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Cosmetic

Tumescent Liposuction without Lidocaine

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Background: Our previous study demonstrated that lidocaine has a negative impact on adipose-derived stem cell (ASC) survival. Currently for large-volume liposuction, patients often undergo general anesthesia; therefore, lidocaine subcutaneous anesthesia is nonessential. We hypothesized that removing lidocaine from tumescent might improve stromal vascular fraction (SVF) and ASC survival from the standard tumescent with lidocaine. Ropivacaine is also a commonly used local anesthetic. The effect of ropivacaine on ASC survival was examined.

Methods: Adults who underwent liposuction on bilateral body areas were included (n = 10). Under general anesthesia, liposuction on 1 area was conducted under standard tumescent with lidocaine. On the contralateral side, liposuction was conducted under the modified tumescent without lidocaine. Five milliliters of lipoaspirate were processed for the isolation of SVF. The adherent ASCs were counted after 24 hours of SVF culture. Apoptosis and necrosis of SVF cells were examined by Annexin/propidium iodide staining and analyzed by flow cytometry.

Results: Average percentage of live SVF cells was 68.0% ± 4.0% (28.5% ± 3.8% of apoptosis and 3.4% ± 1.0% of necrosis) in lidocaine group compared with 86.7% ± 3.7% (11.5% ± 3.1% of apoptosis and 1.8% ± 0.7% of necrosis) in no-lidocaine group (P = 0.002). Average number of viable ASC was also significantly lower (367,000 ± 107) in lidocaine group compared with that (500,000 ± 152) in no-lidocaine group (P = 0.04). No significant difference was found between lidocaine and ropivacaine on ASC cytotoxicity.

Conclusions: Removing lidocaine from tumescent significantly reduced SVF and ASC apoptosis in the lipoaspirate. We recommend tumescent liposuction without lidocaine, particularly if patient's lipoaspirate will be used for fat grafting. *(Plast Reconstr Surg Glob Open 2016;4:e829; doi: 10.1097/GOX.0000000000000830; Published online 9 August 2016.)*

Human lipoaspirate harvested via liposuction is an ideal autologous filler for reconstruction of soft-tissue defects.[1,2] The main drawback of fat grafting is graft resorption, which has shown graft volume loss to vary between 20% and 90% at 1 year after transplantation.[3–5] In recent years, liposuction followed by fat grafting with enrichment of adipose-derived stem cells (ASCs) or stromal vascular fraction (SVF) has gained popularity in aesthetic surgery.[6–9] Although ASCs and SVF are a minor fraction of lipoaspirate, investigators have been encouraged due to their high potential for self-renewal, multilineage differentiation, and higher yield, which may compensate for some of the graft volume loss.[10,11]

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Tumescent anesthesia is widely accepted as one of the standard anesthetic methods used in large-volume liposuction.[12,13] It can be achieved through a method of infusing large volumes of solution, which contain diluted anesthetic with epinephrine. Lidocaine is the most widely used anesthetic in tumescent anesthesia.[14–17] However, our previous study[18] showed that lidocaine used in liposuction can have a negative impact on ASC survival. Currently for large-volume liposuction in conjunction with fat grafting, many patients undergo general anesthesia; therefore, lidocaine subcutaneous anesthesia may be considered nonessential. In this study, we hypothesized that completely removing lidocaine from the tumescent solution might improve SVF and ASC survival from the standard tumescent with lidocaine.

Moreover, ropivacaine is also a commonly used local anesthetic though it is not as popular as lidocaine used

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for liposuction. However, recent studies from others[19–21] have shown that ropivacaine may be less cytotoxic to human mesenchymal stem cells compared with lidocaine. Nevertheless, the effect of ropivacaine on ASC viability has not been examined in the literature. In this study, we examined and compared the effect of ropivacaine and lidocaine on ASC survival in a cell culture model.

METHODS

Experimental Protocol

The human lipoaspirates were harvested from adults who underwent outpatient, cosmetic, or reconstructive liposuction procedures with associated fat grafting. All of the participants were provided informed consent and agreed to inclusion into the study. The institutional review board at our county hospital approved all of the protocols involving human tissue and cells. Lipoaspirates were harvested via standard liposuction techniques by a single plastic surgeon (R.C.B.). In brief, through a 4-mm incision, wetting solution was infiltrated into the subcutaneous fat at a ratio of 1:1 (infiltrate volume vs aspirate volume). The lipoaspirate was procured using a blunt-tipped 3.7-mm Mercedes cannula and machine suction and collecting the lipoaspirate in a sterile canister for processing. For quality control, individuals who underwent liposuction on bilateral body areas were included in this study. Under general anesthesia, liposuction on one area was conducted under the standard tumescent with lidocaine (1,000 mL of Ringer's solution with 30 mL of 1% lidocaine and 1 µg/mL epinephrine). On the opposite area, liposuction was conducted under the modified tumescent (1,000 mL of Ringer's solution with 1 µg/mL epinephrine) without lidocaine.

Isolation of SVF

The method for SVF isolation has been described in our previous publication.[18,22–25] Briefly, 5 mL of lipoaspirate from each liposuction was processed. Lipoaspirate samples were centrifuged at 430*g* for 10 minutes. After oil removal, the lipid phase of the lipoaspirate from the top of the conical tube was harvested and then diluted with an equal volume of collagenase digestion solution (final concentration: 0.3 U/mL, Collagenase NB 4G proved grade, Serva Electrophoresis, Heidelberg, Germany). After 30 minutes of incubation, an equal volume of Dulbecco's Modified Eagle Medium containing 20% fetal bovine serum was added to stop enzymatic digestion. The floating layer containing adipocytes and the pellet containing SVF were separated by centrifugation. The isolated SVF was filtered through a 100-µm nylon filter and then processed for density gradient by centrifugation with Histopaque-1077 (Sigma-Aldrich, St. Louis, Mo.). The white band (mononuclear cells) remaining at the plasma interface was carefully aspirated and the total number of SVF cells was counted. Harvested SVF cells were then either cultured in nonhematopoietic expansion medium (NHEM; Miltenyi Biotec, Auburn, Calif.) for ASC purification or stained by Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) and analyzed by flow cytometry.

Purification of ASCs through SVF Culture

SVF is highly heterogeneous and contains many cell subsets including ASCs, endothelial cells, hematopoietic cells, etc. One of the characteristics of ASC is that they are adherent to the plastic surface. Therefore, isolation of ASC can be achieved through SVF culture. In brief, all the isolated SVF cells from the lipoaspirate sample were added into a 75-cm^2 cell culture flask containing 15 mL of prewarm NHEM and 1% of penicillin–streptomycin. The flask was cultivated at 37°C, 5% CO_2, and 95% humidity. After 24 hours of culture, the nonadherent cells in the flask were removed by phosphate buffer saline (PBS) washing. Trypsin/ethylenediaminetetraacetic acid 1 mL was added into the flask and incubated at 37°C for 10 minutes. After complete dissociation, the total number of ASC was harvested and counted.

After ASC counting, all of ASCs were moved to a new flask with 15 mL of fresh prewarmed NHEM for continuation of culture. Cell adhesion was examined under an inverted microscope. The cultured ASCs were checked daily and the medium was changed every 3 days. Passage was performed when ASC number had reached more than 2 million.

Dose–Response Study In Vitro

To clarify the cytotoxicity of ropivacaine on ASCs, the effect of ropivacaine and lidocaine on ASC survival was examined and compared in a dose–response study in vitro. In brief, 2×10^5 ASCs were subcultured in each of 10 T-25 flasks. For control flask, 2×10^5 ASCs were cultured with 5 mL of NHEM. For experimental flasks, 2×10^5 ASCs were cultured by 5 mL of NHEM with 0.1, 0.3, or 0.5 mL of 0.5% ropivacaine or 0.5% lidocaine or correspondent PBS. The pH (7–8) was verified for each flask. Passage was then performed at day 4, and the number of adherent ASCs in each of flasks was counted.

Detection of Apoptotic and Necrotic on SVF Populations

The apoptosis and necrosis of SVF cells were detected by to Annexin V-FITC/PI assay.[18,22–25] Briefly, 1×10^5 SVF cells were washed by 1 mL of binding buffer followed by centrifugation. After the supernatant removal, SVF cells were suspended in 100 µL of binding buffer with 10 µL of Annexin V-FITC or without (unstained control). After 15 minutes of incubation in dark, SVF cells were washed again by 1 mL of binding buffer and then centrifugation. After supernatant removal, SVF cells were suspended in 500 µL of binding buffer with 5 µL of PI or without (unstained control). Two tubes were used to set up compensation and quadrants with (1) unstained (2) stained with Annexin V-FITC and PI. Necrosis was determined by PI and apoptosis was determined by Annexin V-FITC. Ten thousand SVF cells from each sample were scanned and analyzed by flow cytometer. Data acquisition and analysis were performed by flow cytometer with BD FACS Aria III software version 6.1.3 (Becton Dickinson, San Jose, Calif.) using an excitation wavelength of 488 nm with an argon laser.

Statistical and Power Analysis

We have analyzed the continuous response variable from matched pairs of study subjects in our previous studies.[18,22–25] Our prior preliminary data based on

7 subjects indicate that the difference in the response of matched pairs is normally distributed with SD 386.7 and the difference between means is 514.3. If the true difference of ASC number in the mean response of matched pairs is 514.3, we will need to study 6 to 8 pairs of subjects to be able to reject the null hypothesis that this response difference is 0 with probability (power) 0.9 (90%). The type I error probability (*P* value) associated with this test of this null hypothesis is 0.05.

RESULTS

The participants (n = 10) were 9 female and 1 male adults. There were no children, no pregnant woman, and prisoner. There were no exclusions based on gender, race/ethnicity, or medical conditions. This study went through 12 months for completion. The average age of the participants was 59.8±2.2 years (±SEM) and the average body mass index was 27.7±2.0 (±SEM). Liposuction sites were located in flank (n = 9), abdomen (n = 4), and thigh (n = 3).

In the Annexin V-FITC/PI assay, we found that lidocaine caused significant SVF apoptosis, but not necrosis (Fig. 1 and Supplemental Digital Content 1, http://links.lww.com/PRSGO/A237). In the tumescent with lidocaine group, the average percentage values of live cells, apoptotic cells, and necrotic cells were 68.0% ± 4.0%, 28.5% ± 3.8%, and 3.4% ± 1.0%, respectively. In the tumescent without lidocaine group, the average percentage values of live cells, apoptotic cells, and necrotic cells were 86.7% ± 3.7%, 11.5% ± 3.1%, and 1.8% ± 0.7%, respectively. The difference was statistically significant between lidocaine and no-lidocaine groups in live cells ($P = 0.002$) and apoptotic cells ($P = 0.002$). However, the percentage of necrotic cells did not show a statistically significant difference between these 2 groups.

The average number of ASCs was also significantly lower ($P = 0.04$) in the tumescent with lidocaine group (367,000±107) compared with that (500,000±152) in the tumescent without lidocaine group (Fig. 2).

To clarify whether ropivacaine is less cytotoxic than lidocaine to human ASCs, a dose–response study was conducted in a cell culture model. We found that ASC survival was significantly lower ($P < 0.007$) in the flasks treated by either lidocaine or ropivacaine and in a dose-dependent manner compared with the correspondent PBS control (Fig. 3). No significant difference was found between lidocaine and ropivacaine on ASC cytotoxicity.

DISCUSSION

Tumescent anesthesia is widely accepted as one of the standard anesthetic methods used for large-volume liposuction.[12,13] Because patients often undergo general anesthesia, lidocaine subcutaneous anesthesia seems unnecessary because lidocaine could compromise SVF and ASC survival in the lipoaspirate.[18,26,27] For example, in our previous study,[18] we have reported that lidocaine has significant and a negative impact on ASC survival in the lipoaspirate that is used for fat grafting. Girard et al[26] reported that lidocaine has a negative impact on ASCs even when the ASCs were exposed for only 1 or 2 hours. Keck et al[27] found that local anesthetics not only affected the quality of viable ASCs but also influenced ASC's ability to differentiate into adipocytes. In recent years, liposuction followed by fat grafting with SVF enrichment has gained popularity in aesthetic surgery.[6–9] Several studies have showed that SVF is comparably effective as regenerative cell therapy[8,9] in treating chronic conditions ranging from arthritis, diabetes mellitus, chronic wound, breast cancer, and radiation injuries. A major concern is how to keep SVF and ASC survival during liposuction and reduce graft volume loss after fat grafting. The results from this study

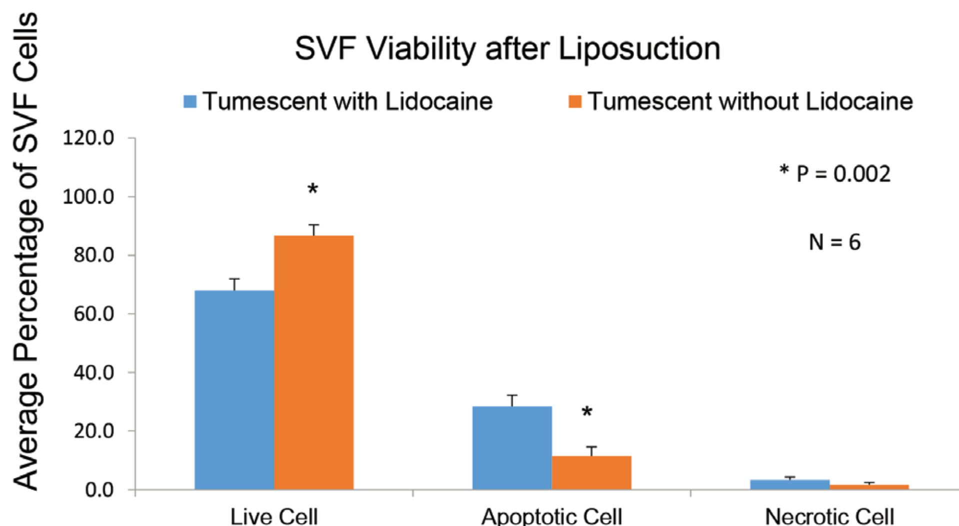

Fig. 1. Average percentage (±SEM) of live, apoptotic, or necrotic SVF cells. Necrosis was determined by PI, apoptosis was determined by Annexin V-FITC, and live cells are negative for both Annexin V-FITC and PI. * indicates statistically significant differences ($P = 0.002$) between tumescent with lidocaine group and tumescent without lidocaine group.

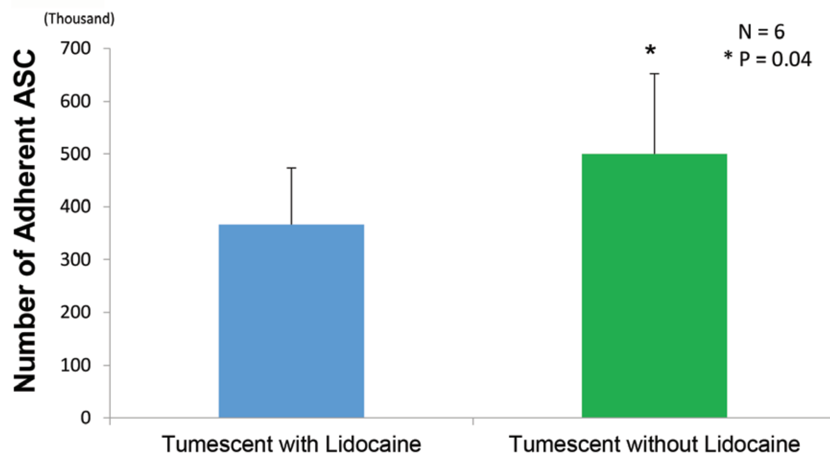

Fig. 2. Average number (±SEM) of ASCs. * indicates statistically significant differences ($P = 0.04$) between tumescent with lidocaine group and tumescent without lidocaine group.

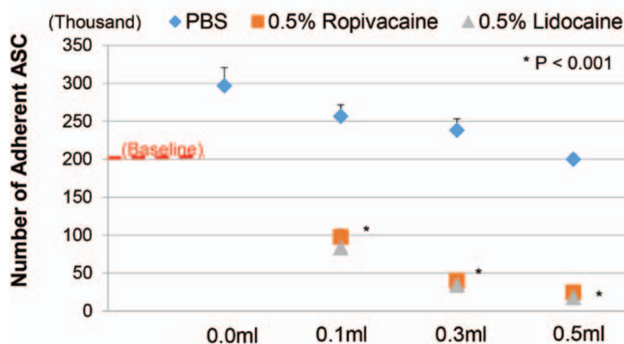

Fig. 3. Average number (±SEM) of survival ASCs after treatment by ropivacaine, lidocaine, or correspondent PBS; 2×10^5 of ASCs were cultured by 5 mL NHEM for 4 d with 0.1, 0.2, or 0.5 mL of 0.5% of ropivacaine or 0.5% of lidocaine or correspondent PBS. * indicates statistically significant differences ($P < 0.001$) between ropivacaine or lidocaine and the correspondent PBS.

indicate that removing lidocaine from tumescent solution significantly improves SVF and ASC survival from the standard tumescent with lidocaine. We strongly recommend performing tumescent liposuction without using lidocaine, particularly if the lipoaspirate will be used for fat grafting. Nevertheless, after lipoaspirate is harvesting, lidocaine may still be injected subcutaneously to reduce postoperative pain.

We are aware that the SVF or ASC in the lipoaspirate can be variable among different heterogeneous populations with various morbidities (ie, overweight vs lean, aged vs young, diabetic vs healthy), different locations (abdomen vs thigh, etc), and different liposuction techniques.[28–30] To reduce these variances, we modified our experiment design. We standardized the liposuction techniques with a single plastic surgeon and excluded the individuals who underwent liposuctions only on unilateral body areas. By this experimental design, the influence of the aforementioned variances is largely diminished or controlled because both samples (with lidocaine or without lidocaine) came from the symmetrical pairs of same individual.

Some investigators have used Trypan blue, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], or [2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide] assays to estimate cell viability in the lipoaspirate.[30–32] However, it is difficult to draw conclusions using these assays because these assays can only stain necrotic cells, but cannot identify apoptotic cells. Current literature indicates that necrosis is no longer considered to be the sole mechanism of cell death.[33,34] Apoptosis might be the initial mode of cell death in the process to ultimate cell death.[35] Cell death mechanisms between necrosis and apoptosis are different. Necrosis is characterized by cell membrane rupture, and the process of necrosis is energy-independent and cannot be blocked.[36] However, apoptosis is characterized by cell membrane shrinkage, and the process of apoptosis is ATP-dependent and can be blocked by specific agents.[37] In this study, we used Annexin/PI assay with the aid of flow cytometry to determine SVF viability. In early apoptosis, phosphatidylserine protein is translocated from the inner side of cell membrane to the outer layer. Annexin is a phospholipid-binding protein with a high affinity for phosphatidylserine; therefore, it can be used as a marker of early apoptosis.[33,34] PI is a vital dye that can stain necrotic cells with broken cell membranes. The results from this study suggest that lidocaine-induced SVF cell death is through a mechanism dominated by apoptosis.

In this study, cell viability of the adherent ASCs was not measured. One of the unique characteristics of ASCs is that they are adherent to the plastic surface. This phenomenon is the foundation for current ongoing ASC research.[38–40] Most nonadherent cells (either non-ASCs or dead ASCs) in the flask were removed by PBS washing after 24-hour culture of SVF cells. In our previous studies,[18,22–25] we found that about 90% of adherent cells to the plastic surface after 24-hour culture of SVF were viable (negative for both Annexin V-FITC and PI) and the ethanol-treated

dead ASCs were unable to adhere to the plastic surface in the culture flask. We believe that the dead ASCs may lose their ability to adhere and could have been removed by PBS washing or medium change. Therefore, the adherent cells from SVF culture can be defined not only as ASCs but also as viable ASCs.

Lidocaine is the most widely used local anesthetic for liposuction.[14–18] The question was whether we can find a local anesthetic that is less cytotoxic than lidocaine. Ropivacaine is a frequently used local anesthetic though it is not commonly used for liposuction. However, recent studies from others[19–21] have shown that ropivacaine may be less cytotoxic to human mesenchymal stem cells compared with lidocaine. For example, Breu et al[19] reported that all local anesthetics showed detrimental cytotoxic effects on human mesenchymal stem cell cultures in a concentration- and time-specific manner. Ropivacaine was significantly less cytotoxic than bupivacaine and mepivacaine. Rahnama et al[20] found that ropivacaine and bupivacaine had limited toxicity in human mesenchymal stem cells compared with lidocaine. Nevertheless, the effect of ropivacaine on human ASC viability has not been addressed in the literature. In this study, we compared the effect of ropivacaine and lidocaine on ASC survival in cell culture model. Unfortunately, the results from this study did not support our original hypothesis. Although ASC number was slightly higher in the flasks treated by ropivacaine than the flasks treated by lidocaine, overall ASC survival was significantly lower in both lidocaine and ropivacaine groups and in a dose-dependent manner compared with the correspondent PBS control. Therefore, we conclude that there is no significant difference between lidocaine and ropivacaine on ASC cytotoxicity.

One of the limitations of this study could be the centrifugation. In this study, the maxima speed we used was 430*g* (1,500 rpm) for 10 minutes. Some investigators may consider that time of centrifugation could be too long. For example, Kim et al[41] found that significant cell destruction could happen when speed exceeded 5,000 rpm and the time longer than 5 minutes. The traditional method described by Pu et al[42] was 1,200*g* (3,000 rpm) for 3 minutes. However, there is no universal agreement on the optimal speed and time for centrifugation. Recently, Ibatici et al[43] conducted a study by comparing the centrifuged versus noncentrifuged methods and suggested that the centrifugation at 600*g* (1,826 rpm) for 10 minutes was safe and feasible and does not impair cell viability. Nevertheless, in this study, both lidocaine and no-lidocaine samples came from the same individual underwent the same process for SVF isolation including same speed/time of centrifugation. We found a statistically significant difference between lidocaine and no-lidocaine groups. Therefore, the speed/time of centrifugation employed in this study may not have significant impact on the results of this study.

SUMMARY

Tumescent anesthesia is widely accepted as one of the standard anesthetic methods used for large-volume liposuction. Because patients are frequently sedated and often undergo general anesthesia, lidocaine subcutaneous anesthesia may be unnecessary and could compromise SVF and ASC survival in the lipoaspirate. The results from this study clearly indicate that completely removing lidocaine from tumescent solution significantly reduced SVF and ASC apoptosis from the standard tumescent liposuction with lidocaine. We strongly recommend performing tumescent liposuction without using lidocaine, particularly if the patient's lipoaspirate will be used for subsequent fat grafting. Nevertheless, after the lipoaspirate is harvested, lidocaine can still be injected subcutaneously to reduce postoperative pain. Moreover, no significant difference was found between lidocaine and ropivacaine on ASC cytotoxicity.

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