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ORIGINAL ARTICLE BASIC SCIENCE/EXPERIMENTAL

Effect of a Formulation Containing Low-Dose Sodium Deoxycholate on Local Fat Reduction

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Abstract

Background Synthetic deoxycholic acid (DCA) has been approved as an injectable drug for the nonsurgical reduction of submental fat.

Objective In this study, we evaluated the fat-reducing effects of a new formula containing a low dose of DCA and fat dissolution by topical application of DCA.

Methods Sodium deoxycholate (99.1% pure) and the new formulation containing 10% DCA were injected or topically applied to the dorsa of obese mice (induced by a high-fat diet). The rate of change in body weight was evaluated, together with comparisons of micro-computed tomography images, body composition measurements, and histology findings.

Results The results showed that the new formula containing low-dose DCA was as effective as the older high-dose formulation with respect to the rate of change in body weight and reductions in subcutaneous fat pad area, body fat weight, and the thickness of the subcutaneous fat layer. Furthermore, topical application of the high-dose, but not the low-dose, formulation yielded promising effects.

Conclusions The development of a better protocol for the high-dose preparation, including dose optimization and application methods that minimize the adverse effects of DCA, merits further study.

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Keywords Deoxycholate · Lipolysis · Subcutaneous fat

Introduction

Obesity is a serious risk factor for many metabolic disorders, including cardiovascular disease and diabetes [1, 2]. Surgical methods are currently used to reduce the amount of fat tissue, whether for medical or aesthetic purposes, and include liposuction and cosmetic surgeries [2–5]. However, liposuction requires anesthesia, has a prolonged recovery time, and poses a risk of contour irregularities, excess skin, infection, and other medical complications, even leading to death on rare occasions [2, 4, 6]. Consequently, nonsurgical options are preferable [4, 5, 7].

Endogenous deoxycholic acid (DCA) is a secondary bile acid that solubilizes dietary fat, thereby contributing to its absorption within the gastrointestinal tract [4, 8, 9]. Cell culture studies have reported reductions in the accumulation of localized fat in DCA-mediated adipocyte lysis. Protein-poor tissue, such as fat, is more sensitive to DCA than relatively protein-rich tissue, such as skin and muscle [4, 9, 10]. In 2015, a synthetic form of DCA, ATX-101 (Kybella, Kythera Biopharmaceuticals, Inc., Westlake Village, CA, USA), was approved as a first-in-class injectable drug for patients with moderate-to-severe convexity or fullness associated with submental fat [5, 11].

In this study, we evaluated a new low-dose DCA-containing formulation with respect to fat reduction in

subcutaneous adipose tissue and compared it to ATX-1, containing a high dose of synthetic DCA. We also investigated possible fat dissolution by topical application rather than local injection of DCA.

Materials and Methods

Reagents

Synthetic DCA (99.1% pure) and a new DCA-containing formulation (ID) were supplied by Natural Skin Natural Life (Wonju, Korea). The ID formula consisted of 10% DCA, water, glycerin, caprylic/capric triglyceride, butylene glycol, tyrosine, *Polygonum fagopyrum* seed extract, *Ginkgo biloba*, and *Nelumbo nucifera* leaf extracts, stearic acid, glyceryl stearate, xanthan gum, and 1,2-hexanediol.

Animal and Experimental Design

The animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee. Six-week-old male ICR mice were purchased from Koatech (Pyeongtaek, Korea). The mice were fed a high-fat diet (HFD) (D12492, Research Diets, New Brunswick, NJ) and supplied with drinking water ad libitum until they reached the desired age (9 weeks) and body weight (32–35 g). The mice were maintained on a 12-h light/12-h dark cycle under semi-SPF conditions.

The 9-week-old HFD-induced obese mice were randomly divided into five groups ($n = 5$ per group): control, DCA injection, DCA topical application, ID injection, and ID topical application. All of the formulations were injected into or applied to the dorsum of each mouse. Body weights were recorded daily during the 10-day experiment. Mice in all groups were fed an HFD from the beginning to the end of the experiment. At the end of the experiment (day 10), micro-computed tomography (CT) imaging and body composition analyses were performed to determine the amount of fat deposition. The mice were then euthanized.

Micro-CT Analyses

Prior to CT scanning, the mice were anesthetized by isoflurane (MNS Korea, Kyungki Do, Korea) inhalation and during the CT scan were maintained under isoflurane narcosis. The fat volume in the anesthetized mice was analyzed by high-resolution in vivo micro-CT. Total fat, visceral fat, and subcutaneous fat from the neck to the bottom of the mice were determined by analyzing the images using CTAn software (SkyScan, Bruker, Billerica, MA, USA)

Body Composition Analyses

In vivo analyses of body lean mass, fat mass, and water content were performed in conscious, restrained mice by nuclear magnetic resonance (NMR) imaging, scanning the mice using the Minispec live mouse analyzer (model mq7.5, "LF50"; Bruker Optics, Inc, Billerica, MA). The Minispec is a time-domain NMR system that acquires radiofrequency signals generated by the hydrogen spins from soft tissues, such as adipose and muscle. Then, it uses the contrast in relaxation times of the hydrogen spins, or the amplitude, duration, and spatial distribution of these NMR signals, from the different tissues to estimate composition. On each day of testing, a quality-control check of internal voltages, temperature, magnets, and NMR parameters was performed using a standard provided by the manufacturer. The mice were placed in a clear, plastic cylinder (50 mm diameter) and kept immobile by insertion of a tight-fitting plunger into the cylinder. Then, the tube was lowered into the sample chamber of the instrument for approximately 2 min, the duration of the scan.

Histological Analyses

Samples from the skin and adipose tissues were freshly collected from the euthanized mice and fixed in 4% paraformaldehyde in phosphate-buffered saline. Tissue samples were processed, embedded in paraffin, and sectioned at 4 μm thickness. Hematoxylin and eosin (H&E) staining was performed on the harvested samples. For comparison of the thickness of subcutaneous fat layers, tissue samples were harvested from the dorsum of mice at same anatomical region, from level L1 to L3. The stained slides were scanned using a Leica SCN400 slide scanner (Leica Biosystems, Wetzlar, Germany).

Figure 4b shows the H&E-stained sections of the subcutaneous fat layers in the five groups of mice. The reduction in the thickness of the subcutaneous fat pads was significant in all of the experimental groups compared to the control group ($p < 0.001$) (Fig. 4c).

Statistical Analyses

All data are expressed as the mean ± standard deviation (SD) and were analyzed using a two-way Student's t test or one-way analysis of variance (ANOVA). The statistical analyses were conducted using GraphPad Prism software version 5.0 (GraphPad, La Jolla, CA, USA). A p value < 0.05 was considered to indicate statistical significance.

Results

Changes in Body Weight During the Experimental Period

As shown in Fig. 1a, the body weight of the mice in all experimental groups decreased significantly throughout the experimental period ($p < 0.05$). Compared to the control group (41 ± 2.0 g), the body weight of mice in the DCA (34 ± 2.3 g) and ID (36 ± 0.5 g) injection groups markedly decreased ($p < 0.001$). The decrease was greater than in the DCA topical application group (37 ± 1.8; $p < 0.05$), whereas topical ID application had no effect ($p = 0.12$) (Fig. 1b).

Quantitative Micro-CT

Changes in subcutaneous fat in the experimental groups were evaluated by in vivo micro-CT analyses on day 10 of the experimental period (Fig. 2a). Quantitative measurements showed significantly less subcutaneous fat in the DCA injection ($p = 0.002$), DCA application (0 = 0.001), ID injection ($p = 0.005$), and ID application ($p = 0.001$) groups than in the control group (Fig. 2b).

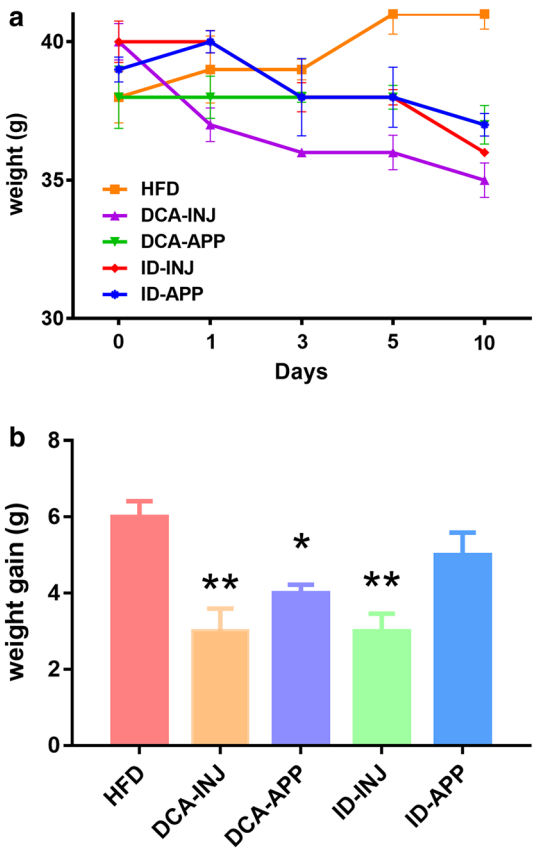

Fig. 1 Change in body weight. **a** The body weight of the mice was reduced in all treatment groups during the experimental period. **b** Differences in the rate of change in body weights between all treatment groups and the control group were significant. *$p < 0.05$; **$p < 0.001$

NMR Evaluation

The average percentage of body fat of the mice in all experimental groups remained proportional to their lean body mass and amount of free body fluid (Fig. 3a). However, compared to the control group, the percentage of body fat at the end of the 10-day experiment was less in the DCA injection and DCA application groups ($p < 0.001$) as well as in the ID injection group ($p < 0.05$) (Fig. 3b).

Fat Histopathological Examination

The histological changes after 10 days are shown in Fig. 4a. Marked blurring and dissolution of the adipocyte cell membrane and disruption of the normal circular architecture of the cells were seen in the DCA injection and ID injection groups compared to the control group. There was no disruption of the muscle fibers or changes in the structure of the epidermis, dermis, or muscle.

Figure 4b shows the H&E-stained sections of the subcutaneous fat layers in the five groups of mice. The reduction in the thickness of the subcutaneous fat pads was significant in all of the experimental groups compared to the control group ($p < 0.001$) (Fig. 4c).

Discussion

The efficiency of nonsurgical procedures in reducing localized submental fat deposits by adipocyte lysis has been recently demonstrated [12]. Among the many advantages of nonsurgical versus surgical fat reduction are that it does not require anesthesia or hospitalization, there are fewer costs, scarring is reduced, and the procedure is simple and fast [13]. However, safety and long-term effectiveness are still the most important issues [2, 6].

ATX-101 (injectable DCA) was approved in 2015 as a first-in-class injectable drug for appearance improvement based on a reduction of submental fat [11]. The development of ATX-101 as a pharmacologic treatment for fat reduction was based on the findings of Rotunda et al. [8], who demonstrated localized fat dissolution using an injectable phosphatidylcholine formulation. Based on their study, DCA was identified as the major active component responsible for the reduction in the amount of adipose tissue [8, 9, 14, 15].

DCA is a commonly used laboratory detergent, which raised concern that its use can cause the nonspecific destruction of cells other than the targeted adipocytes

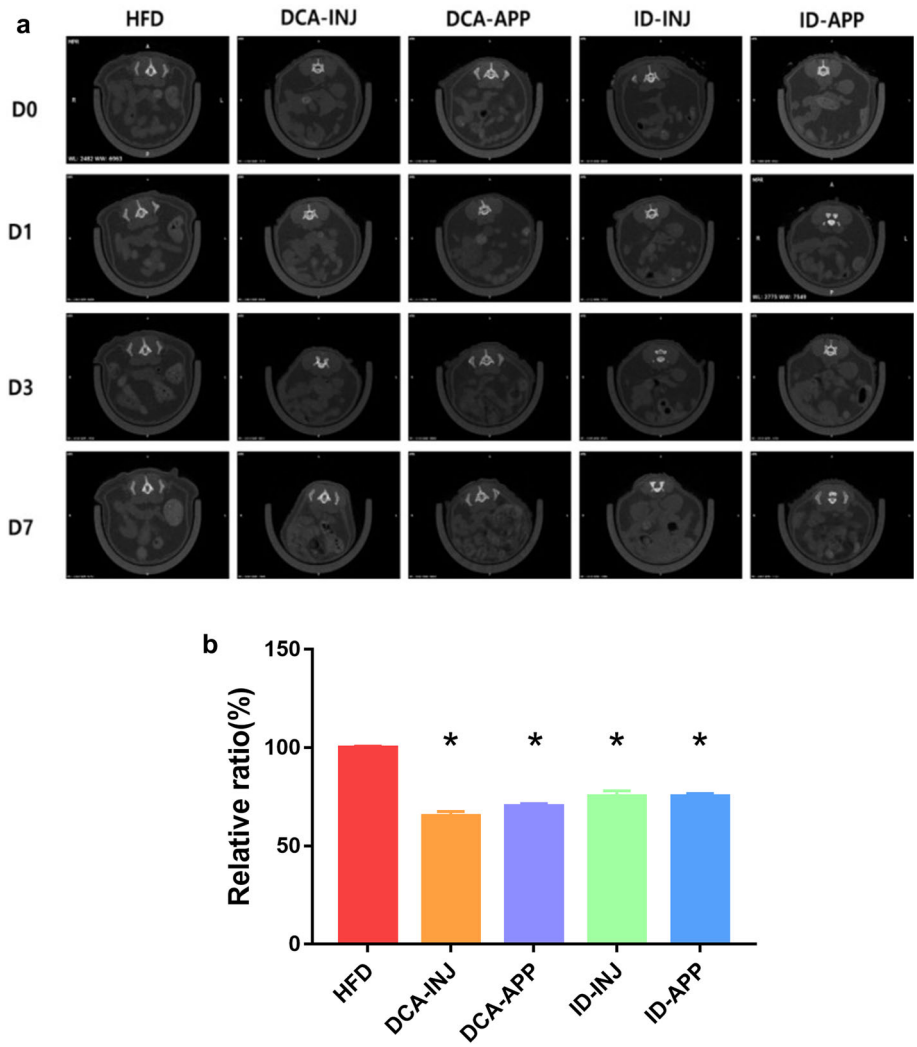

Fig. 2 Micro-computed tomography (CT) analyses. **a** Micro-CT images of adipose tissue during the experimental period. **b** Quantitative determination of the subcutaneous fat pad area after 10 days. $*p < 0.05$

[2, 9, 16]. Possible inflammatory responses and the injury of other vital tissues, such as muscle or nerve, were also of concern. Histological analyses of DCA-treated tissue showed that adipocyte lysis occurred on day 1 followed by acute local neutrophilic inflammation on day 3 and macrophage infiltration by day 7. The local inflammation was largely resolved by day 28 [5, 17]. These responses clearly demonstrated that DCA induces an inflammatory response. In clinical trials, an inflammatory response, including swelling, erythema, and bruises, occurred after DCA injection and multiple injections were shown to lead to repeated inflammation, and thus potentially to fibrosis [2, 11, 18, 19]. It was therefore recommended that ATX-101 treatment be administered in 28-day intervals to allow a resolution of the induced inflammation. In addition, the unintentional injection of other tissues besides adipose tissue may lead to tissue necrosis [7, 8]. In one study, marginal mandibular nerve paresis was reported in 4.3% of ATX-101-treated patients versus only 0.4% of patients injected with a placebo [11].

Overall, the side effects seen in clinical trials were mostly minor or moderate in intensity, temporary, and resolved without sequelae [4, 11, 17, 19]. They were also consistent with the form of treatment and its effects, i.e., subcutaneous injection, cell lysis, and induced inflammation [13, 17]. Therefore, it is important to minimize the risk of complications using a strict protocol of ATX-101 injection.

Safety is a critical requirement for nonsurgical therapeutic fat reduction agents. To reduce the complications associated with DCA, we tested a new formula (ID) based on a lower dose and mixed with other substances commonly used in cosmetic products. In addition, to avoid the adverse effects of subcutaneous injection, we compared the injection and topical application of DCA and ID.

The comparison of DCA and ID injection showed that the latter was sufficient, as the effect on the rate of change in body weights as well as reductions in subcutaneous fat pad area, body fat weight, and the thickness of subcutaneous fat layer was significant. However, the topical

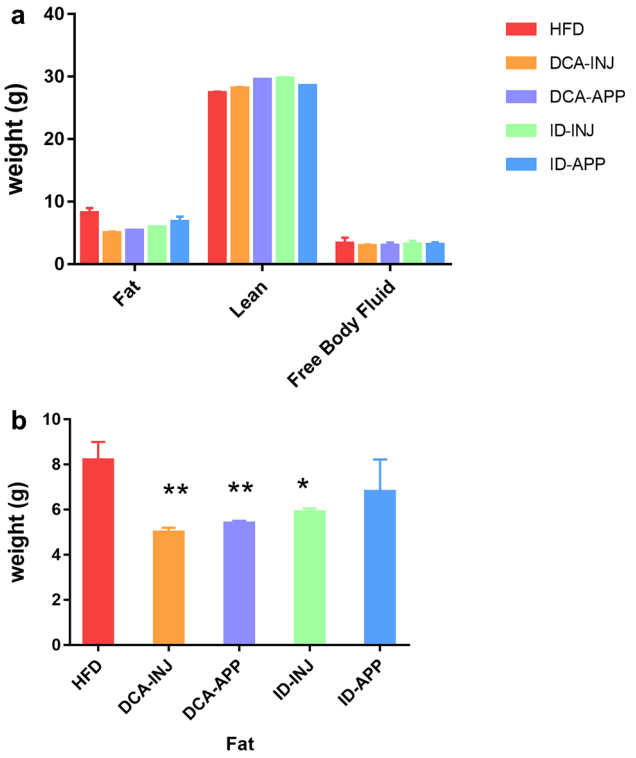

Fig. 3 Body composition analyses. **a** Percentages of body fat, lean body mass, and free body fluid in all groups as determined by nuclear magnetic resonance (NMR) imaging. **b** Body fat weight was significantly reduced in the deoxycholic acid (DCA) injection and DCA application groups as well as in the ID injection group compared to the control group. *$p < 0.05$; **$p < 0.001$

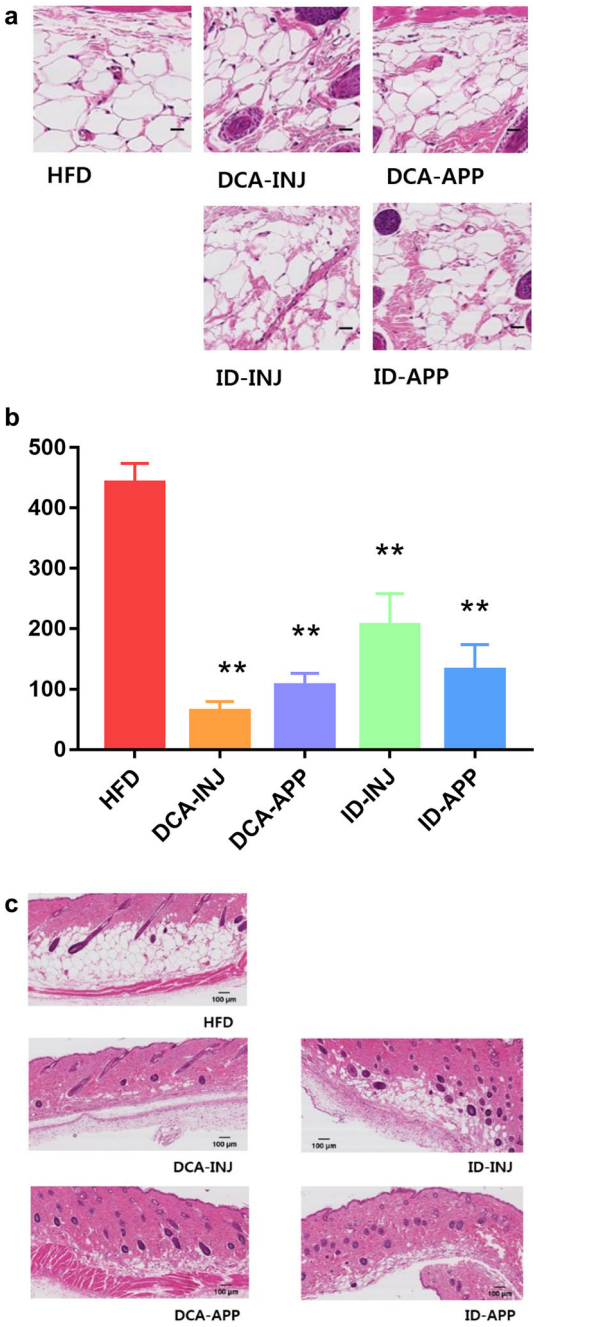

Fig. 4 Histological analyses. **a** Hematoxylin and eosin (H&E)-stained sections of subcutaneous fat layers in all groups (scale bars = 300 μm). **b** The thickness of the subcutaneous fat pad in all groups as determined from H&E-stained sections. **c** Quantitative determination of the thickness of the subcutaneous fat layer after 10 days of treatment. **$p < 0.001$

application of ID did not yield any effect, whereas the effects of topical DCA application were promising. Although not as effective as DCA or ID injection, topical DCA application induced a reduction in most of the measured fat related parameters.

The efficacy of a lower dose of DCA can minimize the adverse effects of pure DCA, by decreasing the intensity of the inflammatory reaction and preventing injuries to tissues other than the targeted adipocytes. The clinical application of DCA injection is limited to submental fat and a treatment interval of 28 days is mandatory, due to the inevitable inflammatory effects. By lowering the dose of DCA, more frequent application and a more regional injection may be possible, which would extend the indications for DCA and therefore its practical use.

However, to minimize the adverse effects of DCA, its topical application may be the best and safest option. Although topical application of DCA showed some activity in this study, the mechanism of its effect on the subcutaneous fat layer while avoiding injury to the epidermis and dermis remains to be determined.

Our study had several limitations. A new DCA-containing formulation consisted of 10% DCA and other compounds (glycerin, caprylic/capric triglyceride, butylene glycol, tyrosine, *Polygonum fagopyrum* seed extract, *Ginkgo biloba*, and *Nelumbo nucifera* leaf extracts, stearic acid, glyceryl stearate, xanthan gum, and 1,2-hexanediol), which may have a compounded effect on fat loss. Further study with additional control group with 10% DCA without

other compounds is required. The experimental period was short and was based on a limited number of mice. Procedures including injection and topical application were performed only once during the experimental period, as we assumed that one injection would be sufficient to observe an effect of DCA and ID injection, but topical application may require a more frequent or even daily regimen. Further studies with longer experimental period, larger number of animals, and different doses of DCA are therefore needed. In addition, we used p values for statistical analysis. However, p value alone is insufficient for comparison between groups and cannot be compared to each other.

In this study, we evaluated the fat-reducing reaction of subcutaneous adipose tissue to a new formulation containing low-dose DCA as well as possible fat dissolution by topical DCA/ID application. The lower dose of DCA (10%) in the ID formulation was as effective as DCA regarding the rate of change in body weight, as well as the reductions in subcutaneous fat pad area, body fat weight, and the thickness of the subcutaneous fat layer. Furthermore, topical application of DCA showed promising effects in fat reduction, as determined by most of the analyzed parameters. The development of a better protocol for DCA application, including optimization of the dose and the application method so as to minimize possible adverse effects, requires further study.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest to disclose.

Human and Animal Rights The animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee.

Informed Consent For this type of study, informed consent is not required.

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