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Abstract

Intestinal ischemia, also called mesenteric ischemia, is a severe gastrointestinal and vascular medical emergency caused by a sudden decrease of blood flow through the mesenteric vessels. It generates hypoperfusion of intestinal tissues and can rapidly progress to intestinal wall infarction, systemic inflammation or even death if not treated in time. The mortality of this condition is still considerably high despite all the medical advances of the past few years. This is partially due to the difficulty of diagnosing early stage mesenteric ischemia. Indeed, a speedy and correct diagnosis is decisive for suitable medical care. However, early symptoms are unspecific and conventional clinical markers are neither specific nor sensitive enough. In the last few years, significant clinical and preclinical efforts have been made to find biomarkers which could predict gastrointestinal damage before it becomes irreversible. Here, the gut-derived hormone glucagon-like peptide-1 (GLP-1) is described as a potential early biomarker of this severe condition. Indeed, GLP-1 plasma levels rise rapidly in both mice and humans with intestinal ischemia. This discovery could counter the cruel lack of clinical biomarkers available to diagnose and therefore manage intestinal ischemia efficiently in the early stages. GLP-1 could thus become part of a panel of biomarkers for intestinal ischemia and could help to reduce the associated high mortality rates.

Key words

Intestinal ischemia, mesenteric ischemia, glucagon-like peptide-1, inflammation, lipopolysaccharides, biomarker

1. Introduction

Intestinal ischemia, also called mesenteric ischemia, is a severe gastrointestinal and vascular medical emergency caused by a sudden decrease or cessation of blood flow through the mesenteric vessels. Four types of vascular dysfunction can generate this condition: embolic arterial occlusion (40-50 %), thrombotic arterial occlusion (20-35 %), non-occlusive forms (5-15 %) and venous mesenteric thrombosis (5-15 %) [1]. Intestinal ischemia can originate from certain diseases (mainly atherosclerosis), trauma, shock, surgery or organ transplantation. It generates hypoperfusion of intestinal tissues and can rapidly progress to intestinal wall necrosis and infarction, local and systemic inflammation or even death if not treated in time. The mortality of intestinal ischemia is still considerably high despite all the medical advances of the past few years, but improvements in outcome would require more rapid diagnosis and early appropriate medical care. Indeed, a prospective pilot study involving 18 patients has shown that early multimodal management of intestinal viability resulted in a significant reduction in intestinal necrosis and resection [2], the 2-year survival rate was 89% in these patients. One of the main issues concerning intestinal ischemia is the lack of specific, sensitive and early stage biomarkers to predict this condition prior to deadly complications. The gut hormone glucagon-like peptide-1 (GLP-1) could be a promising early biomarker for intestinal ischemia. Originally described for its role in glucose metabolism, GLP-1 may have other functions, particularly in relation to gut inflammation [3,4]. Indeed, bacterial-derived pro-inflammatory lipopolysaccharides (LPS) are able to stimulate the secretion of GLP-1, and this phenomenon occurs rapidly after the onset of intestinal ischemia [5,6]. In this review, we first describe intestinal ischemia, its current and investigative diagnostic tools. We then present GLP-1 as a potential biomarker for the rapid diagnosis of intestinal ischemia, which would contribute to a reduction in mortality.

2. Intestinal ischemia

2.1 Pathophysiology

From an anatomical point of view, a large part of the gut (the entire small intestine and ascending colon) is vascularized by the superior mesenteric artery. It supplies the arterioles and the sub-mucosal capillary networks which irrigate the intestinal mucosa up to the tip of the villi. Figure 1 presents the pathophysiology of intestinal ischemia. Ischemic damage starts with desquamation and bleeding of mucosal villi, which is highly sensitive to

hypoperfusion and hypoxia. The intestinal epithelium acts as a barrier against the translocation of luminal components. Rupture of this intestinal barrier rapidly triggers local and systemic inflammation with, respectively, the recruitment of inflammatory mediators and the passing of pro-inflammatory bacterial endotoxins and other microbial components into the bloodstream. This bacterial translocation can result in the development of systemic inflammation response syndrome (SIRS), which potentiates intestinal hypoperfusion and damage. The rapid reversal of ischemia through the reperfusion of digestive vessels is absolutely required to avoid irreversible necrosis and resection of intestinal tissue. However, and paradoxically, reperfusion of ischemic intestinal tissue may further aggravate the severity of the damage. Indeed, restoration of blood flow abruptly reintroduces oxygen that stimulates the production of free radicals, generates oxidative stress and accelerates necrosis [7]. This "vicious circle" can result in lethal intestinal ischemia-reperfusion complications with the dysfunction of additional organs. Intestinal ischemia is considered as a root cause of multiple organ failure (MOF) [8] and ultimately death which explains the urgency of the condition and the need for rapid medical care.

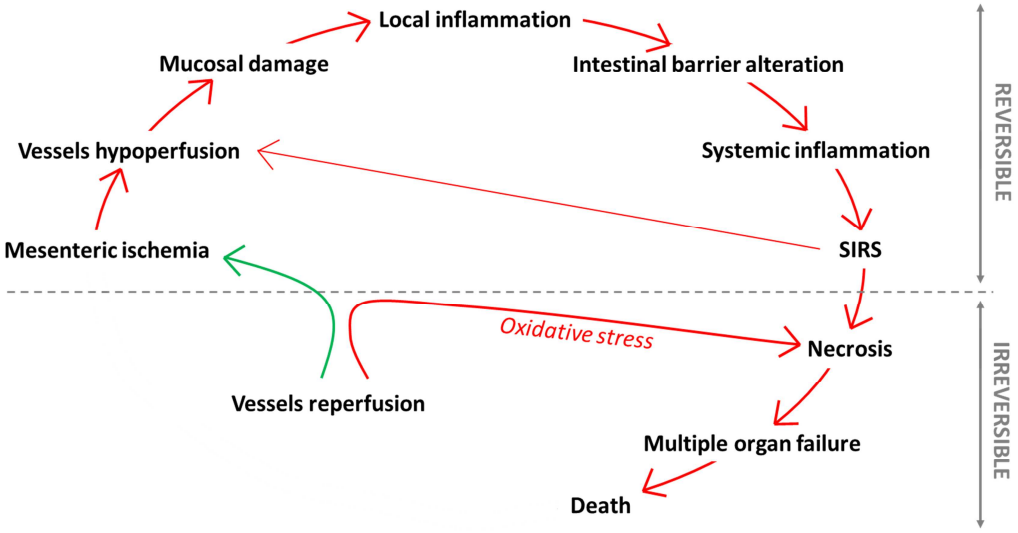

FIGURE 1: Pathophysiology of intestinal ischemia – reperfusion
Several pathophysiological and scalable steps occur after the onset of mesenteric ischemia. Some of them are reversible (above the dotted line), but disease progression is certain and irreversible once intestinal necrosis has begun (below the dotted line). On the one hand, vessel reperfusion is needed to counteract ischemia (green arrow), but it also generates oxidative stress which contributes to irreversible necrosis of tissues. Abbreviations: SIRS, systemic inflammatory response syndrome.

2.2 Epidemiology and current diagnostic tools

In large part because of the association with atherosclerosis, intestinal ischemia is commonly considered a disease that affects older people, with the typical age of onset being over 60 years [9]. However, because this pathology can occur in both sick and healthy vessels, cases have also been reported in younger people [10]. Despite significant medical advances, the high mortality for intestinal ischemia has not changed since the 1940s, and it concerns about 60% to 80% of patients [11]. It is assumed that mesenteric ischemia represents 1/1000 hospitalizations worldwide [12], but this proportion is certainly underestimated [13] due to delays in diagnosis and treatment. The high mortality is also partially due to the difficulty of diagnosing early stage mesenteric ischemia. Indeed, a speedy and correct diagnosis is decisive for suitable medical care and the resulting patient prognoses. Before warning signs such as peritonitis appear, symptoms are unspecific (*e.g.* abdominal pain, nausea, vomiting) and conventional clinical markers such as serum lactate levels or white blood cells numbers are neither specific nor sensitive enough [14]. Until now, there has been no biological marker that could diagnose intestinal ischemia at an early stage. Computerized tomography and angiography are radiological imaging techniques that may also be used and which are helpful diagnostic tests. However, they are costly, more invasive and can generate medical complications [15]. Most of all, it is difficult to manage radiological investigations in emergency settings and critical care medicine. Accordingly, there is an obvious need for specific and sensitive plasma biomarkers that would help clinicians to diagnose intestinal ischemia in the early stages.

2.3 Investigative plasma biomarkers

A recent study conducted in a specialized intestinal stroke centre demonstrated that, during intestinal ischemia, irreversible intestinal ischemic damage could be prevented or delayed through multidisciplinary management of the condition: preserving intestinal viability improves patient outcomes and survival [16]. This development is definitely a great step forward for the medical care of intestinal ischemia, but rapid diagnosis is still needed. In the last few years, significant clinical and preclinical efforts have been made to find biomarkers which could predict gastrointestinal damage before it becomes irreversible and spreads to the rest of the body. D-lactate [17], intestinal fatty acid binding protein (I-FABP) [18], α-glutathione S-transferase [19], albumin [20], D-dimer [21], procalcitonin [22], endothelin-1 [23] or smooth muscle protein 22 [24] have been studied as potential biomarkers for

mesenteric ischemia. Some are certainly promising while others lack specificity, sensitivity or rapidity, but all require in depth study to assess their diagnostic accuracy [14].

3. GLP-1: a promising early biomarker

3.1 Glucagon-like peptide-1 (GLP-1)

GLP-1 is a hormone produced by enteroendocrine L cells which are found primarily in the epithelium of the distal ileum and colon. These cells express proglucagon as well as prohormone convertase 1/3, a specific peptidase able to cleave proglucagon into several peptides including GLP-1. For some years now, GLP-1 has generated great clinical interest for the treatment of metabolic disorders such as type 2 diabetes because of its role in the regulation of glucose homeostasis [3]. Initially described for its incretin function, and hence its insulinotropic role in pancreatic beta cells [25], GLP-1 has subsequently been shown to have many other functions in glucose metabolism. Indeed, this gut-derived hormone is also able to stimulate insulin biosynthesis [26], inhibit glucagon production [25], delay gastric emptying [27] or even induce satiety and reduce food intake [28]. Secretion of GLP-1 from intestinal L cells is mostly triggered by ingested nutrients and involves various and intricate molecular mechanisms [29,30]. Carbohydrates, lipids and proteins are all able to stimulate GLP-1 secretion through direct interaction with their specific membrane receptors on the surface of L cells (Figure 2). However, the densest population of L cells is located in the distal gut, and postprandial plasma GLP-1 levels increase rapidly, even before nutrients reach this part of the gut [31]. This physiological discrepancy suggests the implication of other secretion mechanisms, and, accordingly, the existence of hormonal and neural secretion pathways has been previously studied. Overall, GLP-1 secretion can be activated by a wide range of L cell membrane receptors that recognize nutrients but also molecules such as neurotransmitters, hormones or even inflammatory compounds (Figure 2).

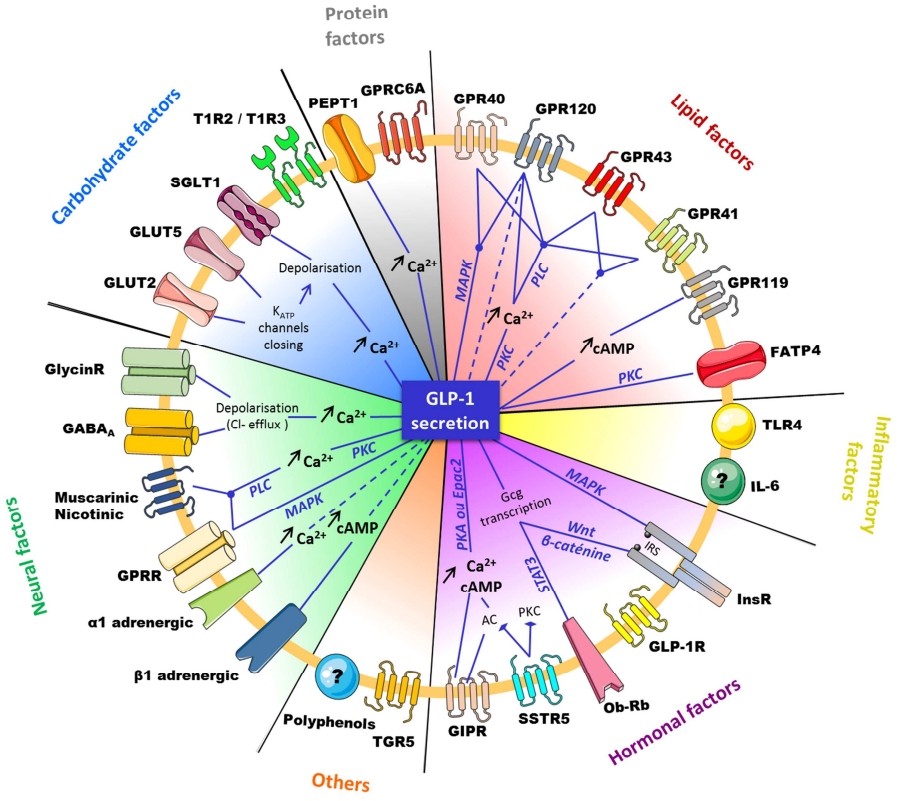

FIGURE 2: Cellular signalling pathways responsible for GLP-1 secretion

GLP-1 secretion from enteroendocrine L cells can be stimulated by different factors (nervous, protein, inflammatory…). Each of these pathways involves various membrane receptors. Some of them are well-described, some are still controversial and some are completely unknown yet (polyphenols and IL-6). Abbreviations: TLR4, toll-like receptor 4; IL-6, interleukin 6; GLP-1, glucagon-like peptide-1; GLUT, glucose transporter; SGLT1, sodium glucose linked transporter 1; T1R, type 1 taste G-protein coupled receptors; PEPT1, peptide transporter 1; GPRC6A, G protein-coupled receptor family c group 6 subtype A; GPR, G protein coupled receptor; FATP4, fatty-acid binding protein 4; InsR, insulin receptor; GLP-1R, GLP-1 receptor; Ob-Rb, leptin receptor; SSTR5, somatostatin receptor type 5; GIPR, glucose-dependent insulinotropic polypeptide receptor; TGR5, membrane-type receptor for bile acids; GPRR, gastrin-releasing peptide receptor; $GABA_A$, γ-aminobutyric acid receptor; GlycinR, glycin receptor; PK, protein kinase; MAPK, mitogen-activated protein kinase; PLC, phospholipase C; cAMP, cyclic adenosine monophosphate; Epac2, exchange protein activated by cAMP 2.

3.2 GLP-1, lipopolysaccharides and inflammation

Besides its originally described glucoregulatory role, GLP-1 has also more recently been investigated for its anti-inflammatory properties and its link with sepsis and critical illness. Previous studies have demonstrated that the endogenous GLP-1 system is activated during sepsis [32], have associated GLP-1 levels with predicted mortality [32,33] and have correlated increased levels of circulating GLP-1 with the extent of concomitant critical illness [33–35]. GLP-1 may exert both distant and local beneficial anti-inflammatory effects. For instance, it has been described as protective for the heart, lung or liver, and some studies highlight a role for GLP-1 in maintaining gut homeostasis [3,4]. GLP-1 based drugs, initially studied and used in the context of glucose homeostasis, are now investigated in the treatment of various inflammatory disorders (gallego-colon 2018, Drucker 2018, Insuela 2017). This potential anti-inflammatory role of GLP-1 is in line with its recently described inflammatory-induced secretion. LPS are pro-inflammatory molecules found at the surface of Gram negative bacteria and in huge amounts within the gut microbiota. *In vivo*, LPS were shown to induce glucose-stimulated insulin secretion through an increase in GLP-1 plasma levels. The insulinotropic effect of LPS was strongly diminished when mice were pretreated with a GLP-1 receptor antagonist or when GLP-1 receptor deficient mice were used [6]. Among the inflammatory mediators that have been shown to increase GLP-1 plasma levels, pro-inflammatory cytokine IL-6 increases insulin secretion *in vivo* through the stimulation of GLP-1 synthesis and secretion in the intestines [36]. Moreover, it was recently shown that LPS molecules are able to directly stimulate GLP-1 secretion through activation of the toll-like receptor 4 (TLR4) of enteroendocrine L cells [5].

3.3 GLP-1 as a biomarker of intestinal ischemia

In inflammatory settings, intestinal ischemia is associated with a prompt and significant increase in GLP-1 plasma levels. As soon as intestinal barrier begins to disorganize, which is right after the onset of experimental mesenteric ischemia in mice, luminal LPS (and certainly other molecules) have more access to epithelial enteroendocrine L cells and can trigger GLP-1 secretion. The LPS / GLP-1 cascade, first described in mice [5,6,35], works similarly in humans. Injection of a low dose of LPS led to a significant rise in GLP-1 plasma levels in healthy volunteers, and GLP-1 secretion was rose rapidly in an experimental model of intestinal ischemia in humans [5]. These results suggest that GLP-1 could be a promising

biomarker for early stage intestinal ischemia which could help to identify the condition in the critical first, reversible steps. Unlike other promising biomarkers which are released only after intestinal cell death (*e.g.* I-FABP) or systemically generalized inflammation (*e.g.* D-lactate or procalcitonin), the increase in GLP-1 secretion occurs earlier and is the physiological response of functional enteroendocrine L cells to the luminal compounds which are usually kept at a distance. GLP-1 secretion is therefore quicker and more sensitive, making GLP-1 a potential biomarker for intestinal ischemia and opening new perspectives in diagnosis and medical care of this fatal illness. GLP-1 meets all the criteria necessary to be an optimal marker of intestinal pain and distress: 1) it is produced by mucosal intestinal cells, 2) hepatic proteolytic activity is insignificant since GLP-1 is quantified under its total (active and inactive) form, and 3) it is detectable and quantifiable in systemic circulation. This discovery could counter the cruel lack of clinical biomarkers available to diagnose and therefore manage intestinal ischemia efficiently in the early stages. GLP-1 could thus become part of a panel of biomarkers for intestinal ischemia and could help to reduce the associated high mortality rates. In addition, GLP-1 testing could become a routine laboratory test provided as part of the ELISA kit already on the market.

4. Conclusion

A large number of preclinical and clinical studies aim to improve the diagnosis and outcomes of intestinal ischemia through identification of specific and sensitive biomarkers which are able to predict the disease in the early stages. Here, the gut-derived hormone GLP-1 is described as a potential early biomarker of this severe condition: GLP-1 plasma levels rise rapidly in both mice and humans with intestinal ischemia. In the context of clinical care, further work is needed to 1) assess whether GLP-1 levels reflect the severity of gastrointestinal damage in humans and 2) confirm whether GLP-1 can efficiently diagnose intestinal ischemia. Since GLP-1 is not clinically used to diagnose any pathology so far, future clinical investigations are also needed to determine a reference plasma level for GLP-1 which would be used for comparison purposes. Indeed, GLP-1 levels can vary inter- and intra-individually (fasting or postprandial state). However, consistently with our preliminary results described here, we assume that the pathological increase in GLP-1 levels would be greater than the physiological one and therefore easily differentiated. Finally, from a fundamental research point of view, it would also be interesting to investigate the role of

GLP-1 secretion after intestinal ischemia. After the discovery of GLP-1's anti-inflammatory properties, we could assume that it promotes the restoration of mucosa and reduces inflammation.

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Author contributions

Writing – original draft and visualization, L.J.L. and J.G.; writing – review & editing, L.J.L. and J.G.; supervision, J.G.; declarations of interest, none.

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