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Red blood cell ghosts as promising drug carriers to target wound infections

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ABSTRACT

Autologous red blood cell ghosts (RBC ghosts) can carry cytokines to the sites of inflammation. The targeting moiety of the RBC ghosts is associated with the nature of purulent inflammation, where the erythrocytes are phagocyted and encapsulated drugs are released. In the present study we have investigated the healing potential of RBC ghosts loaded with cytokine IL-1β and antibiotic. Additionally, the pharmacokinetic properties of RBC ghosts loaded with IL-1β were studied. 35 Male Wistar rats (250–300 g) were used in the pharmacokinetic study and in a wound infection model where a suspension of *Staphylococcus aureus* was placed into a surgical cut of the skin and subcutaneous tissue in the femoral region. In order to monitor progression of the wound repair processes, wound swabs or aspiration biopsies were taken for analyses on the 1st–6th days. Wound repair dynamics assessment was based on suppression of *S. aureus* growth, signs of pain, time of disappearance of pus and infiltration around the wound. Visual observations, as well as microbiological and cytological analysis of wound exudates demonstrated a significant acceleration of healing processes in a group of animals treated with a local injection of IL-1β and ceftriaxone encapsulated into RBC ghosts when compared to the animals treated either with a local or IM injection of free drugs. For the pharmacokinetic study, single IV injections of either free or encapsulated IL-1β were made and the concentration of IL-1β in serum samples and tissue homogenates were determined. Encapsulation in RBC ghosts improved pharmacokinetic profiles of IL-1β by increasing the half-life, reducing its clearance, and increasing the deposition of the drug in the liver, spleen and lungs. These data suggest that RBC ghosts are effective drug carriers for targeted delivery of cytokines to the sites of inflammation, and have a potential for improving the treatment outcomes of purulent diseases.

1. Introduction

Despite significant advances in modern medicine and the development of new antibiotics the struggle against wound infections still remains relevant [1]. One group of patients who are at high risk for developing untreatable wound infections are those affected by diabetes mellitus [2]. Statistically, approximately 20–25% of diabetic patients develop foot ulceration as disease progresses and around 25% of these patients develop infections that result in amputation [2]. There is also an increase in number of surgical site infections (SSIs) which are associated with antibiotic-resistant pathogens [3]. Disease related inhibition of the immune system is a major factor in the development of chronic and recurrent purulent surgical infections and diabetic foot ulcers And, in this regard, the enhancement of immune functions with interleukins is an attractive therapeutic tool against bacterial complications in immunocompromised patients [4].

It has been shown that experimental application of ointment containing IL-1β resulted in increased healing of venous ulcers and infected burns [5,6]. IL-1β is a cytokine, produced by monocytes/macrophages and other cells. It has multiple biological functions, including induction of other cytokines and stimulation of functional activity of various cells involved in development of innate and acquired immunity [7]. In clinical practice IL-1β is used as a part of cytokine replacement therapy to regulate cytokine imbalance, compensating the lack of endogenous IL-1β.

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Further, there is evidence of the high therapeutic potential of IL in treatment of chronic purulent infections when administrated in parallel with antibiotics [4,8]. This potential is diminished by the pharmacokinetics of the standard treatment regimen of IL-1β (once a day intravenously or subcutaneously) in which the high clearance rate precludes the maintenance of concentrations required for prolonged therapeutic action at the infection site [9,10]. There are some other limitations attributed to many recombinant cytokine drugs which prevent their widespread use in clinical settings, including the probability of serious adverse reactions when presented in high concentrations in blood [11]. Thus, the use of closed transport systems to prevent the creation of excessive peak concentrations in blood and to increase the concentration of the therapeutics at the sites of infection is an effective approach.

Of the many drug transport systems studied as potential carriers for targeted delivery of immune-boosting agents and antibiotics to the sites of infection, red blood cell ghosts (RBCs ghosts) have shown the greatest potential for clinical implementation in clinical results [12–14]. The intrinsic targeting moiety of RBC ghosts is associated with the nature of purulent inflammation, where a large number of erythrocytes are phagocyted by immune cells releasing the drugs encapsulated in the RBC ghosts. [15]. Among the many advantages of using RBC ghosts as drug carriers are biocompatibility, immune system evasion, and biodegradability [12–14].

A wide range of chemicals has already been studied and successfully loaded into RBC ghosts [12,14,16]. Multiple studies have shown the efficiency of using RBC ghosts to target particular organs, such as liver and spleen [17,18]. Among other advantages of carrier erythrocytes are the improvement of pharmacokinetic and pharmacodynamics properties of the drugs and significant increased stability of plasma concentrations [12,14,16]. RBC ghosts have also been shown to protect the host from the toxic effects of the drugs, and any undesired immune response against the encapsulated drug [19].

All of the benefits described above, as well as a broad spectrum of available techniques and technologies for transfusion, isolation and handling of red blood cells, make them promising candidates to target wound infections. In this regards, in the present study we have investigated a healing potential of RBC ghosts loaded with cytokine IL-1β and antibiotic, as well as pharmacokinetic properties of RBC ghosts for their further clinical implementation.

2. Materials and methods

2.1. Animals

35 male Wistar rats of 250–300 g body mass were used for this study. The rats were housed in a room with controlled temperature of 24 ± 1 °C, a relative humidity of 55 ± 5%, and a 12-h light/dark cycle (7:00 a.m. to 7:00 p.m.) with free access to water and a standard rat diet.

2.2. Preparation of IL-1β loaded RBCs

Lyophilized powder of Betaleukin (0.5 mg) was from the State Research Institute of Highly Pure Biomaterials (St. Petersburg, Russia). Blood samples (1 ml per animal) were collected from saphenous vein of healthy white Wistar rats by venipuncture and transferred to heparinized polypropylene tubes. After centrifugation at 1000*g* for 10 min, the plasma and buffy coat were separated by aspiration, and the remaining erythrocytes were washed three times with PBS (150 mmol/l NaCl and 5 mmol/l K_2HPO_4, pH 7.4).

A hypotonic preswelling method described and validated by Tajerzadeh and Hamidi was used for loading the erythrocytes with IL-1β [20]. Briefly, 1 ml of PBS containing washed packed erythrocytes was transferred to a test tube, 4 ml of hypotonic PBS with an osmolarity of 0.67 was added, and the resulting cell suspension was gently mixed. The swollen cells were separated by centrifugation at 1000*g* for 10 min, and the supernatant was discarded. A 200 µl aliquot of a hemolysate, prepared by diluting another portion of erythrocytes with distilled water (1:1), was added to the remaining swollen cells, 250 µl of an aqueous solution of IL-1β (50,000 pg/ml) was gently added onto the cell suspension, and the resulting mixture was centrifuged at 1000*g* for 5 min. This procedure was repeated three more times. The RBCs were then resealed by rapid addition of 100 µl of hypertonic PBS with an osmolarity of 10 times that of an isotonic solution, followed by gentle mixing of the suspension and incubation at 37 °C for 30 min. The obtained RBC ghosts were washed three times in PBS to remove free IL-1β and unbound cell constituents.

2.3. Determination the amount of IL-1β irreversibly bound to RBCs

The extent of drug encapsulation was determined using a dialysis-based method. The amount of encapsulated drug (C_i) was calculated as the difference between the initial concentration of drug and the concentration of drug in the dialysate: $C_i = L_0 - L_i$ (1), where L_0 is total concentration of drug loaded into the dialysis system with the RBCs, L_i is concentration of unbound drug after three washings. Drug-loaded RBCs were washed with saline solution thrice, each time with a determination amount of eluted drug. A binding efficiency of IL-1β was calculated according to a formula: $C_i \times 100\%/L_0$ (2). The concentration values of IL-1β in PBS were determined using an ELISA kit (Sigma-Aldrich).

2.4. A modeling of wound infection

All the surgical procedures have been carried out under isoflurane anesthesia. To create a wound infection, a cut of 1.0 cm in the skin and subcutaneous tissue of a pre-shaved area of the femoral region was made. The fascia was then dissected and the muscle fiber longitudinally separated using anatomical forceps. Gauze soaked in 1 ml of microbial suspension containing *Staphylococcus aureus* at a dose of 2×10^6 (as determined by a turbidity standard) was placed in the cut. The skin defect was then stitched with two Dacron sutures (3/0) using an atraumatic needle. Hip abscess in experimental rats was observed in 5–7 days. On day 7 abscesses were opened and sanitized.

Following opening of the abscess animals were divided into two control and one experimental group ($n = 15$). The animals from the experimental group were treated by the following technique: RBC ghosts containing a single dose of ceftriaxone (0.01 g) and cytokine interleukin-1β (0.02 µg) were injected into the edges and bottom of the wound. Then the wound cavity was sutured layer by layer. After 24 and 48 h RBCs loaded with ceftriaxone and cytokine interleukin-1β were reintroduced to the wound edges. The assessment of binding efficiency and pharmacokinetic study of ceftriaxone loaded into RBC ghosts were conducted and reported earlier [21]. Animals in the first control group were treated with a local injection of free drugs. Animals in the second control group were treated with the intramuscular (IM) injection of the antibiotic ceftriaxone (0.01 g twice a day, during 7 days) and topical application of ointment “Levomecol” (combination of Chloramphenicol and Methyluracil).

2.5. Assessment of wound repair dynamics

In order to monitor progression of the repair processes wound swabs or aspiration biopsies were taken immediately post-injury and on the 2nd, the 4th, and the 6th days after surgery for cytological and microbiological analyses. Cytological slides were stained using *Papanicolaou (PAP) stain* and assessed by light microscopy. The efficacy of the treatment was evaluated by the suppression of