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Research

Effect of Metformin on Adipose Tissue Degeneration During Obesity

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Abstract

Obesity is associated with an increased inflammation involved in the development of atherosclerosis and insulin resistance. Macrophages are key in the genesis of these processes. Obesity induces macrophage accumulation in adipose tissue, they produce many of the inflammatory molecules secreted by adipose tissue like TNF-α, interleukins (IL-1β, IL-6, IL-8), as well as acute phase proteins such as haptoglobin (Hp), PAI-1 and C-reactive protein. The presence of these molecules in obesity relates this pathology to various inflammation degrees. The analysis of histological sections obtained from rabbit adipose tissue biopsy samples and processed by the hematoxylin-eosin and immunohistochemistry, showed the effect of metformin, a molecule which acts on the insulin metabolism, by the degeneration of this tissue during obesity. Metformin and its effects on metabolism are such that decreases the inflammation process suffered by adipose tissue, which is reflected by morphological changes in the distribution and migration of cells in the histological structure.

***Keywords:** Inflammation, histological section, monocyte, rabbit.*

Introduction

Adipose tissue is undoubtedly the pathogenic site where obesity-induced insulin resistance initiates locally, before becoming systemic. Its endocrine and paracrine production of bioactive proteins, given its secretory gene expression profile, reflects a generalized inflammatory state in this tissue. Proteins produced by adipocytes that could initiate such a process include TNF-α, IL-6, leptin, adiponectin, PAI-1 and angiotensinogen. On the other hand, recruited immune cells (mainly monocytes and macrophages) express the same vasoactive and pro-inflammatory proteins, with the exception of leptin and adiponectin. Both cell types, recruited adipocytes and macrophages, appear to be coordinately involved in the pathogenesis of inflammation-induced insulin resistance (1). Tumour necrosis factor-alpha (TNF-α) can reduce the amount of adipose tissue, due to its ability to inhibit adipocyte differentiation, inhibit lipogenesis, stimulate lipolysis and induce apoptosis (Lung, 2014). Since virtually all fatty acids are accumulated in adipocytes, it is generally assumed that the inflammatory process is initiated in adipocytes and amplified by macrophages (3).

Several treatments to improve insulin sensitivity and adipose tissue functions for the management of NAFLD have been proposed in several studies, including weight loss, metformin treatment and insulin sensitization by thiazolidinediones (4).

Metformin is a biguanide drug widely used as an anti-diabetic drug that is effective in lowering plasma glucose levels by decreasing hepatic glucose production (gluconeogenesis) and improving lipid metabolism, thereby preventing possible complications such as hepatic steatosis and the development of non-alcoholic fatty liver disease (5).

Metformin enhances insulin action on fat, facilitating glucose uptake and glycogen synthesis. Biguanides increase GLUT4 expression and its transport capacity by facilitating insulin receptor tyrosinase activity. It also reduces circulating levels of free fatty acids and their oxidation by up to 30%. It also causes decrease in LDL triglycerides and decreases hepatic synthesis of VLDL (5).

According to the mechanisms described with the use of metformin, it protects against non-alcoholic fatty liver disease by acting directly on hepatic metabolism and having indirect effects on the inflammatory response and improving the phenotype of adipose tissue by suppressing the action of monocytes and macrophages.

Materials and Method

Animal species

Female New Zealand rabbits provided by the biotherium of the Natural and Exact Sciences Division, University of Guanajuato.

Induction of obesity in rabbits

Over a period of nine weeks, rabbits were fed a high-fat diet (HFD) containing:
12.6% vegetable fat
11.2% unsalted commercial butter 4.2% lard
2.1% commercial sugar

Pre-biopsy anesthesia

Once outside the bioterium, the experimental animals were subjected to different doses of sodium pentobarbital via the intraperitoneal route to provoke an anaesthetic state and proceed with the collection of adipose tissue.

Table 1. Weight/dose ratio administered to each of the rabbits used in the experiment.

The dose used in the DAG rabbits was modified because sodium pentobarbital is fat-soluble and undergoes a redistribution process between adipose tissue and the central nervous system.

Rabbit	Weight	Dose pentobarbital sodium	mL pentobarbital sodium pentobarbital administered
High fat diet	4.150 kg	0.6 mL/kg	2.5 mL
High-fat diet, subsequent treatment with metformin	4.00 kg	0.63 mL/kg	2.5 mL

Several samples of adipose tissue were obtained from the adipose tissue above the adrenal gland by puncturing the middle part of the rabbit's back, as this is where most of this tissue accumulates. The procedure was carried out using an Illinois sternal T-handle/iliac crest aspiration needle, 15 Gx 76 mm, previously sterilized and in an environment away from air currents to avoid possible contamination or subsequent infection of the experimental animals. The above procedure was performed on one rabbit subjected to DAG for nine weeks and one more under this same diet but with a subsequent metformin treatment at a dose of 25mg/kg body weight.

The biopsies obtained from fat tissue were placed in a cassette for the following treatment:

Table 2: Fat tissue biopsy fixation treatment.		
Dehydration		
Number	Process	Time
1	Immersion in 10% formalin (x2)	2 hrs.
2	Formaldehyde- alcohol immersion (1:1)	1 hr.
3	Immersion in 96° alcohol for one hour (x3)	3 hrs.
4	Immersion in absolute alcohol for one hour (x2)	2 hrs.
Clarification		
5	Immersion in xylol for one hour (x2)	2 hrs.
Paraffin impregnation		
6	Immersion in paraffin (liquid) for one hour (x2).	2 hrs.

Solidification of the paraffin was carried out for 20 minutes at room temperature and then in the freezer.

They were processed in the paraffin microtome, 4 to 6 micrometers thick. They were then deposited in a flotation bath containing an aqueous solution of 2 g of containing an aqueous solution of 2 g. of grenetin in 2.5 L of water, at 42 °C.2.5 L of water, at 42 °C.

The cuts were subjected to the rehydration process which consisted of:

Table 3: Sequence of steps for obtaining and staining histological sections of adipose tissue biopsy.		
Number	Process	Time
1	Citrisolv immersion (x2)	10 minutes
2	Absolute alcohol immersion	7 times
3	Immersion in 96° alcohol	7 times
4	Immersion in 80° alcohol	7 times
5	Immersion in 70° alcohol	7 times
6	Immersion in 50° alcohol	7 times
7	Immersion in flowing water	7 times
STAINING		
8	Acetic acid-activated Harris haematoxylin immersion	5 minutes
9	Immersion in flowing water	7 times
	Immersion in 1% hydrochloric acid solution	7 times
10	Immersion in flowing water	7 times

Table continued...

11	Immersion in saturated lithium carbonate solution	7 times
12	Immersion in flowing water	7 times
13	Eosin immersion	5 minutes
14	96° alcohol immersion	7 veces
15	96 alcohol immersion	7 times
16	Absolute alcohol immersion	10 minutes
17	Absolute alcohol immersion	10 minutes
18	Xylol immersion	10 minutes
19	Xylol immersion	10 minutes
Assembly		
Place a drop of synthetic resin on the tissue and a coverslip on the drop, avoiding the formation of bubbles. Observe under a light microscope.		

The tissue is embedded in paraffin, sections are made, and rehydration is stopped until immersion in 50% ethanol, then washed with distilled water for 5 minutes, placed in a humid chamber, so that the histological preparation is suspended, and its edges are free from contact with any surface to avoid spillage of liquids.

The histological preparation is blocked with 5% fat-free milk in TBS [tris (hydroxymethyl) aminomethane] buffer for at least one hour, after which it is discarded and three 10-minute washes with TBS and another three washes with TTBS (same buffer but now with triton X-100) are performed. The antibody was prepared at 1:1000 dilution [monoclonal antibody made in rat against the Cd11b clone of macrophages recruited to adipose tissue]. This antibody is coupled to FITC (fluorescein, green, emitting at 520nm and excited at 494nm). It must be left in the humid chamber, in darkness, for at least two hours, in this case it was left overnight. Subsequently, the antibody is recovered and 3 washes with TTBS and 3 washes with TBS are performed, the preparation should be mounted with an aqueous mounting medium.

Results and Discussions

From the observation of the histological sections, it was possible to observe the infiltration of monocytes (which in connective tissue are known as histiocytes) within the intercellular space of the adipocytes, which can be identified by the presence of an unstained halo due to the small amount of DNA they possess and therefore cannot be strongly stained with hematoxylin. This is consistent with the hypothesis that adipose tissue during obesity generates various interleukins and inflammatory molecules which attract lymphocytes to inhibit adipocyte differentiation and thus gradually decrease inflammation and the development of the obesity process. inflammation and the development of the obesity process.

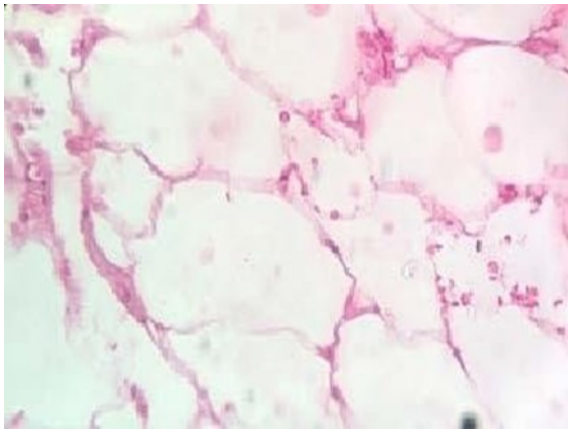

Figure 1: Adipose tissue obtained from rabbits fed nine weeks of high fat diet without metformin treatment (E.H. 40X).

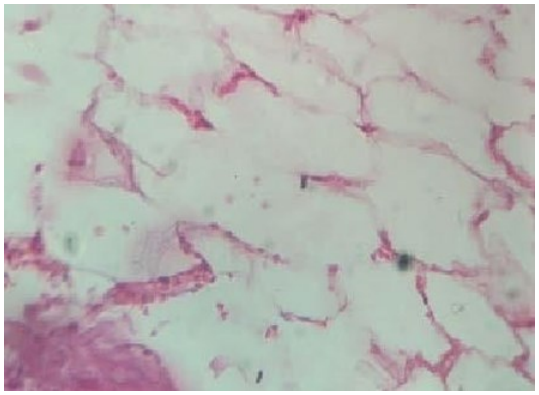

Figure 2: Adipose tissue obtained from rabbits fed nine weeks of high fat diet without metformin treatment (E.H. 40X).

The presence of monocytes in adipose tissue was verified by immunohistochemical techniques, in which monocytes were observed in the space between adipocytes, as shown in Fig. 3.

Histological analysis of biopsies of adipose tissue obtained from rabbits subjected to a high-fat diet and subsequent metformin treatment showed an increase in the size of the space between the adipocytes, which is also consistent with the reported degeneration of this tissue when an organism is subjected to treatment with this drug. The absence of monocytes within the spaces between the adipocytes is also observable.

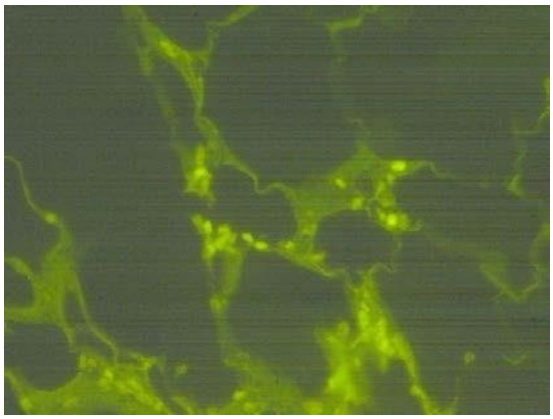

Figure 3: Evidence of monocytes in adipose tissue during obesity (Immunofluorescence, 40X).

Conclusions

By inducing obesity in rabbits and subsequent treatment with metformin in one of them, it has been possible to observe the presence of monocytes in adipose tissue, as well as a reduction in adipocyte size and the absence of monocytes at least in histological studies, which is congruent with the effects reported with the use of metformin in fat tissue. The reduction of adipocyte size and the absence of monocytes at least in histological studies is congruent with the reported effects of metformin use in adipose tissue. Such studies may be of great importance for the further study of the inflammatory reaction at molecular levels and offer new and better treatments for conditions that afflict large numbers of people such as obesity and its complications.

Conflict of Interest

The authors declare no conflict of interest.

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