Red Blood Cells for a New Drug Formulation

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ERYTECH Pharma
Advantages of Erythrocytes for Drug Delivery

- Post mitotic cells
- Biocompatible
- Well understood metabolism and biodistribution
- Well understood and adjustable biological $T_{1/2}$
- Ability to encapsulate macromolecules
- Long history of regulatory experience
- Homogenous cell source
- Easily obtained
- Evolved to transport proteins
- Transit capillary beds easily
Erythrocyte carrying Drugs …

… an innovative cell based medicinal product

Encapsulated

Coupled to the surface
## Methods for drug encapsulation in erythrocytes

|M. Magnani, Biotechnology Intelligence, 2003|

<table>
<thead>
<tr>
<th>Methods</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electroporation</strong></td>
<td>Suitable for low amount of cells</td>
</tr>
<tr>
<td></td>
<td>Best suited for low-Mr substances</td>
</tr>
<tr>
<td><strong>Drug Induced Endocytosis</strong></td>
<td>Inducible only by certain drugs</td>
</tr>
<tr>
<td><strong>Osmotic Pulse</strong></td>
<td></td>
</tr>
<tr>
<td><strong>DMSO</strong></td>
<td>Suitable for low amount of cell</td>
</tr>
<tr>
<td></td>
<td>Require long time</td>
</tr>
<tr>
<td><strong>Continuous-flow</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Suitable for large amount of cell</td>
</tr>
<tr>
<td></td>
<td>Require long time</td>
</tr>
<tr>
<td><strong>Hypotonic hemolysis</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Dilutional</strong></td>
<td>Simple and fast</td>
</tr>
<tr>
<td></td>
<td>Suitable for low-Mr substances</td>
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<tr>
<td></td>
<td>Loss of erythrocyte content</td>
</tr>
<tr>
<td></td>
<td>Low percentage encapsulation</td>
</tr>
<tr>
<td><strong>Preswell Dilutional</strong></td>
<td>Simple and fast</td>
</tr>
<tr>
<td></td>
<td>Good in vivo survival</td>
</tr>
<tr>
<td></td>
<td>Low percentage encapsulation</td>
</tr>
<tr>
<td><strong>Dialysis</strong></td>
<td>Simple</td>
</tr>
<tr>
<td></td>
<td>High percentage encapsulation</td>
</tr>
<tr>
<td></td>
<td>Good in vivo survival</td>
</tr>
<tr>
<td></td>
<td>Good cell recovery</td>
</tr>
<tr>
<td></td>
<td>Large scale procedures available</td>
</tr>
<tr>
<td><strong>Preswell Dilutional with Concentration</strong></td>
<td>Require short time (2 hours)</td>
</tr>
<tr>
<td></td>
<td>Suitable for clinical use starting from 50 ml blood</td>
</tr>
<tr>
<td></td>
<td>Good in vivo survival</td>
</tr>
<tr>
<td></td>
<td>Good cell recovery</td>
</tr>
<tr>
<td></td>
<td>Good percentage encapsulation</td>
</tr>
</tbody>
</table>
Hypotonic reversible hemolysis method

Controlled lysis (hypotonic stress)

Resealing (hypertonic stress)
Erythrocyte loaded Drugs = Long circulating and systemic action

Membrane treated Erythrocyte loaded Drugs = Targetting to macrophages/Dendretic cells

thanks to erythrophagocytosis by the Reticulo Endothelial System
A large broad of applications

- Enzyme Replacement Therapy
- Tissue Oxygenation enhancement
- Drug Targeting (REs)
- Thrombolytic Vehicle
- Small Molecules Release
- Immunotherapy (vaccine)
- Specific Protein Tolerance induction
An unique mode of action …

… for erythrocyte encapsulated enzymes
A large broad of applications

Enzyme Cancer Therapy
Enzyme in cancer therapy: Ex. GRASPA

Stage of Development: European Pivotal Phase II/III

By ERYTECH Pharma, France
Improved Pharmacokinetics/Pharmacodynamic

Half-Life
(free) Asparaginase $\approx 24$ hours
Erythrocytes Encapsulated Asparaginase $\approx 30$ days

Duration of plasmatic asparaginase depletion
(free) Asparaginase $\approx 3$ days
Erythrocytes Encapsulated Asparaginase $\approx 18$ days

## Tolerability of GRASPA vs. Free L-Asparaginase

Comparative table of adverse effects collected on patients treated with GRASPA and patients treated with free L-Asparaginase (native E.Coli)

<table>
<thead>
<tr>
<th></th>
<th>TOTAL GRASPA (%)</th>
<th>TOTAL L-ASPA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All doses (50, 100 and 150 IU/kg)</td>
<td></td>
</tr>
<tr>
<td>N patients</td>
<td>39</td>
<td>16</td>
</tr>
<tr>
<td>Anaphylactic shock</td>
<td>0.0%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Drug hypersensitivity</td>
<td>5.1%</td>
<td>62.5%</td>
</tr>
<tr>
<td>Clinical Thrombosis</td>
<td>2.6%</td>
<td>6.3%</td>
</tr>
<tr>
<td>Antithrombin III decreased</td>
<td>2.6%</td>
<td>43.8%</td>
</tr>
<tr>
<td>Other Coagulation disorder</td>
<td>28.2%</td>
<td>37.5%</td>
</tr>
<tr>
<td>Protein synthesis disorders</td>
<td>17.9%</td>
<td>25.0%</td>
</tr>
<tr>
<td>Transaminases increased</td>
<td>46.2%</td>
<td>37.5%</td>
</tr>
<tr>
<td>Clinical Pancreatitis</td>
<td>7.7%</td>
<td>6.3%</td>
</tr>
<tr>
<td>Pancreatic enzymes increased</td>
<td>20.5%</td>
<td>25.0%</td>
</tr>
</tbody>
</table>
A large broad of applications

Enzyme Replacement Therapy
Severe combined immunodeficiency due to

**Adenosine Deaminase (ADA) deficiency**

⇒ Increase in deoxyadenosine triphosphate (dATP)

⇒ Decrease in S-adenosylhomocysteine hydrolase (SAHH)
  Vital mediator of transmethylation reactions

**Stage of Development: Proof of concept in Patients**
Developed by Dr Bridget Bax (St Georges Hospital, London, UK)

One-off therapy for a patient with no other treatment options
A patient has been treated since 1997…

- Erythrocyte ADA activity on diagnosis <1 nmol/hr/mg Hb
- Normal control range = 40 -100 nmol/hr/mg Hb
- Trough circulating erythrocyte ADA maintained at supra-physiological level

Bax et al, European Journal of Haematology, 2007; 79, 338-348
Erythrocyte dATP level and SAHH activity

- From 153 months onwards, achieved metabolic correction of the dATP levels

- Past 12 months achieved activities at the lower end of the normal range (3 - 12 nmol/hr/mg Hb)
• CD4⁺ CD8⁺, CD3⁺ T cells and C16⁺ natural killer cells all increased in numbers over the last 12 months

• Not been able to expand the peripheral blood lymphocyte populations to levels seen in healthy individuals

• Maintained adequate immunity for over 13 years in terms of preventing hospital admissions for respiratory disease
**Mitochondrial NeuroGastro-Intestinal Encephalomyopathy** (MNGIE) due to

**Thymidine Phosphorylase Deficiency**

⇒ Pathological Tissue and Plasma accumulation of Thymidine and Deoxyuridine

No treatment available.

Patients die in the second and third decades of life

**Stage of Development: Proof of concept in Patients**
*Developped by Dr Bridget Bax (St Georges Hospital, London, UK)*
• 2006 approached by neurologist - seriously ill patient who had been recently diagnosed with MNGIE

• Obtained ethical approval for a one off administration of enzyme-loaded erythrocytes on compassionate grounds

• Administered 1020 IU TP encapsulated in 20.3 x10¹⁰ cells

• 3 days post infusion: urinary excretion of thymidine and deoxyuridine ↓ to 6% and 13%, respectively of amounts excreted pre-therapy

• In parallel, plasma metabolites ↓ first 3 days after infusion

• Preliminary data demonstrated the feasibility of this therapeutic strategy in reducing plasma and urine [thymidine and deoxyuridine]

• Patient died from pneumonia 21 days later

Moran NF, Bain MD and Bax BE. Neurology. 2008; 71, 686-688

To date, two other patients have been successfully treated for 2 years
Tissue Oxygenation enhancement
Enhancement of tissue Oxygenation

Three-fold increase of oxygenation capacity of RBCs

Physiological conditions « Normal RBCs »

Encapsulation process of Inositol Hexaphosphate (IHP)

IHP-loaded RBCs

Entrapment of Inositol Hexaphosphate increases oxygen release by 3 folds compared to normal RBCs
Application in Sickle Cell Anemia

IHP-RBCs (ENHOXY) Infusion = enhanced oxygenation

- Vaso-occlusive crisis
- RBC sickling
- Vaso-constriction: Increase of EDN1 peptide
- Vascular adhesion: Increase of THBS1
- Inflammation: Release of interleukins and acute phase response proteins
- Cascade of vascular events

Hyoxia
In vivo evaluation in SAD mice

Partial RBC exchange
- Control RBCs
- IHP-RBCs

Hypoxia 4h/Reoxygenation stress

Sacrifice at 6h of reoxygenation

RT-PCR analysis of the lungs

**Bourgeaux et al, British Journal of Hematology, 2012**
Small Molecules
Slow Release
Ex: Phosphate coupled Dexamethasone

Stage of development: Phase III
Developed by Prof. Magnani (ERYDEL, University of Urbino, Italy)
Already tried for Crohn Disease, Ulcerative Colitis, Cystic Fibrosis, COPD
Semilogarithmic plot of plasma concentration of Dexamethasone in patient n. 9 at times 0 (immediately post infusions) and 1, 9, 16 and 28 days post two successive infusions of erythrocytes loaded with 10.76 and 15.47 mg of Dex 21-P, respectively.

Rossi et al, Blood Cell Mol Dis., 2004
A large broad of applications

Thrombolytic Vehicle
Example of Plasminogen Activators

Developed by Prof. V. Muzykantov (UPenn, Philadelphia, USA)
Stage of development: In vivo proof of concept in animals done
Plasminogen activators (PAs) are used to treat life-threatening thrombosis, but not for thromboprophylaxis because of rapid clearance, risk of bleeding, and central nervous system (CNS) toxicity. Erythrocyte encapsulation improve the pharmacokinetics, the targetting to clots and reduce the toxicity.

Muzikantov, Expert Opin Drug Deliver., 2010
Drug Targetting (RES)
Biodistribution depending on RBC surface treatment

Spleen

Liver
Specific Protein Tolerance induction
Immune tolerance induction to proteins

Inducing antigen-specific Immune Tolerance

This strategy is based on:

- The physiological properties of Red Blood Cells (RBCs) to be removed from the circulation by antigen-presenting cells (APCs)

- Specific treatment of RBCs allowing targeting preferentially in the liver, known to favor the induction of immune tolerance

Developed by ERYTECH Pharma
Stage of development: several demonstration in animal with different allergenic therapeutic proteins.
Immune tolerance induction to proteins

Immune tolerance induction: 3 intravenous (IV) injections of OVA: free or RBC-loaded (100µg)
Protocol of immunization: 2 IV injections of OVA (170µg) and Poly(I:C) (32µg)
or 2 intradermal (ID) injections of OVA (25µg) and Cholera Toxin (CT, 1µg)

Tolerance induction

Free OVA RBC-OVA
(with targeting or not)

Immunization

Challenge
OVA + Poly(I:C) (IV) or
OVA + CT (ID)

OVA-specific T
and B cell response
analysis

T and B cell responses inhibition

% of OVA-specific in vivo lysis

% of OVA-specific IgG titers

IV Challenge
RBC-OVA with targeting + IV Challenge
Free OVA + IV Challenge
Vehicle

p < 0.03
p < 0.04
A large broad of applications

Immunotherapy
(vaccine)
Therapeutic vaccination by *in situ* targeting of DCs

Antigens and/or Adjuvant are encapsulated in erythrocyte and then modified in a way to enhance their captation by specific DCs.
Tyrosinase-related protein 2 (TRP-2), is a peptide able to control the growth of B16F10 tumor cells expressing TRP-2. TRP-2 is a tissue differentiation antigen expressed by normal and malignant melanocytes.

*Vaccinal Immunotherapy: Animal proof of concept*

*Banz et al, Immunotherapy 2012 (In press)*
Animal proof of principal

Banz et al, Immunotherapy 2012 (In press)
The Erythrocyte-Drug product has to be release by a qualified person based on quality control results. The quantity of encapsulated drug has to be reproducible between batches and it has to be controlled before release.

Even if it is a one-to-one cell therapy product, this has to be manufactured at an industrial scale (automatisation of the process) to be economically relevant.

One-to-one…..

…..until the universal cultured red cells are available.