Tissue-Selective Effects of Injected Deoxycholate

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BACKGROUND   Recent studies suggest that the principal active ingredient in phosphatidylcholine-containing injectable fat-reduction formulations is actually deoxycholate (DC). This bile acid acts as a detergent to rapidly disrupt cell membranes. Thus, it is not obvious why DC would preferentially target fat.

OBJECTIVE   To investigate possible mechanisms for the selectivity of DC for fat tissue using in vivo and in vitro models.

METHODS AND MATERIALS   Histology, drug distribution studies, and cell viability assays were used to examine possible mechanisms contributing to DC selectivity.

RESULTS   In vitro, DC caused the lysis of all cell types tested within the tested concentration range. DC injected into fat tissue caused adipocyte death, whereas other cell types appeared less affected. Physiological concentrations of albumin or protein-rich tissues decrease the ability of DC to lyse cells. Furthermore, DC relocated to the gastrointestinal tract in animals within hours of injection. This suggests that similar mechanisms may be present in humans.

CONCLUSION   We report observations that provide a possible explanation for the in vivo preferential fat targeting by DC. Fat tissue, being deficient in cell-associated proteins and interstitial albumin, may be unable to sufficiently neutralize the detergent activity of DC, possibly making fat uniquely sensitive to DC.

Phosphatidylcholine (PC) injections have become an increasingly used modality for removal of excess fat.[1] This procedure is marketed under many names (e.g., Lipostabil, Lipodissolve, [Sanofi-aventis, Bridgewater, NJ], mesotherapy, injection lipolysis), but all of these variations involve patients receiving dozens of emulsified phosphatidylcholine injections over several sessions, with the desired end result being reduction of localized fat.

PC is an endogenous phospholipid that can be injected intravenously for the treatment of posttraumatic intra-arterial fat emboli.[2] Based on the assumption that PC could solubilize triglycerides within living adipocytes in the same way it solubilizes intravascular fat emboli, clinicians began injecting cosmetically undesirable subcutaneous fat tissue with PC-containing solutions developed for intravenous use.[1,3] Rittes first reported improvements in the appearance of lower eyelid fat pads after injecting a PC-containing solution directly into these fat pads in 30 patients.[4] After this initial report, several subsequent publications reported that PC injections produced aesthetic improvements by reducing subcutaneous fat.[5] These studies described variations of a technique in which a PC-containing solution was periodically injected directly into subcutaneous fat pads over several months.[3,6–8] These treatments were reported to remove subcutaneous fat (as inferred from clinical photographic images) and to induce fat cell necrosis (as observed in histological sections harvested from injection sites),[9] although no injectable formulation has received

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approval from any regulatory agency for cosmetic fat reduction.

Recent observations from our laboratory[10] and others[7,11] suggest that the active component in PC-containing fat reduction formulations is not PC but rather sodium deoxycholate (DC), the bile salt invariably included in these formulations to emulsify the otherwise self-aggregating PC molecules. Lipomas injected with DC or the combination of PC and DC showed rapid damage to adipocytes consistent with DC-mediated membrane destabilization.[12–14] Moreover, in a head-to-head clinical trial, DC alone produced a similar reduction in subcutaneous fat pad volume as did a conventional PC/DC formulation,[15] suggesting that PC did not contribute to clinical efficacy.

DC is an endogenous bile acid detergent that emulsifies dietary lipids in the human gastrointestinal tract.[16] Although DC is predominantly associated with proteins or lipids in vivo,[17] cultured cells exposed to high in vitro concentrations of DC (>2 mM) die rapidly through membrane disruption.[10] Because DC destroys cells through a lytic mechanism, it seems unlikely that DC would be cell-type specific and that it should therefore also destroy non-fat tissue near the injection site. If so, it is not directly apparent why DC injections are clinically well tolerated or why the drug should preferentially reduce adipose tissue while sparing non-fat tissues such as muscle and skin.

We report observations that provide an explanation as to why injection of DC into fat deposits is clinically well tolerated, despite DC being a detergent that lyses cells. Our findings suggest specific mechanisms that spatially limit DC-mediated cell lysis to the injection site within fat tissue and may also explain the differential susceptibility of body tissues to DC-mediated injury. First, we report that physiological concentrations of albumin or protein-rich tissues (e.g., muscle or skin) inactivate DC-mediated cell lysis. Because fat is protein poorer than most body tissues, fat tissue may be highly sensitive to DC-mediated injury. Second, we demonstrate that, after subcutaneous injection, DC is transported to the gastrointestinal tract, preventing accumulation at the injection site.

Our findings are consistent with a model in which DC, injected into fat, is rapidly neutralized through interactions with albumin or with insoluble, tissue-associated proteins. DC bound to insoluble proteins may be transferred to albumin, driven by the high affinity of albumin for DC[17] and the high concentration of albumin in plasma. Albumin-DC complexes then enter systemic circulation and migrate to the liver, where bile acid transporters on the surface of liver cells remove DC from circulation. The extracted DC then joins the endogenous bile acid pool. We suggest that the ability of albumin to neutralize and transport DC confers features that may make this detergent biocompatible.

Materials and Methods

Histology

Genetically obese mice (ob/ob) ($n = 4$) were injected with 200 μL of 1% DC or vehicle into the left gluteal fat pad on days 1, 3, and 5 and sacrificed 20 days after the first injection. Lean mice received a single injection of 100 μL of 0.5% DC or vehicle into the subcutaneous fat compartment in their tail and were sacrificed 20 days later, at which time tissue at the injection site was harvested. Samples were fixed in formalin and 4-mm punch biopsies were taken. Samples were then prepared for staining with hematoxylin-eosin or osmium tetroxide. Hematoxylin-eosin staining was performed by paraffin embedding of the formalin-fixed tissue followed by hematoxylin-eosin staining. Samples for osmium tetroxide staining were fixed in formalin as described and then placed in a 1% osmium tetroxide solution followed by dehydration in graded ethanol solutions (75%, 95%, 100%). The specimens were then transferred into propylene oxide and then infiltrated with a 1:1 solution of propylene oxide and Epon resin (Polysciences, Inc., Warrington, PA). Specimens were

then infiltrated with 100% Epon. Smaller sections were cut and counter-stained with toluidine blue.

Cell Culture

Ninety-six-well plates containing primary human subcutaneous adipocytes and human dermal fibroblasts were obtained from Zen-Bio, Inc. (Research Triangle Park, NC). Human epidermal keratinocytes were obtained from Cell Applications, Inc. (San Diego, CA). Cells were incubated for 24 hours after arrival at 37°C in the presence of 5% carbon dioxide (CO_2). Human skeletal muscle cells were also obtained from Cell Applications and incubated in differentiation media (Cell Applications) for 5 days after arrival at 37°C in the presence of 5% CO_2.

In Vitro Cell Lysis by DC

Ninety-six-well plates of cells were washed twice at room temperature with phosphate buffered saline (PBS) and then incubated in various concentrations of DC (0%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.06%, and 0.10%) obtained from Sigma-Aldrich (St. Louis, MO) in serum-free RPMI medium obtained from Mediatech, Inc. (Manassas, VA). After a 1-hour incubation at 37°C, the medium was removed, and 100 μL of CellTiter 96 AQ$_{ueous}$ 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymehtoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay reagent obtained from Promega Corporation (Madison, WI) was diluted 1:25 in RPMI and added to each well. Each plate was incubated at 37°C with 5% CO_2 until an orange color developed in control wells without DC, indicating 100% cell survival. The time required for color development varied according to cell type. Cell viability was determined by measuring the optical density (OD) at 490 nm (OD_{490}) using a UVMax Kinetic Microplate Reader (Molecular Devices, Sunnyvale, CA).

Albumin Incubation

Primary human adipocytes were maintained as previously described in 96-well plates. Stock solutions (2 ×) of 8%, 2.6%, and 1.4% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO) were prepared in RPMI. Stock solutions (2 ×) of DC were also prepared at concentrations of 0.20%, 0.12%, 0.08%, 0.06%, 0.04%, 0.02% and 0.01%. Medium was removed from plates, and the cells were washed with 1 × PBS. A fixed volume (40 μL) of the BSA stock solutions was added to the corresponding well in a 96-well plate. An equal volume of the DC stock solutions was then added to each well such that the final concentrations of BSA and DC were 1 × in each well. Cells were incubated at 37°C in the presence of 5% CO_2 for 45 minutes and then treated with MTS reagent as previously described to determine OD_{490}.

Tissue Incubation with DC

Cells were maintained at 37°C in an incubator containing 5% CO_2. Primary human adipocytes were cultured for a week before the performance of in vitro assays. A375M cells (an immortalized human melanoma cell line provided by R. O. Hynes, Massachusetts Institute of Technology, Cambridge, MA) were maintained in Dulbecco's modified Eagle medium (DMEM, Mediatech, Manassas, VA) with glucose, L-glutamine, without sodium pyruvate with 10% fetal bovine serum (Omega Scientific, Tarzana, CA), and 0.5 mg/ml of Normocine (InvivoGen, San Diego, CA). A solution of 1% DC was prepared in DMEM (10% serum) and diluted 10 times to obtain 0.1% DC (in DMEM and 1% serum). Porcine tissue taken from a pig sacrificed less than 24 hours previously was dissected to yield 50-mg tissue samples derived from specific tissue types (fat, muscle, and skin). Skin samples were de-epithelialized. Increasing numbers of these samples (1–4) were added to 1.5-mL microcentrifuge tubes containing 1 mL of 0.1% DC such that the 1 mL of 0.1% DC was exposed to 50, 100, 150, or 200 mg of each tissue type. The samples were gently mixed for 1 hour in a rotary mixer at 37°C and then spin-filtered to remove insoluble components. Samples of each filtrate (200 μL) were then tested for cytotoxicity against adipocytes, as described above.

Cell lysis by DC was quantitatively measured as follows. Cells were grown to confluence under the conditions listed previously. Growth medium on cell plates was aspirated completely and replaced with the 0.1% DC solutions that had been pre-incubated with various amounts of the porcine tissues and incubated for 30 minutes at 37°C sealed under parafilm. After incubation, DC solutions were aspirated, and 100 μL of 1:25 PBS-diluted MTS assay reagent was added to each well. The plate was resealed with parafilm and incubated at 37°C for 120 minutes. Cell viability was determined by obtaining the OD$_{490}$.

Radio-Labeled Drug Distribution

Twenty-one Sprague-Dawley rats weighing 250 to 300 g were treated with [^{3}H]-DC, a sodium salt, in ethanol (American Radio-labeled Chemicals, Inc., St. Louis, MO). On the day of DC administration, 2.25 mL of a 1-μC$_i$/mL solution of [^{3}H-]-DC was transferred into a vial and dried under a stream of nitrogen. Non-radioactive DC (149.8 mg) and sterile water for injection were added to the vial such that the final volume of the dose solution was 15 mL. The contents of the vial were mixed and then sterile-filtered using a 0.22-μm mixed cellulose filter (Whatman, Florham Park, NJ). A final concentration of 1% [^{3}H]-DC ($\sim$150 μC$_i$/mL) at a dose volume of 1 mL/kg was administered to each animal as a subcutaneous injection in the intrascapular region of the dorsal thorax. Interim blood samples were collected at 0.25, 1, 2, 6, 8, and 12 hours after dosing. Terminal blood samples were collected just before scheduled euthanasia at 0.5, 4, 24, 28, 72, 120, and 168 hours after dosing. Tissues were collected from each animal after euthanasia and rinsed with 0.9% sodium chloride, blotted dry, and then weighed. Sample combustion was performed using a Model 307 sample oxidizer (PerkinElmer, Inc., Boston, MA). The resulting trihydrate was trapped and mixed in 15 Monophose scintillation fluid (Perkin-Elmer). Samples that were analyzed directly were mixed with 15 mL of Emulsifier-Safe scintillation fluid (PerkinElmer). Samples were then analyzed for

total radioactivity using liquid scintillation counting for 2 minutes in a Model LS 6000 or LS 6500 liquid scintillation counter (Beckman Coulter, Inc., Fullerton, CA).

Results

Injection of DC Destroys Fat in Obese Mice

To demonstrate that DC alone is sufficient to destroy fat cells, 200 μL of 1% DC was injected into the left gluteal fat pad of genetically obese mice; a similar volume of vehicle was injected on the contralateral side. Mice were injected on days 1, 3, and 5. Animals were sacrificed 20 days after the first injection, and standard paraffin histology sections were prepared and stained with hematoxylin and eosin. In contrast to the normal-appearing adipose tissue architecture seen in the saline-injected side (Figure 1A), the injected area on the DC-treated fat pads showed few viable adipocytes (Figure 1B). DC treatment caused adipocyte destruction and triggered an immune incursion involving numerous histiocytes that appeared "foamy" in high-power images (Figure 1C). Triglyceride release was also evident in DC-treated tissue. Released triglycerides were stabilized by treating the tissue with osmium tetroxide to prevent the extraction of extracellular triglycerides during tissue processing (Figure 1D).

Localized Injection of DC Preferentially Targets Adipose Tissue in the Mouse Tail

The mouse tail contains subcutaneous adipocytes mixed with muscle fascicles and cartilage. Because these tissues exist in a small, confined volume, we used the mouse tail as a model to determine whether DC differentially affected specific tissues. A volume (100 μL) of DC (0.5%) or saline vehicle was injected into the mouse tail 1 cm caudal to its base. Mice were sacrificed 20 days after injection, and standard histological sections were cut perpendicular to the long axis of the tail within 5 mm of the injection site. The saline-injected mouse tail showed normal histology, with a layer of adipocytes beneath the dermis (Figure 2A). In the DC-injected tail (Figure 2B), most
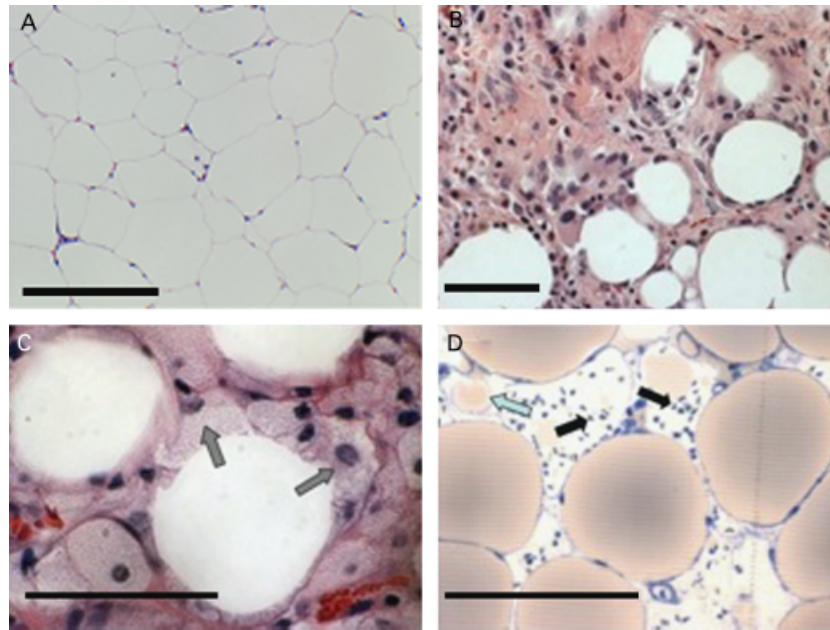
Figure 1. Hematoxylin and eosin staining of *ob/ob* mouse gluteal fat pad injected 20 days previously with (A) saline or (B) 1% deoxycholate (DC). (C) Higher magnification of hematoxylin and eosin–stained adipocytes treated with DC showing foamy macrophages (grey arrows). (D) Osmium tetroxide staining of DC-treated adipocytes indicates the presence of free lipid droplets (blue arrow) and remnants of destroyed adipocytes (black arrows). Bar represents 50 μm.

of the subcutaneous fat tissue showed loss of margins between the cells, with an inflammatory infiltrate. In contrast to fat, muscle fascicles and epidermis showed little apparent damage from the injection. The tissue architecture of the muscle and remaining skin layers were intact, with no signs of necrosis and little to no inflammatory infiltrate.

All Cell Types Tested Showed Similar DC Sensitivity In Vitro

Treatment of cultured primary human adipocytes with DC resulted in dose-dependent cytotoxicity. As shown in Figure 3, the concentration of DC necessary to kill half the adipocytes (LD_{50}) was approximately 0.045% (1.0 mM). A375M melanoma cells were similarly sensitive to DC as were adipocytes. Human fibroblasts and human skeletal muscle cells appeared to be somewhat less susceptible to DC-mediated toxicity, with LD_{50} values of approximately 0.055% (1.22 mM). In contrast, human keratinocytes were more susceptible to DC-meditated

cytolysis, with an LD_{50} of approximately 0.015% (300 μM; Figure 3). Although there were slight differences in the ability of DC to lyse different cell types, DC was cytotoxic to all cell types tested in this in vitro assay.

Physiological Concentrations of Albumin Attenuate DC Cytolytic Activity

DC is known to bind serum albumin with high affinity.[17] We hypothesized that DC injected directly into fat to trigger adipocyte lysis might bind to albumin molecules present in the extracellular space and thus become unavailable to mediate cell lysis. To determine whether albumin could protect adipocytes from DC, adipocytes were pre-incubated with physiologic concentrations of albumin and then challenged with DC in vitro. Figure 4 demonstrates that the ability of DC to kill cultured adipocytes was less in the presence of 0.7% albumin, a concentration similar to the amount present in interstitial fluids surrounding human fat cells,[18] as demonstrated by a
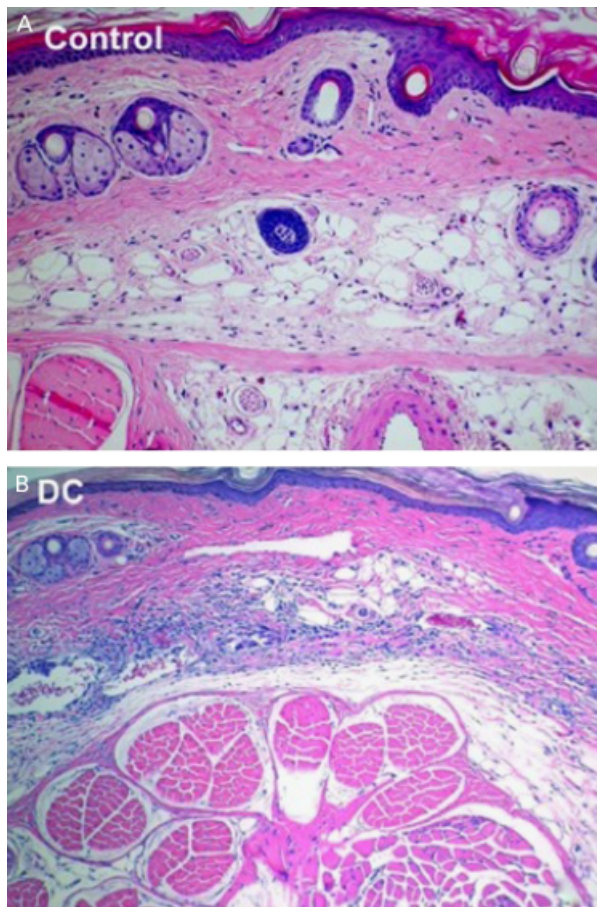
Figure 2. Hematoxylin and eosin staining of a mouse tail 20 days post-injection of (A) saline vehicle or (B) 0.5% deoxycholate showing necrosis and inflammatory infiltrate of the subcutaneous fat in the treated tail. (B) The tissue architecture of the muscle and skin layers remains preserved in the treated tail, with no signs of necrosis and scant inflammation.

Figure 3. Viability of cultured primary human cells after treatment with deoxycholate (DC). Keratinocytes (X), adipocytes (●), A375M (◆), skeletal muscle cells (▲), and fibroblasts (■) were treated with increasing concentrations of DC, and cell viability was assessed using an MTS colorimetric assay. All cell types show a dose-dependent decrease in viability. Error bars represent standard error of the mean.

Attenuation of DC-Mediated Cytolysis by Tissue

To better understand tissue-specific differences in susceptibility to DC-induced cytolysis, we compared the ability of various porcine tissues to attenuate DC-mediated cell lysis. The indicated wet weight of tissue (50, 100, 150, and 200 mg) was incubated with 1.5 mL of 1% DC in PBS for 1 hour. The supernatant was then removed and tested for adipolytic activity, as described above. As shown in Figure 5, fat was significantly less effective than skin or muscle at attenuating DC-mediated cell lysis.

DC Injected into Subcutaneous Fat Rejoins the Endogenous Bile Acid Pool

The fate and disposition of DC injected into fat tissue was investigated by subcutaneous injection of [3H]-labeled DC into the intrascapular fat pad of Sprague-Dawley rats. Figure 6 displays radioactivity as a function of time within the plasma and tissues of injected rats. More than 45% of the [3H]-DC migrated to the intestinal contents within 24 hours of intra-adipose injection (Figure 6), suggesting that

small shift in the LD_{50} from 0.05% to approximately 0.075%. Additionally, when these cells were incubated in medium containing 1.3% albumin (the approximate concentration of albumin present in the interstitial fluid surrounding human skeletal muscle cells),[18] there was a two-fold shift in the LD_{50} of adipocytes for DC, from approximately 0.05% to 0.10%. Pre-incubation of cells with 4% albumin, the concentration in human plasma, completely protected cells from 0.1% DC, a DC concentration that is instantly cytotoxic to cells in the absence of albumin (Figure 4).
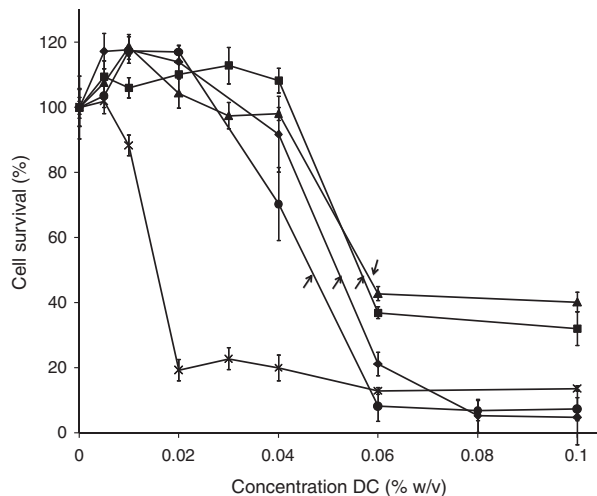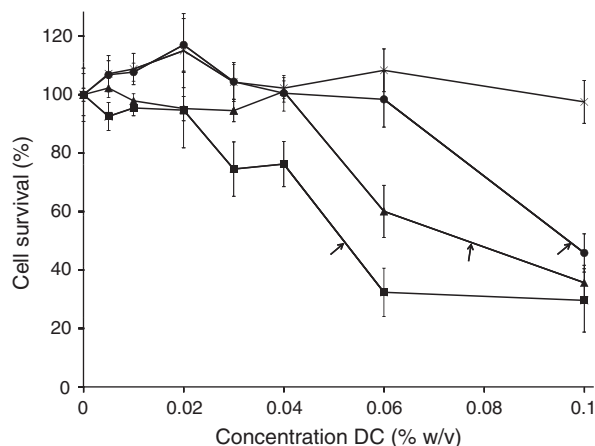
Figure 4. Viability of cultured primary human adipocytes treated with deoxycholate (DC) in the presence of increasing concentrations of BSA. Cells were incubated with 0% BSA (■), 0.7% BSA (▲), 1.3% BSA (●) and 4% BSA (x) and then challenged with increasing concentrations of DC. Cell survival was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymehtoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium colorimetric assay for cell viability. The concentrations of bovine serum albumin used are representative of the concentrations found physiologically in and around the site of injection. Albumin was demonstrated to attenuate DC-mediated cytolysis in a concentration-dependent manner. Error bars represent standard error of the mean.

Figure 5. The relative ability of various porcine tissues to attenuate deoxycholate (DC)-mediated cell lysis. The indicated wet weight of tissue (50, 100, 150, and 200 mg) was incubated with 1.5 mL of 1% DC in phosphate buffered saline for 1 hour. The supernatant was then removed and tested for adipolytic activity. Muscle (▲), skin (●), and fat (■) were tested. Pre-incubation of DC with muscle and skin tissue resulted in greater attenuation of cytolysis than pre-incubation with fat tissue. Error bars represent standard errors of the mean.

DC injected into fat joins the endogenous bile acid pool within 24 hours. By 48 hours, half of the radiolabel that had accumulated in the intestinal contents was eliminated from the animal, although an additional 5 days was required for the remainder of the [3H]-DC in the intestines to be eliminated, reflecting fecal elimination kinetics typical of DC.[19,20] There was also a noticeable accumulation of [3H]-DC in the small intestine that peaked 4 hours after treatment and was eliminated by 120 hours. The remainder of the [3H]-DC was distributed throughout various other tissues in trace amounts. Figure 6 shows those tissues with detectable levels of [3H]-DC.

Discussion

We investigated why reducing aesthetically undesirable fat deposits with DC is possibly selective for adipose tissues. Although DC is acutely toxic to cultured cells in a nonspecific manner, it is
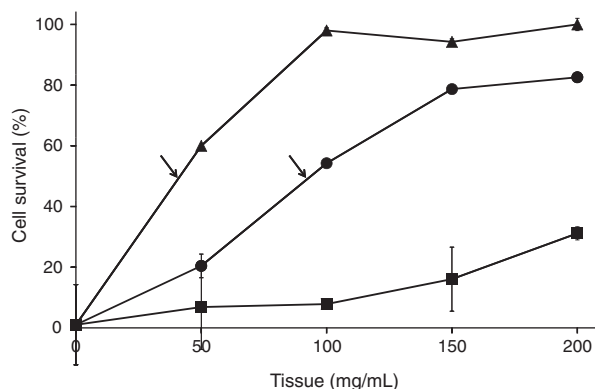
nontoxic when serving its role in the human body solubilizing dietary lipids. In addition, DC has been clinically demonstrated to be safe when used as an additive to emulsify intravenous drug formulations. Because endogenous DC causes no acute harm as a result of contact with cells of the small bowel, enterohepatic vessels, or liver, some mechanism(s) must protect animal cells from the detergent effects of DC. Several possible mechanisms could explain the ability of DC to destroy fat while sparing surrounding tissues. The most direct explanation would be that adipocytes are uniquely sensitive to the membrane destabilizing effects of DC, but our in vitro results show that DC is similarly toxic to numerous cell types (including adipocytes) cultured in the laboratory (Figure 3). Therefore, we hypothesized that neutralizing substances could protect nonadipose cells from DC, perhaps because of mechanisms analogous to those that protect the portal circulation from high concentrations of bile acids. This was supported by our observations in mice, which demonstrated that DC treatment killed adipocytes while apparently sparing other cell types. However, the biology of subcutaneous fat in rodents, particularly
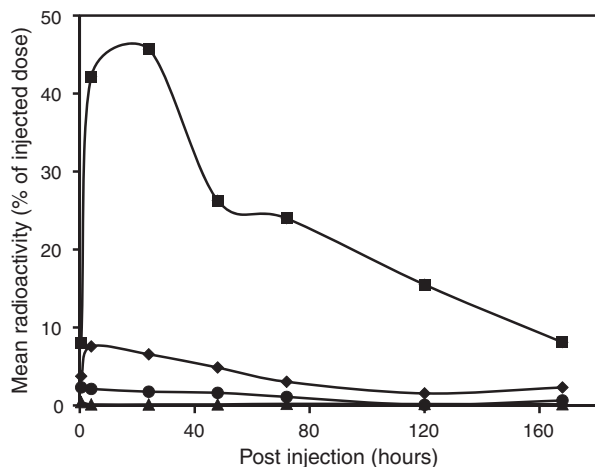
Figure 6. Tissue distribution over time of [^{3}H]-deoxycholate (DC) injected into intrascapular fat in rats. After injection, rats were sacrificed at variable time points, and tissues and organs were harvested. Intestinal contents and wash (■), small intestine (◆), liver (●), plasma (▲). Radioactivity in these tissues and organs was measured using scintillation counting and calculated as a percentage of the total radioactivity injected. Radiolabeled DC was transported to the intestinal contents within 4 hours. The small intestine and liver also showed accumulation of radiolabeled DC within this time period, albeit at lower levels than in the intestinal wash.

genetically obese mice, is not identical to that in humans, so differences may be observed. Sequestration and inactivation of DC may occur by protein binding at the injection site, which may limit damage to nonadipose tissues near the injection site. In addition, PC present in cell membranes and physical barriers, such as insoluble proteins, may also limit DC's distribution. The concentrations of DC used in the in vitro studies described here are 10 to 50 times lower than the concentrations of DC injected in clinical practice. If in vitro experiments are performed at the DC concentrations in clinical use, one does not observe as much albumin- or tissue-mediated attenuation of DC action. We contend that the observations made at these lower DC concentrations are relevant to clinical practice, because DC dilutes rapidly after injection as it diffuses away from the needle tip. The attenuation phenomena observed at lower DC concentrations may be relevant to controlling the actual size of DC-mediated lesions.

Given the high affinity of DC for certain hydrophobic protein domains, a protein that binds DC with an affinity stronger than its LD_{50} could potentially protect cells from DC toxicity. The LD_{50} of DC for adipocytes is approximately 0.5 mM. Each albumin molecule can bind four DC molecules with an affinity of 0.015 mM,[17] indicating that the albumin interaction is sufficiently strong to attenuate DC-mediated cell killing. Thus, albumin can eliminate the ability of DC to lyse adipocytes if present in a 1:4 or greater molar ratio. In addition, albumin contains numerous low-affinity DC binding sites that bind DC at concentrations higher than the LD_{50} of DC (0.5 mM). Although these sites would only weakly inhibit toxicity, low-affinity binding may effectively slow the spread of locally injected DC to adjacent tissues. Gupta and colleagues have obtained lower LD_{50} values for adipocytes and other cell types.[21] A possible explanation for this might be that their group used 3T3-LI preadipocytes and neonatal foreskin fibroblasts in their experiments, whereas we used subcutaneous adipocytes and human dermal fibroblasts. Furthermore, their group used different media for each cell line during incubation with the drugs, whereas we incubated our cells in serum-free medium during exposure to DC. The complete medium for each cell type they used may have contained serum and therefore possibly a variable amount of albumin, which would have attenuated the effect of DC.

Although our data indicate potential mechanisms through which various tissues can neutralize the toxic effects of DC, the exact mechanism remains unclear. We suggest that the binding of DC to endogenous albumin or to similar proteins may protect non-fat tissues from DC-induced lysis much in the way that endogenous DC is rendered harmless in the human body. Plasma contains approximately 4% albumin (0.59 mM), which is sufficient to neutralize approximately 0.2% DC. Tissues consisting predominantly of stroma, such as dermis, contain abundant albumin, whereas tissues that consist primarily of cells contain much less albumin per volume of tissue.[22] Thus, local

differences in albumin may contribute to the sensitivity of a specific tissue to localized DC injection. Other possible mechanisms may also inhibit the activity of DC in a tissue-specific manner. For example, edema fluid released into better perfused tissues would dilute and neutralize DC more efficiently than poorly perfused tissues.

We have shown that DC injected into subcutaneous fat in the scapular region of rats relocates to the intestines. The ability of exogenous DC injected into subcutaneous tissue to reach the enterohepatic circulation in animals is an important finding and suggests that a similar mechanism may be present in humans. It demonstrates that DC does not accumulate at the site of administration and is transported to the gastrointestinal tract where it joins the endogenous bile acid pool. Transient neutralization of the detergent properties of DC through association with albumin probably facilitates transport to the intestine.

We propose that DC may preferentially destroy fat because fat lacks specific proteins that bind DC and neutralize its toxicity. Although the toxic effects of DC on cultured cells may differ from effects on adipocytes in intact fat, our results suggest that adipocytes are not intrinsically more sensitive to DC than other cells. Rather, we suggest that fat may lack enough DC binding proteins to protect it from the detergent effects of DC after intra-adipose injection. After binding albumin, in edema fluid and neighboring tissues, DC can be absorbed into vessels and transported by the liver to recombine with endogenous bile stores. The rapid neutralization of DC and prompt removal from the systemic circulation can prevent DC from damaging tissues surrounding the injected fat. This further supports the observation that DC does not remain in the treated area and is rapidly returned to enterohepatic circulation.

Through this analysis, we have identified features that make detergents biocompatible; biocompatible detergents should be metabolically inert (i.e., they should be excreted in the same chemical form in which they are administered), or if metabolized, their metabolites should be harmless, and biocompatible detergents should display tissue-specific activity, affecting only the targeted tissue while sparing others. DC exhibits both of these features. In contrast to exogenous or synthetic detergents, which possess unknown metabolism, transport, and elimination properties in vivo, DC is not metabolized in vivo and is fecally eliminated in the same chemical form in which it is administered. DC is endogenous, with an average adult human body containing approximately 0.5 g (including DC in circulation and biliary stores). Lastly, because of the rapid attenuation of DC detergent activity by proteins and the transport of DC to the endogenous bile acid pool, the activity of DC is spatially limited, targeting the injected tissue. We contend that these features make DC a biocompatible detergent with potential use as a therapeutic agent.

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