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Abundance and turnover of GLP-1 producing L-cells in ileal mucosa are not different in patients with and without type 2 diabetes

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ABSTRACT

Introduction. The gastrointestinal hormone GLP-1 is released from enteroendocrine L-cells and augments postprandial insulin secretion. In patients with type 2 diabetes, the incretin effect is markedly diminished. It is unclear, whether this is due to a reduction in the abundance of L-cells in the intestine.

Methods. Ileal tissue samples from 10 patients with and 10 patients without diabetes that underwent surgery for the removal of colon tumors were included. Tissue sections were stained for GLP-1, Ki67, TUNEL and chromogranin A.

Results. The number of L-cells was not different between patients with and without diabetes in either crypts (1.81 ± 0.21% vs. 1.49 ± 0.24%, respectively; $p = 0.31$) or villi (1.07 ± 0.16% vs. 0.83 ± 0.10%, respectively; $p = 0.23$). L-cell number was higher in crypts than in villi ($p < 0.0001$). L-cell replication was detected rarely and not different between the groups. L-cell apoptosis was similar in patients with and without diabetes in both crypts (7.84 ± 2.77% vs. 8.65 ± 3.77%, $p = 0.85$) and villi (4.48 ± 2.89% vs. 8.62 ± 4.64%, $p = 0.42$). Chromogranin A staining was found in a subset of L-cells only.

Conclusions. Intestinal L-cell density is higher in crypts than in villi. Chromogranin A is not a prerequisite for GLP-1 production. L-cell density and turnover are not different between patients with and without diabetes. Thus, alterations in the number of GLP-1 producing cells do not explain the reduced incretin effect in patients with type 2 diabetes.

1. Introduction

The incretin hormone glucagon-like peptide 1 (GLP-1) is released from enteroendocrine L-cells, which are located in the gut mucosa, in response to meal ingestion [1,2]. GLP-1 primarily acts to augment postprandial insulin responses, thereby contributing to the so called "incretin effect" [3]. Furthermore, the peptide has important physiological roles in the regulation of glucagon secretion, appetite control and gastrointestinal motility and secretion [4,5]. While earlier studies have suggested that GLP-1 secreting L-cells are predominantly located in the lower intestine (ileum and colon) [6], more recent studies have revealed a rather homogeneous occurrence of L-cells throughout the intestine

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as well as a large degree of co-staining between GLP-1 and the other incretin hormone GIP within the same cells [7]. Furthermore, L-cells are known to secrete peptide YY along with GLP-1 and other proglucagon-derived peptide hormones [7].

In recent years, L-cells have gained increasing interest because of the therapeutic role of GLP-1 in type 2 diabetes [8]. Thus, a number of GLP-1 receptor agonists and inhibitors of the degrading enzyme dipeptidyl-peptidase 4 (DPP-4) have already become available for the treatment of type 2 diabetes, and various approaches to stimulate L-cell secretion (e.g. activators of G-protein-coupled receptors) are currently being explored [9].

There is also an ongoing controversy as to the role of the incretin hormones in the pathogenesis of type 2 diabetes. Thus, in patients with type 2 diabetes, the incretin effect is markedly reduced [10]. The reasons underlying this defect are still widely debated. A common view states that deficient GLP-1 secretion in patients with type 2 diabetes contributes to the loss of incretin activity in type 2 diabetes [11,12], although this finding has not been uniformly reported across all studies [13]. A recent meta-analysis has suggested that reduced GLP-1 concentrations are found especially in patients at more advanced stages of type 2 diabetes [14]. Indeed, high glucose and glucagon concentrations have been shown to diminish postprandial GLP-1 secretion [15,16].

An alternative explanation for impaired GLP-1 secretion would be a reduction in the intestinal abundance of L-cells in patients with type 2 diabetes. However, the amount of L-cells in patients with established diabetes has not yet been thoroughly examined.

Therefore, in the present study, we addressed the question (1) does the abundance of intestinal L-cells differ between patients with and without type 2 diabetes and (2) are there differences in the turnover (apoptosis and replication) of L-cells between patients with and without type 2 diabetes.

2. Patients and Methods

2.1. Study Design

Ileal tissue samples from 10 patients with type 2 diabetes and 10 healthy patients without diabetes mellitus 2 were included. Eligible patients were identified retrospectively from a review of patients with the primary or secondary diagnosis of colon cancer, who underwent right hemicolectomy and/or ileum resection between January 2008 and May 2011 in the Department of Surgery, St. Josef-Hospital, Ruhr-University Bochum. Patients were included only if sufficient information on the presence or absence of type 2 diabetes prior to surgery was available and if concomitant diseases affecting gut mucosal integrity (e.g. gastroenteritis, chronic inflammatory bowel disease) could be excluded. Paraffin-embedded gut samples were available from all patients. For morphometric analyses, only tumor-free tissue samples (at least 2 cm distant from the tumor margin) were included. The study was approved by the ethics committee of the Ruhr-University Bochum (registration number 4667-13).

2.1.1. Patient Characteristics

The group of patients with type 2 diabetes comprised 6 males and 4 females. Their mean age was 67.8 ± 11.7 years. The control group comprised 5 male and 5 female patients. The mean age was 72.7 ± 9.3 years. In the control group, diabetes was excluded based on the patient history and the lack of glucose-lowering medication. In all control patients, non-fasting glucose concentrations determined during the hospital stay were in the non-diabetic range (i.e. less than 200 mg/dl). Mean (±SD) non-fasting glucose levels were 103.2 ± 25.6 mg/dl. Furthermore, fasting glucose levels, determined during surgery were in the non-diabetic range (i.e. less than 126 mg/dl) in all controls, except for one patient, who received parenteral glucose infusion. Mean fasting glucose levels were 102.3 ± 22.1 mg/dl, when this patient was included, and 97.8 ± 17.8 mg/dl, when this patient was excluded.

Patients with diabetes were treated with metformin only in one case, glimepiride monotherapy in one case, insulin in two cases, metformin plus sitagliptin in one case, and diet plus exercise in three cases. In two cases, no information on previous glucose-lowering therapy was available. The mean HbA_{1c} (available in 8 cases only) was 7.4 ± 1.4%. All patients with type 2 diabetes underwent hemicolectomy for the removal of malignant tumors of the cecum or ascending colon. In the control group, nine patients underwent surgery because of malignant tumors of the cecum or ascending colon, and one patient was treated with an ileal resection because of intestinal adhesions.

2.2. Tissue Preparation

Human ileal tissue was fixed in formaldehyde and embedded in paraffin. 5 μm thick sections were cut, mounted on charged glass slides and processed for immunohistochemistry. The sections were stained as follows: (1) for GLP-1, Ki67 and DNA for immunofluorescence, (2) for GLP-1, TUNEL (apoptosis) and DNA for immunofluorescence, (3) for GLP-1, chromogranin A and DNA for immunofluorescence.

2.2.1. Staining Procedures

All primary and secondary antibodies were diluted in Dako Antibody Diluent with Background Reducing Components (Dako, Golstup, Denmark; #S3022).

2.2.1.1. Immunofluorescence Staining for GLP-1, Ki67 and DNA. After heating sections at 37 °C overnight, sections were deparaffinized using Xylene twice for 10 min and hydrated by EtOH thrice (100%, 96%, 80%, 70%) for 5 min (each step) and distilled water for another 5 min. The sections were permeabilized by heating in the microwave in DakoCytomation Target Retrieval Solution pH 9.0 (Dako, Golstup, Denmark; #S2367) for 10 min. After down cooling for 30 min and rising in distilled water, sections were blocked for unspecific protein binding with CAS-Block (Invitrogen, Darmstadt, Germany; #00-8120) for 15 min. For the detection of GLP-1, sections were incubated with the rabbit primary antibody against human GLP-1 (diluted 1:800; Phoenix Pharmaceuticals; Karlsruhe, Germany; #H-028-11) overnight at 4 °C. Afterwards, slides were washed in TBS (Dako, Golstrup, Denmark; #S3006) and the GLP-1 antigen was detected by the use of a secondary antibody Cy3-conjugated goat anti-rabbit (diluted 1:400; Jackson ImmunoResearch Europe, Newmarket, UK; #111-165-003) for 30 min at room temperature. After a brief rinse in TBS, co-staining with Ki67 was performed as