Overexpression of leptin mRNA in mesenteric adipose tissue in inflammatory bowel diseases

Maryse BARBIER (1), Hubert VIDAL (2), Pierre DESREUMAUX (3), Laurent DUBUQUOY (3), Arnaud BOURREILLE (1), Jean-François COLOMBEL (3), Christine CHERBUT (1), Jean-Paul GALMICHE (1)

(1) Pôle Digestif et UC-INSERM, CHU Nantes et INRA, 44033 Nantes Cedex 1; (2) INSERM U549, Faculté de Médecine Librairie, 63373 Lyon; (3) Laboratoire de Recherche sur les Maladies Inflammatoires Hépatiocoliques, INSERM U114, CHU Nantes, 59037 Lille.

SUMMARY

Background — Leptin, a protein with a cytokine-like structure, is produced predominantly by adipocytes. It appears to play a key role in immune responses by increasing the secretion of Th1 and pro-inflammatory cytokines. As fat-wrapping is a characteristic feature of Crohn’s disease (CD), and as increased leptin levels have been reported in animal models of intestinal inflammation, this study investigated whether mesenteric adipose tissue could be a source of leptin in human inflammatory bowel disease (IBD).

Aim — To quantify the expression of leptin mRNA in mesenteric adipose tissue of patients with CD or ulcerative colitis (UC).

Methods — Specimens were obtained from mesenteric white adipose tissue of patients with CD or ulcerative colitis (UC).

Results — Leptin mRNA levels were significantly higher in mesenteric adipose tissue of CD and UC patients than in controls (P < 0.05). In CD and UC, concentrations were not significantly different in mesenteric fat specimens, whether contiguous to macroscopically normal or grossly abnormal intestine.

Conclusions — This study provides the first evidence of a novel abnormality of the mesentery of patients with IBD. Overexpression of leptin mRNA in mesenteric adipose tissue may contribute to (a) the inflammatory process, (b) enhancement of mesenteric TNFα expression in CD (as recently reported), and/or (c) the anorexia frequently reported during flares of IBD.

Leptin (the protein product of the ob gene) is an adipocyte-secreted hormone that regulates the size of adipose tissue mass [1]. It acts on specific neuronal targets in the hypothalamus and thereby controls body weight homeostasis, reducing food intake and increasing the metabolic rate [2, 3]. Moreover, clear relationships exist between leptin and cytokines.

The primary sequence of leptin is compatible with a cytokine-like structure [4, 5]. The long isoform of the leptin receptor Ob-Rb bears homology to members of the cytokine receptor superfamily [6-8]. Accordingly, in vivo and in vitro studies have indicated that leptin is implicated in immune responses. Leptin enhances the secretion of pro-inflammatory cytokines (TNF-α, IL-6 and IL-12) by monocytes and macrophages [9, 10] and promotes CD4+ helper T-cell (Th) activity in vitro, which orchestrates most immune responses. In particular, leptin increases Th1 (IFN-γ and IL-2) and suppresses Th2 (IL-4) cytokine production [11].

In Crohn’s disease (CD), mesenteric adipose tissue displays well-recognised hallmarks, such as thickening, stiffness, hypertrophy and fat-wrapping [12, 13], and has been recently shown to express high levels of TNFα [14]. An abnormal expression pattern has been described for PPARγ [14], a member of the nuclear hormone receptor family which is predominantly expressed in adipocytes and involved in adipogenesis [14, 15]. As PPARγ is also a well-known regulator of leptin gene expression [16-18], we hypothesised that leptin could be involved in mesenteric abnormalities. In fact, our previous studies in models of intestinal inflammation in rodents showed a transient increase of plasma leptin levels during the early stages of inflammation [19]. However, controversial results have been published about plasma leptin levels in human inflammatory bowel disease (IBD) [20-22], which suggest that they may be affected by various factors not necessarily reflecting changes in mesenteric adipose tissue. In this context, the present study focused on direct quantification of leptin mRNA expression in the mesentery of patients with either CD or UC (ulcerative colitis) and in control patients.

Patients and methods

Patients and samples

All patients gave their informed consent, and the study was approved by the Ethics Committee of the Huriez Hospital in Lille, France. Study subjects were of normal weight and without diabetes mellitus. The diagnosis of CD and UC was established using previously published criteria [23]. All patients with perforating IBD. Twenty-six patients were included in the study. Fourteen with CD (9 females, 5 males; mean age 31 years old; body mass index (BMI) 22 kg/m²) underwent right ileocolonic resection because of symptomatic ileal stenosis with transmural inflammation. They had not previously received any specific drug therapy. Five subjects had colonic resection for UC (15 females, 1 male; mean age 33 years old; BMI 22 kg/m²) after failure of a short-course of steroids and/or cyclosporin. Seven subjects with carcinoma of the right colon (4 females, 3 males; mean age 73 years old) served as controls. None of these control subjects was obese, but no data on their height were available for BMI calculations.

All patients underwent surgery after an overnight fast. The first step in the surgical procedure consisted of taking biopsy specimens from mesenteric white adipose tissue. As far as possible, fat samples were obtained from mesenteric close both to inflamed and non-inflamed segments. In patients with carcinoma, mesenteric fat samples were obtained in front of normal intestine at a sufficient minimal distance from tumour. Adipose tissue samples were immediately frozen in liquid nitrogen and stored at - 80°C for subsequent mRNA analysis.

RNA analysis by reverse transcription-competitive polymerase chain reaction (RT-cPCR)

RNA preparation

Adipose tissue samples (about 200 mg of frozen tissue) were pulverised in liquid nitrogen, and total RNA was prepared from the frozen...
Rescent dye (Eurogentec, Seraing, Belgium), and the amplified products
sample indicated the high reproducibility of the assay, with a coefficient
assays for leptin mRNAs were previously described in detail [24, 25].
sequence of the primers for leptin, and the validation of the RT-cPCR
leptin mRNA was determined at the competition equivalence point, as
using an ALF-Express DNA sequencer (Pharmacia, Uppsala, Sweden)
°
°
µ
5
µ
L of leptin DNA competitor at four different known concentrations.
°
°
µ
L of water were added to the RT medium, from which 20 µL were
< 0.05.
Results
As shown in figure 1, the RT-competitive PCR used in this study was able to detect leptin mRNA in the mesentery of all IBD
and control patients. In central mesenteric fat, leptin transcripts
were expressed at remarkably (and constantly) low levels, with no value exceeding 1.3 amol/g total RNA (figure 2). In patients
with CD, levels of leptin mRNAs were significantly increased in
mesenteric adipose tissue contiguous to inflamed intestine as
compared with controls (median 2.4, n = 5, vs controls, P = 0.001),
although a slight increase was observed in CD, no statistically significant differences were detected between CD and UC patients. Increased levels were also measured in samples taken close to apparently normal segments
in CD (median 4.15 vs controls, P = 0.004) and UC (median 2.05, n = 4, vs controls, P = 0.073). Leptin mRNA concentrations
were not significantly different between mesenteric fat specimens
contiguous to normal intestine and those contiguous to the
inflamed intestine in CD and UC. Finally, no correlation was
found between mesenteric leptin mRNA levels and gender, age
or BMI in IBD patients (data not shown).
Discussion
This study using a sensitive quantitative RT-PCR approach enabled us to detect and quantify leptin mRNA expression in
mesenteric adipose tissue. Although remarkably low levels of
leptin mRNA were found in normal mesenterity, a dramatic
increase was observed in both CD and UC, with or without intact
inflammation contiguous to the fat biopsy sample.
The quantitative RT-PCR method used in this study was pre-
viously validated and used to study leptin mRNA expression in
small samples obtained from abdominal subcutaneous and vis-
ceral adipose tissues of lean, obese and type II diabetic subjects
[26, 27, 28]. As very low levels of leptin mRNA can be quantified
only minimal amounts of sample material, this is the method of
choice for investigating ob gene expression in IBD patients when
only small tissue samples can be obtained. It was previously
determined that the level of leptin mRNA is about twice as low in
omental adipose tissue as in subcutaneous abdominal fat
[27, 29, 30]. Our results confirm that the expression level of ob
gene is very low in mesenteric fat in humans. With the same
RT-PCR methodology, the mean value measured in control
mesenteric adipose tissues was about 10 times lower than in
subcutaneous abdominal fat of healthy lean subjects [24]. This
suggests that leptin, if secreted by mesenteric adipose tissue, is
only likely to increase circulating concentrations of leptin very slightly.
Increased leptin mRNA levels were observed in the
mesenteric fat of IBD patients. Obesity, which is known to be a
factor in leptin overexpression in subcutaneous and omental fats
[27], could be excluded since our IBD patients and control sub-
jects were not obese (BMI ranging from 19 to 25 in both CD and
UC). The difference of age between patients and controls
is unlikely to play a role as plasma leptin levels are not correlated
to age [31]. Interestingly, this overexpression of leptin mRNA
appeared to be non-specific since similar increases were
observed in both CD and UC. Moreover, increased expression of
leptin was observed in tissue samples contiguous to or distant
from inflamed segments, which suggests that IBD are associated
with a general increase in leptin expression in the mesentery.
The effect of previous drug therapy could be excluded because
the same findings were observed in patients who did not receive
drug therapy and those (usually UC patients) who failed to
Overexpression of leptin mRNA in mesenteric adipose tissue in inflammatory bowel diseases

responder to a previous course of steroids and immunosuppressive therapy.

Although controversial results have been published concerning plasma leptin levels in IBD [20-22], the correlation between BMI and plasma leptin is usually preserved, as in other conditions without bowel inflammation [32]. A lack of correlation between leptin mRNA levels and plasma leptin levels has already been reported [33, 34], which tends to support the approach adopted in our study. The repetition of plasma leptin assessment would not appear to be useful in clarifying the discrepancies in the literature. In our study, mesenteric leptin mRNA levels were not correlated with BMI, possibly because the subjects were all lean. This may also reinforce the notion that leptin produced by mesenteric fat does not increase the plasma level of the hormone but rather contributes to a local paracrine effect in the intestine.

What pathophysiological relevance do our findings have?

Overexpression of leptin in mesenteric fat must be interpreted in the context of other abnormalities of mesenteric tissue recently reported in both animal models and humans. Desreumaux et al. [14] found high levels of TNFα in the mesentery of CD patients. Similarly, we observed increased TNFα expression in the mesentery of indomethacin- and TNBS-induced ileitis in rats (unpublished data). This increase in mesenteric TNFα could lead to higher leptin expression in CD (but not in UC), as suggested by many studies [35-40]. Finally, given the interactions between leptin and the immune system, it would appear that leptin produced by mesenteric fat does not increase the plasma level of the hormone but rather contributes to a local paracrine effect in the intestine.

What pathophysiological relevance do our findings have?

Overexpression of leptin in mesenteric fat must be interpreted in the context of other abnormalities of mesenteric tissue recently reported in both animal models and humans. Desreumaux et al. [14] found high levels of TNFα in the mesentery of CD patients. Similarly, we observed increased TNFα expression in the mesentery of indomethacin- and TNBS-induced ileitis in rats (unpublished data). This increase in mesenteric TNFα could lead to higher leptin expression in CD (but not in UC), as suggested by many studies [35-40]. Finally, given the interactions between leptin and the immune system, it would appear that leptin produced by mesenteric fat does not increase the plasma level of the hormone but rather contributes to a local paracrine effect in the intestine.

What pathophysiological relevance do our findings have?

Overexpression of leptin mRNA in mesenteric adipose tissue in inflammatory bowel diseases

respond to a previous course of steroids and immunosuppressive therapy.

Although controversial results have been published concerning plasma leptin levels in IBD [20-22], the correlation between BMI and plasma leptin is usually preserved, as in other conditions without bowel inflammation [32]. A lack of correlation between leptin mRNA levels and plasma leptin levels has already been reported [33, 34], which tends to support the approach adopted in our study. The repetition of plasma leptin assessment would not appear to be useful in clarifying the discrepancies in the literature. In our study, mesenteric leptin mRNA levels were not correlated with BMI, possibly because the subjects were all lean. This may also reinforce the notion that leptin produced by mesenteric fat does not increase the plasma level of the hormone but rather contributes to a local paracrine effect in the intestine.

What pathophysiological relevance do our findings have?

Overexpression of leptin in mesenteric fat must be interpreted in the context of other abnormalities of mesenteric tissue recently reported in both animal models and humans. Desreumaux et al. [14] found high levels of TNFα in the mesentery of CD patients. Similarly, we observed increased TNFα expression in the mesentery of indomethacin- and TNBS-induced ileitis in rats (unpublished data). This increase in mesenteric TNFα could lead to higher leptin expression in CD (but not in UC), as suggested by many studies [35-40]. Finally, given the interactions between leptin and the immune system, it would appear that leptin produced by mesenteric fat does not increase the plasma level of the hormone but rather contributes to a local paracrine effect in the intestine.

What pathophysiological relevance do our findings have?

Overexpression of leptin in mesenteric fat must be interpreted in the context of other abnormalities of mesenteric tissue recently reported in both animal models and humans. Desreumaux et al. [14] found high levels of TNFα in the mesentery of CD patients. Similarly, we observed increased TNFα expression in the mesentery of indomethacin- and TNBS-induced ileitis in rats (unpublished data). This increase in mesenteric TNFα could lead to higher leptin expression in CD (but not in UC), as suggested by many studies [35-40]. Finally, given the interactions between leptin and the immune system, it would appear that leptin produced by mesenteric fat does not increase the plasma level of the hormone but rather contributes to a local paracrine effect in the intestine.

What pathophysiological relevance do our findings have?

Overexpression of leptin in mesenteric fat must be interpreted in the context of other abnormalities of mesenteric tissue recently reported in both animal models and humans. Desreumaux et al. [14] found high levels of TNFα in the mesentery of CD patients. Similarly, we observed increased TNFα expression in the mesentery of indomethacin- and TNBS-induced ileitis in rats (unpublished data). This increase in mesenteric TNFα could lead to higher leptin expression in CD (but not in UC), as suggested by many studies [35-40]. Finally, given the interactions between leptin and the immune system, it would appear that leptin produced by mesenteric fat does not increase the plasma level of the hormone but rather contributes to a local paracrine effect in the intestine. © 2020 Elsevier Masson SAS. Tous droits réservés. - Document téléchargé le 20/06/2020 Il est interdit et illégal de diffuser ce document.
In summary, this study is the first report of high mRNA levels of leptin in the mesenteries of patients with CD and UC. Further studies are required to determine whether this biological abnormality contributes to the inflammatory process by releasing other cytokines (or enhancing their effect) or is a factor in metabolic disturbances and denutrition.

ACKNOWLEDGEMENTS - The authors are grateful to Dr M. Laville (INSERM U449, Lyon) for assistance with RT-PCR analysis.

REFERENCES


Overexpression of leptin mRNA in mesenteric adipose tissue in inflammatory bowel diseases