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Designing dapsone polymer conjugates for controlled drug delivery

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ABSTRACT

Polymer–drug conjugates have significantly influenced polymer therapeutics over the last decade via controlled pharmacokinetics. Dapsone (4,4′-diamino diphenylsulphone) is not only widely used in the treatment of leprosy but forms an essential component in the treatment of autoimmune inflammatory diseases and malaria. However, its low bioavailability and non-specific distribution in the body leads to absorption throughout organs including skin, liver, and kidneys that can cause serious side effects. Thus, in this study we report the synthesis of polymer–drug conjugates of dapsone covalently bonded to macromolecular chains towards the development of new bioactive polymeric formulations with anti-inflammatory properties. Dapsone was functionalised with an acrylic moiety in which the acrylamide residue was directly bonded to one of the aromatic rings of dapsone. This functionalisation yielded an unsymmetrical dapsone methacrylamide (DapMA) structure, which on free radical polymerisation and co-polymerisation with HEMA yielded polymers of hydrocarbon macromolecules with pendant dapsone units. Thermal and size-exclusion chromatographic analysis revealed an increase in thermal stabilisation of the homopolymer (p(DapMA)) in comparison to the copolymer (p(Dap-co-HEMA)) with relatively high average molecular weight. The polymer conjugates exhibited high stability with low dapsone release from the polymeric backbone due to hydrolysis. However, a significant anti-inflammatory activity in a nitric oxide inhibition assay confirmed that this property was the consequence of only the macromolecular composition and not related to the release of low molecular weight compounds. Thus, the conjugation of dapsone to macromolecular systems provides a synthetic route to incorporate this drug into polymeric systems, facilitating their development into new anti-inflammatory therapies.

Statement of Significance

The dapsone-conjugated methacrylic monomer and polymer derivatives with anti-inflammatory properties described are previously unreported. The scientific impact of this work lies in its potential to expand the clinical applications of dapsone toward the development of advanced anti-inflammatory therapies based on polymer-therapeutic approaches. These approaches facilitate the treatment of existing rare auto-immune and other inflammatory related diseases.

1. Introduction

During the last century, sulphone derivatives have been extensively used in medicine because of their versatile bioactive, biocidic and anti-inflammatory properties. Dapsone is structurally one of the simplest sulphones but also recognised as an active therapeutic agent from this family of compounds. As an antibiotic, dapsone acts against bacteria and protozoa inhibiting the synthesis of dihydrofolic acid through competition with para-aminobenzoate for the active site of dihydropteroate synthase [1]. In addition, dapsone has been successfully used as an essential component for the treatment and prophylaxis of leprosy, actinomycetoma, Pneumocystis pneumonia, and malaria. The anti-inflammatory action of the drug is not related with its antibacterial action and is still not fully understood. Dapsone has been reported to interfere with the activation or function of a large number of polymorphonuclear

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leukocytes, predominantly neutrophils and eosinophils, into the affected tissue and shows a noticeable activity in reducing or suppressing the symptoms of many autoimmune [2] and skin [3] related diseases such as acne fulminas, actionomycetoma, brown recluse spider bites, celiac disease, cicatricial pemphigoid, dermatitis herpetiformis, bullous pemphigoid, Behcet's disease, and lupus erythematous.

One of the main disadvantages of oral administration of dapsone stems from its low solubility and non-specific distribution in the body, once absorbed, it is distributed throughout the organs in the body including skin, liver, and kidneys [4]. Dapsone has also been reported to cross the blood–brain barrier and the placenta and is found in breast milk [5,6]. Hence, dapsone therapies are still associated with drawbacks of hemolysis, methemoglobinemia and patient non-compliance [7]. To render the treatment more effective and reduce these side effects, a better control over pharmacokinetics and site specific delivery is needed. Thus far, the design of polymer formulations for controlled delivery of dapsone has been limited to encapsulation within polymeric systems, for which mainly polysaccharides have been used [8,9]. Although these methodologies have improved the pharmacokinetics, the therapeutic effects of these polymer formulations were found to be modest and a reduction of the complications associated with dapsone therapies were not sufficiently achieved.

Recent developments in the field of polymer-conjugates have provided very promising examples of FDA approved and marketed pharmaceutical products [10]. Anti-inflammatory therapies involving the incorporation of low molecular weight drugs into macromolecular systems such as ibuprofen and aspirin polymer–drug conjugates have shown promising advances that enhance the bioavailability and compatibility within the body [11,12]. Nonetheless, biomedical approaches based on dapsone polymer–drug conjugates have not yet been fully exploited. Chemically incorporating 4,4′-diamino diphenylsulphone (dapsone) into a polymer system has only been reported in elastomeric formulations for engineering applications based on its capacity to act as a curing agent in epoxy resins. This results in a high performance elastomeric material with enhanced thermal and mechanical properties, composite materials, and structural adhesives that are unrelated to the current work [13].

In view of the limitations of current dapsone treatment, a polymer system that intrinsically contains the active dapsone molecule anchored to the macromolecular polymer structure will be extremely beneficial and efficacious for therapeutic action. This would lead to enhanced control of pharmacokinetics whilst providing a novel approach for safe use of this promising drug. The covalent linking of dapsone into macromolecular chains is advantageous as it will reduce the migration of dapsone into the surrounding tissues and improve hydrolytic and biological stability.

This study reports for the first time the synthesis and characterisation of a polymerisable derivative of dapsone, dapsone methacrylamide (DapMA) (Fig. 1) and the polymers thereof. These systems have the potential for the development of bioactive polymeric formulations with anti-inflammatory properties by virtue of the dapsone residues anchored to the macromolecular chains, which have not yet been reported.

2. Materials and methods

2.1. Chemicals

Dapsone (97%) was purchased from Aldrich and recrystallised from methanol. Methacryloyl chloride (97%, Aldrich) and triethylamine (98%, Scharlau) were purified by distillation under reduced pressure. 2-Hydroxyethyl methacrylate (HEMA) (97%, Sigma–Aldrich) was purified according to the literature by water–hexane selective extraction and washing [14]. 2,2-Azobisisobutyronitrile (AIBN) (98%, Merck) was recrystallised using methanol. Ethylene glycol dimethacrylate (EGDMA) (98%, Aldrich) was used as received. Solvents used were high performance liquid chromatography (HPLC) grade and all other reagents were analytical grade purchased form Sigma–Aldrich.

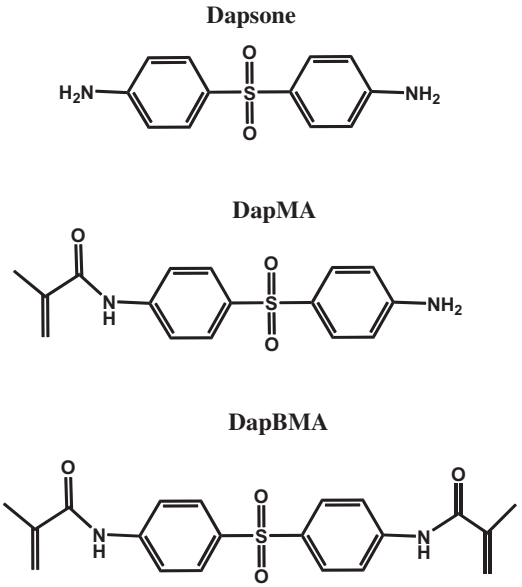

Fig. 1. Chemical structures of dapsone and its derivative monomers dapsone methacrylamide (DapMA) and bis-methacrylamide (DapBMA).

Phosphate-buffered solution (PBS) of pH 7.4 (Sigma) was used as received. Thermanox (TMX) control disks were supplied by Labclinics S.L., and aqueous solutions of Triton X-100 were supplied by Aldrich. Tissue culture media, additives, trypsin, and 3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) were purchased from Sigma.

2.2. Synthesis of dapsone methacrylamide monomer

Dapsone (4 g, 16 mmol) was dissolved in 150 mL of ethyl acetate containing triethylamine (2.8 mL, 20 mmol). A stoichiometric amount of methacryloyl chloride (1.2 mL, 16 mmol) dissolved in 10 mL of ethyl acetate was added dropwise with constant stirring at room temperature under nitrogen atmosphere, followed by stirring the reaction mixture for a further 24 h at room temperature. The reaction medium was filtered to remove the triethylamine hydrochloride and any unreacted reagents were removed by successive extraction with 0.5 M HCl and saturated solutions of $HNaCO_3$ and NaCl. After drying over anhydrous Na_2SO_4, the solvent was removed by distillation under reduced pressure and the crude product was purified by a triple recrystallisation in methanol, ethyl acetate, followed by diethyl ether and dried under vacuum until constant weight.

2.3. Synthesis of poly(dapsone methacrylate) homopolymer and poly (hydroxyethyl methacrylate-co-dapsone methacrylate) co-polymer

Homopolymer p(DapMA) and co-polymer p(DapMA-co-HEMA) (50% feed molar ratio) were synthesised by free radical polymerisation and named P100 and P50, respectively. The appropriate co-monomer mixtures were dissolved in DMF ($[M] = 0.25$ mol L^{-1}) containing AIBN (1.5×10^{-2} mol L^{-1}), and the reaction mixtures were deoxygenated with N_2 for 15 min and the reaction containers were then transferred to an oven at 50 °C for 24 h. After this period, the reaction mixture was

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