Preparation and In Vitro Evaluation of Primaquine-Conjugated Gum Arabic Microspheres

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Abstract:

Gum arabic, a branched polysaccharide was oxidized using periodate to generate reactive aldehdye groups on the biopolymer. Primaquine, an 8-aminoquinoline was covalently coupled onto oxidized gum arabic via imine bond and simultaneously fabricated into microspheres of less than 2 µm in size by heat denaturation in a reverse emulsion of 1:1 light paraffin oil and toluene stabilized by sorbitan sesquioleate as the surfactant. The covalent binding of primaquine to the polysaccharide using the clinically used water-soluble form of the drug, primaquine phosphate was achieved in the presence of borate buffer of pH 11. Up to 35% of the drug could be bound to the polymer backbone depending on the concentration of the drug employed initially and the degree of oxidation of the polysaccharide. Interestingly, both the aliphatic and the hindered aromatic amino groups of primaquine were found to react with the aldehdye functions through Schiff base formation leading to crosslinking of the polysaccharide with the drug itself. In vitro release of the drug from microspheres into phosphate buffer (pH 7.4, 0.1 M) at 37ºC showed that the release of primaquine from the matrix was slow, although gradually increased with time. Maximum released was below 50% of the drug payload even after 10 days. A possible reason for the poor hydrolytic susceptibility of the Schiff linkage is suggested based on the unequal reactivity of the amino groups on primaquine and its relevance in possible therapeutic application of this polymer-drug conjugate discussed.

Key words: gum arabic, polysaccharide, periodate oxidation, microspheres, drug delivery, primaquine, 8-aminoquinoline, sustained release, controlled release, polymer-drug conjugate.

Introduction:

Primaquine, an 8-aminoquinoline and some of its derivatives are potent drugs in the treatment of malaria as well as other parasitic diseases such as Leishmaniasis (1 - 8). The clinical use of primaquine however, poses problems due to its toxicity. Primaquine may cause nausea, abdominal pain and oxidant haemolysis with methemoglobinemia,anemia, hemoglobinurea, and is contraindicated in patients with different variants of glucose-6-phosphate dehydrogenase deficiency (1). In order to improve the therapeutic efficacy of the drug and diminish its toxicity, various approaches have been examined. These include linking the drug to a carrier protein such as albumin (9), linking peptide derivatives of the drug onto biodegradable polyacryl starch microspheres

(10), encapsulation in polycyanoacrylate and polylactide nanoparticles (11,3), in erythrocytes (12) and in liposomes (13 - 14).

Polysaccharides constitute an important class of biomaterials as most of them exhibit good biocompatibility and biodegradability and a number of drug delivery approaches have been reported in the literature using polysaccharides as drug carriers (15). To the best of our knowledge, there is very little information in the published literature on the use of polysaccharides as drug delivery vehicles for 8-aminoquinoloines such as primaquine (10). Gum arabic is a water-soluble natural polysaccharide obtained from the exudate of the acacia tree. This highly branched polysaccharide is a complex mixture of Ca, Mg and K salts of arabic acid that contains galactose, rhamnose, glucuronic acid, 4-O-methyl glucuronic acid and arabinose residues (16). The molecular structure of gum arabic consists of mainly three components, the major component being arabinogalactan (90%) having a low (0.5%) protein content, the second being arabinogalactan (< 10%) with a high protein content (10%) and the third component consisting less than 1% includes glycoprotein having around 50% protein content (17). It is extensively used as a food additive (18), and is reported to be fermented and metabolized in the caecum and the colon (19 - 20).

Periodate oxidation of polysaccharides offers a convenient route to synthesize polymer-drug conjugates especially with drugs possessing aliphatic amino functions via imino bonds with the aldehyde groups of the oxidized polysaccharide. In a recent report, the synthesis of soluble amphotericin B-arabinogalactan conjugate of reduced toxicity and enhanced therapeutic efficacy was demonstrated by periodate oxidation of the polysaccharide followed by coupling the amino-group containing antibiotic onto the polymer (21). Primaquine contains an aliphatic primary amino group as well as a hindered aromatic amino group of unequal reactivity (22) and therefore is an interesting candidate for a similar approach in the preparation of a polymeric drug. The Schiff's reaction between the amine and the aldehyde group is a rapid reaction under appropriate conditions and it was of interest to examine how the two different amino groups in primaquine would respond in the formation of the polymer-drug conjugate and its hydrolytic stability, the latter being the key-factor determining the release profile of the drug from the conjugate.

Experimental:

Materials :

Gum Arabic (from acacia tree) of approximate molecular weight 250,000 (Product No.G-9752), primaquine phosphate, sorbitan sesquioleate, borax (sodium tetraborate decahydrate) and sodium *m*-periodate were purchased from Sigma, USA. Dialysis tubing (Spectra Por®, M.W.CO. 6000-8000) was from Spectrum Laboratories Inc., CA, USA. Liquid paraffin (Light, viscosity 18 cP at 30°C) was from S.D. Fine Chemicals, Mumbai, India. All other reagents such as methanol, acetone, toluene, boric acid, disodium hydrogen phosphate, monosodium hydrogen phosphate, sodium chloride etc., were of analytical grade and were procured locally. Borate buffer of pH 11 was prepared by dissolving 6.18 g of boric acid and 9.54 g of borax in 1L distilled water and the pH was adjusted to 11 by the addition of sodium hydroxide. Phosphate buffered saline (PBS, pH 7.4, 0.1 M) was prepared by dissolving 17.97 g of disodium hydrogen phosphate, 5.73 g of monosodium

hydrogen phosphate and 9 g of sodium chloride in 1L distilled water.

Methods:

Oxidation of gum arabic

Gum arabic was oxidized using sodium *m*-periodate. Into 100 ml of a 10% solution of gum arabic prepared in distilled water were introduced different quantities of periodate depending on the degree of oxidation desired and the contents were stirred magnetically at 20°C in the dark for 6 h. The extent of oxidation at the end of 6 h was determined by iodometry (24). After the reaction, the contents were dialysed against distilled water with several changes of water over 2 days till the dialysate was free from periodate (checked with silver nitrate). The solution was then frozen and lyophilized to dryness and stored in the desiccator at 4°C until use. Typical yields ranged from 75 to 80%.

Synthesis of gum arabic-primaquine microspheres

Gum arabic-primaquine microspheres were prepared by thermal denaturation process and finally dehydrated using methanol and acetone. Typically, 2 ml of a 10% solution of oxidized gum arabic in borate buffer, was added to 50 mL of a mixture of liquid paraffin and toluene (1:1) containing 1 g of the oil soluble surfactant, sorbitan sesquioleate in a 100 mL round-bottomed (RB) flask. A reverse emulsion was formed by the dispersion of the aqueous phase in the organic phase. To prepare microspheres of small size (<2 μm), the dispersion was cooled in ice and sonicated using the Q-horn of a sonicator (Model 4710, Cole-Parmer, IL, USA) at a power setting of 7 for 3 min. After sonication, a 20% aqueous solution of primaquine phosphate equivalent to 10, 20, 50 and 70% drug payload was introduced and the contents were stirred at 1000 rev/min with a stainless steel half-moon paddle stirrer in an oil bath maintained at 80°C using a mechanical stirrer (Model RW-20, IKA Labortechnik, Staufen, Germany) for 2 h. The contents were then centrifuged, washed with 5 mL aliquots of toluene (5x) till free from oil, followed by 5 mL aliquots of methanol (4x) and finally with 5 mL aliquots of acetone (2x). The microspheres thus obtained were air-dried. Yields were within 70-75%.

Estimation of drug content

Determination of extend of loading of primaquine in microspheres was carried out as follows. A known amount of drug-loaded microspheres was digested using 5 mL of 6 N HCl over a period of 5 h, the solution was filtered through a 0.45 μm filter and the absorbance was measured at 355 nm spectrophotometrically (2) in a UV-Vis spectrophotometer (Spectronic Genesys 2, Milton Roy, NY, USA).

Electron microscopy

Scanning electron microscopy (SEM) was performed using a Hitachi (Model S-2400, Japan) instrument. Microspheres were sprinkled onto double-sided tape, sputter coated with gold and examined in the microscope.

Particle size analysis

The particle size analysis of the microspheres was carried out using SEM. SEM pictures of microspheres were used to determine the average particle size. The diameter of about 100 microspheres was measured from the photomicrographs and the distribution was plotted.

In vitro drug release

A known quantity of the primaquine-loaded

microspheres was suspended in 50 mL PBS in stoppered Erlenmeyer flasks. The flasks were placed in a bath-shaker (Model SW-22, Julabo Labortechnik, Seelbach, Germany), thermostated at 37±1°C at a speed setting of 100 rpm. Aliquotes of 0.5 mL were with drawn at various time intervals, and analyzed spectrophotometrically for primaquine. A constant volume in each flask was maintained by replacing the aliquots with fresh buffer.

Results and discussion:

Branched polysaccharides are reported to be better candidate materials for periodate oxidation to as opposed to linear polysaccharides such as dextran that undergoes non-specific oxidations leading to considerable reduction in molecular weight after oxidation (25). When arabinogalactan was oxidized using periodate at different molar ratios of periodate and the polysaccharide, the molecular weight of the oxidized product was reported to be stable (21). The high yield of the product after oxidation also is a pointer to a stable molecular weight as small fragments generated by non-specific oxidation of the back bone polymer would freely diffuse through the dialysis membrane leading to drastic reduction in the yield.

The oxidized gum arabic was also found to be soluble in aqueous solutions although the solubility was not quite as high as that of gum arabic. Solutions of oxidized gum arabic at 10% concentration were clear and viscous and these solutions were employed in the synthesis of microspheres. The microspheres were prepared by conjugating the drug to the polymer and simultaneously fabricating the microspheres by heat denaturation. Primaquine was used in its commercially available form, the diphosphate salt, the free primaquine being insoluble in water (22). The reaction was carried out in borate buffer of pH 11 as this buffer has been reported to be most efficient in the formation of Schiff base linkages between the amino group of the drug and the aldehdye group in oxidized arabinogalactan (21).

The microspheres obtained were quite spherical in shape and free flowing. Figure 1 shows the SEM of the microspheres and the particle size distribution of the same as depicted in Fig 2. Virtually all microspheres were below 2 μm in size.

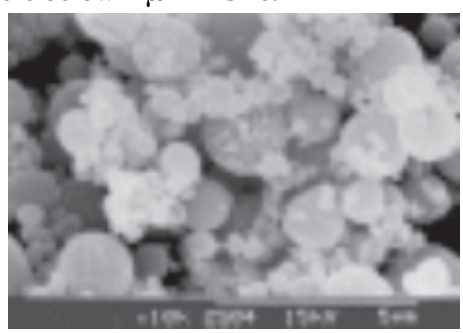

Fig 1: Scanning electron micrograph of microspheres

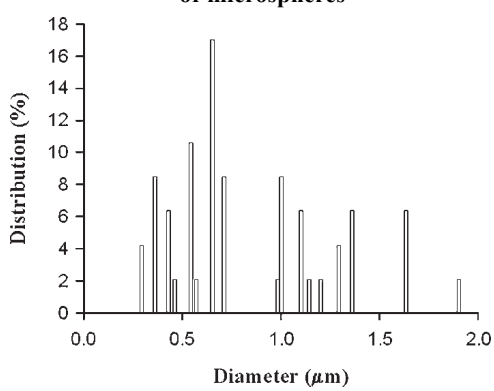

Fig 2: The particle size distribution of the microspheres

Estimation of the drug content of the microspheres after acid digestion showed that the incorporation efficiency the microsphere preparations is not high at all initial drug contents (Table 1).

Table 1: Incorporation efficiency of primaquine in oxidized gum arabic microsphere

Percentagex oxidationof gum arabic	Theoretical drug content (%)	Actual drug content (%)	Incorporation efficiency (%)
50	70	24.7 ± 3.7	35.3 ± 5.2
50	50	8.5 ± 1.3	17.0 ± 2.5
50	20	4.7 ± 0.5	23.5 ± 2.3
20	50	12.8 ± 1.4	25.6 ± 2.6
20	10	3.5 ± 0.4	35.0 ± 2.8

At the alkaline pH, the free primaquine dissociated from the diphosphate salt would enter into reaction with the aldehdye groups of the oxidized polysaccharide leading to the formation of Schiff base linkage between the drug and the polysaccharide and we expected the covalent immobilization of the drug onto the polymer to be high. During the incorporation process, at the relatively high temperature employed, it was seen that the free primaquine generated by the dissociation of the phosphate salt migrating into the non-aqueous dispersion medium as evidenced by the yellow coloration of the medium. However, reasonable, therapeutically significant loadings were obtained by the process employed.

In vitro release of primaquine from the microspheres was examined in phosphate buffer at 37°C. Release from 50% oxidized gum arabic was slower (Fig 3) compared to the release from 20% oxidized gum arabic (Fig 4). For instance, while only about 10% of the drug was released from 50% oxidized matrix having a drug pay load of 4.7%, over 30% was released from 20% oxidized matrix having similar pay load. With both matrices, as expected, increased release was seen with increase in amount of drug initially present. We believe that slow release observed from matrices having a higher degree of oxidation is due to the better drug conjugation onto the polymer because of the large number of aldehyde groups present.

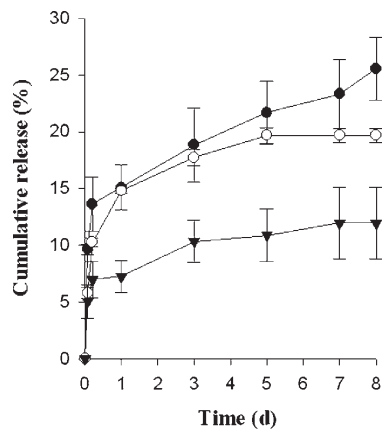

Fig 3: Release of primaquine from microspheres obtained from 50% oxidized gum arabic

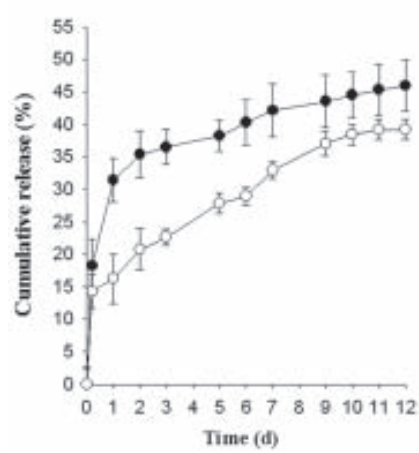

Fig 4: Release of primaquine from microspheres obtained from 20% oxidized gum Arabic

The slow release seen in the case of all microsphere preparations into phosphate buffer pointed to the rather poor hydrolytic susceptibility of the Schiff linkages between the drug and the polymer. Primaquine contains two amino groups and both are not equally reactive. When primaquine was reacted with a highly reactive diacid chloride such as sebacoyl chloride in chloroform, instead of a polyamide, the product obtained was diprimaquine sebacamide demonstrating that the hindered aromatic amino group did not enter into reaction with the acid chloride (22). In case of Schiff base formation between the aldhehyde and the amino groups, it is known that both primary and secondary amino groups are reactive and when there is an aryl group on the nitrogen, the resultant products are reported to be quite stable (26). In order to examine whether both the amino groups are reacting with the aldehdye groups in oxidized gum arabic, we simply mixed primaquine phosphate and oxidized gum arabic in borate buffer and obtained a crosslinked gel that was insoluble in water. This observation suggested that primaquine actually crosslinks the oxidized polysaccharide. Conjugation only through the aliphatic amino group onto the polymer should have resulted in a soluble polymer-drug conjugate as in the case of amphotericin B and arabinogalactan reported by earlier workers (21).

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