

Injectable Therapies for Localized Fat Loss: State of the Art

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KEYWORDS

- Phosphatidylcholine • Deoxycholate • Injection lipolysis
- Adipolysis • Lipodissolve

KEY POINTS

1. The US Food and Drug Administration (FDA) or any regulatory body worldwide has not approved injectable therapies to remove small quantities of fat.
2. Lipodissolve and mesotherapy are unapproved combinations of unregulated compounded medication associated with adverse events and controversy.
3. An adipolytic medication, sodium deoxycholate, is in registration trials for the reduction of submental fat.
4. A lipolytic medication, a combination of a beta agonist (solmeterol xinafoate) and a steroid (fluticasone propionate), is in registration trials for the reduction of abdominal fat.
5. The clinical applications of the (currently) unapproved medications presented in this review represent the authors experiences and a summary of the literature, not the outcome of clinical data generated through the ongoing pharmaceutical development of adipolytic or lipolytic formulations.

Since Rittes developed the procedure of injecting subcutaneous fat for localized reduction, a decade of erratic progress and setbacks in the use and understanding of injectable therapies for fat has passed. After being received with initial enthusiasm earlier in the decade, by 2007

mesotherapy and injectable methods for fat loss (termed most often as Lipodissolve, injection lipolysis, and injection adipolysis) were the subjects of critical scrutiny by the media and the US Food and Drug Administration. Despite the fact that the process of liposuction developed in much the same way (first tried on patients and then studied in the laboratory), the reputation of injectable fat loss therapies remains tarnished whereas, liposuction is the second most popular aesthetic procedure in the United States.

Liposuction removes fat by mechanical avulsion. The process has been enhanced by ultrasound, vibration, laser assistance, and radiofrequency heating. Nonsurgical fat reduction options include cryolipolysis, various types of external radiofrequency and ultrasound, low-level light therapy, and various injection methods. Although traditional mesotherapy remains unproven as a fat reducer, multiple researchers have confirmed the efficacy of injecting deoxycholate-based compounds, with recent focus on clarifying the exact mechanism of action as well as optimizing safety. The two most commonly used injectable formulas are phosphatidylcholine/deoxycholate (PC/DC) and deoxycholate (DC) alone. The use of additives or cocktail-type formulas, which defined traditional mesotherapy, has become less and less popular as proof of their lack of therapeutic efficacy and the presence of side effects has become well

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Disclosure: Dr Rotunda is the co-inventor of several patents that describe methods to reduce subcutaneous fat with deoxycholate.

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known. Therefore, the focus of this article rests primarily on injectable PC/DC and DC as methods to locally reduce fat.

Despite nonapproval by any regulatory body worldwide for the purpose of localized fat loss, practitioners have been using approved PC/DC combinations, such as Lipostabil (Aventis; Frankfurt, Germany) or Lipobean (Amipharm; Seoul, Korea), off-label for subcutaneous fat reduction in Europe and Korea, respectively. Clinicians using PC/DC combination or DC alone elsewhere, including the United States, obtain their medications from compounding pharmacies. Over the past decade, clinical practice has changed in that PC/DC or DC formulas have been replaced with more dilute solutions in an effort to increase safety and reduce side effects. Furthermore, although US users tend to treat small areas, such as the neck and jawline, lipomas, and bra rolls, other high-volume users, such as Korean physicians, treat larger areas, such as the abdomen, arms, and thighs. Low treatment costs and the availability of a standard, pharmaceutical-grade formula make injection lipolysis a popular fat reduction treatment in Korea, although the rest of the world has not seen a similar rise in popularity.

MECHANISM OF ACTION

Membrane Disintegration

The mechanism of adipocyte lysis following injection of phosphatidylcholine or deoxycholate has been the subject of debate for many years. Several aspects of the controversy persist.

The mechanics of adipocyte lysis, the lytic agent, and the role of phosphatidylcholine, if any, are still conjecture to many practitioners, despite publication of scientifically proven illustrations of mechanism of action over a 5-year period.^{1,2} Speculation spoken as truth has led many clinicians to think and propagate the notion that rather than fat cell lysis caused by disintegration of cell membrane by detergent (DC), the scientifically proven mechanism, that adipocyte lysis following PC/DC or DC injections is induced by apoptosis,³ stimulation of hormone sensitive lipase,⁴ or the beta-adrenergic-stimulated egress of glycerol and fatty acids,⁵ all unsubstantiated theories.

The process of oncosis reveals the mechanism of action of detergent substrates on fatty tissue.⁶ Oncosis differs from the term *necrosis* as the method of cell death in that necrosis describes what happens to cells only after death. The process of oncosis, a term revived by cellular pathologists in 1995, was originally described by von Recklinghausen in 1910. The term *oncosis* is based on the Greek word for swelling, *onkos*. This process is

usually seen after an anoxic event, which can be precipitated by an infarct or by a sudden cutoff of cellular oxygen. Clinically, the process is usually a regional one, localized to the affected tissue. It is characterized by sudden onset of profound cellular swelling and subsequent formation of blebs or mechanical insults in the cell wall, which cause an increase in membrane permeability. A sharp drop in regional pH is seen in the region caused by cellular oxygen deprivation. There is a subsequent shift to anaerobic glycogen metabolism. Glycolysis produces lactic acid, which in severely ischemic tissues is unable to be removed because of the lack of local circulation. If local circulation is restored at this point, cell death does not occur. If cellular respiration is not restored, lysosomes leak hydrolase into the favorable acid environment, causing further damage to the cell membrane. Irreversible cell destruction continues as the cell wall undergoes lysis. The detergent effect of sodium deoxycholate histologically creates the appearance of moth eaten holes in the adipocyte membranes⁷ immediately upon injection, perhaps promoting the lysosome hydrolase activity. Although the cells can repair small areas of membrane injury, larger areas of damage create an irreparable cascade of events leading to cell death.⁸

Subsequent loss of mitochondria function, with subsequent insufficient adenosine triphosphate reduces cell functioning, sodium-potassium ion pump activity, and further regional swelling. Cellular and soft-tissue swelling reduce local circulation, with closing pressure of venules leading to the no-reflow phenomenon, as is seen in tissue affected by the sudden failure of venous circulation in a free flaps.⁹ Profound localized swelling creates an opportunity for extensive fat necrosis and even overlying skin loss is at risk (see later discussion).

Apoptosis

Claims of an apoptotic mechanism of cell death following PC/DC injections began with Peckitt in 2006,³ who describes a complex caspase cascade. Apoptosis is an important means of regulating cell populations and is characterized by noninflammatory cell shrinkage followed by phagocytosis. Studies performed in Regensburg¹⁰ tested tissue injected with a phosphatidylcholine/deoxycholate formula for apoptotic markers, which were found to be present. The process of apoptosis, characterized by noninflammatory shrinkage of affected cells, does not clinically or histologically correlate with the tissue reaction generated by deoxycholate injection. Furthermore, as of this writing (Bechara FG, unpublished data, 2011) there is unpublished

experimental data to support a lytic, nonapoptotic mechanism of PC/DC-induced cell death.

Two types of cell death can be seen following a single subcutaneous injection of a toxic substance.¹¹ Histologically, the region that stains pink under a hematoxylin and eosin (H&E) preparation indicates death by oncosis. Karyolytic nuclei are another oncotic marker. At the periphery of the region of coagulation necrosis, along the margin of live and dead cells, the occasional histologic presence of half moon nuclei marking apoptotic cell death is observed. These cells are few, and are only noted at the edge of the much larger region of oncotic tissue. If an apoptotic index (ratio of counted oncolytic dead cells vs apoptotic dead cells per hundred dead cells) is counted, the index is quite low (0 to 3 cells per 100) depending entirely on the region counted. Apoptosis is, by definition, a nonmassive reaction. The only current reproducible method of generating large regions of apoptosis is the repeated freezing and thawing of regional tissue, as is seen in cryolipolysis.

Although causative factors may vary, oncosis is generally induced by situations producing anoxia; whereas, apoptosis is either programmed because of cell signaling or by thermal shock. Histologic evaluation of detergent injected fat can clearly define both. Along with swelling, oncosis is accompanied by the presence of inflammation, specifically a neutrophilic infiltrate, and a later migration of macrophages in to the region.

IDENTIFICATION OF LYTIC AGENT

Deoxycholate was initially isolated from PC in 2004 and identified as the predominant lytic agent in the PC/DC formulation.¹ Early literature supporting the role of DC as the lytic agent has been independently supported by a stem cell study performed by numerous studies.¹² Occasionally, publications persist the notions that phosphatidylcholine is the active agent in PC/DC treatments.^{13,14} The difficulty in identifying the true lytic agent is that phosphatidylcholine is not significantly water soluble and therefore isolation of PC in water-based cell lysis experimental models have been technically difficult to reproduce.^{15–22} This problem was solved by identifying an inert solvent that was used to isolate PC as a single agent.²³ The study performed at the McGowan research institute in Pittsburgh isolated PC, as well as other common constituents of Lipostabil and compounded PC/DC mixtures, to determine cytotoxicity and lipolytic activity of each constituent, using cultured adipocytes derived from stem cells. Cytotoxicity was calculated using lactate dehydrogenase and oil red O. Lipolytic activity (as opposed to cell lysis,

lipolysis maintains cell integrity) was measured using a glycerol and triglyceride assay. The measure of permanent destruction of adipocytes is important, as many lipolytic agents only cause temporary egress of glycerol and triglyceride, and therefore only temporary results can be achieved. **Table 1** shows the absence of any lytic activity by isolated PC and the results of the lysis assays. The only agent that causes adipolysis in a standard Lipostabil formula, or compounded PC/DC formulation, is sodium deoxycholate.

UTILITY OF DC ALONE VERSUS PC/DC

Phosphatidylcholine, a phospholipid comprising a significant percentage of mammalian cell membranes, lacks detergent or adipolytic activity, as previously discussed; it would be counterintuitive to think that a substance that comprises most of the biphospholipid structure of a cell membrane could induce membrane disintegration.

There is great variation in the degree of tissue response, the dispersion of adipocyte lysis, and the onset of cell reaction between PC/DC and DC formulations. Previous studies have demonstrated that subcutaneous injection of deoxycholate and PC/DC both produce localized inflammatory reactions, with DC (**Fig. 1**) appearing to produce more inflammation and cell lysis compared with PC/DC mixtures. Much if not all of these differences can be accounted for the fact that PC has an apparent buffering effect upon DC, thereby minimizing inflammation/tissue

Table 1
Qualitative levels of adipolysis after incubation with various agents

Test Solution	Adipocyte Cell Lysis Obtained with this Solution
PC50/DC42	++
Deoxycholate 1.0%	+++
Deoxycholate 2.4%	+++
Phosphatidylcholine 5.0% in mineral oil	0
Isuprel 0.08% injectable	0
Local anesthetic 5.0%	0
Saline 0.9% (control)	0
Benzyl alcohol	0

PC in isolation from DC does not cause adipolysis (fat cell lysis) or lipolysis (triglyceride breakdown). These data are the first to experimentally confirm prior deductions that PC will not reduce fat without DC.

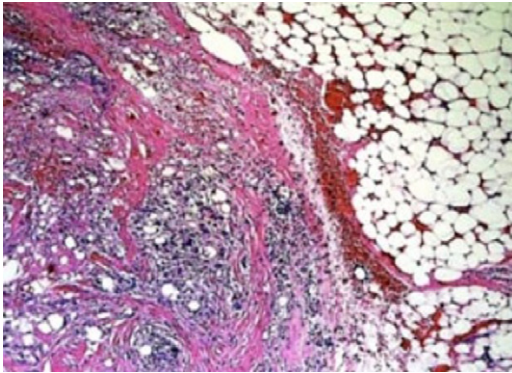


Fig. 1. Histologic findings of an excised lipoma 48 hours after subcutaneous infiltration with DC (1%), revealing a well-demarcated area of acute inflammation, extravasated erythrocytes, and necrosis adjacent to unaffected adipose tissue (hematoxylin and eosin, original magnification x10).

damage. This theory has been supported by recent experimental data, which demonstrates attenuation of DC LD₅₀ (the concentration of a substance at which 50% of cells die) by the addition of PC. Additional studies reveal that PC/DC combinations produce more dispersion (**Figs. 2, 3**) relative to DC alone. As increasing concentrations of deoxycholate are introduced into PC/DC combinations, the onset of adipocyte lysis is hastened.

Isolated deoxycholate as well as PC/DC combinations reduce localized fat, and when injected with correct technique, are safe and efficacious. Clinical indications should direct which one of these compounds should be used for each condition. When a small, localized fatty deposit is present and near total removal is the desired outcome, DC alone (at 1% or less) would be indicated. The best illustration of this would be treatment of submental fat (**Fig. 4**), bra strap fat, and lipomas. When a broader region is the target, some clinicians add PC thinking that it permits a more even dispersion of the solution. If a large, broad, or thick region of fat is the desired treatment region, some clinicians increase the PC/DC ratio to 1:<1 to minimize the PC-induced cholinergic side effects or significantly dilute PC/DC solutions. The thought that PC is inert, or perhaps even a buffer that inhibits the lytic



Fig. 2. Dispersion pattern, sodium deoxycholate 42 mg/mL at 10 minutes.



Fig. 3. Dispersion pattern, PC 50 mg/mL and DC 42 mg/mL at 10 minutes.

activity of DC motivates most clinicians to consider using DC formula without PC, at low concentrations.

TISSUE SPECIFICITY OF DEOXYCHOLATE

In cell cultures, a significant, dose-dependent nonspecific toxic effect of deoxycholate, as well as formulas containing PC/DC, has been reported (**Fig. 5**). Adipocytes, melanoma cells, skeletal muscle cells, keratinocytes, and fibroblasts are more or less uniformly destroyed with DC, although keratinocytes appear to be more susceptible to DC relative to the others.

In an effort to track the body's processing of injected DC radioisotope-labeled DC was injected into the fat pads of mice. Almost half of the DC injected was transported to the intestinal tract within 24 hours of injection into fat tissue. A peak accumulation in the small intestine was noted at 4 hours, and at 5 days, the remaining DC was eliminated in the feces.²¹

Several mechanisms are theorized to account for why DC injections (and similarly PC/DC) are not completely ablative to all surrounding tissue. It has been determined, using albumin in the experimental model, that protein is protective of adipocytes to the cell lytic effects of DC. Investigators demonstrated that albumin neutralizes or binds DC in nonadipose tissue, given the lack of tissue damage seen by endogenous DC (ie, present in the gut and circulatory system).²¹ Nevertheless, skin necrosis following direct dermal or superficial subcutaneous injections with PC/DC has been reported and is attributable to deoxycholate because no other active ingredients in the formula have been identified.

Another group of investigators tested the effect of intra-neural injections of 0.1 mL of Lipostabil into the left posterior tibial nerve of 10 rats.²⁴ Ten other rats were injected with a saline control in the same region. The rats were observed on a walking track for 21 days. No statistically significant clinical signs of nerve damage were noted. At 21 days the animals were sacrificed and the treated and control nerves were subjected to

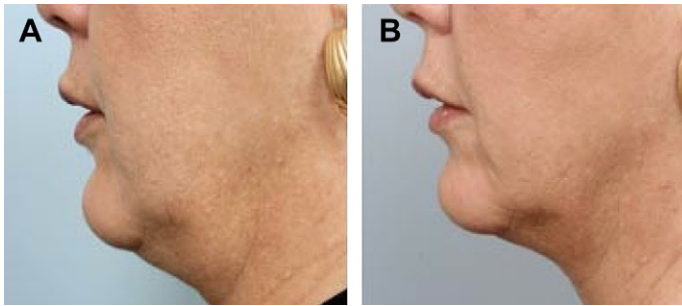


Fig. 4. Patient profile (A) before and (B) 2 months after 5 monthly injection sessions with 1.0 mL of DC (1%) into the submental fat.

histologic examination and also to electron microscopy. Grossly and histologically (including H&E as well as electron microscopy), the treated and nontreated nerves looked similar with surprisingly minimal inflammation.

One of the early studies of the detergent effects of sodium deoxycholate showed that in vitro, porcine fat and muscle was ablated by high-dose (5%) DC, as well as PC/DC (5.0%/2.5%) injection.¹ Further, Thuangtong and colleagues²¹ showed a cytotoxic response in cell culture by 4 different cell types to PC/DC. Jancke²⁴ also found that Lipostabil had dose-related cytolytic effects on adipose tissue, vascular smooth muscle cells, renal epithelial cells, and myocytes. Schuller-Petrovic²⁵ found that injection of subcutaneous fat in rats caused fibrosis in cutaneous muscle, with partial muscle loss. Changes were reported to be dose related. Cytotoxic necrosis was noted in both adipose tissue and in the walls of adjacent blood vessels. The visible histologic progression of events that occurs following detergent injections is well documented.^{26,27} Depending on the

agent used, gradually inflammation and subsequent cell membrane rupture, or immediate cell wall lysis (with deoxycholate) can be seen. The presence of foamy macrophages is noted with both DC and PC/DC.

The takeaway lesson from all studies should be that when injecting PC/DC or DC alone for the purpose of fat reduction, the injector should be keenly aware of the location of the tip of the needle. Only subcutaneous injections not adjacent to skin or muscle will be safely tolerated. As DC effects are clearly dose related, a lower dose (concentration and volume) of DC per injection site will reduce the risk of undesired sequelae.

CELL SIGNALING AND CYTOKINES

Not only is there signaling within the damaged adipocytes but also messages among cells. Bechara²⁸ has studied the influence of TNF- α and cytokines. Seven subjects with lipomas were treated with Lipostabil. Analysis of biopsy specimens noted upregulated levels of tumor necrosis factor (TNF)- α , as well as interleukin (IL)-4, IL-6, and IL-10, which are proinflammatory cytokines, in the early postinjection phase. TNF- α is cited as the mediator of the immediate and visible histamine response, noted as immediate and sometimes profound swelling, erythema, and regional warmth and discomfort. No increase in IL-2, IL-5, and eosinophilic granulocytes was noted. Occasionally, macrophages can become killer cells when appropriately signaled. The elevated TNF- α levels signal macrophages to bind to receptors on a target adipocyte, and will induce apoptosis. As macrophages are also involved in phagocytosis of necrotic cells, the presence of apoptotic fat cells along the periphery of the lipolytic reaction can be explained as TNF- α generated.²⁹

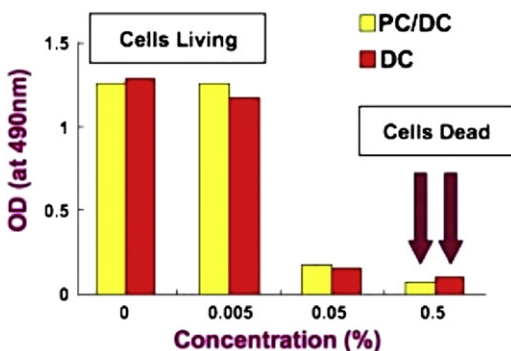


Fig. 5. MTS cell viability assay measuring living keratinocytes exposed to phosphatidylcholine/deoxycholate and deoxycholate. Absorbance (OD) is directly related to cell viability. Increasing concentration of either PC/DC or DC alone produces cell death. DC alone profoundly reduces cell viability, with PC producing minimal effect.

REGULATORY ISSUES

The development of new technologies is usually begun as an in vitro process in the laboratory,

followed by animal testing, and then by FDA submission and subsequent clinical trials. Although the time from research initiation to FDA approval is usually 3 to 5 years for devices (or less), depending on their regulatory path, drugs have a much longer (5–10+ years) and more costly testing and approval process. Although the FDA has approved many fillers as devices, review by the FDA³⁰ has clearly designated phosphatidylcholine and deoxycholate as drugs. According to the FDA a substance is considered a drug if the purpose of the substance is to change the structure or function of an animal or human.²⁹ The chemical, even though found in endogenous tissue, is also considered a drug if administered exogenously. According to Rittes, personal communications, August 2010, Sanofi Aventis, the manufacturers of Lipostabil in Brazil, declined to seek National Health Surveillance Agency (ANVISA, the Brazilian equivalent of the FDA) approval for use of their formula for reducing subcutaneous fat. In fact, the company issued a statement that it did not support the use of the drug for this purpose. Moreover, use of Lipostabil is off-label *only* in countries where Lipostabil is approved for other indications (ie, for dyslipidemia, angina). Any combination of PC with DC or DC alone is *not off-label* in the United States, because these have not been approved for any other indication.

Currently, almost all of PC/DC, the most popular injected formula worldwide, is being compounded in the United States and, in select European countries, compounded or used in the form of Lipostabil, the pharmaceutical medication. However, according to its package insert, the manufacturer does not support the use of subcutaneous injections of Lipostabil.³¹ Because of the extremely high cost of new drug development and lack of patent protection, existing phosphatidylcholine/deoxycholate combinations have not found a pharmaceutical developer.

KYTHERA Biopharmaceuticals (Calabasas, California), is currently seeking approval for a deoxycholate-based fat-reducing formula for submental fat; as of this writing, they are entering phase III (the last phase of clinical drug development) outside the United States and entering phase IIb (dose and tolerability testing) in the United States. KYTHERA Biopharmaceutical is seeking regulatory approval in the United States and worldwide for a low-dose (1% or less DC) formulation called ATX-101. This strategy is consistent with the experiences of experienced injectors, which suggests that low-dose formulations of PC/DC or DC alone is a prudent approach to yield gratifying results while minimizing risks. The company has sublicensed the sale and marketing of ATX-101 to Intendis (Berlin,

Germany, a subsidiary of Bayer HealthCare) outside the United States. As previously noted, the results of registration studies will ultimately determine the fate of ATX-101.

Lithera, Inc (San Diego, CA, USA) is developing a novel injectable combination of a steroid plus a beta-adrenergic agent, also for fat localized (see details later). Completion of rigorous clinical testing and regulatory approval will determine if any of these companies are successful at marketing their products and making it into clinics throughout the world.

LIPOLYSIS

LIPO-102 is a novel injectable treatment created by Lithera, Inc and currently in registration trials to achieve local, selective fat-tissue reduction (termed according to the company “injection lipoplasty”).³² Using FDA-registered drugs proven safe and effective in other indications, LIPO-102 targets and stimulates natural fat metabolism to produce nonablative, nonsurgical fat-tissue reduction in specific locations. In clinical testing, using sophisticated imaging technology (Canfield Vectra analysis) weekly injections of LIPO-102 for 4 to 8 weeks produce significant reductions in abdominal circumference and volume.

LIPO-102 is a combination of salmeterol xinafoate and fluticasone propionate. Salmeterol xinafoate is a highly selective, long-acting β_2 -adrenergic receptor agonist. Fluticasone propionate is a synthetic trifluorinated glucocorticoid. The two drug products currently under development are formulated as either sterile solutions or as separate lyophiles. The single-use, sterile, preservative-free solutions will be mixed and diluted with saline if necessary, to form LIPO-102.

Salmeterol xinafoate is a highly selective, long-acting β_2 -adrenergic receptor agonist. Adrenergic receptors play a major role in the regulation of several processes in the body, including fat-cell metabolism. Activation of β_2 -adrenergic receptors located on human fat cells by salmeterol triggers the breakdown of triglycerides in these cells to free fatty acids and glycerol by means of lipolysis (no adipolysis, as the fat cells remain intact). Fluticasone propionate is a synthetic trifluorinated glucocorticoid with potent antiinflammatory activity. Glucocorticoids, such as fluticasone, have an important permissive effect on β -adrenergic receptor function in vivo. Glucocorticoids enhance β -adrenergic receptor-mediated responses by regulating the coupling of β -adrenergic receptors to G proteins and the resulting activation of adenylate cyclase and by preventing downregulation of β -adrenergic receptors caused by chronic receptor stimulation

(eg, by salmeterol). In simpler terms, salmeterol stimulates lipolysis through activation of β_2 -adrenergic receptors on fat cells and fluticasone upregulates the cellular machinery/pathways turned on by salmeterol.

OTHER PC/DC FORMULATIONS IN USE WORLDWIDE

Amipharm's (Seoul, Korea) PC/DC formulation, Lipobean, is approved by the Korean FDA for use in treating hepatic coma, yet commonly used off-label in the country for localized fat reduction.³³ The brand name is based upon the source of phosphatidylcholine, the soybean. The formulation consists of PC 50 mg/mL and DC 24 mg/mL, similar to Lipostabil available in the United Kingdom. Standard practice in Korea is to dilute each 5 mL vial with 5 mL of normal saline, thus achieving a concentration of PC 25/DC 12. The solution is well tolerated, as swelling and postinjection discomfort is not as profound as with PC 50/DC 42, the United States standard compounding pharmacy ratio (with higher DC).

Charlatan use of any drug can cause widespread problems resulting in a bad reputation of an otherwise effective treatment. As long as there is unregulated availability of injectable lipolytics online, this will be a continuing problem and will cause damage to the reputation of any effective formulation. A recent report notes complications following self-injected Lipostabil,³⁴ which is easily available to any person wishing to purchase it online.^{35–37} The target audience for many of these websites is bodybuilders who cannot achieve definition in diet-resistant and exercise-resistant areas. With no medical evaluation or supervised treatment, as well as in some cases, self-treatment by patients alone, abuse of these medication may continue.

REGIONAL GLOBAL EXPERIENCE

The routine use of targeted fat-reducing injections varies throughout the world. Viewpoints vary widely from country to country. The procedure remains banned in Brazil³⁸ and is forbidden in Turkey because of patient complications following charlatan use.³⁹ In Europe, injection lipolysis remains popular in Austria as well as in Germany,⁴⁰ and is quietly practiced in the United Kingdom.⁴¹ According to Dr Mark Palmer, a UK physician, only German-produced Lipostabil is approved, but for the purposes of medical, intravenous use only. Although possible to use in an off-label manner, there are significant risks and a degree of medical-legal complexity in the importation and use of this product for aesthetic purposes.

Asian countries see much more use as Asian patients try to avoid a surgical approach if at all possible. The popularity of the procedure has declined dramatically in the United States after the collapse of several chains of providers in 2007 and 2008, but according to the Network Lipolysis, more than 12,000 treatments were performed in 2009.²³

In Korea, injection adipolysis is reported to be more popular than Botox injections, although this is anecdotal. Amipharm sells approximately 115,000 vials of Lipobean per month, and this number has seen a dramatic recent increase despite the current recession or perhaps because of it. Korean practitioners attribute the popularity of injection adipolysis to its cost effectiveness, efficacy, and the Asian ethnic antipathy toward surgical incisions of any kind. There is a high cultural acceptance of cosmetic procedures in Korea, but the results must look natural and not extreme. Their product is approved by the Korean FDA for use in treating hepatic coma, so the use of PC/DC is therefore considered off-label.

Amipharm's largest customer is an obesity clinic that routinely administers these injections. After dilution of their 5.0% PC/2.4% DC formulation with 5 mL saline, the final injected ratio of PC/DC ratio is 2.5% PC/ 1.2% DC. Compounded medication is illegal in Korea, so the practice of PC/DC is limited to the use of Lipobean. According to the largest Korean obesity clinic chain, 365 MC, the most common treatment region is the abdomen. There is a marked difference in body mass index of the average Korean patient versus the average American patient. In most Koreans, the surface area and depth of fat pad is small, and therefore is more amenable to treatment with PC/DC. Patients at the obesity clinic report high satisfaction with treatment in the abdominal region, arms, thighs, and also the submental chin.

CLINICAL PRACTICE

The preface to this section is that there is no absolute standard of injection for injectable fat-reducing methods. The authors' guidelines are based on their experience only, not from registration study guidelines, which ultimately are the most appropriate guidelines to follow, should any of these medications be approved. Furthermore, the authors' experiences are based on medications legally available through compounding pharmacies. The authors suggest that clinicians interested in using fat reducing medications strongly consider waiting until an FDA approved pharmaceutical medication becomes available. State laws and local practices determine the indemnity risks

associated with using compounding medications and 'standard of care,' respectively.

The best indication for treatment of a region with injection lipolysis or adipolysis is reduction of small, localized, regions of soft, not fibrous fat. Although broader regions of fat can be treated, results are not as dramatic after a single treatment. Multiple treatments of larger areas are often necessary. It is optimal to use a more dilute formulation in larger areas, both to reduce side effects and to minimize post-treatment swelling.

Experience from both authors has been generally positive. One author (DD) has performed several thousand patient treatments since 2004, and has observed it to be an excellent noninvasive treatment for the reduction of small focal areas of subcutaneous fat, especially associated with moderate skin tightening. It is particularly useful for facial contouring and volume reduction, especially for jowl reduction and jawline contouring. This procedure can have a hugely rejuvenating effect on patients' aging face and can delay or prevent the need for a more invasive intervention. The other author (AMR) has been using PC/DC and DC formulations since 2003, primarily in clinical trial and experimental laboratory settings, but has similarly found its benefits along the jawline and submental region gratifying.

Dr Duncan has used normal saline as the diluents, with the dilution with a dilution of PC 25 mg/mL, DC 12 mg/mL for the past 4 years; this ratio has minimized discomfort and produced a tolerable procedure. Similarly, Dr Rotunda uses a dilute formulation, approximately 0.75% DC (formulated with 3:1 ratio of 1% DC and 1% lidocaine without epinephrine). Most patients require 2 or 3 treatments for facial contouring. These formulations are particularly effective for defining the mandibular angle, jawline, and reducing submental fat. The results appear to be permanent and it is also highly useful for the reduction of small, localized subcutaneous areas of fat and volume in other body areas, including the chin, neck, inner knees, and inner and outer thighs where other more invasive treatment options may be unsuitable or avoidable. A volume of 0.5 mL (PC/DC) should be injected subcutaneously at a spacing of approximately 1.5-cm intervals into areas needing volume reduction or 0.2 mL per 1-cm space for the DC-only formulation. Areas where patients would not benefit from volume reduction or that need volume augmentation should be carefully avoided. A reduced volume of 0.2 to 0.3 mL (PC/DC) per injection should be used in areas close to areas of insufficient volume, such as nasolabial mounds lateral to the nasolabial fold, to avoid spread into the valley of the fold

itself. With all adipolytic treatments, success outcome depends on success recovery; Dr Duncan treats every 8 weeks, Dr Rotunda treats every 4 to 6 weeks. For patients receiving jowls and marionette treatment, the wait period can be at least 10 weeks because overcorrection in these areas is difficult to repair.

In the upper face, small volumes of 0.05 to 0.1 mL per injection can be used, injected just subcutaneously, to successfully treat redundancy and skin laxity the malar areas, although these areas are prone to significant swelling, therefore the physician should proceed with caution and patients should be advised to expect that multiple treatments will be needed to obtain successful results.

In more than 6 to 7 years of clinical practice of these techniques and many thousands of patient treatments, the authors have not seen any adverse effect other than the expected temporary minor swelling and focal tenderness; therefore, it is one of the safest cosmetic medical interventions that the authors provide to their patients.

Basic Technique

Depth of injection is extremely important. The effect of these injections is a local one; the solution only diffuses in a 1- to 2-cm radius, depending on the volume and dose of the injection. Lower-face and neck injections are generally performed using a 6-mm depth meso needle and 13-mm BD needle (BD, Franklin Lakes, NJ, USA) injected halfway. It is not wise to use a longer needle as multiple injections are needed, and guesstimates of needle depth are often incorrect. One author (AR) uses a 30-gauge needle, although the other author (DD) prefers a 27-gauge needle, with the thought that high resistance to injection is encountered and therefore difficulty in determining whether dermis or fat is being injected. It is best to pinch the skin thereby lifting the fat from the deeper connective tissue, and thereafter inject the solution into the pinched tissue (pinch and prick).

The volume of each injection should be 0.1 to 0.6 mL to obtain the best dispersion with the fewest skip areas. Larger volumes have been shown to track up to the skin or deeper toward fascia. Smaller volumes are recommended as treatment regions become more superficial. The distance between injections is not recommended to exceed 1.5 cm. If injection sites are about 1 in, or 2.5 cm apart, skip areas are noted and the result can be lumpy.

COMPLICATIONS AND ADVERSE EVENTS

The rate of reported complications has dramatically decreased since the wane in popularity of

Lipodissolve. Safety issues persist, however, because of the lack of understanding of the physiologic basis of action of the injections, upper limits of dose (volume and concentration), lack of standardized formulation and pharmaceutical-grade preparations. Swelling can be profound and is an anticipated, unavoidable side effect. The anoxia caused by swelling is a trigger for oncotic induction of cell death. Cells not immediately killed by a direct chemical effect are affected by local swelling and external pressure on adipocyte circulation. Extracellular fluid accumulates and causes a jellylike consistency of treated tissue. Regions that already have poor circulation, such as distal extremities, and regions that have undergone previous surgery are at high risk for skin loss or extreme fat necrosis. Localized areas that have had aggressive liposuction may not be able to swell because of fibrosis of the subcutaneous compartment. In fact, a compartment syndrome can occur if swelling is prevented. Cases of skin loss and draining of liquefied necrotic fat have been reported in instances of previous inner-thigh liposuction, wearing of tight compression garments post-treatment, and even by patients wearing tight jeans following injection. Because of the marked temporary swelling deformity, many patients are reluctant to undergo more than 1 injection session, and may request that a large surface area or multiple regions be treated at one time. Published guidelines recommend limiting treatment to a region no larger than 12×15 cm if bilateral. Clinicians should not inject multiple regions (ie, abdomen, flanks, hips), unless quite small (ie, chin and jowls). The total dose of PC should not exceed 2500 mg, and further limiting this dose will reduce postinjection cholinergic side effects (nausea, vomiting, diarrhea), which can be accomplished by diluting the injected solution by as much as 50%. Further, dilution may markedly affect the efficacy of treatments. One author (AR) uses DC only and has not had patients experience cholinergic side effects; in fact, AR treats submental fat, jowls, and lipomas only.

Avoiding postinjection compression garments will decrease the risk of focal skin loss. Other more common causes of skin necrosis include intradermal or immediate subdermal injection, injecting the same site twice, and injection with a Mesorelle-type multiple needle device. Not commonly available in the United States, Mesorelle (BMA Biomedica, Italy) injection devices are widely used in Europe and Asia. The 3- to 5-needle attachment to a syringe theoretically reduces the number of times that patients feel a needle, and

also reduces the number of times the physician makes an injection. However, safety of injections is greatly dependent on maintaining a uniform depth of injection. When tissue is injected, a curved surface rather than a flat surface is usually present. Therefore, the central tissue is injected at a deeper level than the peripheral tissue. A case report of focal skin loss in a male flank resulting from a Mesorelle-aided injection attests to the wisdom of avoiding their use.

A specific needle length is advised when performing injections. It is almost impossible to insert a long needle only part way into the tissue at a consistent depth. One author (DD) prefers 6-mm, 27-gauge meso needles for the face; whereas, 10-mm and 13-mm needles can be used when treating other body parts. Another author (AR) uses 13-mm BD, 30 gauge uniformly on the face and neck. It is unwise to use a needle too short (4 mm), or even a 13-mm needle in patients with thinner fat deposits. Again, the pinch-and-prick method (gently pinching the skin and injecting the pinched tissue) is recommended. Case reports of intramuscular injection with resultant fibrotic nodules and restriction of range of motion have been attributed to the use of a 13-mm needle for injections in a thin woman's thighs. A 4-mm needle has been reported to cause focal skin loss in a patient who received multiple superficial injections by a practitioner of mesotherapy.

Volume of injections also influences the safety profile. Dispersion studies show pooling and migration of fluid volumes more than 0.6 mL when injections are spaced 1.5 cm apart. On the other hand, injecting 1 mL of solution 2.5 cm apart is not encouraged, as a large volume of solution can migrate down to fascia, collect as a firm persistent subcutaneous nodule, or if placed too superficial, into the dermis. If the distance between injections is too great, skip areas are seen, and contour irregularities may result.

Other complications include hyperpigmentation, persistent nodules of focal fat necrosis, contour irregularities, and less-than-hoped-for fat reduction. Hyperpigmentation has been attributed to hemosiderin deposition, and is temporary in many cases. However, permanent discoloration of the treatment region can occur, and it typically does not respond to hydroquinone or kojic acid. Neovascularization of treatment regions can also occur, especially in areas with circulatory compromise. The formation of blood vessels under the skin of a treatment region does respond to intense pulsed light but damage to a previously unblemished skin surface generates significant unhappiness in these patients.

CONTRAINDICATIONS

No children under 18 years of age are treated. Patients who are breastfeeding are asked to wait to be treated for 6 weeks after they cease lactating. This procedure is not meant to be a rapid weight-loss program. Therefore, patients who are obese are not treated. No treatments should be performed for the purpose of breast reduction. Clearly, an allergy to soy products or other formula components, such as benzyl alcohol, is a contraindication to treatment with the injections when PC is incorporated in the formula. If in doubt, a small patch test can be done 1 week before the anticipated treatment. Most injectors do not treat patients who are diabetic, especially in the distal extremities. Microangiopathy and vascular insufficiency are also contraindications. Although Lipostabil is licensed in Germany for intravenous use in cases of coronary artery compromise, many physicians avoid treating patients who are hypertensive and cardiac. Patients with severe chronic illness should not be injected, especially those who are immunocompromised. It is not wise to treat patients with autoimmune disease, especially those with scleroderma. Two exceptions to this guideline are patients with Hashimoto's thyroiditis and rheumatoid arthritis. Many of these patients have been treated with good results and few post-injection problems. Although some physicians avoid treating patients who are HIV positive, many other patients get adequate results injecting the buffalo hump that frequently occurs with HIV medication. The majority of physicians avoid injecting patients on blood thinners, such as warfarin sodium (Coumadin) or clopidogrel bisulfate (Plavix). Although some avoid treating patients taking nonsteroidal antiinflammatory drugs, most do not see their use as an absolute contraindication. Patients on chemotherapy should not be treated until their immune system has recovered. Those on prednisone or another steroid regimen should also defer treatment with this technique. Local skin conditions may preclude treatments with

any cutaneous injections. An ulcer or infection near the treatment region should negate treatment. Many respondents do not treat patients with active eczema or psoriasis.

Unrealistic expectations are also a relative contraindication to treatment. This treatment results in subtle improvement, not total elimination, of the localized fatty accumulation. An average of about 5% of patients do not respond to treatment, although with careful patient selection and proper dose, spacing, and injection depth, the great majority of treated patients will have some visible improvement. If patients are noncompliant or show up for only 1 treatment, they will not be satisfied with the outcome. Also, those patients who will not adhere to a diet and exercise plan and see the injections as a quick fix will be poor candidates for this treatment.

NEW USES AND CASE EXAMPLE

The new clinical use that has developed in the injectable fat loss arena is that of a redundant tissue and contour problem solver. When combined with collagenase, PC/DC injections or DC alone can be used to reduce contour irregularities following liposuction, and to reduce protrusions and adjacent depressed scars (**Fig. 6**).

Correction of a contour irregularity with these nonsurgical formulations can frequently produce a better result than revision surgery can (**Fig. 7**). By injecting the scar itself at multiple levels with collagenase 250 units/mL (Masterpharm, Richmond Hills, NY, USA), the tethering effect of the scar upon the surrounding tissue is reduced. Collagenase is enzymatically specific for collagen only; it has no effect on adipose tissue. The adjacent protrusions of fat are injected with PC/DC or DC, thereby serving as a combination technique. Tapering doses at the periphery of the protuberance will improve results; larger protrusions are injected with slightly more of the adipolytic agent, and minor protrusions with less. Some visible

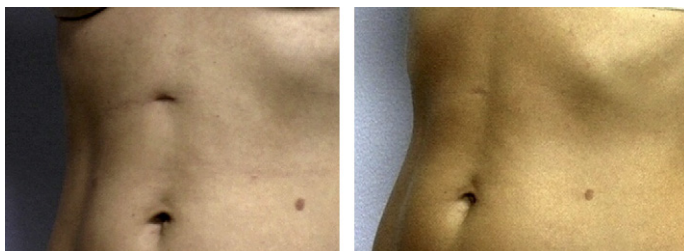


Fig. 6. (Left) Patient following liposuction with residual depressed laparoscopic cholecystectomy scar. (Right) Six weeks following injection at base of scar with 0.2 mL collagenase and PC/DC.



Fig. 7. A 53-year-old woman with dog bite scar along her right marionette line, with ptotic marionette fold. Improvement at 2 weeks following injection with 0.2 mL PC/DC centrally and 0.3 mL collagenase at base of scar. Ulthera microfocused ultrasound the same day.

effects can be seen as early as 3 days because of the rapidity with which collagenase works.

Another excellent use for adipolysis is nonsurgical contouring of small regions in combination with other treatments, such as microfocused-fractionated ultrasound (**Fig. 8**). The combination treatment resulted in good correction of the volume excess and redundancy in the lower face and neck region.

A third potential niche indication for injection adipolysis is focal skin tightening, although there is some unpublished data demonstrating DC alone as having minimal effect on submental skin laxity. An example of potential use for this treatment is residual periumbilical laxity following abdominoplasty. Many women who have had children have

the most extreme stretch and subsequent skin damage in the midline periumbilical region. This problem can be treated as a standalone process, or it can be combined with abdominoplasty. If combined, it is best to wait at least 3 months after abdominoplasty to allow most of the swelling resolve. A small amount of fat must be present underneath the lax skin in order for this process to be effective. The following case illustrates this point.

A 37-year-old woman underwent an abdominoplasty with initial good results. She returned 8 months postoperatively complaining of loose skin in the supraumbilical region and some periumbilical edema, a problem that is not uncommon, as many patients with postpartum deformities have striae



Fig. 8. A 38-year-old patient with heavy neck and a ptotic face beset by gravity and heredity. Eight weeks following combination treatment of adipolysis with PC/DC followed by Ulthera (Mesa, Arizona) microfocused ultrasound in the neck and lower face.

and damaged skin the periumbilical region. Women tend to develop a guitar-shaped region of lipodystrophy during and after childbearing. Unless liposuction accompanies the abdominoplasty, many patients are likely to have a mild postabdominoplasty residual of central lipodystrophy and loose, sometimes pendulous skin. Dermolipectomy and liposuction will not correct damaged skin that exists preoperatively. Even if patients understand this, patient may still express this postoperatively. It is difficult to surgically improve this situation, as a purse-string type suture around the umbilicus does not look aesthetically attractive. Excision of skin above the umbilicus can be done, but unless the problem is minimal, residual laxity or a vertical scar may result. A nonsurgical solution to this problem can be achieved with injection adipolysis. After the treatment region is delineated, a grid marking injection sites 1 cm apart is drawn. Injection depth is superficial, about 6 mm. Injection volume per site is 0.2 mL of PC 50/DC 42 is used. The region will profoundly swell immediately after injection, and may take 8 to 10 weeks for the early improvement to be seen. Care must be taken during the injection process to make sure the needle tip is in the subcutaneous layer and is not intradermal because skin loss can occur.

SUMMARY

The use of injectable fat-reducing solutions is evolving as we learn more about the mechanisms of action and more about the risks and limitations of treatment. When the process was first created, there were no apparent boundaries or limitations. Both laboratory research and clinical research and experience have contributed greatly to the compendium of knowledge regarding direct injectable treatment of fatty deposits.

Because of the initial lack of approved formulations in many countries, unscientific cocktail compounds were used, causing adverse sequelae and some serious regional complications. As the field of knowledge widens, and speculation is replaced with science, the industry is expected to grow. Proper research and development has led to safe and effective formulations that are emerging. After the sudden rise of popularity of Lipodissolve was followed by an equally rapid demise, a secondary slower growth phase is expected as lipolytic and adipolytic compounds are carried through the FDA regulatory process.

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