FGF receptor antagonism does not affect adipose tissue development in nutritionally induced obesity

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Introduction

In early stage development of adipose tissue, adipogenesis is tightly associated with angiogenesis.1 It is widely accepted that vascular endothelial growth factor (VEGF) accounts for much of the angiogenic activity of adipose tissue.1-3 However, also the fibroblast growth factor (FGF)–FGF receptor (FGFR) signaling axis is important for vascular development. FGFs are proteins with diverse functions in development, repair, and metabolism. The human FGF gene family with 22 members can be classified into three groups, canonical, intracellular, and hormone-like Fgf genes. Canonical FGFs mediate their biological responses as extracellular proteins by binding to and activating cell surface tyrosine kinase Fgf receptors (FGFRs) with heparin/heparan sulfate as a cofactor. They act as local signaling molecules in an autocrine/paracrine manner. In the development of multicellular organisms, various signaling pathways are activated in a highly coordinated manner to ensure proper morphogenesis.4 FGF is pro-angiogenic and enhances adipocyte differentiation in vivo.5 FGF-2 is expressed in adipose tissues of mice kept on normal chow or on high fat diet.3 Moreover, administration of a FGFR1 blocking antibody to rodents and monkeys resulted in rapid dose-dependent weight loss, induced primarily by decreased energy intake, suggesting that blocking FGF signaling through FGFR1 (c-splice form) may lead to weight loss.6

Low molecular weight synthetic molecules have been derived that potently and selectively block the FGFR system upon oral administration. SSR128129E (SSR) binds to the extracellular part of the receptor. It does not compete with FGF for binding to FGFR but inhibits FGF-induced signaling linked to FGFR internalization in an allosteric manner.7 Therefore, the rationale for the present study was to investigate whether SSR affects adipose tissue related angiogenesis and fat development in a murine model of nutritionally induced obesity.

Results

A preliminary experiment revealed that administration of SSR was associated with markedly reduced food intake (1.8 ± 0.09 vs. 3.2 ± 0.19 g/mouse/day; n = 5). Therefore, the food of the mice, receiving placebo was restricted to the amount consumed by the group receiving SSR (pair-feeding).

SC and GON adipose tissue of mice treated with SSR showed a significantly decreased ratio of P-ERK/ERK expression as compared with placebo treatment (Fig. 1). HPLC analysis confirmed detectable levels of SSR in extracts of treated adipose tissues (about 4 ng/mg SC or GON fat).

Compared with pair-fed mice on HFD, the evolution of body weight and fat mass was comparable under SSR treatment (Table 1). The weight of main organs was not affected by SSR treatment (data not shown). Administration of SSR resulted in enhanced adipocyte size and lower density in GON but not in SC adipose tissue. Blood vessel size in SC and GON fat was comparable in both groups, whereas blood vessel density was lower in the GON adipose tissue of inhibitor treated mice. However, no differences were observed in the normalized blood vessel density
SSR inhibits fibroblast growth factor receptor signaling in adipose tissue of mice kept on high fat diet. (A) Expression of P-ERK and ERK in subcutaneous (SC) and gonadal (GON) adipose tissue of mice treated with SSR or placebo normalized to β-actin expression and shown as ratio P-ERK/ERK. Data are means ± SEM of 4 extracts for each condition; *P < 0.05 vs. placebo-treated mice. (B) Representative western blots of P-ERK and ERK both shown as bands of 42 kDa and 44 kDa. Lane M represents the protein calibration ladder.

**Figure 1.** SSR inhibits fibroblast growth factor receptor signaling in adipose tissue of mice kept on high fat diet. (A) Expression of P-ERK and ERK in subcutaneous (SC) and gonadal (GON) adipose tissue of mice treated with SSR or placebo normalized to β-actin expression and shown as ratio P-ERK/ERK. Data are means ± SEM of 4 extracts for each condition; *P < 0.05 vs. placebo-treated mice. (B) Representative western blots of P-ERK and ERK both shown as bands of 42 kDa and 44 kDa. Lane M represents the protein calibration ladder.

Table 1. Effect of 5-week SSR treatment on adipose tissue weight and composition of mice fed a high fat diet for 8 weeks

|                      | Placebo (n = 9) | SSR (n = 10) |
|----------------------|----------------|-------------|
| **Body weight (g)**  |                |             |
| Start                | 18 ± 0.74      | 18 ± 0.70   |
| End                  | 24 ± 0.50      | 25 ± 0.70   |
| **SC fat**           |                |             |
| Weight (mg)          | 472 ± 48       | 510 ± 57    |
| Adipocyte size (μm²) | 2530 ± 225     | 2670 ± 280  |
| Adipocyte density (×10³/μm²) | 430 ± 48 | 450 ± 49 |
| Blood vessel size (μm²) | 66 ± 8.3      | 57 ± 3.5    |
| Blood vessel density (×10³/μm²) | 550 ± 75 | 560 ± 65 |
| **GON fat**          |                |             |
| Weight (mg)          | 590 ± 100      | 744 ± 69    |
| Adipocyte size (μm²) | 3100 ± 200     | 4150 ± 470* |
| Adipocyte density (×10³/μm²) | 350 ± 27 | 270 ± 16* |
| Blood vessel size (μm²) | 74 ± 8.4      | 68 ± 5.1    |
| Blood vessel density (×10³/μm²) | 490 ± 53 | 340 ± 15* |

Data are means ± SEM of n experiments. SC, subcutaneous GON, gonadal; *P < 0.005 vs. pair-fed mice receiving placebo.

mice inhibits angiogenesis, inflammation, and bone resorption in arthritis, and delays tumor growth and metastasis. In our study, oral administration of SSR (30 mg/kg per day) to wild-type mice effectively inhibited FGF signaling in adipose tissues, as confirmed by a decreased P-ERK/ERK expression ratio. It was previously shown that SSR inhibits FGF-2 driven activation of ERK1/2 in vitro.

In order to investigate whether SSR may affect regulation of appetite or of energy intake or expenditure by affecting hypothalamic gene expression, we have used a DNA microarray that profiles the expression of 84 genes encoding known orexigenic or anorectic peptides, hormones, and receptors, as well as central and peripheral signaling molecules involved in energy expenditure. Using this approach we have not been able to identify a potential target for SSR in the hypothalamus.

When mice were pair-fed, no significant effect of SSR was observed on body weight or SC or GON fat mass. The effect of the inhibitor on adipocyte or blood vessel size and density was divergent. Adipocytes were larger in GON, but not in SC, adipose tissue of mice treated with the inhibitor, associated with lower adipocyte density. Our data thus do not indicate a marked effect of SSR on adipocytes, but effects on stromal–vascular cells cannot be excluded. Blood vessel size or normalized blood vessel density were not affected. The lack of effect of SSR on adipose tissue angiogenesis was further supported by comparable levels of endoglin (CD105) in SC adipose tissue of both groups, whereas these were apparently higher in SSR-treated as compared with placebo-treated GON adipose tissues. It should be noted, however, that endoglin levels in placebo-treated GON fat were 2-fold lower than in SC fat. Endoglin is a type 1 transmembrane glycoprotein that is predominantly expressed on proliferating endothelial cells and angiogenic blood vessels in vivo. Differences in characteristics of SC and GON fat have been reported previously.

Discussion

Previous studies have suggested the potential to impair adipose tissue development by inhibition of angiogenesis. Because of the emerging role of FGF in promoting adipose tissue related angiogenesis, it may represent an attractive target to affect obesity. In the present study we have investigated the effect of SSR, a low molecular weight FGFR inhibitor, on adipose tissue-related angiogenesis and fat development in a murine model of diet-induced obesity. Binding of SSR induces a conformational change of the receptor, which blocks FGFR signaling by inhibiting ERK1/2 activation. In vivo, orally administered SSR to
Table 2. Effect of 5-week SSR treatment on metabolic parameters of mice fed a high fat diet for 8 weeks

|                     | Placebo (n = 9) | SSR (n = 10) |
|---------------------|----------------|-------------|
| Glucose (mg/dl)     | 110 ± 15       | 180 ± 10*   |
| Total cholesterol (mg/dl) | 120 ± 6.4     | 170 ± 11*   |
| HDL (mg/dl)         | 97 ± 12        | 165 ± 11**  |
| LDL (mg/dl)         | 21 ± 1.6       | 33 ± 32.9*  |
| Triglycerides (mg/dl) | 39 ± 7.3       | 44 ± 4.3    |
| AST (U/l)           | 158 ± 21       | 148 ± 21    |
| ALT (U/l)           | 95 ± 19        | 98 ± 18     |
| Leptin (ng/ml)      | 9.1 ± 2.0      | 10 ± 2.4    |
| Insulin (ng/ml)     | 3.2 ± 1.0      | 3.3 ± 0.34  |

Data are means ± SEM of n experiments. *P < 0.005; **P < 0.001 vs. pair-fed mice receiving placebo. ALT, alanine transferase; AST, asparagine transferase.

and may be due to different patterns of protein expression. Comparison of metabolic parameters revealed enhanced levels of glucose and of total, HDL, and LDL cholesterol upon inhibitor treatment. It was previously shown that suppressed ERK activation in the liver contributes to alcohol-induced hypercholesterolemia in rats, indicating a relation between ERK signaling and plasma cholesterol levels.

In conclusion, our data do not demonstrate a marked effect of SSR on adipose tissue development or on adipose related angiogenesis in mice kept on HFD for 8 weeks.

Materials and Methods

Animal model

Five-week-old male wild-type (WT) mice (genetic background 100% C57Bl/6) were kept on a high fat diet (HFD; TD88137, Zeist, containing 42% kcal as fat, with a caloric value of 20.1 kJ per g) for 3 weeks, as described previously. Then, one group of mice (n = 10) was given SSR during 5 weeks at a dose of 30 mg/kg/day by oral gavage, and the other group (n = 9) received the same volume of solvent (0.6% methylcellulose, placebo). Based on preliminary experiments, the food of the mice receiving placebo was restricted to the amount consumed by the group receiving SSR (pair-feeding).

At the end of the experiment, the mice were killed by i.p. injection of 60 mg/kg sodium pentobarbital (Abbott Laboratories). Intra-abdominal (gonadal, GON) and inguinal subcutaneous (SC) fat pads were removed and weighed; portions were snap-frozen in liquid nitrogen for protein extraction and paraffin sections (10 μm) were prepared for histology.

All animal experiments were approved by the KU Leuven ethical committee.

Assays

The number and size of adipocytes and blood vessels in adipose tissue sections was determined by computer-assisted image analysis. Blood vessel density was also normalized to the adipocyte density.

Leptin, endoglin (R&D Systems Europe), and insulin (Merodia) levels were determined with commercially available ELISAs.

Expression in the hypothalamus of 84 genes known to be related to obesity was monitored using the Mouse Obesity RT2 Profiler™ PCR Array (SA Bioscience, Qiagen B.V.).

Protein extracts (50 μg) from subcutaneous (SC) and gonadal (GON) adipose tissue were used to monitor ERK and Phospho (P)-ERK expression by western blot analysis.

Statistical analysis

Data are reported as means ± SEM. Statistical significance is analyzed by non-parametric Mann–Whitney U test. Values of P < 0.05 are considered statistically significant.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Author Contributions

I.S. performed experiments, analyzed data, and generated the figures. C.V. performed experiments. H.R.L. was involved in the study design and data interpretation. All authors were involved in writing the paper and had final approval of the submitted manuscript.

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