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Short communication

Ex vivo analysis of serum chemerin activity in murine models of obesity

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ABSTRACT

Objectives: Chemerin is an adipokine with established roles in inflammation, adipogenesis and the regulation of glucose and lipid homeostasis. Extracellular proteolytic processing of chemerin generates a spectrum of isoforms that differ significantly with respect to the ability to activate the cognate receptors chemokine-like receptor 1 (CMKLR1) and G-protein-coupled receptor 1 (GPR1). Increased total serum chemerin has been widely reported in obese humans as well as in preclinical rodent models of adiposity. However, very little information is available regarding the correspondence, if any, of changes in total serum chemerin protein with chemerin bioactivity.
Methods: Total serum chemerin and *ex vivo* CMKLR1 and GPR1 activation was compared using two widely used murine obesity models: high fat diet feeding (HFD) and leptin deficiency (ob/ob).
Results: Total serum chemerin levels and *ex vivo* CMKLR1 and GPR1 activation were significantly induced in HFD. The bioactivity ratio (bioactive chemerin/total chemerin) was also increased when measured with CMKLR1, but not GPR1. In contrast, while ob/ob mice exhibited increased total serum chemerin protein, *ex vivo* receptor activation was observed with GPR1, but not CMKLR1. There was no change in bioactivity ratio for either receptor. Of note, GPR1 but not CMKLR1 bioactivity positively correlated with adipose tissue inflammation.
Conclusions: While increased total serum chemerin is a consistent finding among rodent obesity models, this may not accurately reflect changes in chemerin bioactivity which is the major determinant of biological effects.

1. Introduction

Chemerin is produced primarily by adipocytes and hepatocytes as an inactive precursor. It is subsequently processed by extracellular proteases to generate several products with a wide spectrum of biological activity. In particular, several C-terminally truncated chemerin isoforms are produced which vary in their ability to activate the chemokine-like receptor 1 (CMKLR1) and/or G-protein coupled receptor 1 (GPR1) [1–3]. Binding of human chemerin (Chem-157, a highly active chemerin isoform) to CMKLR1 and GPR1 induces the recruitment of β-arrestin 1 and 2 [2]. RhoA and rho-associated protein kinase are involved in the activation of serum response factor-mediated pathways by both receptors [4]. The biologic function and signaling properties of further chemerin isoforms have received considerably less study.

Chemerin is an attractant for immune cells, such as dendritic cells and macrophages, and has established roles in the regulation of adipogenesis, lipid and glucose metabolism [1,3]. In concert with the function of chemerin in metabolic processes its systemic levels are rised in human and murine obesity [1,3]. Almost all studies quantify chemerin by Enzyme-linked Immunosorbent Assays (ELISAs), which usually do not distinguish the different isoforms [1,3,5]. Thus, it has not been resolved whether higher chemerin serum concentrations are accompanied by increased levels of active chemerin isoforms and activation of CMKLR1 and/or GPR1. Total chemerin protein levels are not correlated with CMKLR1 activation in 3T3-L1 adipocytes upon exposure to tumor necrosis factor [6]. Circulating chemerin is mostly derived from adipocytes [1,3,5] and discrepancies between total and activated chemerin may also exist in the blood compartment. Indeed, activation of CMKLR1 is comparable in lean and obese women while total chemerin levels are increased in serum of the latter [5]. In plasma of obese patients short chemerin isoforms are elevated which do most likely not activate CMKLR1 and GPR1 [7,8]. To assume higher activation of chemerin receptors from increased total chemerin protein is, therefore, not justified.

The aim of the present study was a comparative analysis of obesity-induced changes in total serum chemerin protein levels with serum

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chemerin bioactivity as determined by *ex vivo* CMKLR1 or GPR1 activation. Analysis of total chemerin protein was done to confirm higher levels in the obesity models used. Activity of CMKLR1 and GPR1 using the corresponding sera was normalized to total chemerin protein to evaluate whether both measures are accordingly changed. Two widely employed preclinical rodent models of obesity were used: HFD feeding and leptin-deficiency [9].

2. Materials and methods

2.1. Animals

Male mice (Charles River Laboratories, Sulzfeld, Germany) had free access to food and water and were housed in a 21 ± 1 °C controlled room under a 12 h light-dark cycle. Rising concentrations of CO_2 produced loss of consciousness and mice were killed by cervical dislocation. Procedures complied with the German Law on Animal Protection and the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals, 1999. Experiments were conducted according to institutional and governmental regulations for animal use (Government of the Oberpfalz). Fourteen-week-old C57BL/6 mice were kept on a high fat diet (ssniff® EF R/M, D12451, 42% of energy from fat) or standard chow (ssniff® EF acc. D12450B (I) mod.) for 14 weeks. Leptin deficient ob/ob mice and the respective control mice were killed at an age of 13 weeks. The mRNA expression of F4/80 in adipose tissues of these mice has been already published [10]. Analysis of serum glucose, insulin, triglycerides and cholesterol was done as described [11].

2.2. Quantification of (total) serum chemerin

ELISA to measure murine chemerin was from R&D Systems (Wiesbaden-Nordenstadt, Germany), and was performed as recommended by the distributor. Mouse serum was diluted 1000-fold for chemerin analysis.

2.3. Measurement of CMKLR1 and GPR1 activity

This method is described in detail in Supplementary methods.

2.4. Statistics

Data are shown as box plots (median, lower and upper quartiles and range of the values). Statistical tests used were two-tailed Mann-Whitney U Test and Spearman correlation (SPSS Statistics 19.0 program, IBM, Leibniz Rechenzentrum, Munich, Germany) and a value of $p < 0.05$ was regarded as significant.

3. Results

3.1. C57BL/6 mice on a high fat diet

The median body weight of the mice fed the standard diet (SD) was 27.3 (25.0–30.5) g and 37.7 (31.7–40.3) g in the HFD group. In the latter group median serum chemerin was 224.5 (129.4–321.9) ng/ml compared to 121.9 (106.1–186.0) ng/ml in the mice fed the SD ($p = 0.015$). In line with previous findings, serum total chemerin protein was elevated approximately 1.8-fold in the HFD group [1,3]. Serum chemerin bioactivity (Chemerin156-equivalents) as determined by *ex vivo* CMKLR1 activation was 14.8 (11.9–19.1) nM for mice fed a SD and 33.8 (23.7–49.2) nM for the HFD group, and was approximately 2.3-fold higher in the overweight mice (Fig. 1A). Further, serum chemerin bioactivity as determined by *ex vivo* GPR1 activation was 26.0 (22.3–36.3) nM and 38.8 (33.0–45.7) nM in animals fed a SD or HFD, respectively, and was increased 1.5-fold in the serum of the overweight animals (Fig. 1B). Moreover, the chemerin bioactivity ratio (bioactive chemerin/total chemerin) as measured by CMKLR1 activation was significantly higher in the overweight mice compared to the lean mice (Fig. 1C). However, no differences in bioactivity ratio were observed between the groups when GPR1 activation was used for analysis (Fig. 1D). Thus, while high fat feeding increased CMKLR1 bioactivity beyond the assumed activity reflected by total chemerin protein levels, GPR1 activation increased proportionally to total circulating chemerin protein.

3.2. Leptin deficient ob/ob mice

In a second experiment chemerin, CMKLR1 and GPR1 activation were analyzed in the leptin deficient ob/ob mice which suffer from extreme adiposity (median body weight was 51.6 (47.4–55.4) g compared to 23.2 (21.6–27.4) g of the control animals). Similar to the HFD mice, obesity in this model was associated with approximately 1.6-fold higher median serum chemerin protein levels as described previously [12]. However, serum chemerin bioactivity as determined by *ex vivo* CMKLR1 activation was 22.4 (18.4–28.1) nM and 26.5 (13.1–36.4) nM (Fig. 2A) and was not different between the lean and obese groups. In comparison, serum chemerin bioactivity as determined by *ex vivo* GPR1 activation was 39.6 (27.7–50.0) nM for the lean and 59.8 (37.3–63.5) nM for the ob/ob mice. However, this apparent 1.5-fold increase of serum chemerin bioactivity for the obese mice was not significant ($p = 0.056$; Fig. 2B). In contrast to diet induced obesity, leptin-deficiency was not associated with an increased chemerin bioactivity ratio in the obese group as measured by *ex vivo* CMKLR1 activation (Fig. 2C). Moreover, no differences in bioactivity ratio were observed between the groups when GPR1 activation was used for analysis (Fig. 2D).

3.3. Correlation analysis

Associations of systemic chemerin with insulin resistance and circulating lipids have been described [13,14]. In mice fed the HFD total serum chemerin, CMKLR1 and GPR1 activity did not correlate with serum glucose, insulin, homoeostasis model of assessment of insulin resistance, triglycerides or cholesterol (data not shown). In the ob/ob mice there was no association of total serum chemerin, CMKLR1 or GPR1 activity with systemic triglycerides or cholesterol (data not shown).

Adipose tissue inflammation is a hallmark of obesity [3]. The mRNA expression of the macrophage marker F4/80 was induced in subcutaneous fat of ob/ob mice [10] and was positively correlated with total chemerin protein ($r = 0.817$, $p = 0.004$) (data not shown). While *ex vivo* serum GPR1 activity was associated with F4/80 mRNA ($r = 0.884$, $p = 0.001$) the correlation with CMKLR1 activity was not significant ($r = 0.537$, $p = 0.110$) (Fig. 2E and F).

4. Discussion

The present analyses demonstrate that total serum chemerin protein does not consistently correspond with the more biologically meaningful measure of chemerin bioactivity. In the context of diet induced obesity, the relative increase of *ex vivo* CMKLR1 activation exceeded the rise in total serum chemerin protein levels as reflected by the increased bioactivity ratio. In contrast, while ob/ob mice exhibited similar increases in total serum chemerin protein compared to obese HFD mice, there was no significant increase in *ex vivo* CMKLR1 activation and a trend towards a decreased bioactivation ratio. In both models of obesity, ex vivo activation of GPR1 generally corresponded with the increase of serum total chemerin protein resulting in similar bioactivity ratios.

Chemerin-157, the human homolog of murine Chemerin-156, is generated by the proteolytic cleavage of the six C-terminal amino acids from prochemerin. Overall, this isoform exhibits the highest bioactivity, followed by Chemerin-155 and -158 which are considered to have low