A) TITLE

USE OF BLOOD PLATELETS AS siRNA VECTORS FOR SILENCING THE BCR-ABL FUSION GENE IN CHRONIC MYELOID LEUKEMIA (CML)

B) INTRODUCTION

The BCR-ABL fusion gene is the triggering molecular pathogenic event in chronic myeloid leukemia (CML). The resulting BCR-ABL protein exhibits constitutively active tyrosine kinase activity leading to the permanent activation of several downstream targets resulting in oncogenic growth and inhibition of apoptosis of the leukemic cells (1). In recent years, a variety of tyrosine kinase inhibitors (TKIs) targeting BCR–ABL have been developed for the treatment of CML. However, 20–30% of patients develop TKIs resistance and do not respond optimally to TKIs (2).

Short interfering RNAs (siRNA) have emerged as potential cancer therapeutics and evidence has been provided that siRNAs directed to BCR-ABL reduce protein expression in vitro (3) and in vivo (4). A BCR-ABL siRNA was used as a therapeutic approach in an Imatinib-resistant CML patient obtaining inhibition of the BCR-ABL oncogene. However, the patient became resistant after the third siRNA administration suggesting the emergence of siRNA resistance or transfection failure (5). Indeed, the therapeutic use of naked siRNA is limited due to induction of serum RNase, immune response and by non-targeted effects. Therefore, the development of optimal delivery methods with the use of efficient siRNA carriers is required before getting to the widespread clinical application of siRNA therapeutics in vivo. siRNA carriers should cross cell membranes efficiently, have few or no off-target effects, protect siRNAs from degradation/elimination and have low toxicity (6).

The use of platelets as siRNA carriers could overcome the problems of toxicity, immune system response and low efficiency of interaction of RNAi drugs with the target cell because of their peculiar properties, and in particular for the close interactions that platelets take with cancer cells. In fact, human blood platelets the main cellular effectors of hemostasis and thrombosis, have a strong attitude to closely interact with other cells, including CML cells (7), and play a crucial role in cancer growth, metastasis and angiogenesis (8). The ability of platelets to deliver miRNAs to recipient cells, namely to white blood cells (9) or endothelial cells (10), has been recently demonstrated. Despite several attempts to achieve platelet transfection with siRNA using common methods, so far only a low transfection efficiency (less than 9%) has been obtained (11). In our laboratory we have recently developed a novel technique for platelet transfection (patent filed) obtaining a high and reproducible transfection rate of siRNA into human platelets (97% efficiency) within 5 minutes of incubation and we have shown that siRNAs transfected in human platelets are efficiently transferred to monocytes and endothelial cells with which platelets get in contact.

C) AIMS OF THE PROJECT

General aim of the project is to employ our original transfection method to introduce siRNAs directed against the BCR-ABL junction sequence in platelets and to use the latter as carriers to transfer the siRNAs to CML cells thus silencing the BCR-ABL fusion transcript.

Specific aims/steps of the project are:

1) Evaluate of the release of siRNAs from human platelets to other cell types
2) Evaluate the localization of platelets carried siRNA in vivo in animal models
3) Generate siRNA molecules for the silencing of the BCR-ABL fusion gene.
4) Assess whether blood platelets deliver siRNAs effectively and functionally to CML cells in vitro.
D) METHODS

1) Co-incubation of siRNA-transfected platelets with monocytic cell line (THP1) and human immortalised myelogenous leukemia cell line (K562) will be carried out. Human platelets, isolated from healthy donors and transfected with a fluorescent-labeled siRNA, will be used to test the efficiency of siRNA delivery to the THP1 and K562 cell lines after co-incubation for different time intervals at different ratios. Efficiency of siRNA delivery will be assessed by flow cytometry and fluorescence microscopy.

2) Studies of localization of the release of the fluorescent siRNA in mice (CD1) will be carried out under physiological conditions. Stimuli that generate siRNA release from platelets will also be investigated.

3) Different siRNA molecules directed against the BCR-ABL junction sequence will be tested for their efficiency in silencing the BCR-ABL fusion transcript by transfecting them in the chronic myeloid leukemia cell line K562 using Lipofectamine2000™ (Invitrogen). BCR-ABL transcript level will be assessed by Real Time PCR.

4) The most effective siRNA towards the BCR-ABL fusion gene will be transfected in blood platelets from healthy donors and transfected platelets will be co-incubated with the K562 cell line. The BCR-ABL transcript level in the K562 cell line will be assessed by Real Time PCR and apoptosis by flow cytometry.

White blood cells (WBC) derived from ph+ CML patients will be co-incubated with siRNA transfected platelets in vitro for different times at different ratios. The BCR-ABL transcript level in ph+ CML WBC will be assessed by Real Time PCR and apoptosis by flow cytometry.

Common statistical methods will be applied; number of replications, for in vitro/in vivo studies, will be calculated a priori using sample size calculators.

Expected results are:

- Define the timing of co-incubation and the platelet/drug ratio able to achieve a therapeutic effect in vitro
- Define the timing of co-incubation and the platelet/drug ratio able to achieve a therapeutic effect in vivo
- Obtain a pool of siRNAs potentially able to efficiently silence the BCR-ABL fusion gene.
- Show that efficient silencing of BCR-ABL in CML cells can be obtained by the interaction of siRNA-transfected platelets with CML cells in vitro.

The siRNA-based strategy, using platelets as carriers, may in perspective prove useful not only for the treatment of CML but also for other forms of leukemia and also for solid tumors for which no small-molecule-targeted chemotherapy is currently available or for which development of resistance has been shown, and for which a clear target for a siRNA-based therapy is identifiable. In the future it will be possible to apply this original strategy to re-infuse transfected platelets intravenously in the same subject from which they were collected, thus eliminating the risk of alloimmunization and rejection of transfected platelets. Given the short time required to obtain platelet transfection and its high efficiency, contrarily to what can be obtained transfecting WBCs, platelet carrier-siRNA therapy can be envisaged to be applied to the single patient by the use of an extravascular circulation apparatus.

E) REFERENCES