several hours, CuBr was readily soluble in DMF and acetone with added Bpy. Although these crude solubility studies were conducted at ambient temperature and slightly higher ratio of ligand to CuBr (~2.4-2.7:1), they indicated a greater solubility of CuBr in DMF. This may be consistent with the observation that DMF is not considered an optimal solvent for ATRP [14] because of possible competitive chelation effects. Nonetheless, one reaction was conducted in DMF (Table 1) but only low molecular weight polymer was isolated.

The reaction was then conducted in acetone with a target molecular weight, M_{target}=9,150 g/mol (i.e. 55:1:0.5:1 monomer:initiator:CuBr:Bpy) and a 95% yield of polymer **7** was isolated. Molecular weights of about 20,000-25,000 g/mol were desired during this investigation to ensure that any conjugates derived from the polymeric precursor **7** would exhibit prolonged circulation times. Although higher molecular weights of polymer **7** would be insoluble in acetone, a polymerisation was conducted with a target M_{target}=18,300 g/mol (i.e. 100:1:0.5:1 monomer:initiator:CuBr:Bpy). But polymer **7** precipitated from the reaction mixture while giving only a 30% isolated yield.

It was evident that maintaining a homogeneous solution throughout the reaction was required for the polymerisation to go to completion. Although DMF may not be considered a good solvent for ATRP, it was expected that polymer **7** would be soluble in DMF.[12] Also the suitability of DMF as a solvent for ATRP was not certain for reactive monomers such as **6**, hence further reactions were conducted in DMF. Although we were concerned about competitive thermal initiation [15], the temperature for the polymerisation was increased to 130 °C to ensure the solution remained homogeneous at the monomer concentrations which were examined and as a means to possibly increase the propagation to better compete with competitive chelation effects of DMF. Using M_{target}=18,300 g/mol (i.e. 100:1:0.5:1 monomer:initiator:CuBr:Bpy), polymerisations on a 1.0 g scale were conducted where the monomer:DMF weight ratio was decreased from 10:1 to 0.5:1. A maximum yield of 50% of the polymer was isolated after precipitation into acetone when the monomer:DMF ratio was 1.6:1. When the monomer ratio was above 3:1 a polymeric solid formed which could not be solvated and no polymer was observed when the ratio was 0.5:1. These reactions in DMF did not give high yields of polymer at the target molecular weight.

Polymer **7** was also expected to be soluble in DMSO, so exploratory polymerisations were conducted at 130 °C with a M_{target}=20,679 g/mol (i.e. 113:1:0.5:1 monomer:initiator:CuBr:Bpy). It was found that a yield of 85-95% of polymer **7** was isolated after 2 hours with a monomer:DMSO ratio of 1.3:1. This polymerisation is reproducible at this M_{target} and has so far been conducted on a scale of 2 g in monomer **6**. Decreasing the monomer:DMSO ratio to 1:1 then to 0.7:1 resulted in lower yields of polymer (52 and 40% respectively). The polymeric precursor **7** was isolated as a white powdery solid directly by precipitation into acetone. The acetone solution turned a green colour during precipitation of polymer **7** which was consistent with the earlier observation that CuBr/Bpy is soluble in acetone. It is not yet known how much copper is still associated with polymer **7**, precipitation of this polymer from DMSO/DMF solutions containing Bpy ligand into acetone may offer a viable alternative to alumina chromatography for the removal of copper.

The reaction of 1-amino-2-propanol with the polymeric precursor **7** gave water soluble HPMA homopolymer **8** (M_n= 16,700 g/mol, PD=1.3; GPC, phosphate buffered solution (PBS), PEG standards) which was indistinguishable by infrared spectroscopy with HPMA homopolymer (M_n=11,000 g/mol, PD=3.1) that had been prepared by conventional free radical polymerisation (AIBN at 50 °C in acetone). The M_{target} of the polymeric precursor **7** was 20,679 g/mol. Adjusting for the substitution of the 1-amino-2-propanol side chain, the M_{target} was 16,159 g/mol for the final polymer **8**. At this preliminary stage, the suitability of using PEG standards for GPC is being investigated using MALDI-MS to determine the absolute molecular weights of polymers **7** and **8**.

Conclusions

An active ester polymeric precursor **7** with narrow molecular weight distribution (PD=1.1 to 1.3) was prepared by ATRP. Subsequent reaction of precursor **7** with 1-amino-2-propanol gave the poly(meth)acrylamide, HPMA homopolymer, **8**. The precursor **7** is designed to provide a wide range of water soluble polymer-drug conjugates for study that have the same molecular weight characteristics. This strategy may also have potential for the general preparation of narrow molecular weight poly(meth)acrylamides.[16]

Experimental

*Synthesis of polymeric precursor **7**.* In a typical polymerisation, copper(I)bromide catalyst (14.2 mg, 0.1 mmol), 2-2'-bipyridine (31 mg, 0.2 mmol), 2-bromo-2-methyl-(2-hydroxyethyl)propanoate (21 mg, 0.1 mmol) and monomer **6** (2.05 g, 11.2 mmol) were added to a pressure tube with a screw-cap lid. The reactants were then solubilised by addition of DMSO (1.54 g). The resulting solution was purged with argon for approximately 5 minutes, sealed and heated at 130 °C for 2 hr. The polymeric product was isolated by addition of 3-4 ml of DMF to the cooled viscous reaction mixture and precipitating the polymer into acetone (20 ml) to give a white solid product. Conversion was calculated from the mass of the polymer which was isolated (1.81 g, 88.3%).

*Synthesis of HPMA homopolymer **8** by conjugation of 1-amino-2-propanol to the polymeric precursor **7**.* To poly(methacryloxy succinimide) **7** (0.2 g) in DMF (3 ml) was added 1-amino-2-propanol (0.16 ml, 2.1 mmol) dropwise under stirring at 0 °C. The solution was allowed to warm to room temperature and then heated to 50 °C for 16 hr. The reaction mixture was cooled to room temperature and slowly added to acetone (20 ml) to precipitate a solid product. The product was further purified by a second precipitation into 60:40 (v/v) acetone:diethyl ether to give a water soluble polymer as a white solid after drying in vacuum (M_n= 16,700 g/mol, PD=1.3; GPC, PBS, PEG standards).

Acknowledgement. We are grateful for support from the EPSRC and studentship funding for AG from the School of Pharmacy.

References

(1) Matsumura, Y.; Maeda, H. *Cancer Res.* **1986**, *6*, 6387-6392.
(2) Noguchi, Y.; Wu, J.; Duncan, R.; Strohalm, J.; Ulbrich, k.; Akaike, T.; Maeda, H. *Jpn. J. Cancer Res.* **1998**, *89*, 307-314.
(3) deDuve, C.; deBarsy, T.; Poole, B.; Trouet, A.; Tulkens, P.; van-Hoof, F. *Biochem. Pharmacol.* **1974**, *23*, 2495-2531.
(4) Duncan, R. *Anti-Cancer Drugs* **1992**, *3*, 175-210.
(5) Soyez, H.; Schacht, E.; Vanderkerken, S. *Adv. Drug Delivery Rev.* **1996**, *21*, 81-106.
(6) Brocchini, S.; Duncan, R. In *The Encyclopedia of Controlled Drug Delivery*; Mathiowitz, E., Ed.; Wiley: New York, 1999, p 786-816.
(7) Vasey, P.; Twelves, C.; Kaye, S.; Wilson, P.; Morrison, R.; Duncan, R.; Thomson, A.; Hilditch, T.; Murray, T.; Burtles, S.; Cassidy, J. *Clin. Cancer Res.* **1999**, *5*, 83-94.
(8) Putnam, D.; Kopecek, J. *Adv.Polym.Sci.* **1995**, *122*, 55-123.
(9) Matyjaszewski, K. *Pure Appl. Chem.* **1997**, *A34*, 1785-1801.
(10) Arshady, R. *Adv. Polym. Sci.* **1994**, *111*, 1.
(11) Batz, H.; Franzmann, G.; Ringsdorf, H. *Angew. Chem. Internat. Edit.* **1972**, *11*, 1103-1104.
(12) Ferruti, P.; Bettelli, A.; Fere, A. *Polymer* **1972**, *13*, 462-464.
(13) Hermanson, G. *Bioconjugate Techniques*; New York, **1996**.
(14) Beers, K.; Boo, S.; Gaynor, S.; Matyjaszewski, K. *Macromolecules* **1999**, *32*, 5772-5776.
(15) Pascual, S.; Coutin, B.; Tardi, M.; Polton, A.; Vairon, J. *Macromolecules* **1999**, *32*, 1432-1437.
(16) Teodorescu, M.; Matyjaszewski, K. *Macromolecules* **1999**, *32*, 4826-4831.