**Title:** The role of cytochromes P450 in intestinal barrier function: possible involvement in the pathogenesis of Leaky Gut Syndrome

**Short title:** Cytochromes P450 and gut barrier

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**Abstract:** The intestinal barrier constitutes the largest surface of the human body communicating with the external environment. As a complex system, it developed numerous mechanisms allowing the organism to interact with various stimuli, such as residual microbiome. Alterations affecting elements of intestinal wall may lead to increased intestinal permeability and resulting translocation of bacteria or its components to the bloodstream in the form of the “leaky gut syndrome” (LGS). One of the most common causes of LGS is the disruption of tight junctions (TJ) maintained by tight junction proteins (TJP). LGS and associated alterations in TJP are observed in numerous gastrointestinal (GI) diseases characterized by chronic and relapsing inflammation affecting the GI tract, including inflammatory bowel diseases (IBD) such as Crohn’s disease (CD) and ulcerative colitis (UC). Current literature indicates the key role of LGS in many pathological processes further emphasizing the need for effective pharmacological approaches to treat this syndrome. One of the potential pharmacological targets in LGS treatment are members of the cytochrome P450 (CYP450) superfamily responsible for the metabolism of various endo- and exogenous compounds. Recent studies show that CYP450s are involved in the pathophysiology of the GI by influencing inflammation and intestinal barrier function. This review summarizes the findings on the role of CYP450 isoforms in intestinal hyperpermeability and their potential involvement in the pathophysiology of LGS.

**Keywords:** Cytochrome P450,intestinal permeability, leaky gut syndrome, tight junction, inflammatory bowel disease

**Text:**

**Introduction**

The intestinal barrier is a multilayer system of the gastrointestinal (GI) tract. Mucosa, the luminal layer measures 200-300m2 and constitutes the largest surface of the human body communicating with the external environment [1]. Therefore, the intestinal barrier developed complex mechanisms allowing the organism to interact with numerous stimuli. This system facilitates the selective permeability of nutrients, prevents excessive water and electrolyte loss, and enables the exchange of molecules between the organism and environment. Moreover, these structures prevent bacterial translocation and protect from exogenous, potentially detrimental factors [2,3]. The intestinal barrier consists of two parts: the upper (physical) barrier and the lower (functional) barrier. In a healthy gut, their interaction allows to maintain a balanced permeability [4].

The upper part comprises resident gut microbiota which competes for nutritional resources with pathogens, mucus containing antimicrobial products and IgA, and a monolayer of intestinal epithelial cells. The upper barrier modulates the functions of the lower part. The lower barrier includes a network of immune cells known as “gut-associated lymphoid tissue” GALT which is responsible for immunological responses. It comprises up to 70% immunocytes of the body [5,6]. Alterations affecting any of these elements may lead to increased intestinal permeability and resulting translocation of bacteria or its components to the bloodstream in the form of the “leaky gut syndrome” (LGS) [7]. One of the most common causes of LGS is the disruption of tight junctions (TJ) [8]. Physiologically, TJ reduce paracellular permeability by forming tight connections between epithelial cells. The main components maintaining TJ are tight junction proteins (TJP), including occludin (OCLN), claudins (CLDN), and zonula occludens (ZO) proteins [9]. TJP form complex architecture playing pivotal roles in many intra-, extra- and paracellular processes, such as interactions with cytoskeleton-associated proteins. Therefore, TJP considerably influence the intestinal permeability [10,11].

LGS and associated alterations in TJP are observed in numerous GI diseases, including Crohn’s disease (CD) and ulcerative colitis (UC) belonging to inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), celiac disease, and liver disorders such as nonalcoholic fatty liver disease (NAFLD). Moreover, increased intestinal permeability plays a role in the development of type I and type II diabetes mellitus, obesity and Parkinson's disease [2,12]. Despite the plethora of studies on LGS as a factor associated with other pathological processes, there is still a lack of effective pharmacological approaches to treat this syndrome. Therefore, it is crucial to search for novel LGS therapies since current treatment is based mainly on dietary recommendations [13].

One of the potential pharmacological targets in LGS treatment are members of the cytochrome P450 (CYP450) superfamily. CYP450s are characterized by an intense absorption band at 450 nm that was used to be described as “pigment” 450 in the early 1960s. The enzymes are made up of 400-500 amino acids and contain a single active heme iron center. As heme enzymes, they belong to the group of oxygenases, which are responsible for the metabolism of various molecules within cells. Although the members of the CYP450 superfamily are similar in their tertiary structure, they differ significantly in the shape and size of their active sites. Among the superfamily, the families share >40% amino acid sequence similarity, and subfamilies show >55% such homology. The 57 isoforms of CYP450 catalyze approximately 75% of the biotransformation reactions of drugs and 95% of the oxidation-reduction processes of all xenobiotics [14,15]. CYP450s are primarily expressed in the liver, but also other human and mouse organs, such as the brain, heart, kidneys, and intestine [16,17]. Recent studies have shown that enzymes belonging to the CYP450 group are involved in the pathophysiology of the GI tract with its important roles in the development of colitis [18]. Our review summarizes the results of studies regarding the influence of CYP450 isoforms on TJP and epithelial barrier integrity, including intestinal hyperpermeability observed in GI tract disorders (Table 1.).

**CYP1A**

The CYP1A family consists of 2 isoenzymes: CYP1A1 and CYP1A2. The main isoform CYP1A is expressed in the liver and small intestine. The concentration of the enzyme in the rat proximal small intestine is approximately 4 times higher than in its distal part being the highest in the duodenum, close to the pylorus [19,20]. CYP1A2 accounts for approximately 12% of total hepatic human P450 content [21]. Moreover, some studies provide information about the presence of CYP1As in the human intestinal tissue [22]. The enzymes were found to be responsible for most of the caffeine hepatic biotransformation to paraxanthine, theobromine, and theophylline, which constitute the main caffeine metabolites [19]. CYP1As are regulated mainly by the aryl hydrocarbon receptor (AHR) and play a crucial role in the activation and detoxification of procarcinogens as well as the metabolism of drugs [20,21,23,24]. Several studies focused on the role of CYP1A1 in the GI pathophysiology. It was shown that mRNA expression of this enzyme in the intestinal tract is induced by serotonin which is produced by enterochromaffin cells located in the gut and is often elevated in individuals experiencing IBD symptoms, such as visceral pain [25,26]. Immunohistochemical staining of intestinal samples obtained from IBD patients showed significantly increased levels of CYP1A1 in CD and UC compared to controls [27]. Other research, showed similar results only for CD, while CYP1A1 expression in UC was decreased [28]. Despite the very limited knowledge about the effects of CYP1A1 on inflamed intestinal tissue, studies suggest a link between CYP1As and inflammation in sepsis [29,30]. In animal models of sepsis, xenobiotic receptors such as pregnane X receptor (PXR) and AHR are downregulated. These receptors influence the functions of drug-metabolizing enzymes (DMEs), including the activity of CYP1A subfamily [31,32]. It was shown that inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNFα), released during sepsis inhibit PXR and AHR expression activity leading to alterations in DMEs functions. It results in the decreased expression of CYP1A2[29]. Additionally, Crawford et al. [30]showed that the inhibition of CYP1A2 in sepsis rats caused exacerbation of proinflammatory responses, tissue hypoxia, and hepatic necrosis. Moreover, the level of CYP1A2 was considerably lower in sepsis rats. Other studies showed that one of the regulators of the CYP1A subfamily, PXR might play a crucial role as a future target in IBD therapy through modulating functions of inflammatory agents, including NF-κB, TLR, NOD2, and T lymphocytes. These properties enable PXR to maintain and restore balance between inflammation and immunological response, possibly alleviating colitis symptoms and slowing the progression of IBD [33]

**CYP2A6**

CYP2A6, CYP2A7, and CYP2A13 are members of the CYP2A subfamily. The best characterized of them, CYP2A6, catalyzes the metabolism of several pharmaceuticals and toxins, such as aflatoxin B1 and nitrosamines. Moreover, it takes part in metabolizing nicotine[34]. CYP2A6 is expressed mainly in the liver and is not found in human intestinal tissue [35,36]. However, some studies suggest its influence on IBD and the intestinal barrier. Satka et al. [37] evaluated the role of murine CYP2A5, the mouse analog of CYP2A6, in the model of DSS-induced colitis. This report showed that CYP2A5 is responsible for metabolizing metronidazole, which is an antibiotic used in IBD therapy. It was also found that the mRNA expression of hepatic CYP2A5 is significantly decreased in DSS-induced colitis. The enzyme activity was decreased by approximately 50% during inflammation, resulting in a higher concentration of metronidazole in murine blood. Moreover, administration of butyrate before DSS reversed that effect, causing a better antibiotic metabolism. Furthermore, a rat study showed a link between CYP2A6 and intestinal hyperpermeability. Animals were exposed to aflatoxin B1 (AFB1). This substance increases duodenal permeability by damaging epithelial cells through its metabolite, aflatoxin B1-8,9-epoxide (AFBO). AFBO is produced through CYP450 metabolism, mainly as a result of CYP2A6 and CYP3A4 activity. The administration of ferulic acid (FA), an inhibitor of CYP2A6 and CYP3A4, suppressed the bio-activation of AFB1 and prevented duodenal barrier dysfunction. The use of FA resulted in increased expression of CLDN-1 and ZO-1. Moreover, it reduced duodenal epithelial cell apoptosis and decreased the expression of Rho-associated protein kinase (ROCK) involved in cytoskeleton regulation [38].

The influence of CYP2A6 on intestinal inflammation was also shown in human studies. It was demonstrated that the relative risk of developing UC is lower in smokers compared to non-smokers. The first group also tends to experience a later onset of the disease and its milder form [39]. Although Altarescu et al. [40] in their comparative study showed a lack of significant difference in the course of the disease between smokers and non-smokers, the smokers and former smokers developed UC at a later age. In the smoking group the level of expression of CYP2A6\*1A, an extensive metabolizer of nicotine, was much higher. In contrast, the expression of CYP2A6\*4A, a poor nicotine metabolizer, was considerably lower. The opposite tendency was shown in the non-smoking group. CYP2A6 is thought to influence UC, as CYP2A6-dependent nicotine metabolism leads to the production of toxic intermediates. Moreover, both nicotine and acetylcholine are agonists of the CHRNA3 receptor found in the colonic mucosa. As acetylcholine is the main neurotransmitter of the gut, nicotine may also influence intestinal motility and possibly have an impact on UC development [41].

**CYP2E1**

CYP2E1 is one of the main members of the cytochromes P450 superfamily, which is primarily found in the liver and to a lesser extent, in extrahepatic tissues, including rodent and human intestines [42,43]. CYP2E1 is responsible for the metabolism of many small molecule, hydrophobic compounds. As a result, it produces toxic intermediates, excessive amounts of reactive oxygen species (ROS), and the following process of lipid peroxidation. Therefore, hepatic and extrahepatic CYP2E1 has been implicated in various pathophysiological conditions, including in the GI tract. The enzyme is involved in the metabolism of ethanol and contributes to the development of ethanol-induced liver toxicity [44]. Similarly, CYP2E1-dependent hydroxylation of acetaminophen produces N-acetyl-p-benzoquinone imine (NAPQI), leading to the hepatic tissue injury [45]. Furthermore, studies on samples collected from IBD patients showed alterations in the expression of intestinal CYP2E1. The level of CYP2E1 mRNA is significantly decreased in non-inflamed ascending colon of UC individuals [46]. On the other hand, semi-quantitative immunohistochemical analysis found that the protein level of intestinal CYP2E1 is elevated in colonic tissues during CD and UC [28].

It was also shown that overactivity of intestinal CYP2E1 leads to the loss of intestinal wall integrity and contributes to the development of LGS. The presence of CYP2E1 is necessary for the development of epithelial hyperpermeability in alcohol-treated intestinal cells as well as mice. The monolayers of Caco-2 cells with siRNA knockdown of the CYP2E1 gene were resistant to increased permeability after alcohol treatment. Simultaneously, the lack of CYP2E1 activity prevented the upregulation of circadian rhythm proteins, CLOCK and PER2, which are involved in alcohol-induced LGS [47,48]. Moreover, heavy consumption of alcohol by mice resulted in increased serum endotoxin levels, higher content of enterobacteria in the liver, and microscopic damage of the small intestine. CYP2E1-null mice and wild-type animals treated with selective CYP2E1 inhibitor, chlormethiazole (CMZ), were significantly prevented from these alterations [49]. It is worth noting that alcohol is not only a substrate but also an inducer of CYP2E1. Interestingly, overactivity of CYP2E1, alterations in TJP, and resulting LGS were also observed after treatment with fructose. Fructose in high doses is one of the main dietary factors increasing intestinal permeability, although it is not a substrate for CYP2E1 [50]. Cho et al. showed that fructose increased the permeability of T84 colon cells as assessed by the TEER technique and the measurement of FITC-dextran transport. Additionally, high concentrations of fructose decreased the levels of ZO-1 protein expression. Pretreatment with CMZ protected from elevated permeability of the cell monolayer. Moreover, it was shown that oral administration of 30% fructose solution increases the levels of intestinal CYP2E1 protein in rodents and causes LGS manifested by increased plasma concentrations of FITC-dextran. The high intake of fructose also resulted in intestinal infiltration of inflammatory cells, and decreased expression of TJP: OCLN, CLDN-1, CLDN-4, ZO-1. Moreover, fructose decreased the expression of adherent junction proteins, desmosome plakoglobin, and α-tubulin, while increasing the expression of proteins considered to be markers of apoptosis. CYP2E1-null mice were fully resistant to fructose-induced LGS as well as the other mentioned effects [51]. Taken together, those observations suggest that the use of CYP2E1 inhibitors may display beneficial effects on the gut barrier., Further research on the role of this enzyme is warranted and will likely focus on the exact mechanism trough which it affects the integrity of intestinal tissue.

**CYP2J2**

Although CYP2J2 is mainly expressed in the heart, it is also found in the gut where it is involved in the endocannabinoid system through anandamide (AEA) metabolism. The levels of AEA and other endocannabinoids are increased during colitis. Moreover, fatty acid amide hydrolase (FAAH), which hydrolyzes AEA, is downregulated in IBD patients [52]. Therefore, CYP2J2 which inactivates AEA might be a target for IBD therapy [53]. It was shown in the in vitro conditions, where CYPJ2J is induced by inflammation and has some anti-inflammatory properties. The lack of this cytochrome in the CD macrophages may aggravate CD development [54]. Noteworthily, CYP2J2 was found to convert arachidonic acid (AA) to epoxyeicosatrienoic acids (EETs) which are known to have anti-inflammatory properties [55].

Current literature does not provide any evidence for the involvement of CYP2J2 in the maintenance of the GI tract epithelial integrity. However, some clues for a similar role come from the study by Zhao et al. [56] who observed that endothelial CYP2J2 overexpression maintained the integrity of the blood-retinal barrier (BRB) after ischemia-reperfusion injury. Abundance of CYP2J2 correlated with upregulation of ANXA1 expression which stabilized the distribution of tight and adherens junctions in the endothelium. Of note, it was previously shown that inflamed intestinal epithelial cells overexpress ANXA1 with a beneficial effect on mucosal healing [57]. Therefore, upregulating the expression of CYP2J2 in the gut may lead to the effect similar to this observed in the endothelium what could potentially improve the integrity of the intestinal mucosal barrier.

**CYP3A**

The members of the CYP3A subfamily are the most abundantly expressed CYPs in the human body, with CYP3A4 as a major member of this group. CYP3A4 is expressed in the liver and small intestine, where is estimated to account for approximately 40% (liver) and 82% (small intestine) of total CYP activity in these organs [58]. CYP3A4 is a member of CYP450s characterized by the highest expression in human enterocytes [59]. The second CYP3A in terms of expression, CYP3A5, shares 83% homology to CYP3A4 and is primarily expressed in the kidney [60]

The intestinal form of CYP3A4 is responsible for the first-pass metabolism of orally administered drugs from various groups [61]. Similarly to other isoforms, it is located mainly in the tip of the villous, while absent in crypt cells [62]. The concentration of the enzyme decreases distally, with the highest levels in the duodenum near the pylorus [63]. It is associated with the metabolism of large lipophilic structures, such as steroids or RA (vitamin A) [64]. Moreover, CYP3A4 was indicated to play a crucial role in the activity of hepatic vitamin D-24-hydroxylase and D-25-hydrolase [65]. Additionally, current literature shows that it might be associated with intestinal barrier hyperpermeability. Observations from human studies link CYP3A4 activity with celiac disease, in which intestinal barrier impairment is suggested to be a factor contributing to the induction of the disorder [66]. In the study by Lang et al. 9 patients suffering from celiac disease were given a gluten-free diet. The comparison of small intestine biopsy specimens showed significant alterations of intestinal CYP3A expression caused by the diet. The immunoreactivity of the enzyme was reduced before the treatment and increased as a result of dietary intervention [63]. Another study focused on the level of felodipine in patients with celiac disease and healthy individuals who had previously taken this drug orally. Felodipine, a calcium channel blocker used to treat hypertension, is characterized by low oral bioavailability resulting from its intestinal CYP3A4-dependent metabolism. Its single primary metabolite, dehydrofelodipine, is also metabolized by this enzyme. The study showed that plasma concentrations of felodipine and dehydrofelodipine were significantly higher in individuals with a celiac group with a positive linear dependence on the severity of the disease. These results suggest that patients with celiac disease are particularly exposed to adverse effects from drugs metabolized by CYP3A4, which intestinal levels are decreased during a disease [67]. In addition to the intestinal form, CYP3A4 expressed in the liver is also involved in the gut pathophysiology. Xu et al. [68] evaluated the effects of interaction between gemcitabine hydrochloride and traditional Chinese herbal formula in the rat model of non-small-cell lung cancer and demonstrated that the treatment with gemcitabine resulted in the decreased expression of hepatic CYP3A4. Simultaneously, rats developed increased intestinal permeability visualized by histological assessment and down-regulation of ZO-1 and OCLN expressions. These results suggest a potential link between the expression of the CYP3A family and intestinal hyperpermeability. However, the study did not focus on the exact mechanisms underlying the observed phenomenon.

# **CYP8B1**

CYP8B1, also known as sterol 12alpha-hydroxylase, is expressed in the liver. The enzyme is mainly responsible for cholic acid (CA) synthesis and is inhibited by CA, cholesterol, and insulin. Therefore, it is involved in the maintenance of proper bile composition and the resulting hydrophobicity index of bile acids (BA) [69]. The homeostasis of BA, the final product of cholesterol catabolism, is crucial for the proper functioning of the gut. They act as amphiphilic emulsifiers which facilitate the intestinal absorption of lipids. Numerous studies showed that BA play a pivotal role in the development of intestinal inflammation and hyperpermeability through their direct influence on TJP [70]. Moreover, current literature suggests a link between the expression of CYP8B1 and colitis. The research by Chen et al. [71] showed severe DSS-induced intestinal inflammation in CA-treated and liver Cyp8B1-overexpressing mice. Additionally, the repair of the intestinal barrier was also compromised. DSS-induced colitis led to the activation of CYP8B1, causing excessive production of CA. The study found that CA acted as an inhibitor of peroxisome proliferation-activated receptor alpha (PPARα) causing alterations in fatty acids oxidation and mice Lgr5+ intestinal stem cells (ISC) renewal. When released to the intestinal lumen, CA had a detrimental effect on epithelial regeneration. As a result, it led to more severe inflammatory damage of the gut. The impact of CA on the intestinal barrier was demonstrated by the use of the fluorescein isothiocyanate (FITC)-dextran assay, which showed an increase in intestinal permeability. Moreover, CA significantly decreased mRNA expression of CLDN-1, CLDN-2 and Mucin2, which indicates the direct influence of CA on the epithelial architecture. Contrarily, a decrease of the liver CYP8B1 caused by treatment with farnesoid X receptor (FXR) agonist alleviated DSS-induced colitis. FXR is responsible for the regulation of BA enterohepatic circulation, including the suppression of CYP8B1 expression [72]. All these findings indicate a strong connection between the liver-gut axis, overexpression of CYP8B1, intestinal hyperpermeability, and IBD pathogenesis [71]. Therefore, CYP8B1-targeting drugs might be a promising direction in future research on IBD therapy.

Overactivity of CYP8B1 also contributes to non-intestinal epithelial hyperpermeability, which is manifested by its negative influence on the blood-biliary barrier (BBB). The expression of CYP8B1 is significantly increased in obstructive cholestasis. During the disease, toxic biliary compounds such as BA accumulate in the liver, leading to inflammation, oxidative stress, and injury of the whole organ, including cells of the BBB maintained by TJP [73–75]. The ongoing inflammation increases the production of growth factors and proinflammatory cytokines which are considered to damage TJ and result in the loss of gap junctions in hepatic cells. It was observed that growth factors (EGF and TGF-beta) and proinflammatory cytokine IL-1β cause both downregulation of Cx32 and CLDN-1 and upregulation of CLDN-2, which constitute crucial elements of tight and gap junctions in hepatocytes [76]. These findings indicate the role of CYP8B1 in regulating cellular permeability, which might be worth investigating in intestinal epithelial cells as well.

**CYP26**

The main role of CYP26 genes, such as CYP26A1, CYP26B1, and CYP26C1, is to regulate retinoid acid (RA) levels by breaking down all-trans retinoic acid (atRA), an active metabolite of vitamin A (retinol). This regulation is especially crucial during embryogenesis, as RA participates in the proper development of vertebrae, including patterning of the central nervous system [77]. CYP26B1 primarily influences skeletal development, bone ossification, creation of certain synovial joints, and differentiation of the hindbrain [77,78]. The excessive concentration of RA might be caused by an unbalanced diet or malfunctions of CYP26 enzymes resulting in reduced catabolism. Mutations of CYP26B1 affect the skeletal, dental, nervous, and visual systems [79,80]. Moreover, atRA is crucial for the maintenance of epithelial cells, regulation of apoptosis, embryogenesis, and immune system activity [81]. It functions as a ligand for nuclear retinoic acid receptors (RARα, RARβ, or RARγ) and binds to certain DNA elements (RAREs) located in the promoter regions of atRA-targeted genes, such as the CYP26 family which is responsible for most of atRA degradation [82,83]. Recent studies have shown a link between RA metabolism and inflammation, including IBD development. Changes in atRA-induced CYP26B1 expression in mesenteric lymph nodes (MLN) T-cells and Payer’s patches (PP) may lead to alteration in T-cell differentiation in the gut [84]. Kang et al. [85] showed that both high and low levels of vitamin A result in the amelioration of intestinal inflammation in SAMP1/YP mice. These positive effects were observed due to the induction of distinct FoxP3+ T cell subsets depending on the vitamin A availability. Moreover, a possible association was found between the homozygous carriers of (T) allele in the CYP26B1 rs2241057 polymorphism and Crohn’s disease (CD) development. As CD is thought to be connected to Th17 cells’ activity, the CYP26B1 polymorphism may change atRA metabolism and influence the homing of Th17 cells within the IL-23/Th17 pathway leading to the development of this disease [86]. Another research showed that a Leu-to-Ser substitution in position 264 in CYP26B1 substantially increases its atRA catabolism capacity what may lead to the development of intestinal inflammation and atherosclerosis [87,88].

Retinoids are also essential for epithelial cell differentiation and maintenance of epithelial integrity. Their interaction with RARs was found to promote the expression of numerous TJP and the formation of TJ in F9 embryonal carcinoma cells [89]. Osanai et al. [90] observed that RA promoted the epithelial barrier functions in vitro and in vivo. They evaluated the effect of atRA in genetically modified Madin-Darby canine kidney (MDCK) cellular monolayers. As a result, MDCK transfected with an empty vector and treated with atRA increased their TEER, whereas cells overexpressing CYP26A1 were resistant to these changes. The use of ketoconazole, an inhibitor of CYP26, partially prevented CYP26A1-dependent resistance to the effect of atRA. It was manifested by increased TEER and decreased permeability to inulin and mannitol. The influence of atRA on cellular permeability was associated with significantly increased expressions of OCLN, CLDN-1, CLDN-4, and ZO-1, which was not observed to the same extent in the cells with active CYP26A1. The in vivo part of the study demonstrated the effect of natural and synthetic RAR agonists, atRA and AM580 in the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced model of colitis. Intraperitoneal administration of both compounds prevented the macroscopic manifestations of colonic inflammation. Moreover, RAR agonists significantly reduced the increase in intestinal permeability measured by the FITC-dextran and evaluation of bacterial translocation to the spleen. The study showed a better therapeutic effect of AM580, which is resistant to CYP26 metabolism.

These findings show that CYP26 and retinoid metabolism are involved in the development of intestinal hyperpermeability and inflammation. Diaz et al. [91] evaluated the effects of potential CYP26A1 and CYP26B1 inhibitors using HepG2 liver cells. As a result, they identified inhibitory molecules, which significantly reduced the metabolism of atRA leading to the increased potency of atRA towards RAR. Such agents may be used to treat CYP26-related inflammatory diseases Although further studies must be conducted, CYP26 inhibitors may display beneficial properties as modulators of the TJ functions in diseases associated with GI hyperpermeability.

**CYP27B1**

CYP27B1 gene encodes the 25-hydroxyvitamin D-1 hydroxylase. It is expressed in a wide range of tissues of endodermal, ectodermal, and mesenchymal origin [92]. The enzyme catalyzes the reaction of hydroxylation of 25(OH)D3 in the kidneys and extra-renal tissues leading to the synthesis of a fully active form of vitamin D: 1,25(OH)2D3 [93,94]. This form is beneficial in various autoimmune diseases, which was proven by several studies. In vitro, 1,25(OH)2D3 inhibits the proliferation of T-cells and decreases the production of IL-2, IFN-gamma, and TNFα, while in vivo it delays the hypersensitivity reaction associated with the Th1 cell response [95,96]. Vitamin D deficiency aggravates colitis in IL-10 knockout mice, while the 1,25(OH)2D3 treatment ameliorates IBD symptoms. Therefore, it is suggested that vitamin D might be a crucial factor in the development and regulation of IBD [93]. Similarly, another study showed significantly increased expression of TNFα, the essential cytokine in IBD development, in vitamin D receptor/IL-10 knockout (VDR/IL-10 KO) mice. Moreover, only VDR/IL-10 KO mice had severe IBD with lesions in all sections of the GI, as compared to both VDR KO and IL-10 KO groups [97].In another model of intestinal inflammation, DSS-induced colitis in mice, the approach using adoptive transfer of inflammation-specific monocytes locally synthesizing the active form of vitamin D alleviated inflammation. Such observations were obtained using CD11b+/Gr1+ monocytes and macrophage-specific promoter (Mac1) to control CYP27B1 expression. The study showed that the positive effect of 1,25(OH)2D3 was not associated with hypercalcemia [94]. Infusions of the CD11b+/Gr1+ monocytes overexpressing CYP27B1 led to the T cell differentiation in the colon, which acted antiinflammatory and favored immunological tolerance. 1,25(OH)2D3 synthesized at the inflammatory sites improved most disease parameters, with a particularly beneficial effect on crypt sprouting. This study also showed that infusion of CD11b+/Gr1+ monocytes influenced intestinal permeability by increasing the mRNA expression of CLDN-1 and ZO-1 in the mouse colon. In the in vitro part of the study, 1,25(OH)2D3 decreased intestinal permeability as measured by the flux of FITC-dextran in Caco-2 monolayers [94]. A link between VDR level, CYP27B1 expression, the concentration of vitamin D metabolites, and the development of IBD was observed also in humans. The evaluation of colonic samples showed that VDR protein expression was significantly lower in IBD patients than in the control group. On the other hand, CYP27B1 level was increased in those patients indicating that vitamin D is consumed in the peripheral circulation leading to its reduced level in the gut what contributes to the development of inflammation [98].

**Conclusion and future perspectives**

Intestinal wall impairment is observed in many disorders affecting various systems in the human body. It may be the cause of the development of the disease or occur secondary to it. The proper function of the intestinal barrier is maintained by complex mechanisms occurring in the intestinal tissue, with a key role of connections between epithelial cells maintained by TJP. Current literature indicates the need for further research on intestinal permeability in search of drugs for the LGS. CYP450 family members are potential candidates for drug targets as recent studies show their significant impact on intestinal barrier function. Namely, it has been shown that CYP2E1 inhibitors may be effective in the treatment of LGS and that the activity of intestinal CYP2E1 plays a key role in the development of intestinal barrier impairment caused by dietary factors, such as alcohol and fructose. Since there is no evidence for fructose to interact with CYP2E1 as a direct substrate, these observations suggest a gap in the understanding of the exact CYP450s’ mechanisms of action. Furthermore, future studies should consider the suppression of CA synthesis resulting from CYP8B1 activity, and the inhibition of CYP26B1-dependent atRA metabolism as potential pharmacological approaches to treat LGS. In contrast, local overactivity of CYP27B1 and following overproduction of active vitamin D form were shown to prevent intestinal barrier damage. Another CYP450 expressed in the intestine, CYPJ2J, was suggested to take part in maintaining epithelial integrity. However, these beneficial properties of CYPJ2J were obtained from the study on BRB and were not evaluated in the gut. Lastly, the influence of CYP1A, CYP2A6, and CYP3A on intestinal permeability remains unclear. It raises the need for further research, which may focus on the effects of selective modulators of these isoforms on the intestinal barrier function. Moreover, most human CYP450s have not been tested for their effects on epithelial barrier permeability. Such studies could provide valuable results even if an evaluated isoform is not expressed in the intestinal tract, as it is the case for CYP8B1.

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AM, ZK, and MSw were involved in conceptualization, literature search, interpretation of the data, and drafting of the article. MSa performed critical revision of the article and supervised the study.

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**Declarations:**

The authors declare no conflict of interest.

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