New Therapies for Myocardial Infarction

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Abstract

Adipose tissue derived stem cells (ASCs) are promising candidates for regenerative therapy, like after myocardial infarction. However, when transplanted into the infarcted heart, ASCs are jeopardized by the ischemic environment. Interestingly, it has been shown that multi drug resistance (MDR) proteins like the breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp) have a protective effect in haematopoietic stem cells. In ASCs however, only expression of BCRP was shown until now. In this study we therefore analyzed the expression and functional activity of BCRP and P-gp, and their putative function in ischemia in ASCs. BCRP and P-gp protein expression was studied over time (passage 2-6) using western blot analysis and immunohistochemical staining. MDR activity was analyzed using protein specific substrate extrusion assays. Ischemia was induced using metabolic inhibition. All analyses demonstrated protein expression and activity of BCRP in ASCs. In contrast, only minor expression of P-gp was found, without functional activity. BCRP expression was most prominent in early passage ASCs (p2) and decreased during culture. Furthermore, ischemia induced expression of BCRP. In addition, when BCRP was blocked, a significant increase in dead ASCs was found already after 1 hour of ischemia. In conclusion, ASCs expressed BCRP, especially in early passages. In addition, we now show for the first time that BCRP protects ASCs against ischemia induced cell death. These data therefore indicate that for transplantation of ASCs in an ischemic environment, like myocardial infarction, the optimal stem cell protective effect of BCRP theoretically will be achieved with early culture passages ASCs.

Introduction

Adipose tissue derived stem cells (ASCs) are promising candidates for regenerative therapy, for example after myocardial infarction.[1-4] ASCs are of mesenchymal origin, show high proliferation rates in culture, and in contrast to bone marrow mesenchymal stem cells, can more easily be harvested in larger quantities.[5-7] ASCs also have been shown to have the capacity to differentiate towards several cell types, amongst which endothelial cells and cardiomyocytes [1,8]. Furthermore, we and others have previously shown that *in vivo* ASCs reduce infarct size and improve cardiac function after infarction.[2,3,9] This thus makes ASCs very suitable as a therapeutic agent after myocardial infarction. However, at the same time it is known that after transplantation only a low percentage of the ASCs are retrieved in the infarcted area, due to the ischemic and inflammatory environment in which these cells are injected.[10,11]

Interestingly, it has been shown that multi drug resistant proteins (MDR), like P-glycoprotein (P-gp) and breast cancer resistance protein-1 (BCRP) protect haematopoietic stem cells in harmful environments.[12,13] P-gp (ABCB1, multi drug resistance protein-1), is a 170-kDa membrane associated protein. Like most MDR proteins P-gp is a full transporter, indicating that the transporter consist of two parts, each with a transmembrane domain and a ATP binding region. In contrast to P-gp, the 72-kDa BCRP (ABCG2) is a half transporter, containing only one transmembrane domain and one ATP binding region.[14-16] Both proteins as such play a role in removing a broad range of damaging substances and metabolites.[14,15] Even more BCRP was found to improve survival of haematopoietic stem cells during hypoxia.[17] We therefore wondered whether MDR proteins would also play a protective role in ASCs. In ASCs until now only BCRP expression was demonstrated using FACS analysis.[18,19] However, it has not been studied whether this BCRP protected ASCs. As also P-gp was detected in haematopoietic stem cells, we analyzed in the present study the expression and activity of both BCRP and P-gp in ASCs. We additionally studied whether ischemia affected MDR protein expression and whether these proteins protected ASCs during ischemia.

Materials and Methods

Adipose stem cell isolation and culture

Human subcutaneous adipose tissue samples were obtained as waste material after elective surgery and donated upon informed consent of the patients from three clinics in the Netherlands (Tergooi Ziekenuis, Hilversum; Jan van Goyen Kliniek, Amsterdam; the VU University Medical Center, Amsterdam). Adipose tissue was stored in sterile phosphate-buffered saline (PBS, Braun, Melsungen, AG, USA) at 4°C and processed within 24 hours after surgery as described previously.[8] In brief, adipose tissue was enzymatically digested using 0.1% collagenase A (Roche Diagnostics GmbH, Mannheim, Germany) in PBS containing 1% bovine serum albumin (BSA; Roche Diagnostics) for 45 minutes at 37°C under intermittent shaking. To remove contaminating erythrocytes the cells were subjected to ficoll density centrifugation (lymphoprep, ρ=1.077 g/ml, Osmolarity 280 ± 15 mOsm; Axis-Shield, Oslo, Norway).

Cells were seeded at $1x10^5$ cells/cm^2 and cultured in Dulbecco's Modified Eagle's Medium (DMEM; BioWhittaker, Cambrex, Verviers, Belgium) containing 10% fetal bovine serum (FBS; HyClone, South Logan, UT, USA), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (all from Gibco, Invitrogen, California, USA), in a humidified atmosphere of 5% CO_2 at 37°C. Media were changed twice a week. When reaching 80-90% confluency, cells were detached with 0.5 mM EDTA/0.05% trypsin (Gibco), for 5 minutes at 37°C, and replated. Cells were used between passage 2 and 6, since from passage 2 on a homogenous population of adipose derived stem cells was found.[5]

Culture of control cells

The following cell lines with overexpression of MDR proteins were used as positive controls: MCF-7/MR (BCRP) and SW1573/2R160 (P-gp). Cells were kept under drug exposure by culturing them in mitoxantrone (1.8 mM) for MCF7/MR cells, and doxorubicine (0.16 μM) for SW-1573/2R160 cells.[20] The MCF7/MR cell line was cultured in RPMI medium (BioWhittaker) containing 10% fetal FBS (HyClone), penicillin, streptomycin, and L-glutamine (all from Gibco), whereas SW-1573/2R160 cells were cultured in DMEM (BioWhittaker) containing 10% fetal FBS (HyClone), penicillin, streptomycin, and L-glutamine (all from Gibco).

Immunocytochemistry

Cytospin slides were prepared as described previously, by spinning 10^4 ASCs for 5 min at 500 rpm (Shandon cytospin 3, Thermo Scientific, Waltham, MA, USA).[21] Slides were air-dried overnight and fixed with acetone for 10 minutes. Slides were incubated with either rat antibodies against human BCRP (Bxp53, 1:50, kindly provided by G scheffer, VUMC, the Netherlands), or with mouse antibodies against human P-gp (JSB-1, 1:50, kindly provided by G scheffer, VUMC, the Netherlands) in PBS containing 1% BSA (PBS/BSA) for 1.5 hours at room temperature. Next, slides were incubated with biotin-conjugated rabbit-anti-rat antibodies for BCRP, or rabbit-anti-mouse antibodies for P-gp (1:200, Dako Cytomation, Glostrup, Denmark) for 30 minutes, and then with streptavidin/HRP (1:500, Dako) for 1 hour. Staining was visualized using 3-amino-9-ethylcarbazole (Zymed AEC kit, Invitrogen). Finally the sections were counterstained with hematoxylin and covered. Control sections were incubated with PBS instead of the primary antibody.

Substrate extrusion assays and MDR protein blockers

To study functional activity of MDR proteins, substrate extrusion assays were essentially performed as described previously.[22] In brief, cells were incubated for 2 hours at 37°C in DMEM with protein specific fluorescent substrates (all FITC), and with/without protein specific blockers. Then cells were washed and after 1 hour of extrusion, fluorescence was quantified by flow cytometry using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Substrates used were Bodipy-prazosin (0.025μM) for BCRP, and Syto 16 (0.01μM) for P-gp. Blockers used were respectively KO-143 (0.2μM) for BCRP, and P121 (10μM) for P-gp.

Ischemia

Ischemia was mimicked by metabolic inhibition.[23] Cells were incubated in a metabolic inhibition buffer, containing 0.9 mM $CaCl_2$ -H_2O, 20 mM 2-deoxy-D-glucose (Sigma, St. Louis, MO), 106 mM NaCl, 5 mM NaCN, 3.8 mM $NaHCO_3$, 4.4 mM KCl and 1 mM $MgCl_2$-H_2O in H_2O, pH 6.6 at 37°C in a humidified atmosphere.[23]

Viability assay

Cell viability was analyzed using flow cytometry. Cells (attached- as well as detached cells) were collected and stained with annexin V-FITC (1:40, Bender Med Systems, Vienna, Austria) for 30 minutes at 37°C in serum-free medium. Annexin V was used to assess flip-flop of the cell membrane. Propidium iodide was added immediately prior to measurement (PI; 1:40, Bender Med Systems) and was used to assess membrane permeability, and thus cell death. Cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Cells negative for both annexin V and PI are viable.[23]

Western blot analysis

To analyze MDR protein expression over time, Western blot analysis was performed in passage 2-6 ASCs. To investigate the effect of ischemia on MDR protein expression, cells were subjected to 1 or 5 hours of metabolic inhibition. Western blot analysis was performed as described previously.[24] Per sample, 40 mg of total cell lysate, as quantified using the BCA protein assay kit (Pierce, Rockford, IL, USA), was subjected to SDS-PAGE, and transferred to nitrocellulose membranes. Primary antibodies used were rat antibodies against human BCRP (Bxp53, 1:100, kindly provided by G scheffer, VUMC, the Netherlands), mouse antibodies against human P-gp (JSB-1, 1:200, kindly provided by G scheffer, VUMC, the Netherlands), and mouse antibodies against alpha tubulin (1:1000, ABCAM). Secondary antibodies used were biotin-conjugated rabbit-anti-mouse or swine-anti-rat antibodies (Dako Cytomation, 1:500) for the MDR proteins, and HRP-conjugated rabbit-anti-mouse antibodies for tubulin (Dako Cytomation, 1:1000). Finally for the detection of the MDR-protein expression streptavidin/HRP (1:500, Dako) was used. Blots were visualized by enhanced chemiluminescence (1:40, ECL; Amersham Bioscience AB, Buckinghamshire, UK), and protein quantified using a charge couple device camera (Fuji Science Imaging Systems) in combination with AIDA Image Analyzer software (Isotopenmessgeräte; Staubenhardt, Germany). When quantifying MDR protein expression, expression was corrected for tubulin expression.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 4 software. A t-test, ANOVA or repeated measures with Bonferoni post-test were used for analysis. A p-value smaller than 0.05 was considered to be statistically significant. In the text and figures values are given as mean $\pm$ standard error.

Results

BCRP expression decreases during culture of ASCs

To investigate putative expression of P-gp and BCRP in ASCs, immunocytochemical staining was performed on cytospin slides. Positive control cell lines (MCF-7/MR and SW1573/2R160) with overexpression of respectively BCRP and P-gp stained positive for these particular proteins (Figure 1A+B). Also evident expression of BCRP was found on the majority of ASCs in passage 2 (see Figure 1C), however, expression decreased during culture (Figure 1D, passage 6). It has to be noticed that in passage 6 not only less ASCs were BCRP positive, but there was also a decrease in BCRP expression level per cell. In contrast with the positive control cell line MCF-7/MR, in which BCRP mainly was found on the plasma membrane and cytoplasm, BCRP staining in passage 2 ASCs was most prominent in the (peri)nuclear area (figure 1C). In passage 6 ASCs, however, BCRP was no longer detected in the (peri)nuclear area and was only found as intranuclear dots (Figure 1D). In contrast, staining for P-gp in passage 2 ASCs (Figure 1E), was limited, and was found in only half of the donors. Even then staining was limited to the cytoplasm, and was neither found on the plasma membrane nor in the (peri)nuclear area. At later passages no expression of P-gp was detected (not shown).

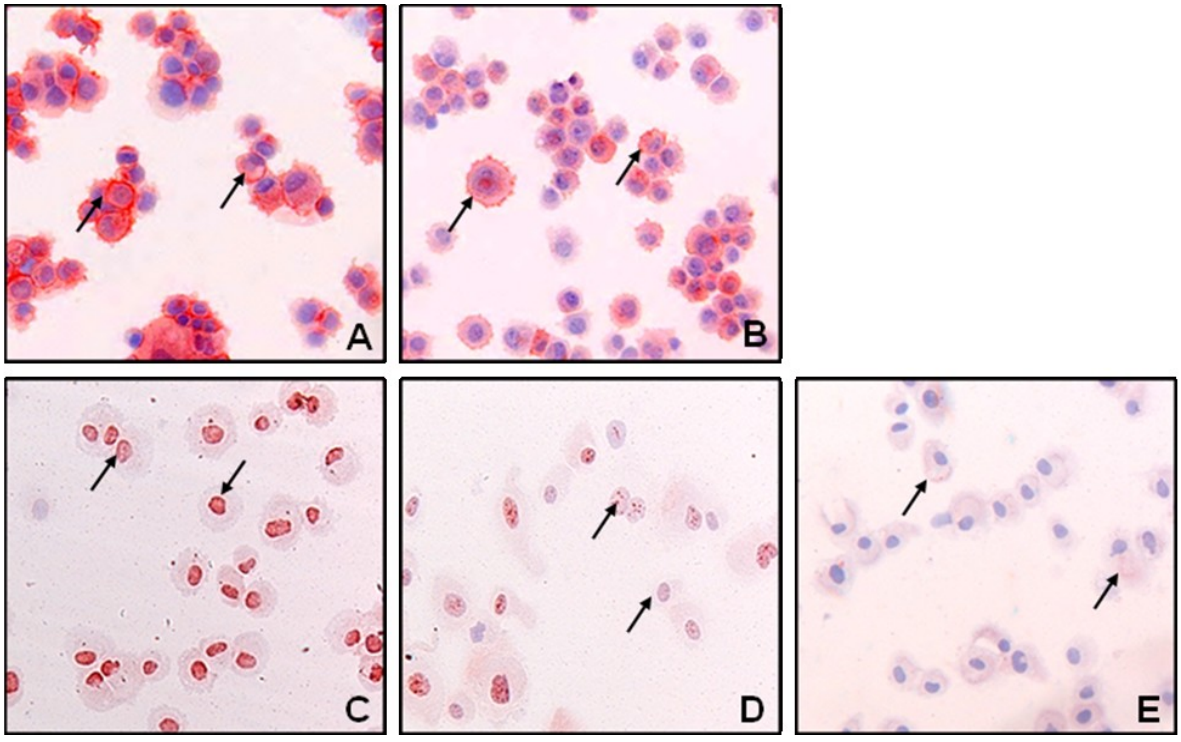

Figure 1: Imunocytochemistry for BCRP and PGP in ASCs. Representative images of immunohistochemical staining for BCRP and PGP in positive control cells, and in ASCs cells from a single donor. A) BCRP staining in MCF-7/MR cells, showing expression of BCRP (arrows). B) PGP staining in SW1573/2R160 cells, showing expression of PGP (arrows). C) BCRP staining in passage 2 ASCs, arrows indicate (peri)nuclear staining, D) BCRP staining in ASCs passage 6 was decreased in the percentage of positive cells, as well as in the positivity per cell, as compared with passage 2 (arrows), E) PGP staining in ASCs passage 2, only limited positive staining for PGP was detected in the cytoplasm of ASCs (arrows). MDR-proteins are shown in red (AEC staining). Nuclei are shown in blue. Original magnification (A-E): 100x.

To further investigate and quantify MDR protein expression over time during culture, Western blot analysis was performed in ASCs derived from passage 2-6, and MDR protein expression was corrected for tubulin expression (Figure 2). Positive control cell lines, MCF-7/MR and SW1573/2R160, showed high expression of respectively BCRP and P-gp (Figure 2). In accordance with immunocytohemical staing, BCRP was mainly found in passage 2 ASCs and decreased during culture (Figure 2A). In contrast, no P-gp protein was detected in ASCs in any of the passages, using western blot analysis (Figure 2B, n=4). To analyze the protein loss of BCRP over time, expression levels at passage 2, 4 and 6 were quantified. A significant decrease was found in BCRP expression at passage 6 compared with passage 2 (decrease 63 ± 19%, Figure 2C, n=7, $p<0.05$). Therefore further functional analysis was performed in passage 2 cells only.

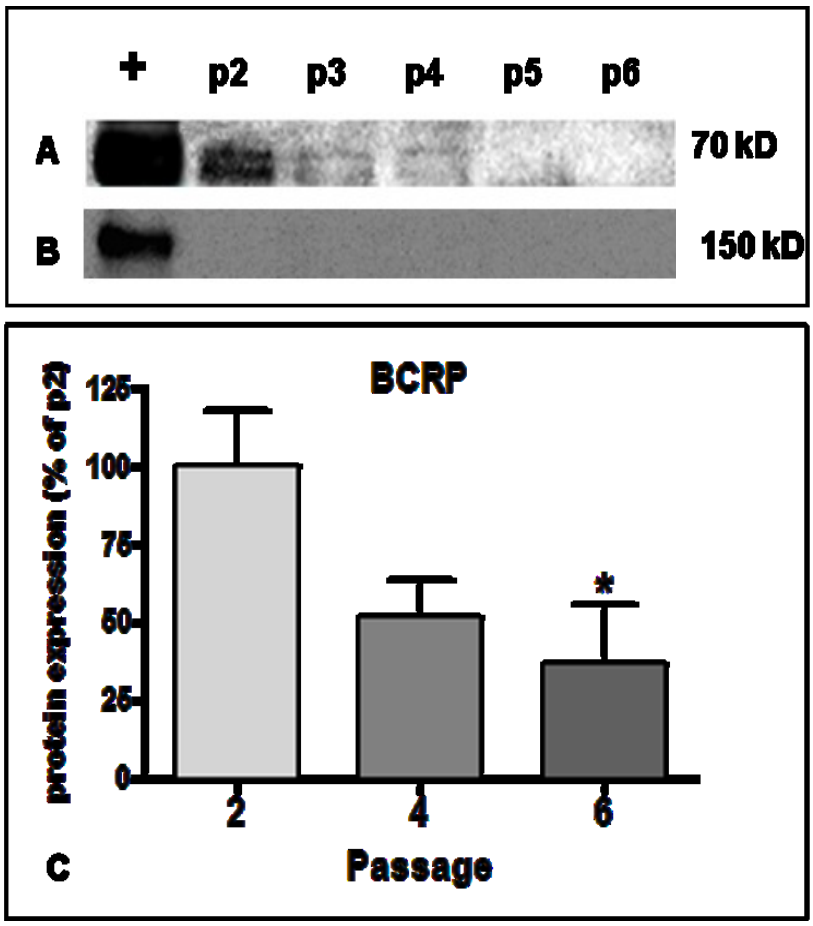

Figure 2: BCRP expression decreases during culture. Western blot images of ASCs from the same donor during culture p2-p6: A) BCRP, and B) PGP. + = positive control (BCRP: MCF-7/MR, PGP: SW1573/2R160), p indicates passage number. C) Relative BCRP protein expression over time as quantified using western blot analysis (expression at p2 is set at 100%), n=7, * $P<0.05$ compared to p2.

We next analyzed functional activity of BCRP and P-gp proteins, using a FACS based extrusion assay.[22] In this assay, it was analyzed whether specific inhibitors of the MDR-proteins could inhibit extrusion of a fluorescent substrate specific for these particular MDR proteins, resulting in an increased intracellular fluorescent signal. In the BCRP overexpressing cell line, its specific inhibitor (KO-143) induced an increase in fluorescent signal of 94 ± 33%. In the P-gp overexpressing cell line, its specific inhibitor (P121) induced an increase of 434±100%. In passage 2 ASCs, we only found a small increase in fluorescent signal of 14 ± 11% after blocking BCRP using KO-143, indicating that only a low amount of protein was extrused from the cell trough BCRP, which might be explained by the peri-nuclear expression of BCRP (n=3). No increase of fluorescence was found after blocking for P-gp (n=3), indicating that P-gp does not play an important active role in ASCs. For this, we suggest that only BCRP plays a role in ASCs.

BCRP protein expression increases after metabolic inhibition

Since BCRP was significantly expressed intracellular, we further focused on BCRP. We wondered whether ischemia, mimicked by metabolic inhibition, would affect BCRP expression, as MDR proteins theoretically can protect cells in harmful environments.[17] We therefore quantified BCRP expression in passage 2 ASCs after 1 and 5 hours of metabolic inhibition, using western blot analysis. We found that expression of BCRP proteins was increased in passage 2 ASCs after 1 hour of metabolic inhibition up to 246 ± 77%, as compared to untreated cells (set to 100%), although this difference was not significant (p=0.12, Figure 3). However, after 5 hours of metabolic inhibition expression of BCRP was significantly increased up to 519 ± 205% as compared to untreated cells (n=8 , p<0.05).

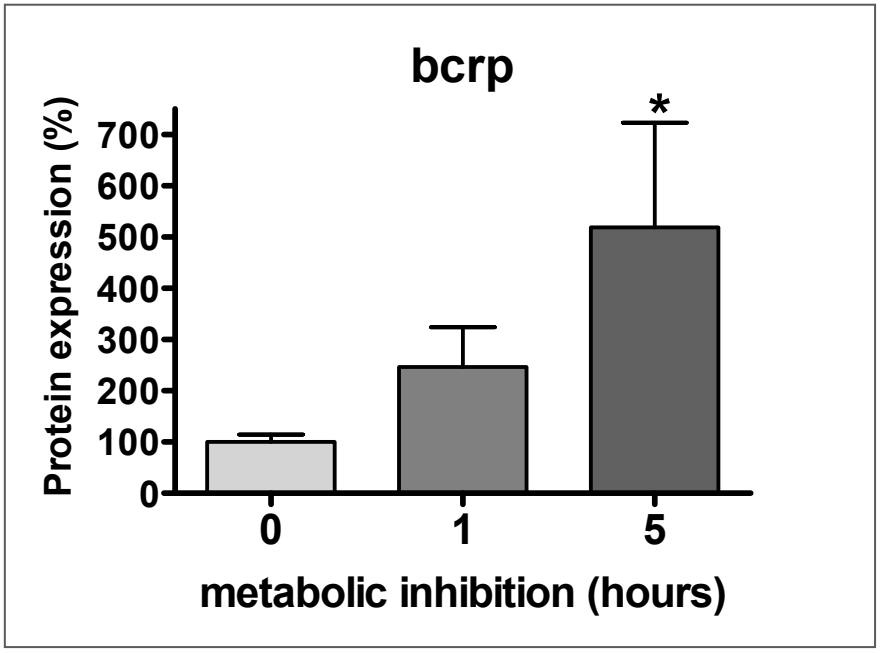

Figure 3: BCRP expression increases during metabolic inhibition. Relative BCRP protein expression after 1 and 5 hours of metabolic inhibition as quantified using western blot analysis (expression of control ASCs was set at 100%), n=8, * P<0.05 compared to control cells.

BCRP protects ASCs against metabolic inhibition

Since BCRP was upregulated after metabolic inhibition, we subsequently analyzed whether BCRP had a functional role in metabolic inhibition. For this we analyzed the effect of blocking BCRP on the number of viable cells after ischemia, by subjecting ASCs to 1 or 5 hours of metabolic inhibition. In control ASCs, cell viability was 80.9 ± 3.6 %. Blocking BCRP using KO-143 did not have an effect on cell viability in these control ASCs (78.0 ± 5.3 %, n=4, p=0.34, Figure 4A). In contrast, one hour of metabolic inhibition significantly reduced the number of viable cells from 80.9 ± 3.6 % in control cells to 63.3 ± 3.9 % after metabolic inhibition ($p<0.05$). No further increase in cell death was found after five hours of metabolic inhibition (not shown). Therefore we analyzed the effect of blocking BCRP during one hour of metabolic inhibition only. When the BCRP blocker (KO-143) was added to ASCs during 1 hour of metabolic inhibition, ASC survival was significantly reduced from 63.3 ± 3.9 % (metabolic inhibition) to 48.4 ± 5.7% (metabolic inhibition + blocker) (n=4, $p<0.05$, Figure 4B). These data thus show that BCRP does play a role in protection of ASCs during metabolic inhibition.

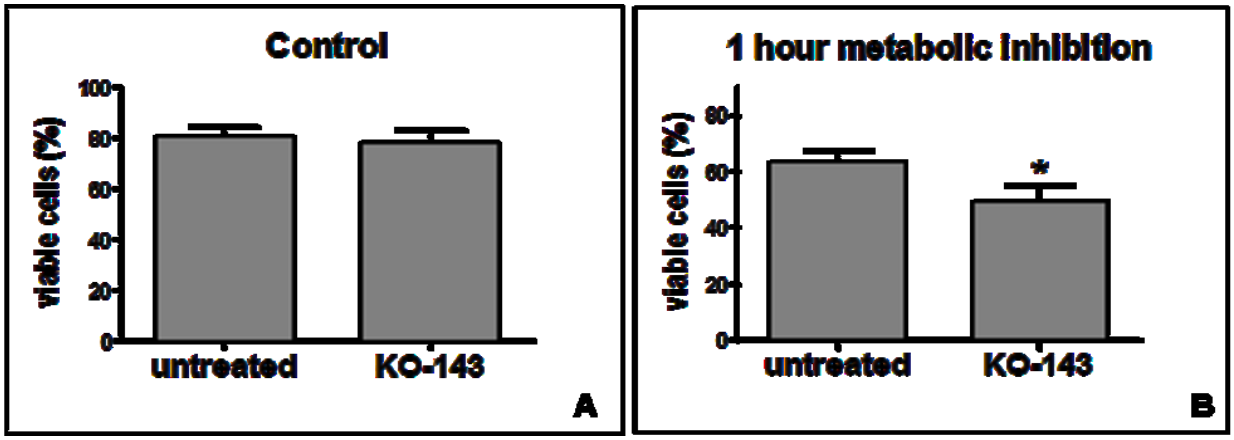

Figure 4: BCRP protects ASCs during metabolic inhibition. Percentage of viable cells as analyzed using Annexin-V and PI in flow cytometry, after 1 hour of metabolic inhibition and/or inhibition of BCRP by KO-143. n=4, * $P<0.05$

Discussion

In this study we analyzed the putative expression and functional role of the MDR-proteins BCRP and P-gp in ASCs. We found that ASCs significantly expressed BCRP (peri)nuclear, which however decreased during culture. In contrast, we did not detect significant amounts of P-gp in ASCs. Interestingly, metabolic inhibition significantly increased BCRP expression. Moreover, blocking of BCRP during ischemia resulted in increased ASC cell death, indicative for a protective role of BCRP during ischemia.

Previous studies have also described protein expression of BCRP in fresh[18] and cultured ASCs.[19] Mitchel et al found BCRP expression in up to 36% of cultured ASCs of passage 2, using FACS analysis.[19] In our studies, we now found BCRP expression in almost all ASCs in passage 2, using immunohistochemistry. In contrast, Katz et al did not find BCRP in ASCs (passage 1-3), neither using FACS

analysis nor in RT-PCR.[25] A possible explanation for these differences could be a difference in culture methods used. It is known that MDR expression is modulated by different culture conditions, including culture serums.[26] [27,28] Katz et al used FBS from Sigma and both we and Mitchel et al from Hyclone. Another explanation could be a difference in assays used to detect the MDR proteins. Both Mitchel et al and Katz et al used immunofluorescently labelled antibodies and FACS analysis, without permeabilisation of the cells. Theoretically, therefore, only membrane bound proteins were detected in the FACS. We, however, performed immunocytochemistry on permeabilized cells, and found mainly an intracellular expression of BCRP. Finally, different antibodies were used. Both Mitchel et al and Katz et al used clone 5D3, which recognizes an external epitope of BCRP, while we used BXP-53, which recognizes an internal epitope of BCRP.

Remarkably, we found that BCRP staining in ASCs was most prominent in the (peri)nuclear area, in contrast to the positive control cell line in which BCRP was predominantly found on the plasma membrane, and partly in the cytoplasm. Previous studies have also described that BCRP is expressed in the perinuclear region, for instance in MDR transfected HeLA cells**.**[29,30] Interestingly, Rajagopal et al have suggested that MDR-proteins expressed at the perinuclear region of transfected HeLa cells do not have an effect on cellular levels of toxins, but instead do decrease nuclear levels of toxins.[30] Therefore, it can be hypothesized that since BCRP generally protects stem cells by extrusion of DNA-damaging substances,[14,15] BCRP could also play this specific role when localized in the perinuclear region of the ASCs. Furthermore, it has been shown for another MDR protein, namely P-gp that this protein can improve cell survival independent of drug-efflux activity in acute myeloblastic leukaemia cells, suggesting a different mechanism for cell protection.[31] Theoretically BCRP also might play a functional role in cell protection independent of drug-efflux activity, and therefore also without membrane bound expression.

In contrast to BCRP, we only found minor positive staining for P-gp in ASCs (cytoplasmic) using immunocytochemistry, but not in western blot analysis. Furthermore we could not detect functional activity of P-gp in ASCs. To the best of our knowledge, no other studies have investigated P-gp expression in ASCs. P-gp, on the other hand, has been detected in haematopoietic stem cells.[12] It indeed is suggested that haematopoietic stem cells have a different MDR gene expression profile compared with other adult stem cells, indicating different cell type specific functions.[32]

It has been shown that exposure to potential harmful environments, like hypoxia related inflammation, affects expression levels of different MDR-proteins in rodent tissues.[33,34] In line with this, we found that ischemia significantly increased BCRP protein expression in ASCs more than 5-fold, and that BCRP protected ASCs against ischemia in vitro. Krishnamurthy et al [17] also found in primary haematopoietic progenitor cells a 7-fold increase in BCRP mRNA expression and in number of BCRP positive cells after exposure to hypoxia. Indeed, also in these primary haematopoietic progenitor cells BCRP protected cells against hypoxia. Our findings thus indicate that BCRP could also protect ASCs in harmful environments like the ischemic heart after myocardial infarction.

Interestingly, we found decreasing BCRP protein levels during culture (decrease 63 $\pm$ 19% between p2 and p6). It has to be noted that not only the percentage of positive cells, but also the expression level per cell decreased during culture. Mitchell et al also found a decreasing percentage of BCRP positive cells during culture, although this decrease was not statistically significant (decrease 60.8% between p2 and p5).[19] It is already known that stem cells change during culture, for instance by losing the ability to differentiate into different lineages.[35,36,11,37] These data thus suggest that when ASCs

are transplanted in harmful environments like myocardial infarction, the optimal stem cell protective effect of BCRP theoretically will be achieved with early culture passages of ASCs.

Taken together, we have now shown that ASCs express perinuclear BCRP, which decreases during culture. Interestingly, BCRP is significantly upregulated in ASCs during ischemia, thereby protecting ASCs. We therefore suggest that transplantation of ASCs in harmful environments, like myocardial infarctions, is optimal when expression of BCRP is high, thus in early passage ASCs (p2).

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References

1. Rangappa S, Fen C, Lee EH, Bongso A and Sim EK. (2003) Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardiomyocytes. Ann.Thorac.Surg. 75:775-779.
2. van Dijk A, Niessen HW, Zandieh Doulabi B, Visser FC and van Milligen FJ. (2008) Differentiation of human adipose-derived stem cells towards cardiomyocytes is facilitated by laminin. Cell Tissue Res. 334:457-467.
3. Zhang DZ, Gai LY, Liu HW, Jin QH, Huang JH and Zhu XY. (2007) Transplantation of autologous adipose-derived stem cells ameliorates cardiac function in rabbits with myocardial infarction. Chin Med.J.(Engl.) 120:300-307.
4. van Dijk A, Niessen HW, Ursem W, Twisk JW, Visser FC and van Milligen FJ. (2008) Accumulation of fibronectin in the heart after myocardial infarction: a putative stimulator of adhesion and proliferation of adipose-derived stem cells. Cell Tissue Res. 332:289-298.
5. Oedayrajsingh-Varma MJ, van Ham SM, Knippenberg M, Helder MN, Klein-Nulend J, Schouten TE, Ritt MJ and van Milligen FJ. (2006) Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. Cytotherapy. 8:166-177.
6. Strem BM, Hicok KC, Zhu M, Wulur I, Alfonso Z, Schreiber RE, Fraser JK and Hedrick MH. (2005) Multipotential differentiation of adipose tissue-derived stem cells. Keio J.Med. 54:132-141.
7. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P and Hedrick MH. (2002) Human adipose tissue is a source of multipotent stem cells. Mol.Biol.Cell 13:4279-4295.
8. Oedayrajsingh-Varma MJ, van Ham SM, Knippenberg M, Helder MN, Klein-Nulend J, Schouten TE, Ritt MJ and van Milligen FJ. (2006) Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. Cytotherapy. 8:166-177.
9. Strem BM, Zhu M, Alfonso Z, Daniels EJ, Schreiber R, Beygui R, MacLellan WR, Hedrick MH and Fraser JK. (2005) Expression of cardiomyocytic markers on adipose tissue-derived cells in a murine model of acute myocardial injury. Cytotherapy. 7:282-291.
10. Danoviz ME, Nakamuta JS, Marques FL, dos SL, Alvarenga EC, dos Santos AA, Antonio EL, Schettert IT, Tucci PJ and Krieger JE. (2010) Rat adipose tissue-derived stem cells transplantation attenuates cardiac dysfunction post infarction and biopolymers enhance cell retention. PLoS.One. 5:e12077.
11. van Dijk A, Naaijkens BA, Jurgens WJ, Nalliah K, Sairras S, van der Pijl RJ, Vo K, Vonk AB, van Rossum AC, Paulus WJ, van Milligen FJ and Niessen HW. (2011) Reduction of infarct size by intravenous injection of uncultured adipose derived stromal cells in a rat model is dependent on the time point of application. Stem Cell Res. 7:219-229.
12. Chaudhary PM, Roninson IB. (1991) Expression and activity of P-glycoprotein, a multidrug efflux pump, in human hematopoietic stem cells. Cell 66:85-94.
13. Scharenberg CW, Harkey MA and Torok-Storb B. (2002) The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. Blood 99:507-512.
14. Abbott BL. (2006) ABCG2 (BCRP): a cytoprotectant in normal and malignant stem cells. Clin.Adv.Hematol.Oncol. 4:63-72.
15. Bunting KD. (2002) ABC transporters as phenotypic markers and functional regulators of stem cells. Stem Cells 20:11-20.
16. Rocchi E, Khodjakov A, Volk EL, Yang CH, Litman T, Bates SE and Schneider E. (2000) The product of the ABC half-transporter gene ABCG2 (BCRP/MXR/ABCP) is expressed in the plasma membrane. Biochem.Biophys.Res.Commun. 271:42-46.
17. Krishnamurthy P, Ross DD, Nakanishi T, Bailey-Dell K, Zhou S, Mercer KE, Sarkadi B, Sorrentino BP and Schuetz JD. (2004) The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through interactions with heme. J.Biol.Chem. 279:24218-24225.
18. Heydarkhan-Hagvall S, Schenke-Layland K, Yang JQ, Heydarkhan S, Xu Y, Zuk PA, MacLellan WR and Beygui RE. (2008) Human adipose stem cells: a potential cell source for cardiovascular tissue engineering. Cells Tissues.Organs 187:263-274.

19. Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Di HY, Storms RW, Goh B, Kilroy G, Wu X and Gimble JM. (2006) Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells 24:376-385.
20. Scheffer GL, Maliepaard M, Pijnenborg AC, van Gastelen MA, de Jong MC, Schroeijers AB, van der Kolk DM, Allen JD, Ross DD, van d, V, Dalton WS, Schellens JH and Scheper RJ. (2000) Breast cancer resistance protein is localized at the plasma membrane in mitoxantrone- and topotecan-resistant cell lines. Cancer Res. 60:2589-2593.
21. Scheffer GL, Kool M, Heijn M, de HM, Pijnenborg AC, Wijnholds J, van HA, de Jong MC, Hooijberg JH, Mol CA, van der Linden M, de Vree JM, van d, V, Elferink RP, Borst P and Scheper RJ. (2000) Specific detection of multidrug resistance proteins MRP1, MRP2, MRP3, MRP5, and MDR3 P-glycoprotein with a panel of monoclonal antibodies. Cancer Res. 60:5269-5277.
22. van der Pol MA, Broxterman HJ, Pater JM, Feller N, van der Maas M, Weijers GW, Scheffer GL, Allen JD, Scheper RJ, van LA, Ossenkoppele GJ and Schuurhuis GJ. (2003) Function of the ABC transporters, P-glycoprotein, multidrug resistance protein and breast cancer resistance protein, in minimal residual disease in acute myeloid leukemia. Haematologica 88:134-147.
23. Krijnen PA, Cillessen SA, Manoe R, Muller A, Visser CA, Meijer CJ, Musters RJ, Hack CE, Aarden LA and Niessen HW. (2005) Clusterin: a protective mediator for ischemic cardiomyocytes? Am.J.Physiol Heart Circ.Physiol 289:H2193-H2202.
24. van Dijk A, Krijnen PA, Vermond RA, Pronk A, Spreeuwenberg M, Visser FC, Berney R, Paulus WJ, Hack CE, van Milligen FJ and Niessen HW. (2009) Inhibition of type 2A secretory phospholipase A2 reduces death of cardiomyocytes in acute myocardial infarction. Apoptosis. 14:753-763.
25. Katz AJ, Tholpady A, Tholpady SS, Shang H and Ogle RC. (2005) Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. Stem Cells 23:412-423.
26. Sarkadi B, Orban TI, Szakacs G, Varady G, Schamberger A, Erdei Z, Szebenyi K, Homolya L and Apati A. (2010) Evaluation of ABCG2 expression in human embryonic stem cells: crossing the same river twice? Stem Cells 28:174-176.
27. Hirsch-Ernst KI, Ziemann C, Schmitz-Salue C, Foth H and Kahl GF. (1995) Modulation of P-glycoprotein and mdr1b mRNA expression by growth factors in primary rat hepatocyte culture. Biochem.Biophys.Res.Commun. 215:179-185.
28. Zhang W, Ling V. (2000) Cell-cycle-dependent turnover of P-glycoprotein in multidrug-resistant cells. J.Cell Physiol 184:17-26.
29. Yeboah D, Kalabis GM, Sun M, Ou RC, Matthews SG and Gibb W. (2008) Expression and localisation of breast cancer resistance protein (BCRP) in human fetal membranes and decidua and the influence of labour at term. Reprod.Fertil.Dev. 20:328-334.
30. Rajagopal A, Simon SM. (2003) Subcellular localization and activity of multidrug resistance proteins. Mol.Biol.Cell 14:3389-3399.
31. Pallis M, Russell N. (2000) P-glycoprotein plays a drug-efflux-independent role in augmenting cell survival in acute myeloblastic leukemia and is associated with modulation of a sphingomyelin-ceramide apoptotic pathway. Blood 95:2897-2904.
32. Tang L, Bergevoet SM, Gilissen C, de WT, Jansen JH, van der Reijden BA and Raymakers RA. (2010) Hematopoietic stem cells exhibit a specific ABC transporter gene expression profile clearly distinct from other stem cells. BMC.Pharmacol. 10:12.
33. Teng S, Piquette-Miller M. (2008) Regulation of transporters by nuclear hormone receptors: implications during inflammation. Mol.Pharm. 5:67-76.
34. Fradette C, Batonga J, Teng S, Piquette-Miller M and du SP. (2007) Animal models of acute moderate hypoxia are associated with a down-regulation of CYP1A1, 1A2, 2B4, 2C5, and 2C16 and up-regulation of CYP3A6 and P-glycoprotein in liver. Drug Metab Dispos. 35:765-771.

35. McIntosh K, Zvonic S, Garrett S, Mitchell JB, Floyd ZE, Hammill L, Kloster A, Di HY, Ting JP, Storms RW, Goh B, Kilroy G, Wu X and Gimble JM. (2006) The immunogenicity of human adipose-derived cells: temporal changes in vitro. Stem Cells 24:1246-1253.
36. Zhu Y, Liu T, Song K, Fan X, Ma X and Cui Z. (2008) Adipose-derived stem cell: a better stem cell than BMSC. Cell Biochem.Funct. 26:664-675.
37. Fehrer C, Lepperdinger G. (2005) Mesenchymal stem cell aging. Exp.Gerontol. 40:926-930.