The Benefit of Dual-frequency Ultrasound in Patients Treated by Injection Lipolysis

[a]IRENE TAUSCH, MD; ILJA KRUGLIKOV, PhD, DrSci
[a]Kosmed Clinic, Kiel, Germany; [b]Wellcomet GmbH, Karlsruhe, Germany

ABSTRACT

Objective: To assess whether dual-frequency ultrasound can improve the treatment results of injection lipolysis (IL). **Design:** Randomized, single-center pilot study. All subjects were treated with a contralateral control: one side of the body received the treatment with IL, whereas the symmetrical contralateral side received the combination of IL with dual-frequency ultrasound in a special form (LDM). Injections were provided subcutaneously in the depth of 8 to 12mm with a distance of nearly 15mm between the single injections. All subjects were treated with LDM once, 1 to 3 hours before the injection and twice per week after the injection. **Setting:** Kosmed Clinic, Kiel, Germany. **Participants:** Seven female healthy subjects with local body contouring problems in femoral or upper arm regions. **Measurements:** Circumference was measured before and 6 to 8 weeks after treatment. Pain, hematoma, itching, burning sensation, redness, swelling, and pressure sensation were evaluated before the first treatment and 6 to 8 weeks for both contralateral sides. **Results:** Application of LDM in combination with IL improved the volume reduction compared to pure IL by 65.6 percent. The difference between IL and IL+LDM treated sides showed very high significance ($p \leq 0.0001$). Significant differences between IL and IL+LDM treated areas were found also in pain ($p \leq 0.01$). No statistical difference was found in all other subjective parameters. **Conclusion:** Application of LDM significantly increases the treatment success after IL and the acceptance of IL procedures by patients.
(*J Clin Aesthet Dermatol.* 2015;8(8):42–46.)

Noninvasive and minimally invasive procedures for local body contouring are of growing interest in aesthetic medicine. Although these procedures are less effective than the surgical lipoplasty, they are generally safer and easier accepted by patients. Different noninvasive procedures, such as high-intensity focused ultrasound, radiofrequency, and cryolipolysis, claim adipocyte death as the main mechanism of their application, which is theoretically necessary to ensure the long-lasting effect of proposed treatment method. For many noninvasive procedures, this is evidently not the case.[1,2]

Injection lipolysis (IL) also belongs to the class of minimally invasive methods. This procedure is based on the subcutaneous injection of lipolytic agents to reduce local fat depots.[3] Applied drugs are generally based on phosphatidylcholine (PPC) mixed with sodium deoxycholate (DOC) converting it into a soluble and injectable medium. It is known that DOC by itself or in combination with PPC causes significant cell lysis.[4] In adipocytes, PPC/DOC is believed to cause inflammatory-mediated necrosis with subsequent resorption of the fat by macrophages as shown by histological investigations.[5]

The main side effects after application of IL are local pain, hematomas, burning sensation, erythema, and edema. All these effects are transient and localized to the area of IL application. However, some of them, especially pain, can significantly reduce the willingness of patients to utilize IL. Therefore, new treatment methods with reduced side effects, but with increased benefits such as those seen with IL, are desired.

The main objective of this study was to find out whether better treatment results can be obtained and pain and pressure sensations reduced by dual-frequency ultrasound assisted lipolysis.

METHODS

Subjects. Altogether, seven healthy female patients

DISCLOSURE: Dr. Tausch reports no relevant conflicts of interest. Dr. Kruglikov is the managing partner of Wellcomet GmbH. A Wellcomet device was used in this study. Dr. Kruglikov was not involved in clinical assessments or statistical data analysis.
ADDRESS CORRESPONDENCE TO: Irene Tausch, MD; E-mail: i.tausch@kosmed-klinik.de

with local body contouring problems in upper arm (Patients 1 and 2) or in femoral (Patients 3–7) regions were medicated. Exclusion criteria included subjects under age 18, willingness to comply with contralateral study protocol, those who were pregnant, those who were breastfeeding, and those with a current illness, immunodeficiency, hypertensivity, cardiac disease, and/or diabetes. The study was carried out in accordance with the guidelines of the Helsinki Declaration. All study participants signed the written informed consent.

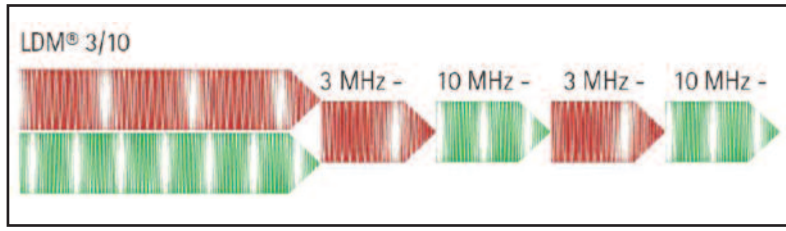

Figure 1. Wave formation in dual-frequency LDM modus

Study design. This was a randomized, single-center, contralateral, controlled, pilot study. Since the participants can clearly identify the treatment with ultrasound waves,[6] neither the participants nor the study nurses and the doctors were blinded. All subjects were treated with a contralateral control—one side of the body received the IL treatment, and the symmetrical contralateral side received the combination of IL with dual-frequency ultrasound LDM (IL+LDM). To avoid a biasing of the results, the choice of the side receiving the combined treatment was randomized. All subjects were treated with ultrasound once 1 to 3 hours before the injection lipolysis and 10 times after injection (twice per week).

Intervention. The formulation of the injected medium was phosphatidylcholine (1.25mL), sodium deoxycholate (0.63mL), benzyl alcohol (0.23mL), sodium chloride (0.09mL), sodium hydroxide (0.63mL), alpha tocopherol (0.38mL), ethanol (0.08mL), and aqua ad injectionem (21.71mL) in a total of 25mL (Euromed Pharmacy, Fürth, Germany). Patients received 0.25mL per injection, resulting in a total of 25mL per treatment. Injections were provided subcutaneously at a depth of 8 to 12mm with a distance of nearly 15mm between the single injections.

Dual-frequency ultrasound was applied by the emitter LDM®-MED (Wellcomet GmbH, Karlsruhe, Germany) producing the ultrasound waves with frequencies of 3MHz and of 10MHz in a special LDM modus. The wave formation is shown in Figure 1.[7]

In this study, LDM was applied in the form of a 5ms application of 3MHz and a 5ms application of 10MHz in a geometrical area of 5cm^2 with spatial average temporal average (SATA) ultrasound intensity of 1.0W/cm^2. Ultrasound treatment required 20 to 30s per spot, most often resulting in a total treatment time of 15 minutes. Ultrasound intensity was controlled by digital ultrasound power meter UPM-DT-10 (Ohmic Instruments Co., Easton, Massachusetts). Ultrasound contact gel (Dispomed, Gelnhausen, Germany) was used as a coupling medium.

Clinical assessment and statistics. Circumference was measured, and pain, hematoma, itching, burning, redness, swelling, and pressure sensations were evaluated and documented before the first treatment and normally after eight weeks for both contralateral sides. Assessment time was six weeks in three patients because of an early improvement.

Circumference was measured with nonstretchable tape in a standardized manner without compressing the soft tissue with an accuracy of 0.5cm. The subjective parameters were assessed by patients and the doctor. A 5-point scale was utilized for this assessment: 1=absent, 2= weak, 3=moderate, 4=strong, 5=very strong.

For statistical evaluations, the authors applied the paired t-test, sign test, and chi-square test, (Fisher's exact test), as well as the non-parametric methods (median). Results with $p \leq 0.05$ were considered as statistically significant.

RESULTS

Circumference. Circumferences, measured before and 6 to 8 weeks after IL treatment, are presented in Table 1. P values were yielded by paired t-test. The difference between IL and IL+LDM treated sides was tested. Circumference measurements before treatment showed no significant difference between the contralateral sides (p=0.393). Treatment resulted in reduced circumference in both sides presenting differences of 1.7±1.5cm on the IL side and 2.7±1.6cm on the IL+LDM side (p=0.016). Combination of IL+LDM showed a greater reduction of circumference (median 2.0) compared to application of IL alone (median 1.0) ($p \leq 0.05$). The high coefficients of variation (CV) (85.6% for IL and 59.1% for IL+LDM) are mainly because of the mixture of different treatment areas. Subjects 1 and 2 were treated on the upper arm, other subjects on the thighs. The means and CV for this latter group of five patients were 2.2±1.4cm, CV=65.5 percent (IL) and 3.2±1.6cm, CV=51.3 percent (IL+LDM), respectively. High significance was received by testing the absolute circumference values after the treatment ($p \leq 0.016$), whereas the difference between IL and IL+LDM treated sides showed even very high significance (p=0.000095).

Volume reduction. Volume reduction with and without supportive application of dual-frequency ultrasound is presented in Figure 2. To make the estimations, the authors have assumed the standard length of the treated area to be 20cm. Patients showed rather different treatment results demonstrating inter-individual variability; however, the intra-individual differences were very significant as proposed by testing

TABLE 1. Circumference measurements (in cm)			
SUBJECT	BEFORE IL/IL+LDM	AFTER IL/IL+LDM	DIFFERENCE IL/IL+LDM
1	30.0 / 31.0	30.0 / 30.0	0.0 / 1.0
2	32.0 / 32.0	31.0 / 30.0	1.0 / 2.0
3	53.5 / 53.5	53.0 / 52.0	0.5 / 1.5
4	62.0 /63.5	59.0 / 59.0	3.0 / 4.5
5	58.0 / 57.0	57.0 / 55.5	1.0 / 1.5
6	64.5 / 65.0	62.0 / 61.5	2.5 / 3.5
7	54.0 / 54.0	50.0 / 49.0	4.0 / 5.0
$p\leq$	not significant	0.05	0.0001

the differences between IL and IL+LDM in Table 1. The difference between the volume reduction by pure IL treatment of 6.1±4.9 percent and by IL+LDM treatment of 10.1±4.7 percent was highly significant (p=0.00062). As a consequence of these two figures, additional LDM application improves IL treatment results for volume reduction by 65.6 percent.

Subjective parameters. Table 2 presents the subjective parameters pain, pressure, itching, and burning as assessed by patients, as well as the criteria hematoma, redness, and swelling assessed by the doctor. Significant differences between IL and IL+LDM treated areas were found in pain with a median of moderate, this being in contrast to strong ($p\leq0.01$). In all other features, chi-squared analysis showed no trend except a slight one in the feeling of pressure (p=0.092). In only IL-treated sides, the sequence of the medians of strength was pressure (very strong), pain and hematoma (strong); itching, redness, and swelling showed the moderate median value. The weakest side event was burning (weak).

In IL+LDM treated areas, pain, pressure, hematoma, and itching presented the median with one grade weaker (e.g., moderate) in contrast to strong in pain and hematoma, compared to only IL treated sides.

DISCUSSION

This is the first clinical study combining IL with dual-frequency ultrasound quickly changing the frequencies of 3MHz and 10MHz (LDM). These waves demonstrate strong anti-inflammatory and wound healing effects.[7–9] It was suggested that these beneficial effects are connected with the ability of dual-frequency ultrasound of 3/10MHz to suppress the activity of matrix metalloproteinases and to activate the heat shock proteins in tissue.[11,12] Furthermore, subcutaneous adipose tissue demonstrated a local loosening after application of LDM as shown by histological investigations.[13]

Theoretically, supportive application of dual-frequency ultrasound can also be beneficial in IL. For example, the distribution of the injected drug would be more homogeneous in a loose rather than rigid tissue, which could be clearly beneficial in all injection methods. Also, tissue loosening is connected with local water retention, which can significantly modify the osmotic relations in the tissue at the moment of injection and influence the absorption of injected substance. Additionally, tissue loosening increases the distance between the blood vessels, thus reducing the probability of tissue damage and the appearance of hematomas.

High inter-patient variability of end results by IL application makes any assessment of the input of supportive method generally difficult. In this study, the CV for circumference reduction in pure IL treatments were high (CV=85.6%). There are two possibilities to overcome this problem: 1) recruitment of a large number of patients; 2) application of contralateral control, which may reduce the inter-patient variability considering the differences in treatment results by the same patient. Since the authors applied the contralateral control to eliminate the inter-individual variations and to reduce the error in subjective assessments of patients, the number of subjects agreeing to enter the study was limited.

Application of IL in combination with LDM has demonstrated a significant improvement of treatment results compared to application of IL alone ($p\leq0.016$, Table 1). After one IL treatment, the circumference was reduced on average by 2.7cm on the IL+LDM treated side, whereas the reduction was only 1.7cm on the IL treated side.

The differences in the circumferences appear small. For example, a decrease of 4.5cm in a circumference of 63.5cm (Patient 4, Table 1) means a reduction of 7.1 percent. In the same patient treated without LDM the reduction effect was only 4.8 percent. It is, however, worthwhile to mention that the difference in the volume is not only a function of the difference of the circumference, but also a function of the circumference itself. This is implicated by the cross section being a function of the square of the radius, whereas the circumference is a function of the radius and not of its square (For readers interested in analysis: $\Delta V= L * C * \Delta C / (2 * \pi)$; V=volume, C=circumference, L=length, Δ=change; so ΔV is a function of C and ΔC). Consequently, the treated volume in the femoral area of the above-mentioned patient was reduced by 9.4 and 13.7 percent when treated with IL and IL+LDM, respectively (Patient 4, Figure 2). Data for volume reduction, presented in Figure 2, are much more impressive than the circumferences demonstrated in Table 1.

Overall, the combined treatment IL+LDM reduced the treated volume an average of 10.1 percent, whereas in IL-treated areas, this reduction was only 6.1 percent. Thus, one can conclude an advantage of 65.6 percent is reached by supportive application of LDM treatment.

The greater volume reduction on the side treated with IL+LDM can be theoretically primarily connected with some loosening of the tissue after LDM application and thus with better distribution of injected drugs. At the same time, the serial application of LDM reduces the water content in the fat tissue producing a washout phenomenon,[14,15] thus causing an additional local reduction of subcutaneous fat tissue volume.

In addition, side effects, such as local pain in the injected areas, were reduced by additional application of LDM. Pain reduction after IL+LDM application seems to be especially important, since this side effect can substantially affect the quality of life of the patients. Observed contralateral differences in pain rating can be explained by anti-inflammatory properties of 10MHz ultrasound, which were demonstrated in different applications.[7–9] The pain relief may also be connected with the activation of some heat shock proteins (HSPs), which was demonstrated after application of dual-frequency ultrasound *in vitro*.[10]

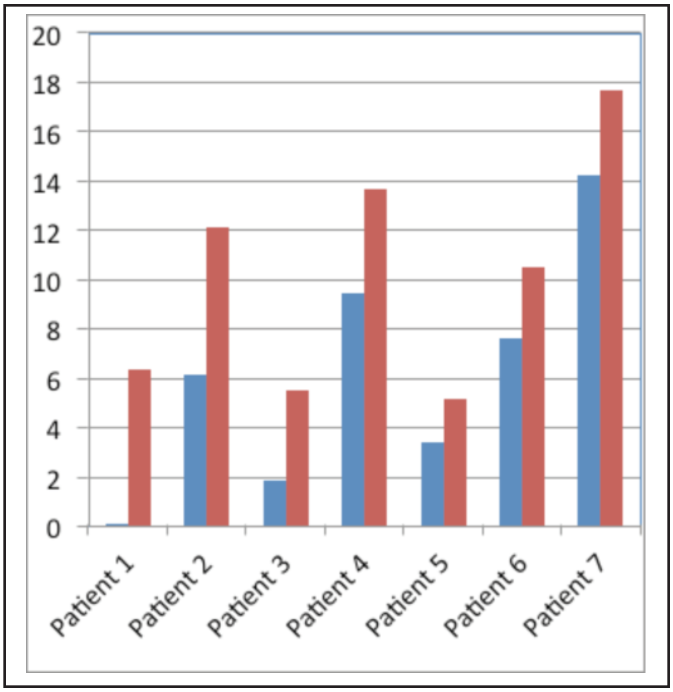

Figure 2. Treatment results presented as a volume reduction, in percent. IL (blue) vs. IL+LDM (red) ($p \leq 0.001$)

TABLE 2. Subjective parameters

SUBJECT	PAIN IL/IL+LDM	ITCHING IL/IL+LDM	BURNING IL/IL+LDM	PRESSURE IL/IL+LDM	HEMATOMA IL/IL+LDM	REDNESS IL/IL+LDM	SWELLING IL/IL+LDM
1	5 / 4	1 / 1	2 / 2	5 / 5	4 / 3	3 / 3	3 / 3
2	5 / 4	4 / 4	2 / 2	5 / 4	5 / 5	4 / 4	5 / 5
3	4 / 3	5 / 5	1 / 1	5 / 5	3 / 3	3 / 3	3 / 3
4	3 / 1	3 / 2	3 / 2	3 / 2	2 / 2	1 / 1	3 / 3
5	5 / 3	2 / 2	2 / 2	5 / 4	4 / 3	2 / 2	5 / 4
6	2 / 1	1 / 1	3 / 2	3 / 2	3 / 2	3 / 3	4 / 3
7	4 / 3	1 / 1	1 / 1	5 / 4	1 / 1	2 / 2	3 / 2
p≤	0.01	not significant	not significant	not significant	not significant	not significant	not significant

It is interesting to note that stimulation of specific HSPs by ultrasound can demonstrate distinct effects depending on the sequence of inflammatory reaction and HSP production. Whereas, induction of these proteins before inflammation normally shows a cytoprotective effect on the treated cells, their expression during pre-existing strong inflammation can lead to significant cytotoxicity known as "heat shock paradox."[16] This paradox can be explained by qualitatively different intracellular (which is believed to be cytoprotective) and extracellular (which is assumed to be cytotoxic) activities of some HSPs.[17] One may speculate that this effect can also be involved in the improvement of volume reduction observed in this study, since LDM was applied before as well as after IL injection, and thus during the strong inflammation phase. This idea, however, could not be directly confirmed in this study since the authors did not provide any histological or HSP examinations of subcutaneous fat tissue.

This study clearly demonstrates that additional application of dual-frequency ultrasound of 3/10MHz improves the treatment results with IL and reduces the level of pain after this treatment. Although no statistical significance could be proven, in IL+LDM treated areas, pressure, hematoma, and itching were one grade weaker (e.g., moderate in contrast to strong in pain and hematoma) when compared to only IL treated sides.

CONCLUSION

The present pilot study demonstrates that the application of dual-frequency ultrasound in combination with injection lipolysis significantly improves the volume reduction compared to injection lipolysis alone by 65.6 percent. Additionally, application of LDM can significantly reduce the pain that often occurs after such treatments, making the application of injection lipolysis more acceptable for the patients. A larger cohort of subjects will be needed to obtain reliable information on other side effects of treatment.

ACKNOWLEDGMENT

The authors gratefully acknowledge Dr. Henseler for his help in statistical evaluation and for discussion of results.

REFERENCES

1. Kruglikov I. General theory of body contouring: 1. Processes of quick and slow modulation of subcutaneous fat tissue. *J Cosmet Dermatol Sci Appl*. 2014;4:107–116.
2. Kruglikov I. General theory of body contouring: 2. Modulation of mechanical properties of subcutaneous fat tissue. *J Cosmet Dermatol Sci Appl*. 2014;4:117–127.
3. Duncan DI, Chubaty R. Clinical safety data and standards of practice for injection lipolysis: a retrospective study. *Aesth Surg J.* 2006;26:575–585.
4. Palmer M, Curran J, Bowler P. Clinical experience and safety using phosphatidylcholine injections for the localized reduction of subcutaneous fat: a multicentre, retrospective UK study. *J Cosm Dermatol.* 2006;5:218–226.
5. Rose PT, Morgan M. Histological changes associated with mesotherapy for fat dissolution. *J Cosmet Laser Ther.* 2005;7:17–19.
6. Watson JM, Kang'ombe AR, Soares MO, et al. Use of weekly, low dose, high frequency ultrasound for hard to heal venous leg ulcers: the VenUS III randomized controlled trial. *Br Med J.* 2011;342:d1092.
7. Kruglikov I, Kruglikova E. Dual treatment strategy by venous ulcers: pilot study to dual-frequency ultrasound application. *J Cosmet Dermatol Sci Appl.* 2011;1:157–163.
8. Meyer-Rogge D, Rösken F, Holzschuh P, et al. Facial skin rejuvenation with high frequency ultrasound: multicentre study of dual-frequency ultrasound. *J Cosmet Dermatol Sci Appl*. 2012;2:68–73.
9. Meyer-Rogge D, Kruglikov I. Pilot study into super-fractionation treatment strategy of acne and rosacea. *J Cosmet Dermatol Sci Appl.* 2013;3:197–202.
10. Sontag W, Kruglikov I. Expression of heat shock proteins after ultrasound exposure in HL-60 cells. *Ultras Med Biol.* 2009;35:1032–1041.
11. Kruglikov I, Sontag W. Ultrasound of 10MHz frequency as a novel strategy for skin anti-aging therapy. *Med Hypoth*. 2010;74:620–621.
13. Kruglikov I, Hoffmann K. [Nicht invasive Umfangreduzierung: Warum bleiben einige Fettablagerungen behandlungs-resistent?] *Kosmet Medizin*. 2011;3:108–113.
14. Kruglikov I. Biophysical basics of body treatments. Is hyaluronan a link that has gone unnoticed? *Am J Cosm Surg.* 2012;2:121–129.
15. Kruglikov I. Biophysical basics of body treatments: a preliminary study into the correlation between washout and circumference reduction. *Am J Cosmet Surg.* 2012;3: 196–202.
16. DeMeester SL, Buchman TG, Cobb JP. The heat shock paradox: does NF-kB determine cell fate? *FASEB J*. 2001;15:270–274.
17. Chen Y, Voegeli TS, Liu PP, et al. Heat shock paradox and a new role of heat shock proteins and their receptors as anti-inflammation targets. *Inflam Allergol - Drug Targets*. 2007;6: 91–100.