Pharmacologically induced intrapneumonal arteriovenous shunting for safe and effective intravenous administration of mesenchymal stem cells

Bachelor thesis

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Abstract
The aim of this study was to shunt cell sized microspheres injected into the jugular vein through the pulmonary circulation to the arterial side using pharmacological intervention. The secondary aim was to observe the systemic effects of the treatments and microsphere injections, with the ultimate goal of finding a viable regimen to use with intravenous mesenchymal stem cell injections as a treatment for type 1 diabetes.

Method: Three vasodilating drugs were tested in a rat model, ET-1β antagonist BQ-788, sodium nitroprusside and imatinib mesylate (gleevec). Microspheres of two colors were used to quantify the degree of passage to the arterial side. Black microspheres were injected prior to drug administration and used as control, whereas green microspheres were injected after the drug administration. The microspheres were later counted in harvested organs and arterial blood.

Results: The drug that yielded the highest percentage of passage was Gleevec which had a 52% shunt quotient to the arterial blood and a 6% quotient to the pancreas. We suggest further investigation into Gleevec therapy.

Introduction
During the last 12 years islet transplantation has been one of the cutting edge techniques for treatment of type 1 diabetes mellitus (Shapiro et al., 2000). Although an effective treatment it’s long term viability is still somewhat unsatisfying, since 90% of the patients that received the transplant again required daily insulin injections 5 years after the transplantation due to partial graft loss (Ryan et al., 2005). Co-transplantation of islets and mesenchymal stem cells has been proposed to improve graft function. Mesenchymal stem cells (MSCs) are multipotent cells that are capable of self renewal and differentiation to various cell types. They can be found in many tissue types such as the skin, bone marrow, adipose tissue, fetal liver and umbilical cord (Shenaq et al., 2010). It is theorized that MSCs could improve graft survival due to their immunomodulatory and angiogenic effects that they have on their surrounding tissue. A recent study showed a possible explanation to the immunomodulation by MSCs (Solari et al., 2009). In this study, it was shown that when islets are co-transplanted with MSCs, the T-Cells in the surrounding lymphoid tissue expressed much lowered levels of the cytokines IFN-γ and IL-10 compared to the T-cells from the group that received the islets alone, a substantial decrease in TNF-α production was also noted. Considering these facts it was concluded that the inflammatory cytokine response is decreased in the presence of MSCs.

An explanation of the angiogenic effect of MSCs has been proposed (Johansson et al., 2008) (Ito et al., 2010). Endothelial cell proliferation increased threefold when cultured together with islets in MSC conditioned medium (Johansson et al., 2008), and there was an increase in VEGF and endothelial von Willebrand factor when islets are co-transplanted with MSCs. (Ito et al., 2010)

Some recent studies show that the mesenchymal stem cells alone are capable of prolonging survival and inducing regeneration of endogenous β-cells (Ciceri and Piemonti, 2010). That coupled with MSCs homing capacity to the pancreas due to cross-talking by chemokines (Sordi, 2009) shows an opportunity for new treatments such as intravenous systemic administration of MSCs, which is a seamlessly effective and safe approach. However this treatment is not without its problems, studies
have shown that intravenously injected MSCs get entrapped in the small blood vessels of the lungs
(Chen et al., 2001).

Figure 1. A mouse that received a bioluminiscent MSC injection into the tail vein. The MSCs are then visualized with the help of an IVIS camera. Colored areas over the lungs show MSC entrapment. Image courtesy of Liza Grapensparr, Uppsala university, Sweden.

To avoid entrapment in the lungs an intracardial approach is preferred, but it still carries serious risk of complications that are associated with intracardial injections.

We the possibility of shunting cell sized particles through the pulmonary circulation with pharmacological intervention prior to cell administration should be investigated. The aim of this study was to determine a pharmacological treatment regimen that will allow shunting of cell sized particles through the pulmonary circulation in a rat model using microspheres.

The microsphere technique has previously been proved valuable for measuring regional blood flow to many different tissues types such as the pancreas under various conditions (Carlsson et al., 1996), (Prinzen and Bassingthwaighte, 2000). Although those studies used smaller-than-cell sized microspheres for blood flow studies, we theorize that using larger microspheres will an adequate model for MSC distribution. The downside of this model is the lack of the innate homing of the MSCs which is not easily replicated.

**Materials and methods**

**Animals.**

14 male Sprague-Dawley rats were obtained from a local colony at Uppsala University Biomedical center. The animals had free access to pelleted food and tap water throughout the experiments. All experiments were approved by the local animal ethical committee at Uppsala University.

**Shunting measurement.**

The rats were randomly divided into 3 groups (n=2x5, 1x4). Each group was subjected to different treatment. The animals were anaesthetized using thiobutabarbitral sodium (Inactin®; 60mg/kg; Sigma Aldrich) and placed on the operating table maintained at body temperature of 38°C.
Polyethene catheters were inserted into the juglar vein and the femoral artery. Fluid (Ringer Acetat, 5ml/kg/h; ACO) were continuously administered through the jugular catheter. The arterial catheter was connected to a pressure transducer allowing continuous mean arterial pressure monitoring. Injection of $9 \times 10^4$ microspheres (15µm diameter) of 2 different colors (black and green) was used to measure the degree of A-V shunting. The use of differently colored microspheres eliminated the need for a control group.

The microspheres were properly agitated before injection. After catheterization, blood pressure was monitored for 10 minutes to obtain baseline recordings after which a suspension containing black microspheres (positive control) was injected into the jugular catheter and blood pressure changes were monitored. Following hemodynamic stabilization, treatment was administered through the abovementioned catheter. After 1 minute an arterial blood sample was obtained during one minute of free flow from the femoral catheter. After the first five seconds of catheter flow a suspension containing the green microspheres was injected through the jugular catheter.

**Drug preparation and administration.**

Each group of animals was assigned one specific therapy. Group 1 received an injection of the ET-1β antagonist BQ-788 (100mg/kg; Sigma Aldrich) dissolved in1% PEG-40 Castor oil (Niccor crop, Japan)and saline.

Group 2 received an injection containing an emulsion of imatinib mesylate (Glivec 7mg/kg; Avartis was prepared using 1% PEG-40 Castor oil (Niccor corp. Japan) in saline. The emulsion was properly agitated prior to injection.

Group 3 received an infusion of Nitroprussid (10µg/kg/min; Sigma Aldrich) dissolved in distilled water and further diluted in saline.

Subsequently to the blood sampling the animals were euthanized by cervical dislocation after which the pancreas, adrenal glands and the lungs were harvested and weighed.

**Sampling**

After weighing the tissue samples were cut into small pieces and put between object slides. They were subjected freeze-thawing for better visualization of the microspheres (Jansson and Hellström et.al 1981). The lungs were suspended in 1M NaOH overnight and later homogenized by sonication.

Microspheres on each slide were counted under a stereomicroscope, the blood and homogenised lung samples were agitated prior to counting of microspheres on filter paper.

**Analysis**

The quantification of the shunt opening was done by calculating a shuntquotient (the amount of microspheres of each color/g tissue divided by amount of microspheres of each color/g lung tissue for each color of microspheres) for all samples.
Results
In these experiments we determined whether it is possible to shunt cell sized particles from the venous side to the arterial with the help of different vasodilating drugs. We used a microsphere technique to quantify the shunting.

After preparation of the tissue samples the amount of microspheres was counted. In the solid samples i.e. the adrenal glands and pancreas all of the microspheres were counted. In the liquid samples i.e. the blood and the lungs the concentration of microspheres was calculated by counting the amount of microspheres in a known volume. The total amount of microspheres in the sample was then calculated by multiplying the concentration by the total volume of the sample. All liquid samples were shaken before counting since the microspheres tend to fall to the bottom of the sample.

Using these values the amount of microspheres per gram tissue was calculated. And the shuntquotient was calculated.

Shuntquotients
The shuntquotient is the percentage of microspheres that passed through the pulmonary circulation to peripheral tissues.

BQ-788
No significant shunting was observed in the group of animals treated with BQ-788 (Figures 2, 3). After four samples were counted and no significant shunting was observed we decided to move on to the next therapy to reduce the number of animals in the experiment.

Figure 2: The Blood/Lung shuntquotient for the group treated with BQ-788. Figure 3: The Pancreas/Lung shuntquotient for the group treated with BQ-788. Note that the shuntquotients for the control spheres were somewhat higher than for the green ones.
**Sodium Nitroprusside**

Somewhat higher shunt quotients were observed in the group of animals treated with sodium nitroprusside (figures 4, 5). Another interesting observation was that three animals that received nitroprusside developed pulmonary edema after approximately five minutes of infusion.

![Figure 4](attachment:image1.png) ![Figure 5](attachment:image2.png)

Figure 4: The blood/lung shunt quotient for the group treated with nitroprusside. Figure 5: The pancreas/lung shunt quotient for the group treated with nitroprusside. Note that subjects NP 1-3 developed pulmonary edema during the infusion of nitroprusside. Notice also that the quotient for the control microspheres is higher than for the green ones.

**Imatinib mesylate**

The only therapy that yielded substantial results, the highest shunt quotient of this study was observed in this group, albeit in only one test subject (Figures 6, 7). One of the test subjects died right after the therapy was administered (GL03).

![Figure 6](attachment:image3.png) ![Figure 7](attachment:image4.png)

Figure 6: The blood/lung shunt quotients of the group treated with gleevec. Figure 7: The pancreas/lung shunt quotients of the group treated with gleevec. Note that the control quotients are below 1%.
Changes in mean arterial pressure

Mean arterial pressure was monitored for the duration of the entire experiment. A baseline measurement was obtained for 10 minutes prior to any procedures. As expected the pressure dropped following the administration of the control microspheres and the treatment since both affect the vascular system. The pressure changes for 3 major events of the experiment were recorded and plotted into the graphs below (Figures 8, 10).

Discussion

The results that were obtained through this study confirmed that cell-sized particles become trapped in the pulmonary circulation. Of the three treatments that were used to diminish this problem only one was potentially successful, imatinib mesylate. It has previously been shown that imatinib mesylate is a promising treatment for pulmonary hypertension (PH) where it acts as a vasodilator and ameliorates the symptoms of this condition (Abe. K et al., 2011). Imatinib mesylate lowers blood pressure by inhibition of myosin light chain phosphatase. When this is inhibited, the smooth muscle cells are unable to contract which in turn lowers vascular resistance. Sodium nitroprusside uses a similar mechanism, and in this study similar effects of sodium nitroprusside and imatinib mesylate were observed. Another similarity was that most of the animals being treated with either imatinib mesylate or sodium nitroprusside developed pulmonary edema a couple of minutes after the drugs
were administered, this can be explained by the rapid blood pressure drop which in turn induced hypovolemic shock. Although they use the same mechanism of action these drugs however yielded different results on the suntquotients. One possible explanation to this is that sodium nitroprusside inhibits the myosin light chain phosphatase through several intermediaries, guanylate cyclase and protein kinase G, whereas imatinib mesylate inhibits the phosphatase directly.

This study have shown that imatinib mesylate is indeed a potent vasodilator and has the potential of assisting larger particles to pass through the pulmonary circulation. This is a useful trait that could be used for intravenous administration of MSCs.

The treatment protocol, however, still needs refining since our study could only produce one successful test subject. We suggest adjusting of the dosage and trying out different administration methods since infusion of a imatinib mesylate emulsion produced high mortality rates in test subjects and imatinib mesylate has a high bioavailability when administered orally although this method of administration is somewhat inconvenient in a rat model.
References


