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Injection of Phosphatidylcholine in Fat Tissue: Experimental Study of Local Action in Rabbits

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Abstract.
Background: Subcutaneous phosphatidylcholine to cause local lipolysis has been performed effectively and safely in the nonsurgical treatment of periorbital fat pads and also in the treatment of localized fat deposits in the abdomen, neck, arms and thighs. However, the studies do not explain the mechanism through which injectable phosphatidylcholine causes localized fat reduction. This study aimed to compare the local action of a phosphatidylcholine formulation with that of a physiologic saline solution in a histologic study investigating the fat tissue of rabbits.
Methods: Using a randomized, blind approach, 10 rabbits were injected with an experimental assay of phosphatidylcholine (the biologic model), and another 10 rabbits were injected with physiologic saline. A histologic study was conducted, and the Mann–Whitney test was applied.
Results: A marked difference was observed between the two groups with respect to necrosis, inflammatory exudation, and fibrosis.
Conclusion: Necrosis of the fat cells in all the phosphatidylcholine-injected animals was observed. Further studies should be performed to clarify and determine the mechanisms of action.

Key words: Experimental study of fat tissue—Phosphatidylcholine injection

Lecithin, a naturally occurring mixture of stearic, palmitic, and oleic acid diglycerides linked to the choline ester of phosphoric acid, commonly called phosphatidylcholine, is found in living plants and animals. Polyunsaturated phosphatidylcholine (PPC), a standardized and highly purified soybean lecithin extract, has been used orally for the treatment and prophylaxis of arteriosclerosis, hyperlipidemia, hepatitis, fat embolism, hypercholesterolemia, and diabetes since 1968 [8].

Lecithin functions as a skin-conditioning agent, surfactant, and emulsifying agent. Product formulation data submitted to the Food and Drug Administration (FDA) in 1997 showed that lecithin was used in 674 cosmetic formulations [2]. The systemic efficacy of PPC or other natural phospholipids as blood lipid–lowering agents has been well described throughout the years [3–5].

Subcutaneous PPC for local lipolysis was performed effectively and safely in the nonsurgical treatment of periorbital fat pads in 2001 [6]. The treatment of localized fat deposits in the abdomen, neck, arms, and thighs was reported in 2003 [5], and in another study attempting to reproduce the technique in 2004 [1].

Although studies regarding the mechanism through which PPC causes localized fat reduction have been undertaken, they do not explain the mechanism, and an increasing number of physicians have been using PPC for this purpose. The current study aimed to investigate, through a histologic study of rabbits, the local action of a phosphatidylcholine formulation in fat tissue.

Methods

A randomized, blind method was used to inject experimental assay in a biologic model (rabbit) after approval to perform animal studies from the Regulatory Board. The models were allocated into two

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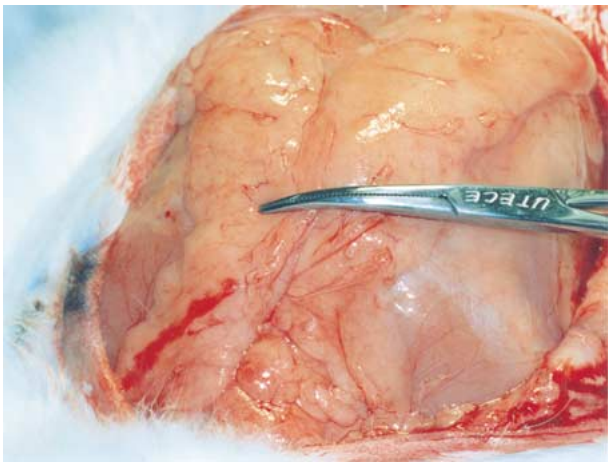


Fig. 1. Fat in the posterior trunk of the animal.

groups (groups A and B), with 10 rabbits in each group.

Group A was injected with 0.8 ml of PPC plus 0.2 ml of lidocaine, and group B was injected with 0.8 ml of physiologic saline plus 0.2 ml of lidocaine.

An animal was dissected before the beginning of the study for better local evaluation with respect to volume and quality of fat because rabbits have excess skin that makes it difficult to find the best fat deposit site. The best fat deposit site was considered to be the posterior trunk of the animal, approximately 5 cm long (Fig. 1).

After shaving and postpalpation of the fat on the back of the rabbit, the area in each group was marked with special pens for tissue in red and blue: blue for the group in which the investigated substance would be injected and red for the physiologic saline solution. The procedure was always repeated at the same original site. Injections were repeated four times each day for 4 days.

At 4 days after the last injections, the animals were anesthetized intramuscularly with acepromazine 5 mg, ketamine 25 mg/kg, and xilozine 10 mg/kg. The marked areas were cut with scissors for visualization of the fat, which was photographed and observed, and samples were removed for histopathologic study. The control group was treated similarly. The histopathologic study was conducted by the pathologist, who did not know which samples were from which rabbits. All the animals were killed with an injection of potassium chloride.

Histopathologic Method

All the specimens were fixed in 10% buffered neutral formalin. After fixation, the tissue was run through the automatic processor for histology, then embedded in paraffin wax. Serial sections were stained with hematoxylin and eosin (H&E).

The histologic study assessed the amount of fat necrosis, the inflammatory activity, and the stage of fibrosis using a semiquantitative scoring system. Inflammatory activity, staging of normal fat, inflammatory exudation, and fibrosis and necrosis were grouped according to the following scores: 0 (none), 1 (<25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%).

Statistical Analysis

The statistical analysis used the Mann–Whitney test to characterize the differences and similarities between groups A and B.

Materials

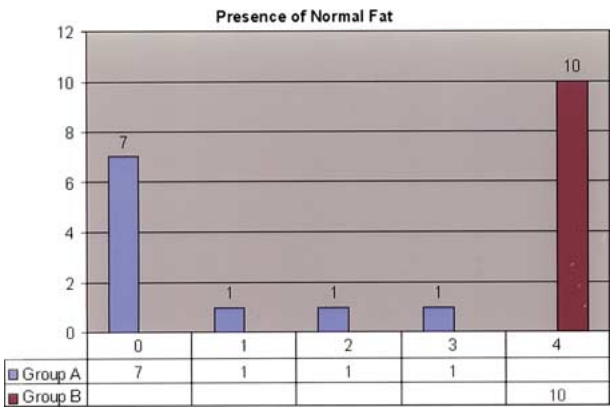
Female New Zealand rabbits were used shortly after they had given birth. The following drugs and substances were used:

Commercial brand	U.S. equivalent	Generic drug name
Aceprona	Promace	1% acepromazine
Francofar	Ketalar	5% ketamine
Rompum	Idem	2% xylazine
Lipostabil	None	50 mg/ml phosphatidylcholine
Physiological saline	Idem	0.9% sodium chloride

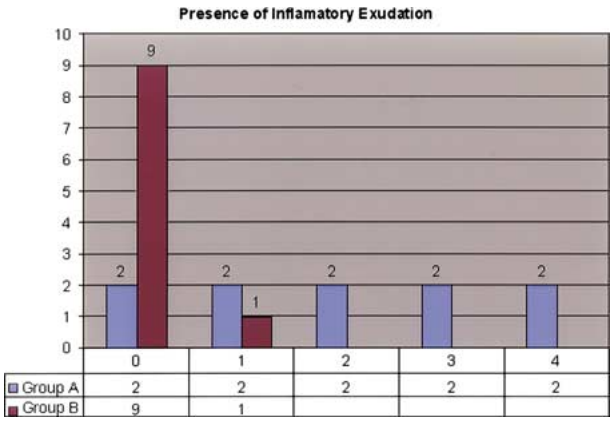
For the injections, 1-ml syringes with 30½-gauge, 13-mm needles were used.

Results

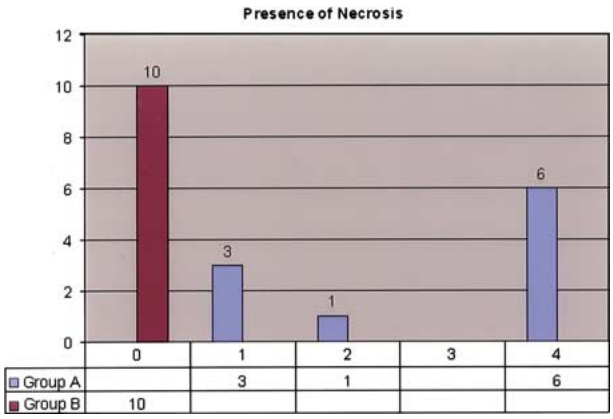
The tables below show the histopathologic differences between groups A and B of rabbits (y axis = number of rabbits; x axis = the score described before).



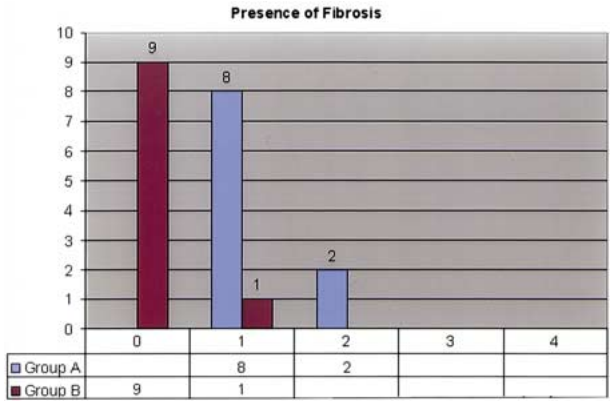
$p < 0.001$, Mann–Whitney test



$p = 0.002$, Mann–Whitney test



$p < 0.001$, Mann–Whitney test



$p < 0.001$, Mann–Whitney test

Histopathologic Results

The most common histologic reaction pattern was extensive fat necrosis and acute suppurative inflammation (Figs. 2, 3, and 4) affecting both septa and lobules. In some cases, we saw a mixed inflammatory cell infiltrate composed predominantly of neutroph-

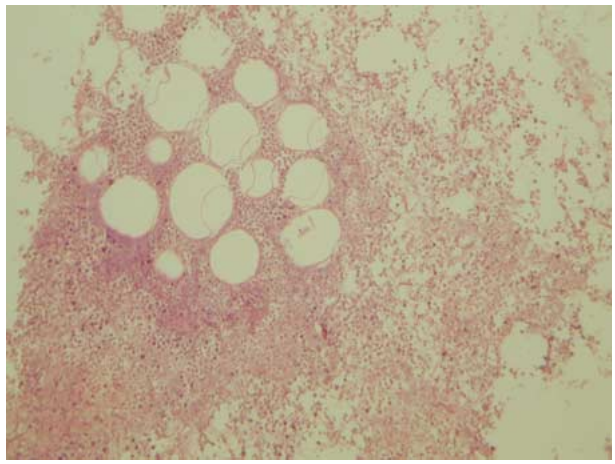


Fig. 2. Histopathology of fat necrosis with neutrophils. (hematoxylin & eosin ×400)

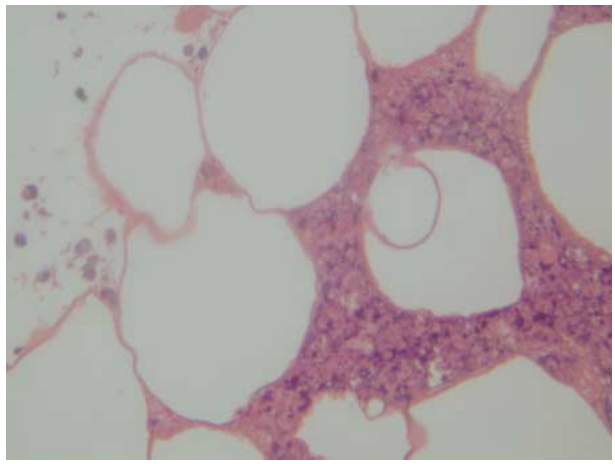


Fig. 3. Histopathology of fat necrosis with neutrophils. (hematoxylin & eosin ×400)

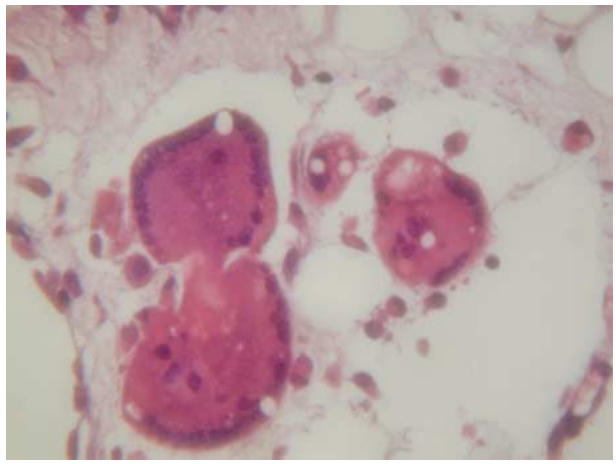


Fig. 4. Histopathology of fat necrosis with giant cell reaction. (hematoxylin & eosin ×400)

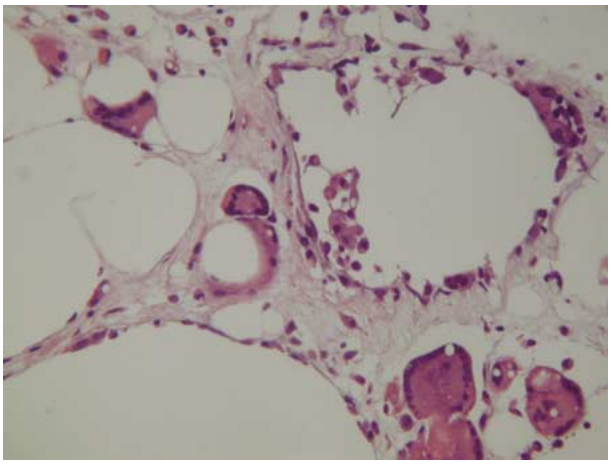


Fig. 5. Histopathology of fat necrosis with histiocytic and giant cell reaction. (hematoxylin & eosin ×400)

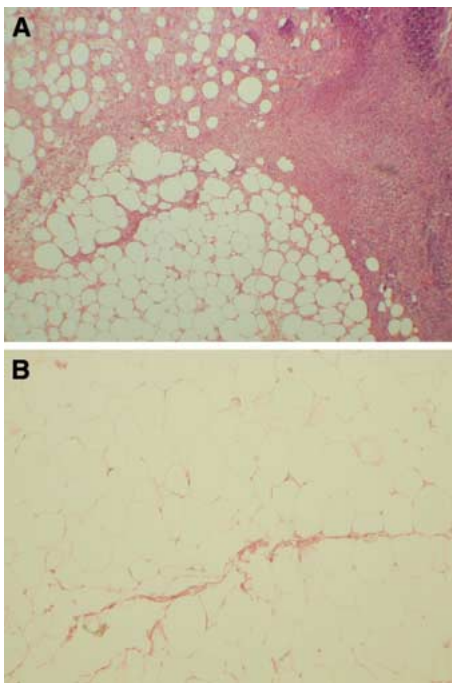


Fig. 6. Histopathology (**A**) of fat necrosis with abundant neutrophilic infiltrate and (**B**) normal fat. (hematoxylin & eosin × 400)

ils, with occasional macrophages, multinucleated giant cells (Figs. 5 and 6), and lymphocytes. Necrosis was present in all injected PPC cases.

Discussion

The conditions under which this study was conducted demonstrated that the injection of phosphatidylcholine into fat tissue leads to significant necrosis, inflammatory exudation, and fibrosis, whereas the injection of physiologic saline results in minimal fibrosis of the fat tissue. Throughout the study, we had difficulty palpating the site for reinjection in the PPC-injected rabbits because, macroscopically, the volume of fat was decreasing. This was not observed in the control group.

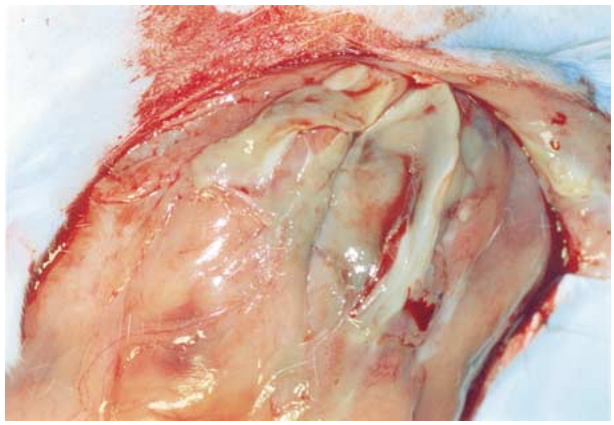


Fig. 7. Modified macroscopic view of the fat tissue.

Macroscopically, the injected PPC fat had various prominent characteristics in comparison with the control condition. It was yellowish (Fig. 7), with reduced volume, and microhard nodules could be felt. No macroscopic change was observed in the surrounding skin and muscle areas in any of the animals.

On the basis of these results, we concluded that immediately after the injection, intensive necrosis occurred in the fat tissue, followed by an inflammatory process, as demonstrated by the presence of inflammatory cells. The evolution of this process might lead to fibrosis, seen clinically as skin retraction, which is interesting if treating skin laxity was the objective. According to Paul Rose's theory [7], the reduction of subcutaneous fat is likely attributable to inflammatory-mediated necrosis and resorption. The presence of giant cells and macrophages containing fat demonstrated that healing probably ends there. The destroyed fat does not move throughout the body, but rather remains at the site and is eliminated by the macrophages.

New studies must be performed to define the mechanism of action for phosphatidylcholine. This study is only the first step toward clarification.

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