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The Opposite Expected Effect of p38 Inhibitors on Fat Graft Survival

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Background: Fat grafting is an increasingly popular method of augmentation/reconstruction of soft tissue defects. However, the clinical unpredictability and high resorption rates of the grafts remain problematic. Cellular stress from the harvest and the ensuing ischemic episode may be the cause of this. Cellular stress activates the p38 mitogen–activated protein kinase (MAPK) signaling pathway. In response to cellular stress, the p38 pathway can lead to apoptosis and can negatively regulate cell proliferation. Inhibition of p38 in ex vivo experiments has been shown to promote the expansion of human cord blood hematopoietic stem cell and improve the adipogenesis process through its upstream regulator, Shp2. Because of its wide-ranging cell regulation and antiinflammatory properties, large-scale clinical trials using p38 inhibitors are also currently being performed, especially for therapeutic effect in chronic obstructive pulmonary disease and asthma. The rationale for our study was that the treatment of fat grafts with p38 inhibitor would (a) prevent apoptosis of adipose-derived stem cells in the fat grafts, (b) increase adipose-derived stem cells proliferation, and (c) stimulate the release of several angiogenic factors and promote revascularization.

Methods: Clinical and histological testing was performed on 5 fat-transplanted (1 mL) CD-1 nude mice compared with the test group of 5 mice, which were injected with a p38 MAPK inhibitor at 1, 3, 6, and 9 days after the fat transplantation.

Results: The weights and volumes of the control group grafts were significantly higher than those of the p38 MAPK inhibitor–treated grafts. Average volume resorption was 36% in the control group and 92% in the test group. Histological evaluation of the grafts revealed significantly improved integration, with a significant reduction of fibrosis and inflammation in the control group versus the treated group.

Conclusions: This preliminary study suggests that as opposed to our hypothesis, inhibition of p38 significantly increases fat graft resorption. The dramatic effects observed in our study may suggest that p38 may act differently on the numerous cell types that constitute the fat graft, and further investigation is necessary.
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Since first attempted by Neuber in 1893, autologous fat transfer has become an increasingly popular and well-established method to enhance/augment soft tissue, for either acquired or congenital malformations. The advantages of this method are the availability and accessibility of the fat tissue; its simplicity and long-term cost-effectiveness; and in addition, when comparing to the foreign body complications of fillers and implants, it is autologous, biocompatible, and nonallergenic.

However, the current major drawback of this method is its clinical unpredictability, with a high rate of the fat graft absorption after its injection, which can reach up to 70%.[1] Studies have reported that reduced fat tissue vascularization and fat cell apoptosis are responsible for this high rate of fat resorption.[1–4] Reduced vascularization is often considered as a consequence of the mechanical stress the fat tissue suffers during the liposuction procedure and

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continues with insufficient vascular supply after transplantation leading to tissue hypoxia.[4–6]

Cellular stress activates the p38 mitogen–activated protein kinase (MAPK) signaling pathway. The stress kinase p38 is activated by phosphorylation and subsequently translocated into the nucleus and involved in the control of expression of various genes. In response to cellular stress, the p38 pathway can lead to apoptosis and can negatively regulate cell proliferation.

The adipose tissue comprises various types of cells, of which only 20% are adipocytes. The others are adipose-derived stem cells (ASCs), stromal/progenitor cells, vascular endothelial cells, and hematopoietic cells.[5]

Recently, several preclinical studies have demonstrated ASCs as an alternative therapeutic approach to effectively enhance the survival and quality of transplanted fat tissue by increasing neovascularization, mainly through the secretion of angiogenic cytokines, such as hepatocyte growth factor and vascular endothelial growth factor, which are important for the survival of the fat graft.[7]

Previous studies have shown that inhibition of p38 in ex vivo experiments promotes the expansion of human cord blood hematopoietic stem cell,[8] and another recent finding showed a crucial role of p38 inhibition, through its upstream regulator, Shp2, in the adipogenesis process.[9]

We have hypothesized that the treatment of fat grafts with p38 inhibitor would (a) prevent apoptosis of ASCs in the fat grafts, (b) increase ASCs' proliferation, and (c) stimulate the release of several angiogenic factors and promote revascularization.

AIM

Our present study was aimed at evaluating the long-term effect of the treatment of fat grafts with p38 inhibitor, particularly the volume, weight, and histological features of the transplanted fat tissue.

METHODS

The study was reviewed and approved by the Institutional Review Board of the Rambam Health Care Campus, Israel, and the Technion Animal Care and Use Committee.

Ten-week-old female CD-1 nude mice (Harlan, Jerusalem, Israel) were housed in cages with artificial 12-hour light/dark cycle at a constant temperature range (24±20°C) and relative humidity (55% ± 10%). The mice were acclimated for 1 week before the study and fed a standard chow and water ad libitum.

Fat was harvested from the lateral thigh of a healthy 50-year-old woman during an elective cosmetic liposuction procedure, performed under general anesthesia. The donor sites were injected using tumescent solution containing lidocaine and adrenaline in Ringer's lactate.[10]

The fat was aspirated using a sterile 20-mL syringe and a 14-gauge 3-hole blunt cannula; shortly after its aspiration, the fat was processed by two sessions of centrifugation, 5 minute each, at 1,200 rpm.[11]

Each centrifugation session was followed by separation of the fat cells from the fluid, blood, and cell debris. All processes were performed under sterile conditions. One milliliter of fat was injected subcutaneously into each mouse's scalp using a 14-G needle while the animal was manually restrained. Grafting into nude mice was within 2 hours from the fat collection, according to previously published protocols.[10–13]

After transplantation, mice were randomly divided into test and control groups. In the control group, the transplanted fat was injected with 110 µL of dimethyl sulfoxide (DMSO) at 100 mg/mL dissolved in phosphate-buffered saline. In the test group, 5 mice were injected 22 µg of p38 MAPK inhibitor SB203580 (dissolved in DMSO at 100 mg/mL) in a total volume of 110 µL at day 0. Additional injections were 1, 3, 6, and 9 days after the fat transplantation, making a total of 5 equal injections of each treatment per fat graft. The use of animals and all the experimental procedures were reviewed and approved by the Technion Animal Care and Use Committee.

Fifteen weeks after transplantation, mice were humanely killed, and the fat grafts were carefully dissected from their scalps. Each fat graft was weighed, and the volume of the fat graft was measured using the liquid overflow method.[13] After weight and volume determination, each fat graft was placed in 4% formalin. The formalin-maintained fat grafts were dehydrated with an increased alcohol gradient and embedded in paraffin; 5-µm-thick sections slices were cut using a Leica microtome and were used for histological examination.

The evaluation of the slides was performed by 2 observers in a single-blind trial under light microscope. Four parameters were evaluated: (a) the extent of integration, (b) the extent of fibrosis, (c) the presence of vacuoles, and (d) the intensity of the inflammatory response. Each criterion was graded on a scale of 0 to 5, where 0 = absence, 1 = minimal presence, 2 = minimal-to-moderate presence, 3 = moderate presence, 4 = moderate-to-extensive presence, and 5 = extensive presence. Microvascular density was evaluated by immunohistochemistry using antihuman CD31 antibody (Abcam, Cambridge, UK).

Means and SE of the means were calculated. Differences between means were analyzed for statistical significance using one-way analysis of variance with the Tukey–Kramer multiple comparisons posttest. *P* values ≤0.05 were considered significant.

RESULTS

All the mice completed the 15-week study period. Representative clinical pictures were taken, hematoxylin and eosin staining was performed, and immunohistochemical analysis with an antibody against the human CD31 was performed (Fig. 1).

The weights and volumes of the control group grafts were significantly higher than those of the SB203580-treated grafts (Fig. 2A). Average volume absorption was 36% in the control group and 92% in the test group, and average weight loss was 79% and 97%, respectively.

Histological evaluation of the grafts revealed significantly improved integration, with a significant reduction

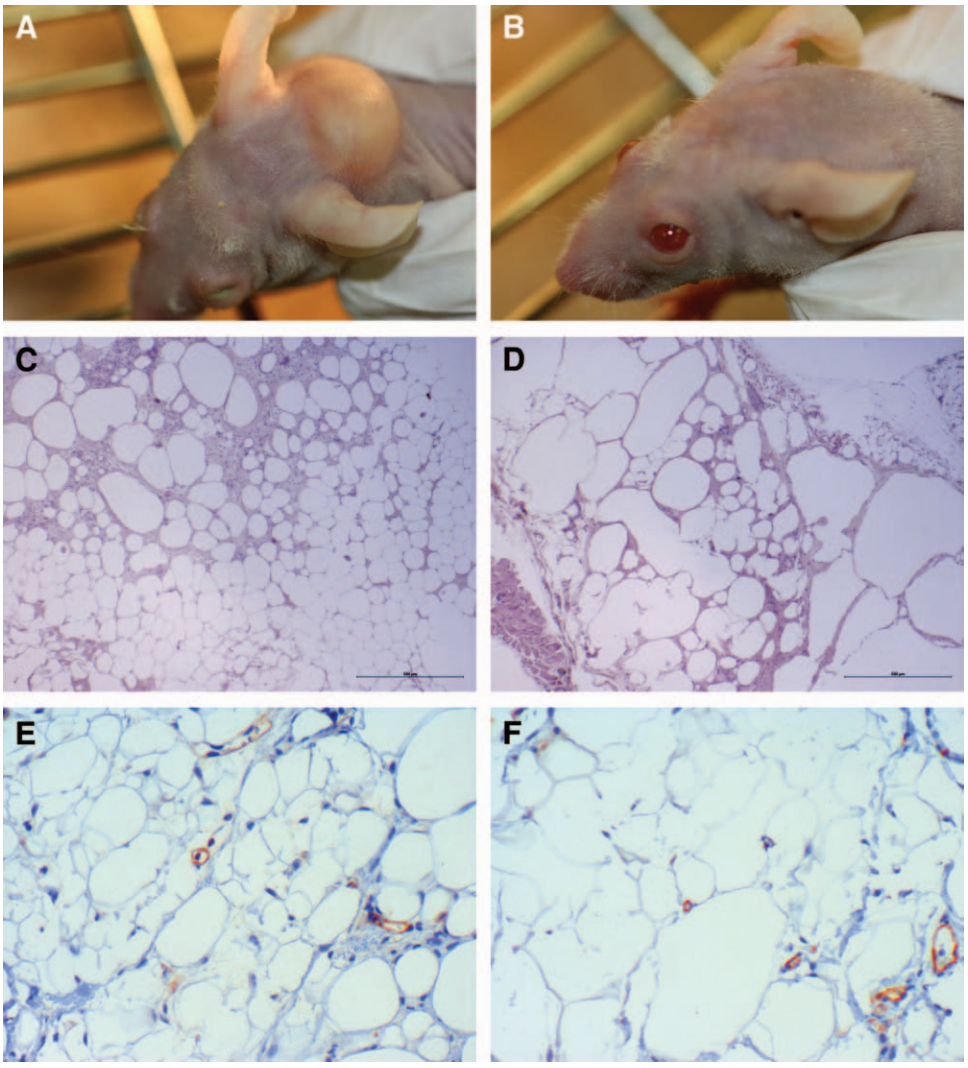

Fig. 1. Photographs of representative mice with fat grafts at the end of the study: DMSO-treated fat grafts (A) and SB203580-treated fat grafts (B). Histological representative sections of the fat grafts at the end of the study (hematoxylin and eosin staining): DMSO-treated fat grafts (C) and SB203580-treated fat grafts (D). Representative sections of CD31 staining: PBS-treated fat grafts (E) and SB203580-treated fat grafts (E).

of fibrosis and inflammation in the control group versus the SB203580-treated group. No statistically significant differences in the vacuoles formation were found between the groups (Fig. 2B).

The microvascular density of the control group fat grafts was significantly higher than that of the SB203580-treated fat grafts (Fig. 2C).

DISCUSSION

The MAPK family includes the p38 kinases, which consist of highly conserved proline-directed serine–threonine protein kinases. The p38 substrates include transcription factors; other protein kinases, which in turn phosphorylate transcription factors; cytoskeletal proteins and translational components; and other enzymes.[14]

The protein p38 MAPK mediates important intracellular mechanisms. It is activated by stress, playing an important role also in immune response and in the regulation of cell survival, differentiation, and apoptosis induction.[14–16] p38 inhibitors are being sought for possible therapeutic effect on acute liver disease,[17] prostate[18] and esophageal cancer,[19] autoimmune diseases, and other inflammatory processes,[14–16] for example, pamapimod.[20] For chronic obstructive pulmonary disease and asthma, numerous clinical trials have even been performed with promising results,[21–23] for example, dilmapimod.

Specifically to fat absorption, the inhibition of p38, in ex vivo experiments, has been shown to promote the expansion of human cord blood hematopoietic stem cell and increase the adipogenesis process, through its upstream regulator, Shp2.[9]

However, contrary to our hypothesis, inhibition of p38 significantly increased fat graft absorption. The antitheti-

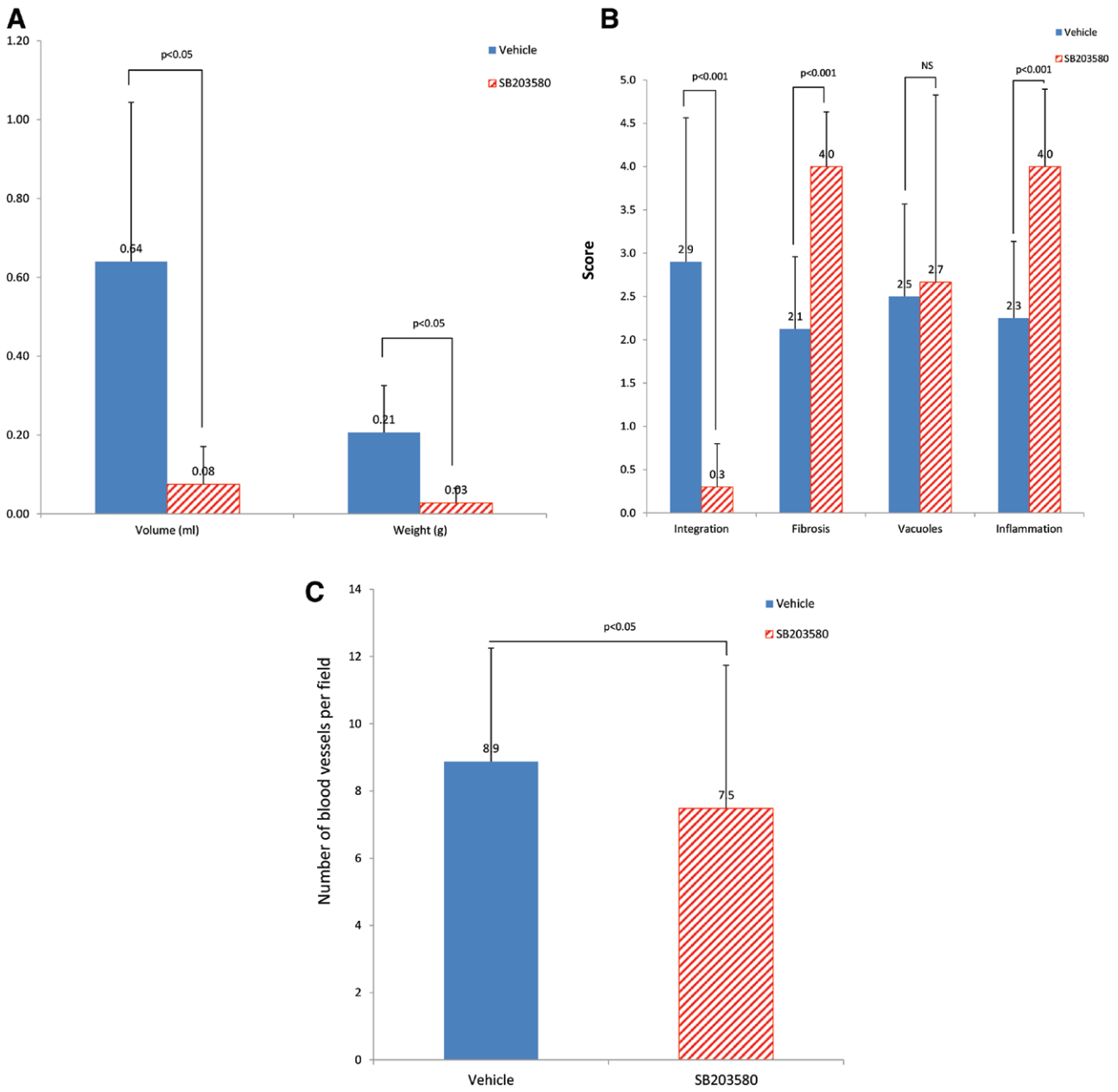

Fig. 2. A–C, Graphic analysis of clinical and histology results.

cal dramatic effects observed in our study may suggest that p38 may act differently on the numerous different cell types and lineages making up the fat graft, and further investigation is necessary. It is possible that there is a negative feedback pathway or dose-related response.

Other than adipose cells, the fat grafts are composed of ASC, stromal/progenitor cells, vascular endothelial cells, and hematopoietic cells. p38 can mediate both cells survival signals and, on the contrary, apoptosis. This all depends on the type of cells that are expressed.

There are many interesting questions raised by the study and different considerations required. Our current plans are for future studies that will be performed to dissect the role of p38 on the different cell types and lineages. As the opposite expected effect was seen with the p38 inhibitor, this study will be repeated by applying p38 to the fat graft. Rather than being injected posttransplant, fat grafts will also be preconditioned with either p38 or p38 inhibitors. The p38 and p38 inhibitor levels will also be measured in the graft samples.

CONCLUSIONS

This preliminary study suggests that as opposed to our hypothesis, inhibition of p38 significantly increases fat graft absorption. The dramatic effects observed in our study may suggest that p38 may act differently on the various cells harboring the fat graft.

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