Nitric oxide and the hyperdynamic circulation in cirrhosis: is there a role for selective intestinal decontamination?

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ABSTRACT

Abnormal vascular tone is responsible for many of the complications seen in cirrhosis making the identification of the pathophysiology of abnormal dilatation a major focus in hepatology research. The study of abnormal vascular tone is complicated by the multiple vascular beds involved (hepatic, splanchnic, peripheral, renal and pulmonary), the differences in the underlying cause of portal hypertension (hepatic versus pre-hepatic) and the slow evolution of the hyperdynamic state. The autonomic nervous system, circulating vasodilators and abnormalities in vascular smooth muscle cells (receptors, ion channels, signalling systems and contraction) have all been implicated. There is overwhelming evidence for an overproduction of NO (nitric oxide) contributing to the peripheral dilatation in both animal models of, and in humans with, cirrhosis and portal hypertension. This review focuses on the proposal that endotoxaemia, possibly from gut-derived bacterial translocation, causes induction of NOS (NO synthase) leading to increased vascular NO production, which is the primary stimulus for the development of vasodilatation in cirrhosis and its accompanying clinical manifestations. The current controversy lies not in whether NO production is elevated, but in which isoform of NOS is responsible. We review the evidence for endotoxaemia in cirrhosis and the factors contributing to gut-derived bacterial translocation, including intestinal motility and permeability, and finally discuss the possible role of selective intestinal decontamination in the management of circulatory abnormalities in cirrhosis.

INTRODUCTION

The initiating factor in the pathogenesis of portal hypertension complicating chronic hepatobiliary injury is an alteration of the hepatic microcirculation, resulting in an increase in intrahepatic resistance. This is generally attributed to morphological distortion of the hepatic vasculature due to fibrosis, thrombosis, hepatocyte enlargement, nodule formation and collagenization of the space of Disse, resulting in a ‘structural’ or ‘fixed’ increase in intrahepatic resistance. There is, however, also a ‘dynamic’ component to intrahepatic vascular resistance, mediated largely by perisinusoidal stellate cells, which undergo transformation due to liver cell injury. These acquire a contractile phenotype with the potential to respond to both local and systemic vasoactive neurohumoral agents, such as noradrenaline (norepinephrine), endothelin, angiotensin II and NO (nitric oxide) [1,2].

Key words: circulation, cirrhosis, intestinal decontamination, nitric oxide, vascular tone.

Abbreviations: IFN-γ, interferon-γ; IL, interleukin; MMC, migrating motor complex; NAC, N-acetylcysteine; NO, nitric oxide; NOS, NO synthase; eNOS, endothelial NOS; iNOS, inducible NOS; PEG, polyethylene glycol; RES, reticulo-endothelial system; SBP, spontaneous bacterial peritonitis; SID, selective intestinal decontamination; TLR, Toll-like receptor; TNF-α, tumour necrosis factor-α.

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Despite the increase in intrahepatic vascular resistance, blood flow into the portal venous system not only continues, but, in many instances, is augmented such that, together with the congestion at the hepatic end, portal venous pressure increases. When this happens chronically over time, the formation of extrahepatic/portosystemic shunts, such as gastroesophageal varices, occur, placing the patient at high risk of portal hypertensive gastrointestinal haemorrhage.

Another result of portosystemic shunting is decreased hepatic clearance of a number of gut-derived vasoactive agents, leading to an increase in their systemic bioavailability and causing systemic arterial vasodilatation. Cross-circulation studies between portal hypertensive and normal animals demonstrate the importance of these transferable humoral factors that accumulate in the circulation due to increased production or reduced hepatic clearance secondary to liver disease and/or portosystemic shunting. Possible contributors include endogenous gastrointestinal hormones such as glucagon, VIP (vasoactive intestinal peptide) and CGRP (calcitonin-gene-related peptide) as well as gut-derived bacterial endotoxins inducing the production of such cytokines as TNF-α (tumour necrosis factor-α), acting either directly on vascular smooth muscle tone or indirectly by their effects on local endothelial-derived factors such as vasodilatory prostaglandins and NO.

Cirrhosis is thus complicated by abnormal blood vessel tone in different regional beds leading to differing important and clinically relevant consequences. Peripheral vasodilatation and the consequent decrease in effective arterial blood volume results in the activation of the renin–angiotensin–aldosterone axis and sympathetic nervous system along with non-osmotic ADH (anti-diuretic hormone) release. These compensatory mechanisms regulate salt and water retention with subsequent plasma volume expansion. Despite these compensatory measures in the decompensated cirrhotic patient, vasodilatation progresses and a hyperdynamic circulatory state develops with elevated cardiac output, decreased systemic arterial pressure, peripheral oedema and the development of ascites [3,4]. The complications are multifactorial; in the renal circulation, the initial stages of the hyperdynamic circulatory state are marked by compensatory afferent arteriolar vasodilatation that preserves renal perfusion and glomerular filtration pressure. As vasodilatation worsens, however, renal perfusion pressure drops and arteriolar vasoconstriction can lead to the development of renal dysfunction (hepatorenal syndrome). In the pulmonary circulation, arteriolar vasodilatation is also observed, increasing pulmonary blood flow and the development of arteriovenous shunts, leading to abnormal gas exchange. In a small proportion of cases, this results in severe hypoxia that is resistant to oxygen supplementation (hepatopulmonary syndrome).

Thus, in essence, abnormal vascular tone is responsible for many of the complications seen in hepatic cirrhosis making the identification of the pathophysiology of abnormal dilatation a major focus in hepatology research. As alluded to above, the study of abnormal vascular tone is complicated by the multiple vascular beds involved (hepatic, splanchnic, peripheral, renal and pulmonary), the differences in the underlying cause of portal hypertension (hepatic versus pre-hepatic) and the slow evolution of the hyperdynamic state. The autonomic nervous system, circulating vasodilators and abnormalities in vascular smooth muscle cells (receptors, ion channels, signalling systems and contraction) have all been implicated.

The role of the endothelium in the control of vascular tone in normal health is well described and the complexities of the role of NO in liver injury [5] and in the cardiovascular complications of cirrhosis [6] have been highlighted recently. In brief, there is overwhelming evidence for an overproduction of NO contributing to the peripheral dilatation in both animal models of, and in humans with, cirrhosis [6] and portal hypertension [7]. The original hypothesis that NO plays a role in cirrhosis came from Vallance and Moncada [8], who proposed that endotoxaemia, possibly from gut-derived bacterial translocation, causes induction of NOS, leading to increased vascular NO production, which is the primary stimulus for the development of vasodilatation in cirrhosis and its accompanying clinical manifestations.

![Diagram](image320x591_to_531x713)

**Figure 1 The Vallance–Moncada hypothesis**

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NO production is elevated, but in which isoform of NO is responsible. Again, the topic is well reviewed elsewhere [6] and will not be reiterated here. Instead, we review the evidence for endotoxaemia in cirrhosis, the factors contributing to gut-derived bacterial translocation and, finally, the possible role of SID (selective intestinal decontamination) in the management of circulatory abnormalities in cirrhosis.

ENDOTOXAEMIA IN CIRRHOSIS

Cirrhosis of the liver is often associated with systemic endotoxaemia in the absence of sepsis [9–11], with the degree of endotoxaemia paralleling the degree of hepatic decompensation [12]. This was the basis for the Vallance and Moncada [8] hypothesis suggesting that the splanchnic/systemic endotoxin load could be the trigger for activating NOS activity with subsequent excess NO production.

Bacterial translocation of gut flora into the portal circulation is well recognized in liver cirrhosis [13,14] and, in the presence of both portosystemic shunting and impaired clearance by the liver, has been implicated in the delivery of endotoxins to the systemic circulation resulting in high local concentrations of biologically active endotoxins capable of activating the cytokine cascade [15]. Cytokines are polypeptides that possess a wide spectrum of inflammatory, metabolic, hematopoietic and immunologic regulatory properties. One of their hallmarks is the extremely pleiotropic nature of their actions. These similarities can be explained by the demonstration that diverse cytokines share the ability to activate identical intracellular signalling and transcription pathways [16]. Cells involved in cytokine production include the macrophage/monocyte system, lymphocytes, neutrophils, endothelial cells and keratinocytes. Although the presence of increased levels of cytokines, such as TNF-α, several ILs (interleukins), including IL-1 and IL-6, and IFN-γ (interferon-γ), in chronic liver diseases such as cirrhosis is well documented [17,18], their functional significance remains unclear [19]. However, it has been proposed that some of these cytokines play a role in the hyperdynamic circulatory syndrome of cirrhosis, with the most studied being TNF-α.

TNF-α is a 17 kDa cytotoxic protein produced by mononuclear cells on activation by bacterial endotoxin and tissue injury [20]. In an elegant study by Riordan et al. [21], up-regulation of peripheral blood mononuclear cells from cirrhotic patients demonstrated expression of TLR2 (Toll-like receptor 2), but not TLR4, which implies, contrary to previous assumptions, an important stimulatory role for Gram-positive microbial components, but not endotoxin. TLR2 probably contributes to increased circulating TNF-α and soluble TNF-receptor levels in cirrhosis.

TNF-α has been implicated as a mediator of angiogenesis and the host immune response in sepsis and neoplasia. This cytokine increases vascular permeability, enhances neutrophil adhesion to vascular endothelium and causes metabolic and structural damage to vascular endothelial cells. It binds specific receptors on the endothelial cell membrane through which the biological response is amplified by the secondary release of factors, including NO. In fact, it has been shown that the hypotension elicited by TNF-α is reversed after inhibition of NO synthesis, suggesting that l-arginine-derived NO is a principal mediator of TNF-α-induced hypotension [22]. Although TNF-α was originally thought to induce NO synthesis primarily through the iNOS (inducible NOS) pathway, it is now known to also increase the activity of eNOS (endothelial NOS) possibly by a novel pathway involving sequential activation of neutral sphingomyelinase, PI3K (phosphoinositide 3-kinase) and Akt [23] or by activating the synthesis of the eNOS cofactor tetrahydrobiopterin, as was observed in experimental cirrhosis [24]. In addition, TNF-α can also induce vasodilatation by way of mechanisms that are independent of its effect on the endothelium, for example by depressing potassium depolarization [25].

Of interest is the report [26] that treatment with a specific monoclonal chimaeric anti-(TNF-α) antibody successfully produced a highly significant early and sustained reduction in hepatic venous pressure gradient, probably through a reduction in cardiac output and intrahepatic resistance in patients with alcoholic hepatitis. These studies parallel those demonstrating the effectiveness of thalidomide in reducing the hepatic venous pressure gradient in stable alcoholic cirrhosis patients, a finding which is thought to be due to the ability of thalidomide to inhibit TNF-α production [27].

FACTORS CONTRIBUTING TO GUT-DERIVED BACTERIAL TRANSLLOCATION

Mucosal injury to the upper gastrointestinal system

Chronic alcohol use has been shown repeatedly to be an important cause of haemorrhagic erosive gastric lesions in humans. The mechanisms, although not fully elucidated, are thought to occur via decreased gastric mucosal prostaglandin formation and increased leukotriene production contributing to mucosal injury [28]. In experimental animals, administration of alcoholic solutions at concentrations corresponding to those commonly available in alcoholic beverages leads to damage of the mucosa of the upper small intestine, which can progress to pronounced exfoliation of the tips of the villi and haemorrhage. It has been suggested that the mechanism of injury may be the induction of villous contraction leading to villous bleb formation, lymphatic obstruction
and, finally, exfoliation of the tips of villi when the blebs rupture [29]. It has been suggested further that the initial event in response to alcohol is the release of noxious mediators such as histamine and leukotrienes, which cause microvascular injury [30]. Thus chronic alcohol exposure to the upper gastrointestinal mucosa leads to mucosal injury which could potentially facilitate transfer of bacteria into the circulation.

**Increased gut permeability to macromolecules**

The mucosal barrier in normal mammals is incomplete, so that small amounts of macromolecules may pass through the mucosa of the small intestine. The escape of minute amounts of lipopolysaccharides from the luminal side across the normal mucosa into the portal vein and intestinal lymphatics, for example, has been demonstrated in experimental models [31]. In these studies, both acute and chronic administration of alcohol results in increased intestinal permeability to macromolecules such as PEG (polyethylene glycol) and 51Cr-labelled EDTA. Similar observations have also been reported in alcoholic patients when 51Cr-labelled EDTA was used as the permeability probe [32]. Cirrhotic patients with portal hypertension show intestinal structural abnormalities characterized by vascular congestion and oedema. In a separate study, Parlesak et al. [33] demonstrated the increased permeability of the intestine to the permeability marker PEG in alcoholic cirrhotic patients, with a strong correlation to serum endotoxin levels. The enhanced permeability of the intestinal mucosa to macromolecules in the setting of chronic alcohol use could result in the absorption of toxic compounds, such as endotoxin and other bacterial toxins, which would normally not be absorbed. This hypothesis is supported by the observation of transient endotoxaemia following acute alcohol consumption in subjects with no evidence of alcoholic liver disease [34].

**Intestinal bacterial overgrowth and bacterial translocation in cirrhosis**

Bacterial translocation is defined as the passage of viable bacteria/endotoxins from the intact gastrointestinal tract mucosa to mesenteric lymph nodes and the portal circulation and has been postulated to be an important step in the pathogenesis of enteric-origin bacterial infections in cirrhotic patients [35].

Although the phenomenon of bacterial translocation has been recognized for more than a century, the precise mechanism of translocation is still unclear. The major mechanisms postulated to promote bacterial translocation are deficiencies in local host immune systems, increased permeability of the intestinal mucosal barrier (such as with chronic alcohol use) and intestinal bacterial overgrowth [11,13]. Regarding the latter, Steffen and Berg [36] demonstrated, in rats, a direct relationship between the increase in Gram-negative bacterial population in the caecum and the magnitude of bacterial translocation, suggesting that bacterial overpopulation in the bowel is one of the major mechanisms that promotes bacterial translocation in experimental models. Guarner et al. [13] have shown that intestinal aerobic bacterial count in caecal stools is increased significantly in cirrhotic rats with bacterial translocation compared with cirrhotic rats without bacterial translocation and normal rats. In another study, Pardo et al. [37] observed that total Gram-positive and Gram-negative jejunal intestinal bacterial overgrowth, as well as total caecal bacterial overgrowth, were significantly higher in ascites from cirrhotic rats with bacterial translocation than in those without bacterial translocation and, importantly, that translocation of a specific organism was almost always associated with intestinal bacterial overgrowth of that organism. The latter two studies strongly suggest that the presence of intestinal bacterial overgrowth might be a prerequisite for the development of bacterial translocation. Furthermore, administration of conjugated oral bile acids to ascitic cirrhotic animals increased bile acid secretion, eliminated intestinal bacterial overgrowth, decreased bacterial translocation and endotoxaemia and increased survival [38]. Translating these findings to the clinical setting, Casafont Morencos et al. [39] demonstrated that 30% of alcoholic cirrhotic patients had intestinal bacterial overgrowth on the basis of breath testing compared with none in the healthy controls and, moreover, the prevalence of spontaneous bacterial peritonitis was significantly higher in patients who had intestinal bacterial overgrowth than in patients who did not. It has also been demonstrated [40] that the incidence of bacterial overgrowth of the small intestine was much higher in cirrhotic patients with bacterial peritonitis than in those without spontaneous bacterial peritonitis. These experimental and human data are strongly suggestive that intestinal bacterial overgrowth favours the development of bacterial translocation from the gut to extra-intestinal sites in cirrhosis.

**RISK FACTORS FOR INTESTINAL BACTERIAL OVERGROWTH IN CIRRHOSIS**

Distal propulsion of luminal contents by intestinal peristalsis is a critical factor in the inhibition of bacterial colonization and replication in the proximal gastrointestinal tract. Bacterial overgrowth frequently develops in the absence of an identifiable structural lesion to account for the impaired intestinal transport. For example, bacterial overgrowth complicates the clinical course of intestinal pseudo-obstruction and is suspected as secondary to intestinal motility dysfunction. In the fasting state, the gastrointestinal tract is characterized by a regular cyclical activity, i.e. the interdigestive MMC (migrating motor...
complex). In humans, the well-defined phase III activity of the MMC occurs every 84–112 min and migrates along the upper intestinal tract at a speed of approx. 6–8 cm/min [41]. This moving band of intense contractions during phase III has been called the ‘intestinal housekeeper’, a sweeping function that clears the gut in association with augmented biliary secretions which act as detergent. Bacteria accumulate in the gut when intestinal motility and transit are impaired. Vantrappen et al. [42] reported that complete or partial absence of the phase III activity is associated with bacterial overgrowth of the small intestine. In support of the above hypothesis, Scott and Cahall [43] reported bacterial overgrowth in the small intestine of the rat in which MMC cycling was disrupted pharmacologically.

Delayed intestinal transit has been demonstrated in cirrhotic rats with portal hypertension [44], and altered small intestinal motility has been documented in patients with cirrhosis [45,46] and appears to be related to the severity of liver failure [47]. In another study by Chang et al. [48], the frequency of MMCs were significantly lower in decompensated cirrhotics compared with compensated cirrhotics and this, in turn, correlated with the degree of intestinal bacterial overgrowth. Possible mechanisms for the dysmotility could include altered smooth muscle contractility, elevated plasma levels of glucagon, dysfunction of the autonomic nervous system and endotoxaemia, although definitive studies clearly identifying the responsible factors have not been reported thus far.

Several other circumstances in cirrhosis that could predispose a patient to intestinal bacterial overgrowth include alcohol abuse [49], malnutrition [50] and deconjugation of bile acids shifting to alkaline pH in gastric juice [51].

Thus multiple factors contribute to the translocation of gut-derived bacteria and bacterial toxins in cirrhosis into regional lymph nodes and the splanchnic circulation, including intestinal bacterial overgrowth and small bowel dysmotility. Importantly chronic alcohol use, still the commonest cause of cirrhosis in many countries, including Australia, can, independently of cirrhosis, facilitate this translocation by inducing upper gut mucosal damage and increasing mucosal permeability to these immunostimulant peptides. This is supported by findings of higher serum endotoxin levels in patients with alcoholic cirrhosis than in patients with non-alcoholic cirrhosis [34,52].

**ROLE OF IMPAIRED LIVER CLEARANCE IN THE SYSTEMIC ENDOTOXAEMIA OF CIRRHOSIS**

In normal conditions, those bacteria and associated toxins that have reached the splanchnic and systemic circulation are destroyed by the mononuclear phagocytic system, known as the RES (reticulo-endothelial system), mainly located in the liver. However, the functional activity of this essential bactericidal system is impaired in some cirrhotic patients. It has been observed that the clearance of $^{99m}$Tc-sulphur colloid (an index of RES function) by the RES is reduced in cirrhotic patients when compared with healthy subjects [53]. Patients with more severe dysfunction of the RES developed more episodes of bacteraemia and/or spontaneous bacterial peritonitis during follow-up and had a shorter survival than patients with preserved or less depressed RES activity. Moreover, it has been demonstrated that, in patients with more advanced chronic liver disease, there was an impaired ability to remove $^{99m}$Tc-labelled microspheres and an increased risk of developing bacterial peritonitis [54]. In this study, survival was shorter in these patients compared with those with a more effective RES. The decreased function of the RES probably favours prolonged episodes of bacteraemia, increasing the probability of seeding of the bacteria in other tissues and ascitic fluid and the consequent development of spontaneous bacterial peritonitis. Once bacteria enter the blood stream, the outcome of blood colonization depends on the capacity of the blood to kill the bacteria. Bacteria must be engulfed by neutrophil leucocytes or macrophages. However, before this occurs, bacteria must be coated or opsonized with IgG and/or the third component of complement. Chemotactic, bactericidal and phagocytic activity of polymorphonuclear leucocytes obtained from cirrhotic patients is abnormal [55]. One of the mechanisms of impaired neutrophil function seems to be a reduced production of oxidative metabolites [56]. Macrophages are the first-line agents in the host response to foreign agents, because of their capacity to recognize and eliminate the IgG-coated organisms; they are located in the peritoneal cavity, liver and spleen. Gomez et al. [57] have found an impaired function of Fcγ-receptors in macrophages from alcoholic cirrhotic patients, similar to that observed in patients with primary biliary cirrhosis [58]. These alterations may predispose to the development of bacteraemia and endotoxaemia in cirrhotic patients.

Thus it is increasingly accepted that gut-derived bacteria/bacterial byproducts, including endotoxins/cytokines, reach the portal circulation via bacterial translocation and, in the setting of cirrhosis, reach the peripheral circulation as a result of impaired clearance by the liver, the presence of porta-systemic shunting and the impaired ability of Kupffer cells to recognize IgG-coated organisms.

From this it is apparent that the source of the endotoxaemia of cirrhosis is the gastrointestinal tract, and that attempts to reduce this may well have clinical implications. This has led to the concept that SID, which is reviewed in more detail below, has the potential to
favourably affect the haemodynamic consequences of cirrhosis of the liver.

SID

SID leads to elimination of aerobic Gram-negative bacilli from the intestinal flora with oral non-absorbable or poorly absorbable antibiotics, whilst preserving the remaining aerobic and the anaerobic bacteria. The antibacterial norfloxacin has been shown to be an appropriate drug for long-term SID since it is incompletely absorbed in the intestine, is highly active against aerobic Gram-negative bacilli, has low activity against anaerobic bacteria and has a low incidence of side effects when administered for long periods. Gines et al. [59] have shown that long-term administration of oral norfloxacin in decompensated cirrhotic patients produces a marked reduction of aerobic Gram-negative bacilli from the faecal flora with no significant side effects on Gram-positive cocci and anaerobic bacteria. In this study, norfloxacin administration was not associated with overgrowth of resistant potentially pathogenic bacteria or with an increase in the faecal concentration of Candida species. Furthermore, the concentration of anaerobic bacteria was not affected by norfloxacin administration. Different mechanisms may contribute to the efficacy of long-term norfloxacin administration in preventing recurrent SBP (spontaneous bacterial peritonitis) caused by Gram-negative bacilli in patients with cirrhosis. The first mechanism is the SID caused by this drug. If most SBP episodes in cirrhosis are caused by the passage of enteric bacteria into the general circulation and thereafter into the ascitic fluid, it is reasonable to assume that eradication of Gram-negative bacilli from the intestinal flora is followed by a reduction in the rate of SBP recurrence caused by these organisms. On the other hand, in patients with cirrhosis and ascites, the trough plasma levels of norfloxacin during long-term prophylaxis have been found to be similar or higher than the 90% minimal inhibitory concentration of norfloxacin for most aerobic Gram-negative bacilli. Therefore the constant presence of bacterial levels of norfloxacin for Gram-negative organisms in serum, and presumably in ascitic fluid, could be an additional mechanism explaining the efficacy of long-term administration of norfloxacin in preventing SBP.

The preservation of lactobacilli in the intestinal flora may be another mechanism contributing to the prevention of SBP by norfloxacin administration [60]. Lactobacilli are an integral part of the normal gastrointestinal microecology and are involved in host metabolism. They possess antimicrobial activities, including the production of bacteriocin and low-molecular-mass antibiotic substances, organic acids and other metabolic end products, in both the human and animal gastrointestinal tract.

Finally, quinolones may have immunoregulatory functions stimulating the bactericidal capacity of the polymorphonuclear cells or decreasing bacterial adhesion to mucosal surfaces.

FLUOROQUINOLONE ANTIBIOTICS

Norfloxacin is a synthetic 6-fluoro-4-quinolone agent (see [61]). The primary bacterial target of norfloxacin is DNA gyrase. Purified DNA gyrase introduces negative superhelical twists into DNA and reversibly separates DNA circles that are interlocked (like links in a chain) by passing one DNA duplex through a transient double-strand break in the other duplex. Within living bacteria, DNA gyrase carries out these same activities and is required for DNA replication, transcription of certain genes and aspects of DNA repair and recombination. These enzymic activities are antagonized by norfloxacin and other quinolone agents. Additionally, norfloxacin induces purified DNA gyrase to cleave duplex DNA on addition of detergent; DNA cleavage also probably occurs intracellularly. Enhanced antagonism of DNA gyrase by norfloxacin appears to account for a substantial portion of its increased potency compared with a sister compound, nalidixic acid.

In healthy subjects, a single 400 mg dose leads to serum concentrations of 1.5 µg/ml in 1–2 h and a terminal serum half-life of 3–4 h. Administration in multiple doses compared with a single dose does not change peak serum concentration or half-life. For an increasing size of a single dose, however, maximum serum concentrations tend not to increase proportionally. Determination of the volume of distribution of norfloxacin has not been possible because of a lack of a drug formulation for intravenous administration. Animal studies, however, are consistent with a large volume of distribution, which, if extrapolated to humans, would be in the range of 30 litres. Peak concentrations of norfloxacin in many tissues and bodily fluids after a 400 mg oral dose are in the 1 µg/ml range. Norfloxacin is excreted, in part, by the kidneys. In the setting of decreased renal clearance, the serum half-life of norfloxacin increases. Non-renal routes of elimination of norfloxacin are not well defined in humans. In patients with moderate impairment of liver function, serum half-life and other pharmacological measures are not altered [62].

Norfloxacin is generally well tolerated [63], as observed in data collected from previous randomized controlled studies. The need to withdraw the drug is extremely uncommon, the major side effects reported include those that involve the gastrointestinal tract (most often nausea) and the central nervous system (most often headache and lightheadedness). Few laboratory abnormalities during prolonged treatment have been reported: when studied in all comparative trials, abnormalities of
blood chemistry and haematology profiles have been equivalent in the norfloxacin and control groups.

**SID, NO AND THE HYPERDYNAMIC CIRCULATION IN CIRRHOSIS**

There are several animal studies demonstrating that norfloxacin can decrease bacterial translocation across the intestinal mucosa [64] and that it does improve outcome in cirrhosis [65]. We have demonstrated that norfloxacin treatment is associated with a reduction in basal forearm blood flow in human cirrhosis, otherwise elevated when compared with age- and sex-matched controls [66]. This finding was associated with a reduced forearm blood flow response to the constricting effects of the NOS inhibitor Nω-monomethyl-L-arginine infused into the brachial artery, suggesting that overproduction of NO was dampened with norfloxacin treatment. Since this report, other investigators have confirmed a positive effect of norfloxacin on vascular resistance [67] and pulmonary shunting [68,69] in cirrhosis. We have since reported on findings that norfloxacin partially reverses the hyperdynamic circulation in cirrhosis in humans [70], a report which was succinctly summarized recently [71]. In essence, norfloxacin significantly decreased endotoxin levels, increased mean arterial blood pressure and systemic vascular resistance and forearm microcirculatory parameters. Trends towards a reduction in cardiac output and hepatic venous pressure gradient, albeit not statistically significant, were observed. In an earlier study by Albillos et al. [67], norfloxacin did significantly improve cardiac index, but only in the subset of patients who had elevated levels of lipopolysaccharide-binding proteins. Although there were some discrepancies between the two studies, both investigations support the continued exploration of the use of SID as adjunct therapy in cirrhosis as both provided proof of the concept that gut-derived bacterial translocation was the primary stimulus for the systemic dilatation and the clinical consequences observed in these patients.

**FUTURE DIRECTIONS**

In his summary, Zucker [71] correctly pointed out that, despite the well-established and well-tolerated profile of long-term norfloxacin administration (as outlined above), the therapy has risks, not least of which is the emergence of bacterial resistance with its continued use [72]. Such concerns have prompted the search for alternative methods for reducing intestinal endotoxin production. The use of oral bile acids to decrease bacterial overgrowth has demonstrated promising results [38]. As well as the possible use of probiotics, live microbial supplements may have beneficial effects. Probiotics are derived from indigenous gut flora and the most widely studied strains are of the *Bifidobacterium* and *Lactobacillus* species. Probiotic therapy is aimed at changing the make-up of the indigenous microflora by administering specific strains of non-pathogenic and potentially beneficial microflora. It is suggested that ingestion of probiotic bacteria has the potential to stabilize intestinal epithelial barrier by reducing local proinflammatory cytokine generation [73]. Preliminary studies report a benefit in reversing some of the immunological changes in inflammatory bowel disease [74] and non-alcoholic fatty liver disease [75] and as prophylaxis in diarrhoeal illnesses of multiple aetiologies [76].

Lactobacilli can inhibit the growth of various potentially pathogenic bacteria, and have been reported to be able to stimulate host immunity, increase host resistance against infection, activate liver and peritoneal macrophages and enhance intestinal immune function [77]. Bacteriotherapy with lactobacilli or other bacterial strains has been reported to be effective in both pseudomembranous and ulcerative colitis [78] and, in fact, to be more effective that standard bowel decontamination in decreasing the rate of post-operative infections following liver transplantation [79]. In the context of the current review, it is noteworthy that *Lactobacillus* administration reduces bacterial translocation in animal models of acute liver injury [80] and synchronous liver resection and colon anastomosis [81], although this has not been demonstrated for experimental models of pre-hepatic portal hypertension [82] or in models of cirrhosis [83], except with the added administration of antioxidants [84]. In the latter study, application of living *Lactobacillus* was demonstrated to significantly decrease endotoxaemia, a finding validated in humans in a single case report study [85]. The finding that co-administration with antioxidants enabled even more pronounced findings, including a decrease in the indices of bacterial translocation, warrants further discussion.

Antioxidants are thought to be useful, since mucosal oxidative damage (due to hypoperfusion and hypoxia) is implicated in the development of an impaired intestinal barrier contributing to bacterial translocation [86,87]. Aside from their effect on the mucosa, however, the potential role of oxidative stress injury in the circulatory changes in portal hypertension is complex. In a study by Fernando et al. [88], oxidant stress injury (measured as urinary F2-isoprostanes) was associated with the hyperdynamic circulation in partial portal vein ligated rats; plasma nitrite and nitrate levels were also significantly higher compared with controls, suggestive of increased endogenous NO production. Cytokines, such as TNFα (discussed above), are a potent stimulus for production of reactive oxygen intermediates, which, in turn, can activate NOS; however, no elevation in TNF-α was observed in this study [88]. Following the chronic administration of intraperitoneal NAC
(N-acetylcysteine), a significant suppression of the enhanced urinary F₂-isoprostanes and endogenous NO markers was observed. In addition, the hyperdynamic circulation and portal hypertension was significantly blunted. NAC is a sulphurated amino acid and has antioxidant activity secondary to its thiol group, which acts both directly as a free-radical scavenger and indirectly by repleting tissue glutathione levels. Although the mechanism of action of NAC is still unclear, it is tempting to speculate that a reduction in oxidative injury might have led to reduced NOS activity. In a follow-up study by the same group [89], the effects of antioxidant therapy were extended to a cirrhotic model. Chronic administration of lipoic acid (a thiol-containing antioxidant) prevented the development of the hyperdynamic circulation and significantly attenuated the rise in portal hypertension in a model of biliary cirrhosis; the enhanced total plasma nitrite/nitrate levels were also reduced following therapy. It was felt by the authors that, since there was no structural change noted in the livers on histology of the treated cirrhotic rats, the reduction in portal pressure was more likely to be secondary to a reduction in the portal venous inflow; this assessment may not be entirely accurate, as potential changes in the dynamic component of intrahepatic vascular resistance were not accounted for.

In spite of the lack of consensus regarding the relative contribution of which NOS isoform is responsible for NO overproduction, there is evidence to suggest that both may be up-regulated by oxidative injury and be inhibited by antioxidants. One of the transcription factors important in activating iNOS gene expression is NFkB (nuclear factor κB) activation, which is strongly inhibited by lipoic acid. Several antioxidants reduce iNOS expression in response to endotoxins in vitro [90] and in vivo, and vitamin E analogues reduce the vascular hyporeactivity observed in lipopolysaccharide-treated rats. eNOS activity is increased in response to hypoxia and shear stress, both of which may be important at a cellular level in cirrhosis. The former leads to the generation of free radicals by mitochondria, and the latter is transduced via extracellular-regulated kinases, which themselves can be activated by reactive oxygen species and inhibited by antioxidants [89].

Whether the potentially beneficial effects of probiotic therapy affect NO production and the hyperdynamic circulation in cirrhosis in man is not known. Whether application of antioxidants, in their own right or as a co-application, is a prerequisite to the effectiveness of probiotic treatment is also unclear and warrants further investigation. As there is a relative paucity in effective pharmacological treatment for the hyperdynamic syndrome and its clinical complications, including portal hypertension, these novel and innovative treatments may provide important alternatives or as adjunct therapy in the clinical management of the cardiovascular complications in cirrhosis.

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