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Expression and regulation of chemerin during rat pregnancy

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ABSTRACT

Background: Chemerin is an adipocytokine that is expressed in different fat deposits and has been shown to play an important role in adaptive and innate immunity due to its activity as a chemoattractant. Chemerin acts as a ligand for the G protein-coupled receptor chemokine - like receptor 1 (CMKLR1). Chemerin has been shown to regulate the development and metabolic function of adipocytes, liver and muscle tissue.
Objective: There is evidence indicating that several adipocytokines play an important role in placenta. This study aimed to investigate the regulation of chemerin in rat and human placentas throughout gestation.
Design and setting: Chemerin was examined in rat and human placentas using immunohistochemistry. The chemerin expression pattern in the placenta and adipose tissue of female Sprague Dawley rats on days 12, 16, 19 and 21 of gestation (each of these days represents a group of 12 rats) was determined using TaqMan probe-based quantitative real-time PCR. Rat chemerin serum levels were analyzed with ELISA on days 8, 12, 16, 19 and 21 and compared to virgin controls.
Results: Chemerin expression was detected in the cytoplasm of rat placental trophoblastic cells and third trimester human placental cytotrophoblast and Hofbauer's cells. The serum chemerin levels of rats decreased significantly as gestation progressed. Furthermore, placental chemerin mRNA levels rose significantly at day 16 of gestation and decreased significantly towards the end of the gestation period.
Conclusion: Taken together, this data suggests that chemerin may be an important regulator of maternal-fetal metabolism and metabolic homeostasis during pregnancy.

1. Introduction

Adipose tissue is one of the largest metabolically active endocrine organs, in which a variety of cytokines known as adipocytokines, are synthesized [1]. Chemerin is a recently identified chemoattractant adipocytokine that acts through the G protein-coupled receptor chemokine-like receptor 1 (CMKLR1). It is expressed mainly by plasmacytoid dendritic cells, macrophages, natural killer cells, and adipocytes, promoting the recruitment of these cells to lymphoid organs and sites of injury [2–5]. Circulating concentrations of chemerin are altered in inflammatory states [3,6,7]. These levels are significantly correlated with dyslipidemia and hypertension, both characteristic features of metabolic syndrome [7]. In addition, serum levels of chemerin correlate significantly with other known factors that are associated with metabolic syndrome, such as TNF-α, IL-6, and C reactive protein [8].

Additionally, chemerin plays an important role in metabolic regulation. High levels of chemerin and CMKLR1 expression have been detected in mouse and human adipocytes and other tissues, suggesting it exerts endocrine, autocrine, and paracrine activities [9,10]. Serum levels of chemerin are decreased substantially by the reduction of its expression in adipose tissue [11]. Previous studies have shown that 3T3-L1 adipocyte cultures secrete chemerin and trigger the activation of signaling pathways not only in white

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adipose tissue but also other CMKLR1–expressing cells [11]. Furthermore, expression and secretion of chemerin increases significantly during adipocyte differentiation [3,6]. Additionally, it has been demonstrated that chemerin increases insulin induced glucose uptake by 3T3-L1 cells [10]. These results confirm that white adipose tissue is a source and target for chemerin, a mediator of the inflammatory signaling pathway. Moreover, chemerin is involved in the regulation of adipose tissue insulin sensitivity [10]. A significant correlation was reported between serum chemerin levels and the proinflammatory cytokines IL-6, TNF-α, and C reactive protein [3,12]. High serum levels are found in patients with morbid obesity, correlating with insulin resistance and its decrease after bariatric surgery [11]. These levels also correlate positively with body mass index, triglyceride concentration, and blood pressure, making chemerin an important target in the study of metabolic diseases [13].

The highest levels of chemerin expression have been detected in visceral, epididymal and brown adipose tissue, and liver tissue [10]. It has been shown that in *db*/*db* mice, the expression of chemerin in adipose tissue is significantly lower compared to wild type controls [10]. Also, different adipocytokines such as leptin, adiponectin, apelin, visfatin, and resistin exert their action on adipose and muscular tissues [1,14]. They are produced and regulated in maternal adipose tissue and the placenta throughout pregnancy with a characteristic expression pattern, and they play an important role in both maternal and fetal metabolic regulation during pregnancy [15]. However, the function of many other recently discovered adipocytokines, such as chemerin, during pregnancy is not well known.

Given that many adipocytokines play an important role in reproduction and that gestation is a hypermetabolic state with a marked increase in food intake, body weight and profound alterations in insulin sensitivity, it was hypothesized that chemerin expression would be affected throughout gestation and by the changes in nutritional status during this period. Thus, the objective was to compare chemerin expression in pregnant rats with and without feeding restriction as well as in controls. Furthermore, expression of chemerin in human placenta was also observed. This study will contribute to the knowledge on the possible role of chemerin in the control of maternal and fetal metabolism throughout gestation.

2. Methods and procedures

2.1. Animals

Female Sprague Dawley rats weighing 250–300 g (10–12 weeks old) were bred in the *Animalario General USC*. The animals were housed in open cages under standard conditions of controlled illumination (12- hour light–dark cycles), at a constant temperature (20–22 °C) and humidity, and with unrestricted access to food and water. The experiments were carried out in accordance to the European Union Laws regarding the protection of laboratory animals after previous approval by the Ethics Committee of the Universidad de Santiago de Compostela.

2.2. Experimental setting

The study of the expression and regulation of mRNA levels of chemerin in placenta, adipose tissue, and the maternal serum chemerin levels during gestation was performed in female rats (70–84 days old). Timed matings were performed on female rats by monitoring their oestrus stage before introducing the males and a stable pattern of vaginal cyclicity was established during a 6 day baseline period. The rats were mated overnight and confirmation of the first day of gestation was determined by the positive presence of spermatozoa after a vaginal smear.

These pregnant rats were randomly assigned to the following experimental dietary groups and housed individually: 1) five groups ($n = 12$ rats/group) of pregnant rats fed *ad-libitum*, with a complete group of rats sacrificed at the end of days 8, 12, 16, 19, and 21 of gestation. 2) Five groups ($n = 12$ rats/group) of pregnant rats subjected to food restriction, also sacrificed in groups at the end of same days previously described. The group of restricted pregnant rats was fed with 30% of the *ad-libitum* intake as previously described [16,17]. A control group of aged matched virgin rats ($n = 12$ rats) was fed *ad–libitum* with a standard rodent's diet. At the end of each period, pregnant and virgin rats were sacrificed in a separate room and the trunk blood sample was collected as previously described [17]. The blood samples were centrifuged at 1500 g for 10 min and the serum harvested and stored at −80 °C until an analysis could be performed. Tissues were collected, frozen at −80 °C, and stored for later molecular analysis.

2.3. Serum chemerin determination

The chemerin levels in rat serum were measured systematically using a commercially available ELISA kit per the manufacturer's instructions (Uscn Life Science Inc. Wuhan -E90945Ra). Serum samples were tested in duplicate within one assay, and the results were expressed in terms of the rat chemerin standard (pg/mL). This assay was specific for the detection of rat chemerin. No significant cross-reactivity or interference between rat chemerin and analogues was reported by the manufacturer. For the chemerin assay, the intra-assay and inter-assay coefficients of variation were 5% and 10% respectively with a detection range for chemerin of 15.6–1000 pg/mL.

2.4. Chemerin immunohistochemistry

Chemerin immunoreactivity was analyzed in embedded paraffin sections of adipose and placental tissues in rats and humans as previously described [18]. Rat placenta at day 19 of gestation and normal human placentas of the third trimester of gestation were immunostained for chemerin. Paraffin-embedded blocks of normal human adipose, placental and testicular seminoma tissues were obtained from the files of the Pathology Department, Universidad Nacional de Colombia. A TIG2 (N-13) antibody was utilized for the detection of chemerin in the tissue samples described. This antibody, which is directed against a peptide near the N-terminus of TIG2 of human origin, is recommended for detection of TIG2 rat and human (Catalog # sc-47483-TIG2 (N-13): Santa Cruz Biotechnology, Inc).

2.5. RNA isolation and real time semi-quantitative RT-PCR

Total RNA was extracted from these rat tissues using TRIzol (Invitrogen, Life Technologies) according to the manufacturer's recommendations. Two micrograms of total RNA was reverse transcribed to obtain the first-strand cDNA synthesis using SuperScript III (Invitrogen) as previously described [19]. The cDNA served as the template for conventional RT-PCR. The PCR reaction was realized in the Thermal Cycle 1000C BIORAD, under the following conditions: 30 cycles of denaturation at 92 °C for 30 s, annealing at 62 °C for 30 s, and 72 °C for 1 min followed by a final extension of 10 min.The real-time PCR (Applied Biosystems 7500/7500 Fast Real - CA, USA) used specific primers and probes for chemerin (Table 1). Real-time PCR was also employed for the analysis of rat hypoxanthine phosphoribosyl transferase mRNA as a control housekeeping gene with the conditions previously described [19]. Relative amounts of the target gene were determined using the comparative delta–delta CT method as described elsewhere [19]. Each experimental group consisted of twelve rats [20].

2.6. Statistical analysis

Data were analyzed using the STATA 10.0 software. Data distribution was tested using the Shapiro–Wilk test. Data were normally distributed and the results are represented as mean ± SEM ($n = 12$ rats/group). Statistical comparisons between groups were estimated by Student's *t*-test (when two groups were compared) or ANOVA and post hoc Bonferroni test (when more than two groups were compared). A p value <0.05 was considered to be statistically significant.

3. Results

3.1. Chemerin expression in human and rat tissues

The expression of chemerin was screened using RT-PCR in visceral and epididymal adipose tissue, placenta, and other tissues as observed in Fig. 1. Additionally, the expression of chemerin in

Table 1
Primers and probe sets used in this study.

Name	Sequence	GenBank accesion number
Chemerin FW	5′-TGTGCAGTGGGCCTTCCA-3	NM_001013427
Chemerin Rv	5′-CAAAGGTGCCAGCTGAGAAGA-3′	
Chemerin Pb	5′-AGA TCG GTG TGG ACA GTG CTG ATG ACC TG-3′	