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Synthesis and evaluation of isometamidium-alginate nanoparticles on equine mononuclear and red blood cells

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ABSTRACT

Isometamidium hydrochloride (ISMM) is an effective drug for the treatment of trypanosomosis, but it causes local and systemic toxicity. Isometamidium hydrochloride has limited therapeutic index and exhibit considerable variation in their prophylactic activities. We developed a trypanocidal nanoformulation using ISMM and polymers sodium alginate/gum acacia to enhance the efficacy of the drug at lower doses, while minimizing undesirable side effects. It was characterized by transmission electron microscopy and infrared spectroscopy for evaluation of size, morphology, functional groups, *etc.* *In vitro* cytotoxicity studies were performed by metabolic resazurin assay at different concentrations of isometamidium-loaded alginate/gum acacia nanoparticles using equine peripheral blood mononuclear cells. Hemolytic assay revealed significantly less toxicity compared to the conventional drug. The results demonstrate that the developed drug delivery module can be evaluated in suitable animal models to evaluate its potency.

1. Introduction

Trypanosomosis (surra) is an economically important disease caused by protozoan parasite *Trypanosoma evansi.* This infection is mainly restricted to animals, but recent reports indicate their ability to infect humans [1]. A great deal of research effort has focused on the development of pharmacological and parasitological techniques, which have advanced our understanding of the efficacy, resistance, disposition and toxicological mechanisms of these drugs. Further research into the existing drugs is a prerequisite for their optimal usage for improving the animal health and productivity through control of trypanosomosis. Isometamidium chloride (ISMM) is an effective trypanocidal drug for the treatment of trypanosomosis, but it causes local and systemic toxicity in animals. Isometamidium chloride (ISMM) has narrow therapeutic indices exhibiting considerable variation in their prophylactic activities [2,3]. In order to extend the period of protection provided by the drug and to decrease local toxicities, different alternative delivery systems have been developed [2]. However, mainly these systems were limited to laboratory animals, the few experiments in cattle gave unsatisfactory results [4]. Nanoformulations have been reported to reduce toxicity and enhance efficacy at lower doses [5,6]. Nanoformulation of ISMM using a biocompatible polymer as a carrier can enhance the drug efficacy at lower doses, with sustained release of the drug while minimizing undesirable side effects. The important methodological advantages of nanoparticles used as drug carriers are increased stability and carrying capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances [7]. The nanoparticles have a higher surface-to-volume ratio as compared with bulk material, and therefore the dose as well as the frequency of the drug administration would be reduced hence increasing patient compliance [8]. It also augments the NP surface available for interaction with cellular components [9]. The pressing need for new, alternative delivery method of trypanocidal drugs is becoming critical. Sodium alginate is a polysaccharide – a long chain, a carbohydrate polymer with a repeated formula of $C_6H_8O_6$. Sodium alginate and gum acacia, a natural gum, are predominately carbohydrate and generally recognized as safe (GRAS), biodegradable and biocompatible. Therefore, a nanoformulation was synthesized using isometamidium hydrochloride and sodium alginate/gum acacia to enhance the efficacy of the drug at lower doses, with sustained release of the drug while minimizing undesirable side effects. The nanoformulation was characterized for size,

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morphology, interaction of various chemicals used during its synthesis, encapsulation efficiency, ISMM loading and *in vitro* drug release. It was also evaluated for cytotoxicity and its effect on peripheral blood mononuclear cells and erythrocytes.

2. Experimental

2.1. Preparation of ISMM-loaded gum acacia and sodium alginate nanoparticles

With an aim to optimize the concentration of carbohydrate polymers alginic acid sodium, gum acacia and cross linker glutarldehyde nanoparticles, twenty various combinations with the constant concentration of ISMM were performed as tabulated in Table 1. Glutarldehyde of different concentrations was added into the aqueous solution of alginic acid sodium (Sigma-Aldrich Chemicals Private Ltd, Bangalore, India) under constant stirring. Nanoparticles were prepared by drop-wise addition of an aqueous solution of gum acacia (GA) into an aqueous solution of alginic acid sodium salt containing isometamidium hydrochloride (ISMM) under constant stirring up to 2 h. The concentration of 15 mg of isometamidium (img/ml) dissolved in aqueous alginic solution is constant. ISMM-loaded alginate/gum acacia nanoparticles (ISMM-NPs) were recovered by centrifugation at 12000 rcf for 45 min, washed two times with deionised water to remove unentrapped ISMM. The optimized batch is lyophilized at −90 °C and 0.0010 mbar pressure for 24 h using D-Mannitol (5%w/v) as a cryoprotectant, For dummy nanoparticles, the same process was carried out without ISMM.

2.2. Characterization of isometamidium-loaded nanoparticles

Isometamidium-loaded nanoparticles were characterized using Transmission Electron Microscopy (TEM), Fourier-transform infrared spectrophotometer (FTIR), and their entrapment efficiency was determined by the UV–vis spectrophotometer. Dynamic light scattering (DLS) was used to measure the average particle size and size distribution (polydispersity index) of formulated nanocapsules at 25 °C using the Zetasizer nano ZS (Malvern instruments, Malvern, UK).

2.3. Morphology and size of the nanoparticle

The morphology and particle size of ISMM-loaded nanoparticles were observed by transmission electron micrography (TEM) (Morgagni 268D, Fei Electron Optics). The lyophilized sample was dispersed ultrasonically in distilled water to read the individual particles. About (20–25 μl) of the suspension was deposited onto a 400-mess copper grids coated with carbon. Approximately 2 min after the deposition, samples were viewed under a transmission electron microscope, in high contrast imaging mode at an acceleration of 100 KV.

2.4. Fourier-transform infrared spectrophotometer (FTIR)

Fourier-transform infrared spectrophotometer (FT-IR) of ISMM-NPs was performed to see the interaction of polymers and cross-linker with drug during nanoparticles formulation. Samples were reduced to powder and analyzed using an IR spectrophotometer (Shimadzu, Japan) taking potassium bromide (KBr) pellets as a reference. Spectral scanning was taken in the wavelength region between the range of 4500–500 cm^{-1}. The same were obtained for ISMM, alginic acid sodium and gum acacia.

2.5. Encapsulation efficiency

ISMM concentration in the sample solution was calculated by the regression equation obtained by preparing a standard curve for various drug concentrations at 320 nm using a UV spectrophotometer (Shimadzu corporation model UV-2450, Japan). The supernatant obtained after centrifugation of the ISMM-NPs was used for determining the residual ISMM content. The percentage encapsulation efficiency was determined using the formula:

$$\%\text{encapsulation} = (\text{TotalISMM} - \text{UnboundISMM})/\text{TotalISMM} \times 100$$

2.6. Cell culture

Peripheral blood mononuclear cells (PBMC) of equines were isolated by density gradient method using Histopaque-1077 Lymphocyte Separation Media (LSM) (density 1.077; *Sigma-Aldrich*) and were cultured in Eagle's minimum essential medium, supplemented with 10% fetal bovine serum, 10 mM HEPES, 2 mM L-glutamine, 25 mM sodium bicarbonate and 1% antibiotic-

Table 1
The effect of formulation variables on particle size and percent encapsulation efficiency of Isometamidium hydrochloride.

No. of runs	Polymer 1 sodium alginate (gm/100 ml)	Polymer 2 gum acacia (mg/ml)	Cross linker glutarldehyde (% v/v)	Particle Size (nm)	Encapsulation Efficiency (%)
1	0.50	0.30	0.03	276	69
2	0.75	0.30	0.03	146	73.3
3	0.75	0.50	0.03	406	63
4	1.00	0.50	0.05	378	86
5	1.00	0.10	0.01	675	69
6	0.75	0.30	0.03	743	79
7	0.50	0.10	0.05	980	90
8	0.50	0.50	0.01	1432	59
9	0.75	0.30	0.03	546	65
10	1.00	0.10	0.05	326	77
11	0.75	0.30	0.03	876	87
12	0.75	0.30	0.05	1098	94
13	0.75	0.10	0.03	1109	61
14	0.75	0.30	0.03	456	60
15	0.75	0.30	0.03	643	71
16	0.50	0.50	0.05	1187	46
17	1.00	0.30	0.03	321	56
18	1.00	0.50	0.01	165	78
19	0.75	0.30	0.01	1109	76
20	0.50	0.10	0.01	987	71