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Polymer complex of WR 2721. Synthesis and radioprotective efficiency

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ABSTRACT

Polymer complex constructed from WR 2721 and poly(hydroxyoxyethylene phosphate) was synthesized. The structure of complex formed was elucidated by ^{1}H-, ^{13}C, ^{31}P NMR and FT-IR spectroscopy. The radioprotector was immobilized via ionic bonds. Radioprotective efficacy was evaluated by clonal survival of stem cells in crypts of mouse small intestine, and incidence and latency of the acute radiation induced bone marrow syndrome. Protection factors were assessed for WR 2721 and for the polymer complex. Protection factors for the polymer complex ranged from 2.6 for intestinal stem cell survival to 1.35 for 30 day survival (LD50) following whole body radiation exposure. In all cases, the polymer complex was a significantly better radiation protector than the parent compound.

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1. Introduction

The research interest in amifostine (WR 2721) continues long after its discovery due to its potential role in reducing the biological effects of ionizing radiation, including lethality, mutagenicity, and carcinogenicity. WR 2721 demonstrated some potential for use as an emergency whole-body radioprotector as well as for use with radiotherapy (Brown et al., 1988). Several studies have demonstrated that amifostine protects normal tissue from both acute and late radiation damage without protecting the tumor, i.e. amifostine is a selective cytoprotector of normal tissues (Burkon et al., 2003; Koukourakis, 2003; Wasserman, 1999; Wasserman and Brizel, 2001). Maximum radioprotection by amifostine is observed when the drug is administered intravenously by a 15 min infusion starting 30–60 min before irradiation (Bukowski, 1996). It is shown that its side-effects, such as hypotension, nausea, and vomiting are significantly augmented upon intravenous administration (Bonner and Shaw, 2002; Cassatt et al., 2002; Schuchter et al., 2002).

A promising approach to improve some characteristics of low molecular weight drugs, already approved and used in practice, as well as to impart new valuable properties is the macromolecular approach, i.e. application of appropriate polymers for drug immobilization, chemically conjugated or physically bound to a polymer chain. Polymer chemistry has contributed in various ways to the present progress in biology, biochemistry, medicine and pharmacy, providing new highly specific materials. Synthetic polymer formulations are becoming more and more attractive as delivery vehicles because of the great flexibility regarding: (i) the type and size of the bioactive molecules/agents delivered, (ii) the degree of carrier loading and (iii) the immobilization techniques applied (Duncan, 1992; Hoste et al., 2004; Ottenbrite et al., 1978; Rihova et al., 2001; Ringsdorf, 1975; Uhrich, 1997; Uhrich et al., 1999). The biodegradable, biocompatible and low toxic polyphosphoesters – a family of polymers including poly(alkylene H-phosphonate)s and derived from them polyphosphates and polyphosphoamidates – are very promising polymers for drug and gene delivery (Tsevi et al., 1993; Georgieva et al., 2002; Huang et al., 2004; Jiang et al., 2007; Troev et al., 2010, 2007; Pencheva et al., 2008; Zhao et al., 2003). Polyphosphoesters have the following advantages (Troev, 2012): (i) relative ease of preparation from commercially available reagents; (ii) the possibility to be constructed from nontoxic and water soluble blocks; (iii) possibility to control the hydrophilic/hydrophobic balance; (iv) relatively narrow molecular weight distributions; (v) the drug-loading capacity is not limited to the end groups; (vi) the reactive P—H group in repeating units allows chemical modification, as well as drug conjugation under

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mild reaction conditions; (vii) the presence of highly polar P═O groups affords possibility for physical immobilization of drugs; (viii) these polymers can be used to prepare gels; (ix) they can be administered over a wider molecular weight range because the PEG blocks will be safely excreted after hydrolysis – important characteristics for intravenous administration; (x) easy to prepare on industrial scale.

The goal of the present study is to evaluate the radioprotective efficiency of a newly synthesized polymer complex composed of poly(hydroxyoxyethylene phosphate) and WR 2721.

2. Material and methods

2.1. Synthetic procedures

2.1.1. Materials

PEG with number average molecular weight 600 g.mol-1 (PEG 600) was purchased from Fluka. It was dried prior to use by an azeotropic distillation with toluene and a subsequent 4 h heating at 120 °C under dynamic vacuum. Dimethyl hydrogen-phosphonate (Fluka) and triethylamine (Flika) were distilled prior to use. 2-[(3-aminopropyl)amino]ethane-1-thiol was obtained from Fluka, while hydrobromic acid (48%), 1,3-diaminopropane (99%) and ethylene sulfide (98%) were Aldrich products. Acetonitrile (Fluka) was dried and distilled before use. Trichloroisocyanuric, 97% (Sigma Aldrich) was used as received. Dowex 50 was purchased from Fluka.

2.1.2. Instrumentation

All ^{1}H, ^{31}P and ^{13}C NMR spectra were measured on a Bruker 250 MHz spectrometer in D_2O or $CDCl_3$ solutions. The infra-red (IR) spectra were recorded on Bruker-Vector 22 FT-IR spectrophotometer in KBr tablets. SEC measurements were performed on a Waters 244 line equipped with four Ultrastyragel columns with pore sizes 100, 100, 500, and 500 Å and tetrahydrofuran as the carrier solvent. The molecular weights were calculated using a conventional calibration with PEG standards.

2.1.3. Synthesis of poly(oxyethylene hydrogen phosphonate)

Poly(oxyethylene hydrogen phosphonate) (POEHP) was obtained from commercial dimethyl hydrogen phosphonate (DMP) (5.50 g, 0.050 mol) and PEG 600 (19.80 g, 0.033 mol) in a two stage reaction. The entire synthesis was carried out in a vacuum distillation set-up. A typical synthetic procedure is described bellow: In the first low temperature transesterification stage, the temperature was slowly increased to 135 °C in N_2 at atmospheric pressure. The reaction was kept at these conditions for 5 h and terminated after the temperature of the methanol vapors dropped down and its distillation visibly stopped. The second polycondensation stage was performed under vacuum of 1 mmHg at elevated temperatures for a total of 4.15 h (4 h at 160 °C, 15 min at 185 °C). The product obtained was wax material.

^{1}H NMR ($CDCl_3$), δ (ppm): 6.93 (d, $^{1}J(P,H)$ = 715.87 Hz, —CH_2 OP(O)(*H*)OCH_2—); 6.86 (d, $^{1}J(P,H)$ = 708.61 Hz, $CH_3OP(O)(H)O$—); 6.79 (d, $^{1}J(P,H)$ = 689.10 Hz, HOP(O)(*H*)OCH_2—); 4.14–4.31 (m, —OP(O)(H)O*C*H_2) and 3.60–3.69 (m, —O*C*H_2*C*H_2O—).

$^{13}C\{H\}$ NMR ($CDCl_3$), δ (ppm): 64.70 (d, $^{2}J(P,C)$ = 5.79 Hz, —*C*H_2 OP(O)(H)O*C*H_2—); 70.18 (d, $^{3}J(P,C)$ = 5.79 Hz, —OP(O)(H)OCH_2*C*H_2—) and 70.58 (s, —O*C*H_2*C*H_2O—).

^{31}P NMR ($CDCl_3$), δ (ppm): 11.21 (d sextet, $^{1}J(P,H)$ = 708.37 Hz, $^{3}J(P,H)$ = 11.74 Hz, —CH_2O*P*(O)(H)OCH_3); 10.51 (d quintet; $^{1}J(P,H)$ = 715.46 Hz and $^{3}J(P,H)$ = 9.82 Hz, —CH_2O*P*(O)(H)OCH_2—); and 7.58 (dt; $^{1}J(P,H)$ = 693.3 Hz, $^{3}J(P,H)$ = 10.32 Hz, —CH_2O*P*(O)(H)OH).

$^{31}P\{H\}$ NMR ($CDCl_3$) δ (ppm): 11.21 (5.4%); 10.51 (90.1%); 7.58 (4.5%).

2.1.4. Synthesis of poly(hydroxyoxyethylene phosphate)

2.1.4.1. Synthesis of poly(oxyethylene chlorophosphate). Poly(oxyethylene chlorophosphate) (POEClP) was obtained from POEHP and trichloroisocyanuric acid. The entire synthesis was carried out under inert atmosphere. To a stirred solution of POEHP (2.69 g, 4.17 mmol) in acetonitrile (9 ml) at room temperature was added in one portion a solution of trichloroisocyanuric acid (0.333 g, 1.39 mmol) in acetonitrile 13.5 ml. The reaction mixture was kept for 1 h. Isocyanuric acid was removed from the solution by filtration.

2.1.4.2. Synthesis of poly(hydroxyoxyethylene phosphate). The synthesis of poly(hydroxyoxyethylene phosphate) (PHOEP) was carried out under inert atmosphere. A typical synthetic procedure is described bellow: To the stirred solution of POEClP in acetonitrile at room temperature water (0.075 g, 4.17 mmol) and triethylamine (0.297 g, 0.41 ml, 4.17 mmol) were added in one portion. The reaction mixture was kept for 30 min. The solution was refrigerated at −12 °C. Triethylamine hydrochloride crystals were removed by filtration. The solvent was evaporated and residue was dissolved in water and was passed through ion exchange resin Dowex 50. After dialysis against deionized water the reaction product was freeze-dried. Yield 80%. The structure of PHOEP was proved by NMR spectroscopy.

^{1}H NMR (D_2O), δ (ppm): 4.00–3.86 (m, $CH_2OP(O)(OH)OCH_2$), 3.60–3.50 (m, CH_2OCH_2); 7.18 ppm, (dt, $^{1}J(P,H)$ = 650.12 Hz, P—H)

$^{13}C\{H\}$NMR (D_2O), δ (ppm): 70.13 (d, $^{3}J(P,C)$ = 8.2 Hz, $POCH_2$ CH_2), 69.59 (CH_2OCH_2), 64.68 (d, $^{2}J(P,C)$ = 6.2 Hz, $POCH_2CH_2$);

^{31}P NMR (D_2O), δ (ppm): 7.62, d, $^{1}J(P,H)$ = 653.12 Hz; 2.17 and 0.90 (phosphate structures).

$^{31}P\{H\}$NMR (D_2O), δ (ppm): 7.62 (1.35%); 2.17 (3.55%) and 0.90 (95.10%);

IR (KBr): 1291 cm^{-1} и 1259 cm^{-1} ν(P═O), 1102 cm^{-1} ν(H_2C—O—CH_2), 1036 cm^{-1} ν (P—OCH_2).

2.1.5. Synthesis of S-2-(3-aminopropylamino) ethylphosphorothioic acid dihydrate (amifostine, WR 2721)

Amifostine was synthesized in two stages following a previously described procedure (Piper and Johnston, 1975). Yield: 77%; m.p. 145 °C. Purity > 98%.

^{1}H NMR (D_2O), δ (ppm): 3.39 (t, $^{3}J(H,H)$ = 5.4 Hz, 2H, CH_2CH_2 NH-); 3.10–3.18 (m, 4H, $H_2NCH_2CH_2CH_2NHCH_2$); 3.04–2.93 (m, 2H, CH_2CH_2S); 2.12 (quintet, $^{3}J(H,H)$ = 7.8 Hz, 2H,$CH_2CH_2CH_2NH$);

$^{13}C\{H\}$ NMR (D_2O), δ (ppm): 53.05 (CH_2NH), 47.27 (NH_2CH_2), 39.41 ($NHCH_2$), 28.40 (d, $^{2}J(P,C)$ = 2.8 Hz, CH_2SP), 26.69 ($CH_2CH_2CH_2$);

^{31}P NMR (D_2O), δ (ppm): 15.77 (t, $^{3}J(P,H)$ = 14.09 Hz);

IR (KBr): (cm^{-1}): 3471 – NH_2 (stretching); 3335 – NH (stretching); 2922–2742 – CH_2 (stretching); 2560 – POH (stretching); 1188 – P═O (stretching).

2.1.6. Immobilization of WR 2721 on PHOEP

A solution of WR 2721 (0.40 g, 1.6 mmol) in 5 ml water was added to an aqueous solution of PHOEP (1.06 g, 1.6 mmol) in 20 ml distilled water and the mixture was freeze-dried.

$^{31}P\{H\}$ NMR (D_2O), δ (ppm): 15.87 (P—SCH_2); 1.88 ($CH_2OP(^{-}O)$ (O)OH); 0.66 ($CH_2OP(^{-}O)(O)$ OCH_2);

IR (KBr): (cm^{-1}): 3660–3200 – NH_2 and NH (stretching); 2887 – CH_2 (stretching); 2677–2495 – POH (stretching), 1643 – NH_2 and NH (bending); 1243 – P═O (stretching); 1101 – P—O—C, C—N and C—O—C (stretching).

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