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Histologic Response to Injected Phosphatidylcholine in Fat Tissue: Experimental Study in a New Rabbit Model

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Abstract. The application of phosphatidylcholine to the fat tissue of humans for aesthetic purposes has recently been in evidence, despite the sparse literature corroboration of this practice. The authors developed a new experimental model to study injection of substances in fat tissue in rabbits. The objective of this particular study was to verify the possible effects of phosphatidylcholine injected in the animals. The animal weight, the fat pad weight, the presence of inflammatory infiltrate, and fibrosis and necrosis at the application sites were observed. Two groups of rabbits received five weekly applications to the dorsal fat pad. The control group received saline solution 0.9%, and the study group received phosphatidylcholine. The removed fat tissue was evaluated 3, 7, 14, and 21 days after the fifth application was completed. The phosphatidylcholine group presented more intense inflammatory infiltrate and fibrosis than the control group ($p = 0.05$). Necrosis was not observed in any animal. There was no statistically significant difference with regard to the weights of the animal or the fat pad. On the basis of this study, the injection of phosphatidylcholine is relatively safe, but no effect was observed regarding the reduction of fat tissue volume. New studies with higher doses are needed to justify the clinical use of this substance.

Key words: Experimental study—Fat tissue—Histology—Phosphatidylcholine—Rabbit

Phosphatidylcholine is a cell membrane lipoprotein that participates in many biologic processes, modulating the transmembrane transportation of lipids. Phosphatidylcholine acts as a source of second messengers in cell signaling, a process by which a signal essential for many functions is transmitted from the outside to the interior of the cell.

Oral and parenteral therapies with phosphatidylcholine have been used in the prevention and modulation of many organic disturbances and biologic vital processes [6,29,30,36]. Phosphatidylcholine plays an important role in brain development and learning, and can be used to enhance sperm motility [8,12,16,17]. In cardiac and hepatic physiology, choline deficiency causes a fatty liver, liver cancer in animals, and a fatty liver in patients receiving total parenteral nutrition. It reduces serum cholesterol and triglyceride levels and participates in metabolism of homocysteine, which increases the risk of cardiovascular diseases [3,5,10,11,15,18–22,24,25,29–36]. Animals fed with choline supplementation had better memory and learning than a control group [7,23].

Soy is the main natural source of phosphatidylcholine [6]. Rittes [27,28] was the first to mention the use of this substance for aesthetic purposes, reporting that injection of phosphatidylcholine reduced the bulging of the lower lid. Later, the treatment of localized fat in the neck, arm, abdomen, and leg also was reported. Reduction of those fat pads was claimed, but only subjective evaluation was used [1,13]. No previous report, however, has demonstrated the objective effect of this substance when injected in fat tissue.

In Brazil, the injection of phosphatidylcholine into pouches of fat tissue quickly achieved wide and popular acceptance, but indiscriminate use with no control was followed by reports of complications such as infection and localized necrosis. The situation forced the National Agency for Sanitary Control (Agência Nacional de Vigilância Sanitária [ANVISA]) to

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prohibit the use of the substance if injected into the subcutaneous tissue, considering that "there is no scientific corroboration that guarantees the quality, security, and efficacy of the use of phosphatidylcholine for aesthetic purposes" [2].

From the medical point of view, it seemed interesting for us to study what really happens in the fat tissue after injection of the substance. For this purpose, an experimental model was developed in rabbits to study these effects and other injected substances that might claim to produce "fat reduction." The objective of this report is to present this experimental model in rabbits, and to evaluate histologic effects of phosphatidylcholine in the fat tissue, based on evidence of necrosis, inflammatory infiltrate, and fibrosis in the application site.

Fig. 1. *Corpus adiposum* interscapulare.

Materials and Methods

Development of the Experimental Model

New Zealand white rabbits were dissected to find a constant fat pad that could be used in an experimental model. A fat tissue pad was identified in the dorsal cervical thoracic region, denominated *corpus adiposum interscapulare* or *corpus adiposum nuchae*, the central part of which can be found at the meeting points of the two scapulas in the dorsal midline [4,26]. This pad has a well-defined cleavage plain and presents four lateral extensions or arms resembling an "X." Two of these arms extend cranially for a length of about 7 cm and a width of about 1 cm each. The two distal arms extend over the scapula with a similar size and format.

This anatomic structure was present as a constant and with identical aspect in 15 dissected rabbits. After the cutaneous incision in the midline, it can be located easily in the scapular area above the rabbit cutaneous muscles or *panniculus carnosum* (Fig. 1). Histologic analysis of the fat pads from three rabbits identified normal fat tissue later used as a control for noninjected fat tissue. The mean weight of these rabbits was 2,374 ± 401.62 g, and the mean weight of the pads was 10.84 ± 4.89 g.

Local Injection of Phosphatidylcholine or Saline Solution in the Fat Tissue

Twelve 3-month-old New Zealand white rabbits weighing from 1,950 to 3,122 g were used, and maintained with water and food *ad libitum*. The rabbits were randomly divided into two groups. Group 1 (n = 7) received phosphatidylcholine injections, whereas group 2 (n = 5) received injections of saline solution 0.9%. The phosphatidylcholine used was available in a concentration of 50 mg/ml.

The rabbits were weighed weekly on digital scales, at the injection and before the biopsies. One injection of 0.5 ml was performed with an insulin needle (30.5 gauge) for each rabbit at weekly intervals during the 5 weeks. The injection site was the vertex of the angle formed by the meeting of the two scapulas on the mid-dorsal line. No anesthesia was needed for the injection procedures. After the injections, group 1 received 125 mg of the substance at the same application site in five sessions of 25 mg each, whereas group 2 received the five applications of saline solution at this site.

Biopsies

The rabbits of each group were randomly chosen for fat pad excision on days 3, 7, 14, or 21 after the last injection. In the control group (n = 5), one rabbit was killed on days 3, 7, and 21, and two rabbits on day 14, whereas in phosphatidylcholine group (n = 7), two rabbits were killed on days 3, 7, and 14 and one rabbit on day 21.

The rabbits were killed under anesthesia. A preanesthesia intramuscular injection was administered in the rabbit's leg. In the sequence, the marginal auricular vein was catheterized for a 10-ml infusion of the same solution corresponding to the lethal dose.

A cranium-caudal incision of about 15 cm was performed in the dorsal suprascapular region on the midline. The *corpus adiposum interscapulare*, or posterior cervical fat pad, was individualized and removed entirely and weighed. The central part of each excised pad was divided into three fragments of 0.5 × 1 cm and sent for histologic analysis (Figs. 2 and 3). The rabbits used in phase 1 were taken as the normal histologic pattern.

Histologic Analysis

Histologic analysis was based on hematoxylin and eosin staining for evaluation of inflammation, and the

Fig. 2. Isolated corpus adiposum interscapulare.

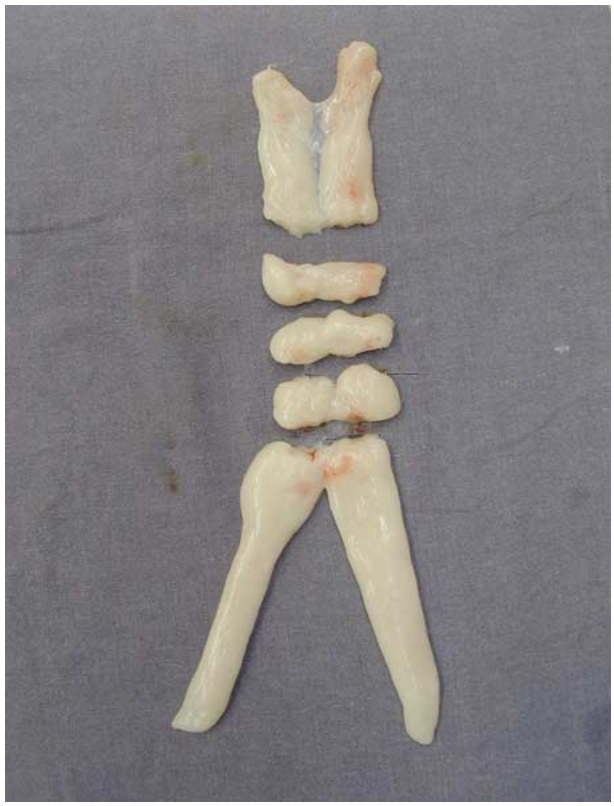

Fig. 3. Fragments sent for analysis.

Masson tricromic staining technique was used to evaluate fibrosis. The pathologist had no previous knowledge of what the injected substance was in the sample. He evaluated and graded using a semiquantitative scale of 0 (absence), 1 (mild), 2 (moderate), 3 (intense), 4 (severe) as well as the degree of inflammatory infiltrate and fibrosis (Figs. 4–7) in each sample. This system is similar to the histologic grading system used for hepatic disturbances [8,13].

The results were statistically analyzed using the nonparametric test of Kruskal–Wallis and Mann–Whitney as well as the Pearson correlation. A p value of 0.05 (5%) was established as significant.

Results

Animal Weight

The weights of the animals presented a large variation, so we compared the percentage variation of the animal's weight. No statistically significant difference was observed between the two groups for any time.

Relation of Fat Pad Weight/Animal Weight

To compare fat pad weights between the two groups, we used the relation of pad weight/rabbit weight in grams. A comparison of the two groups found that the relation of pad weight to animal weight was smaller in the phosphatidylcholine group, but this difference was not considered statistically significant ($p = 0.167$) (Fig. 8).

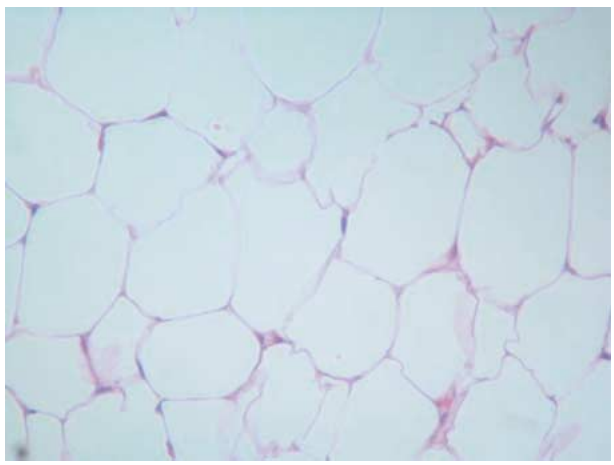

Fig. 4. Absence of inflammation graded 0/4. Sample from group 2 (control).

A Pearson correlation analysis found a 23.6% correlation between the biopsy date and the pad weight/animal weight ratio. Thus, the pad weight/animal weight ratio was not significantly correlated with the date of sample biopsy.

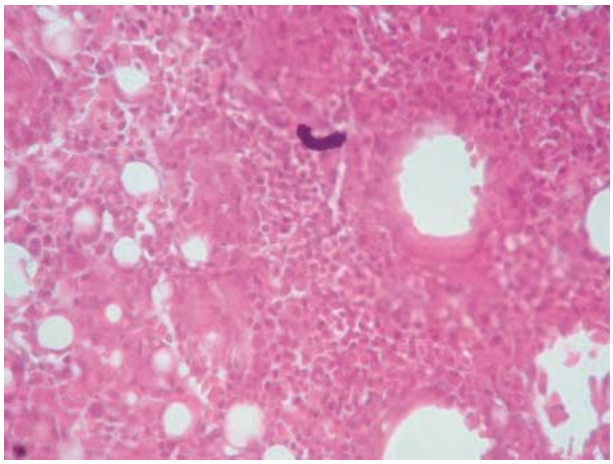

Fig. 5. Severe inflammation graded 4/4. Sample from group 1.

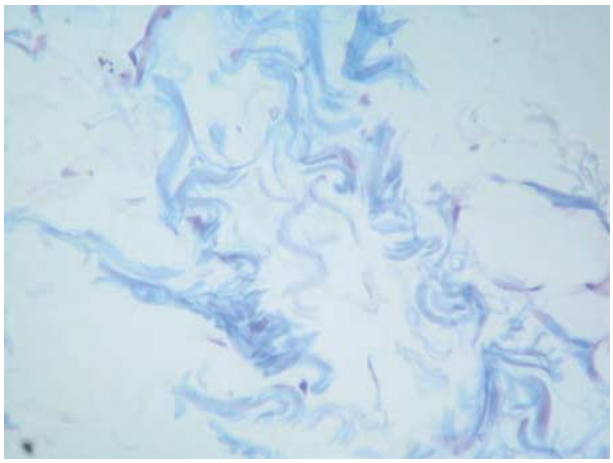

Fig. 6. Mild fibrosis graded 2/4. Sample from group 2 (control).

Histologic Findings

Necrosis was not observed in any sample in any group. In the saline solution group, no inflammatory infiltrate was seen in any biopsy, whereas in the phosphatidylcholine group, an infiltrate with a mean degree of 0.33/4 was observed. This difference was considered to be statistically significant ($p = 0.026$) (Fig. 9).

The presence of inflammatory infiltrate in the phosphatidylcholine group increased progressively from day 3 to 14, but was not seen after day 21. These differences among different biopsy days were not significant.

Some degree of fibrosis was observed in all biopsies of all the rabbits according to the system adopted by the pathologist. The control group presented a mean value of 1.87+/4+, as compared a mean value of 2.81+/4+ in the phosphatidylcholine group. This

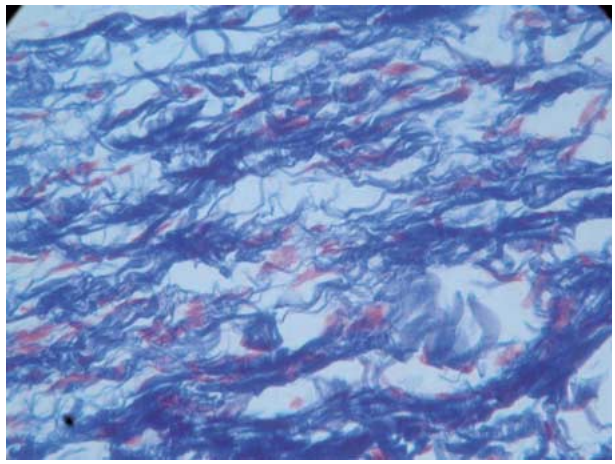

Fig. 7. Severe fibrosis graded 4/4. Sample from group 1.

difference was statistically significant ($p = 0.003$) (Fig. 9). When fibrosis on different biopsy days was compared in the same group, only in the phosphatidylcholine group was there a difference, with less fibrosis seen on day 21. However, this reduction was statistically significant only when compared with days 3 and 14 ($p = 0.028$ and 0.016, respectively).

Discussion

The experimental model in rabbits was developed to simulate clinical conditions. In this first experiment, we studied the effects of phosphatidylcholine injected in fat tissue. The control group received an inert substance: saline solution. Five injections were performed at a weekly frequency using small doses of the substance (25 mg) at the same concentration as that used in the clinical series (20–40 mg). Conversely, we injected only one site per session, whereas in the clinical situation, all the fat areas to be treated would receive multiple injections.

In our study, no change in weight was observed when the group that received phosphatidylcholine was compared with the control group. In the phosphatidylcholine group, the relation of pad weight/rabbit weight was smaller than in the control group, suggesting that the weight of the fat pad was lighter in the phosphatidylcholine group. However, this difference was not statistically significant. The absence of a reduction in weight or fat pad weight does not allow us to state that injection of phosphatidylcholine into fat tissue was or was not effective, because of the small dose used in this study, applied at only one injection site, as compared with multiple injection sites in clinical studies. This was not the purpose of this research.

The phosphatidylcholine group elicited more inflammatory infiltrate and formation of fibrosis than the saline solution group. These findings corroborate those reports in which adverse effects were observed

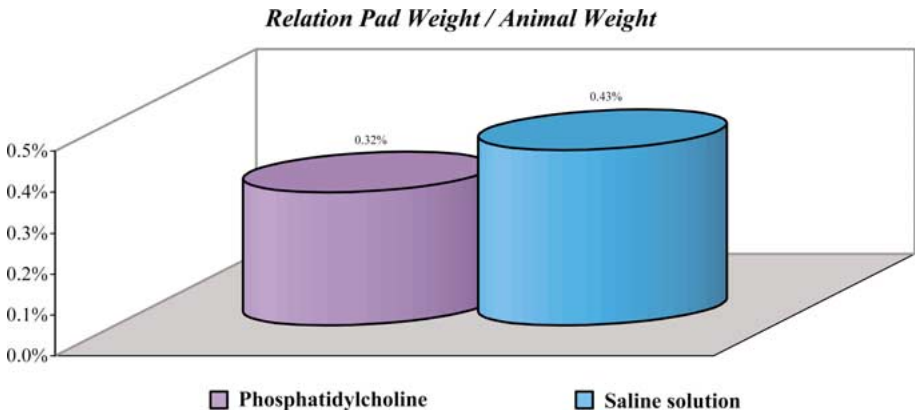

Fig. 8. Relation of fat pad weight/animal weight in the two groups.

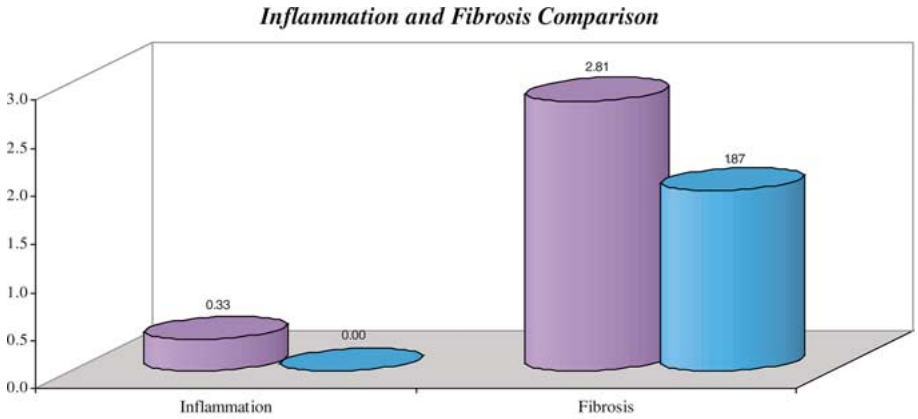

Fig. 9. Inflammation and fibrosis comparison in the two groups.

clinically such as edema, erythema, swelling, and a local burning sensation that could last up to 10 days [1,5,24,25].

Necrosis was not observed in any group. In the published clinical series, necrosis also was not reported. The safety of the procedure probably is dependent on the use of small doses, regularly distributed in multiple sites in multiple sessions.

The results of this study have not helped us to explain how phosphatidylcholine, although relatively safe, could be clinically justifiable. New studies are necessary to establish the action of phosphatidylcholine in reducing human fat tissue.

This new experimental model in rabbits was satisfactory for studying fat tissue changes after direct injections of substances. It can be useful for testing the response of fat tissue to other substances.

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