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Liver regeneration: a spotlight on the novel role of platelets and serotonin

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Summary

The development of novel approaches in liver surgery in the last decade has saved the lives of a large number of patients via resection of liver tumours previously thought to be non-resectable. Concurrently, living donor liver transplantation has emerged as one of the ways of lowering mortality on the waiting lists. These breakthroughs demanded a rigorous understanding of the mechanisms of liver regeneration after partial hepatectomy. Based on our previous studies on blood platelets and cold ischaemic injury, platelets and serotonin have attracted attention due to their theoretical potential contribution to liver regeneration. Both platelets and serotonin have been proven to be crucially involved in liver regeneration after partial hepatectomy. This review article provides an overview on the process of liver regeneration, with emphasis on its molecular basis and the coordinate contribution of several cells to restoring the organ's original volume and function. The role of platelets and serotonin is highlighted as novel contributors in this process.

Key words: liver regeneration; platelets; serotonin

Introduction

There have been many breakthroughs in the field of liver surgery over the past decade with the realisation that liver resection is the only cure for liver tumours in many patients. This has resulted in novel surgical strategies involving removal of more than 70% of the liver, with the need in some cases sometimes to manipulate the liver volume prior to surgery to enable growth of the healthy liver and atrophy of the part containing a tumour [1]. Similar progress has occurred in the field of liver transplantation, where the lack of available organs and the increasing number of patients with liver diseases dying on the waiting list has triggered a search for alternative strategies [2]. Living donor liver transplantation has emerged as an effective means of increasing the organ pool. This procedure involves a major liver resection (right hemiliver = 50–60% of the liver) in a healthy adult [3]. While the liver has the unique ability to regenerate in a few days or weeks after major tissue loss, major liver resection both in patients with liver tumours or for organ donation involves the risk of liver failure due to insufficient liver mass. Below a certain threshold (about 20–25%) the remnant liver cannot regenerate, leading to the liver failure called "small-for-size syndrome" – meaning that a remnant liver is too small for the size of an individual [1, 4].

Small-for-size syndrome is the single most important limiting factor in liver surgery and transplantation. Strategies to prevent liver failure have been the focus of many groups, and a breakthrough will likewise occur only through an improved understanding of the many pathways of liver regeneration. We have summarised the current advances in liver surgery in a recent review article published in the New England Journal of Medicine [1]. This has been a major focus for our laboratory and the clinical research of my groups

Abbreviations:

EGF	epidermal growth factor
HGF	hepatocyte growth factor
IL-6	interleukin-6
PDGF	platelet-derived growth factor
NF-κ B	nuclear factor-kappa B
SERT	serotonin reuptake transporter
STAT 3	signal transducer and activator of transcription 3
TPO	thrombopoietin
TGF-α & β	transforming growth factor alpha & beta
TPH	tryptophan hydroxylase
TNF-α	tumour necrosis factor alpha
VEGF	vascular endothelial growth factor

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for about a decade. In this article, I will first cover some current knowledge of liver regeneration and then present our recent and ongoing work on the role of serotonin and platelets in initiating liver regeneration.

An overview of liver regeneration

The human body responds to partial hepatectomy by reestablishment of the original volume of the organ thanks to the unique ability of the liver cells to replicate and increase the remnant segments. The typical scenario of liver volume restoration commences with hyperplasia of various types of intrahepatic cells followed by a phase of cellular hypertrophy [1]. This phenomenon is traditionally known as liver regeneration despite the fact that, in purely biological terminology, neither hyperplasia nor hypertrophy is a synonym for regeneration [5].

Fundamental characteristics of liver regeneration

Several experimental methodologies have been applied to the study of liver regeneration. Rodent models of partial hepatectomy have provided the most convenient approach to study this process, given their close resemblance to the human situation, absence of immediate tissue injury or inflammation and precise definition of the initial time point when liver regeneration is triggered [1, 6]. In a normal murine liver mature differentiated hepatocytes exhibit minimal turnover [7] with only one out of 2000–3000 hepatocytes dividing under physiological conditions to maintain normal liver mass [8]. The extent of the resection and tissue damage remarkably influence the initiation and synchronisation of replication in different types of hepatic cells. For instance, minor damage (eg toxic or ischaemic injury) or a relatively small resection (removal of less than 30% of the liver) results in a less powerful replication rate, which is also less synchronised in comparison with a major resection (removal of 70% of the liver) [8, 9]. After a massive resection, up to 90% of the hepatocytes appear to replicate [9]. There is significant variation among species with regard to the onset and peak of hepatocyte replication [1]. In human beings, replication of hepatocytes generally begins within 1 day after a major resection. Endothelial cells, Kupffer cells and bile duct cells replicate in delayed fashion. Several additional days are then required for growth, during which enhancement of cell size occurs [1]. In mice, deoxyribonucleic acid (DNA) replication after partial hepatectomy peaks 44–48 hours after the operation, regardless of the time of the day at which the procedure has been performed (fig. 1). The entry of cells that had replicated their DNA into mitosis always occurs at the same time of day [10–12]. It has been suggested that proliferating hepatocytes provide the mitogenic stimuli required for proliferation of the other cells [6]. Hence nonparenchymal cells, such as endothelial cells, Kupffer cells, and biliary-duct cells, replicate in delayed fashion [1]. DNA synthesis is completed in 72 hours. At this time the inhibitory action of TGF β_1 on liver regeneration predominates [13]; allowing for changes in liver histology [6]. At post-hepatectomy day 3 to 4, clumps of small hepatocytes penetrated by processes from Ito cells are observed around newly formed capillaries [14]. These small hepatocyte clumps are to be reorganised into the characteristic hepatocyte plates of the mature liver, while the capillaries change into sinusoids lined by fenestrated endothelium and Kupffer cells [6]. Eventually the liver should reach the normal liver/body mass ratio to enable proper function, even if a small-sized graft is implanted into a larger recipient [15–17].

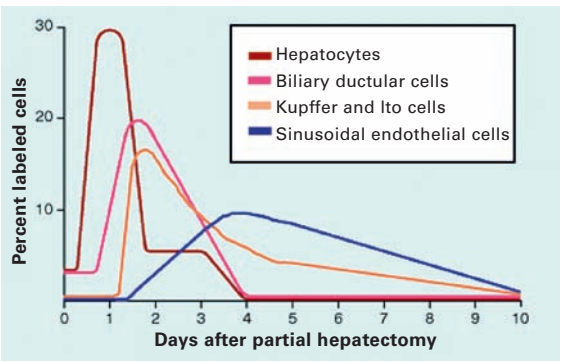

Figure 1

Kinetics of DNA synthesis in hepatocytes, biliary ductular cells, Kupffer, Ito and sinusoidal endothelial cells. [Adapted from: Michalopoulos GK, DeFrances MC. Liver regeneration. Science. 1997;276 (5309):60–6 and reprinted with permission.]

Basic molecular pathways of liver regeneration

Liver regeneration encompasses activation of many intra- and extracellular molecules and pathways (fig. 2). The current concept is that there are cytokine and growth factor-mediated networks promoting regeneration of various types of intrahepatic cells. Many factors pertaining to liver regeneration have been extensively studied, among which serotonin and platelets have recently made exciting advances and will be discussed in later sections. Precise integration of growth signals is required for full and synchronised regeneration. Failure to activate these signaling cascades may result in delay of the onset of regeneration, inadequate recovery of liver volume and eventually clinical signs of liver failure [18].

Cytokine-mediated pathways

Partial hepatectomy stimulates expression of many genes which are involved in a cytokine network. Several studies have demonstrated that the release of cytokines during the early post-hepatectomy period is critical for initiation of liver regeneration. The cytokine action is initiated through

binding of tumour necrosis factor-α (TNF-α) to its receptor TNFR1, resulting in activation of NF-κ B (nuclear factor-κ B) in non-parenchymal cells. In addition it causes release of interleukin-6 (IL-6) and activation of signal transducer and activator of transcription 3 (STAT3) in hepatocytes [19]. Lipopolysaccharide (LPS) which is released from enteric bacteria into the portal circulation has been suggested as a principal trigger of cytokine network activation [20]. Rats with restricted production of LPS and LPS-hyporesponsive mice have shown delayed regeneration after partial hepatectomy [21].

Tumour necrosis factor-α (TNF-α)

Plasma levels of TNF-α which is produced mainly by Kupffer cells increase after partial hepatectomy. Antibodies against TNF-α administered at the time of hepatectomy lower the regenerative response [22]. Liver regeneration is blocked after partial hepatectomy in mice with genetic deletions of the TNFR1 [18, 23, 24]. TNF-α is not a direct stimulant of normal hepatocyte replication and does not induce DNA synthesis either in primary cultures of hepatocytes or in vivo [25]. Furthermore, TNF-α enhances the effects of direct mitogens such as HGF [26]. Despite convincing evidence indicating a crucial role of TNF-α in liver regeneration, TNF-α represents only one out of the many other important molecules which are concurrently activated and contribute to orchestrating the early events after partial hepatectomy [25].

Interleukin-6 (IL -6)

IL-6 is chiefly produced by Kupffer cells, which represent resident hepatic macrophages [27]. Following partial hepatectomy plasma levels are transiently increased [28]. Several studies have shown that IL-6 is necessary for proper liver regeneration [29, 30]. However, since restoration of liver mass is only delayed in the absence of IL-6, it does not appear to be the main inducer of this process [29]. IL-6 is not a direct mitogen for hepatocytes and does not enhance the mitogenic effect of other growth factors. Nonetheless, IL-6 exerts a mitogenic influence on biliary cells [31] and rescues failure of liver regeneration in the ischaemic [32] and fatty liver [33]. Moreover, IL-6 influences the integrity of the intrahepatic biliary tree by regulating production of small proline-rich proteins by cholangiocytes [34, 35]. Hence, similarly to TNF-α, IL-6 is probably a contributory factor in optimising but not initiating the processes of liver regeneration.

Growth factor-mediated pathways

There is a consensus that no single molecule is able to induce liver regeneration after partial hepatectomy. Several growth factors are involved in stimulating the liver to regenerate, eg hepatocyte growth factor (HGF), epidermal growth factor

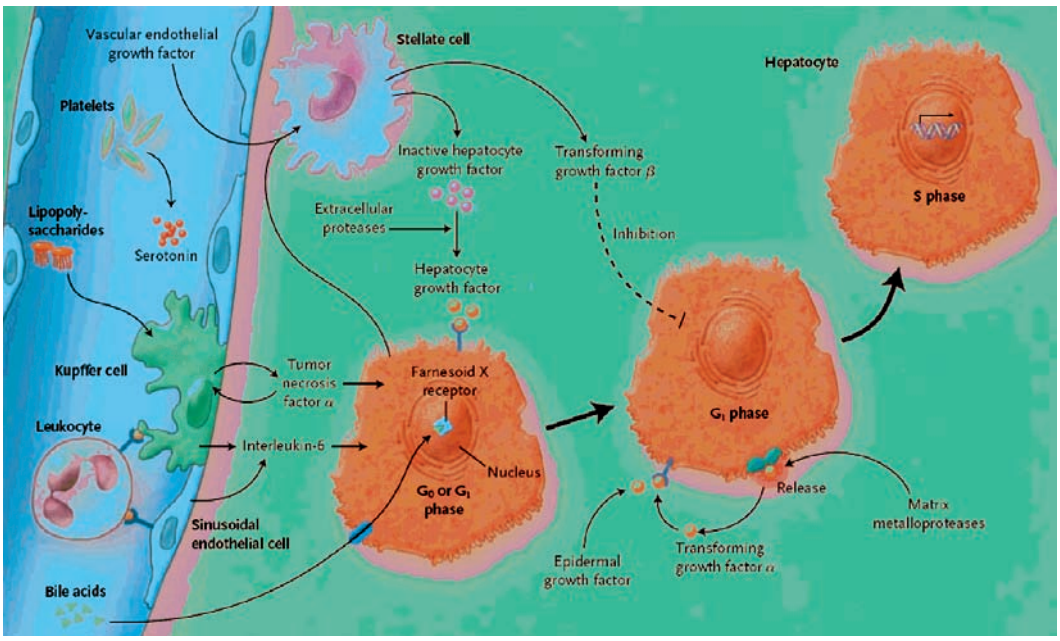

Figure 2

After hepatectomy, nonparenchymal cells, such as stellate cells, Kupffer cells, leukocytes, and platelets, are activated by soluble factors such as vascular endothelial growth factor and lipopolysaccharide. Interaction between activated vascular components, including platelets, leukocytes, sinusoidal endothelial cells and Kupffer cells, results in the release of tumour necrosis factor α, interleukin-6, and serotonin. The cytokines cause priming of the remnant hepatocytes and, concurrently, extracellular proteases such as urokinase-type plasminogen activator convert inactive hepatocyte growth factor to its active form. Inactive hepatocyte growth factor, which is secreted by stellate cells, is a mitogen that induces hepatocyte proliferation. Matrix metalloproteases convert membrane-bound transforming growth factor α into the soluble form. In an autocrine loop, transforming growth factor, along with endothelial growth factor, signals through the endothelial growth factor receptor. The cytokines and the growth factors act in concert to initiate the reentry of quiescent hepatocytes (in the G_0 phase) into the cell cycle from the G_1 phase to the S phase, resulting in DNA synthesis and hepatocyte proliferation. To signal the end of proliferation, transforming growth factor β blocks further replication. The metabolic load resulting from the loss of hepatocytes is indicated by the accumulation of bile acids in the blood. The bile acids enter the hepatocytes and drive bile acid receptors such as the farnesoid X receptor, resulting in increased protein and DNA synthesis [Adapted from: Clavien PA, et al. Strategies for safer liver surgery and partial liver transplantation. N Engl J Med. 2007;356(15):1545–59;]

(EGF), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Several investigators have identified TGF β_1 as the main inhibitor of liver regeneration. We will focus on HGF, EGF and TGF β_1 as the most important key players.

Hepatocyte growth factor (HGF)

HGF is synthesised by non-parenchymal cells, particularly stellate cells, and therefore affects hepatocytes in a paracrine manner [9]. It is present in the extracellular matrix in relatively large quantities as inactive precursor [36, 37]. Activation by proteases results in a 10- to 20-fold increase in plasma HGF levels [38]. Systemically injected HGF is trapped mainly by the liver [39]. HGF injection into the portal vein of normal rats and mice causes proliferation of hepatocytes and enlargement of the liver [40, 41]. The intrahepatic reserve of HGF is consumed during the first three hours after partial hepatectomy, followed by new HGF synthesis [42]. HGF exerts a direct mitogenic effect on hepatocytes. HGF administration results in events similar to those occurring during liver regeneration, including massive hepatic enlargement, and triggers a strong mitogenic response and clonal expansion of hepatocytes in culture [43]. HGF signals through cMet (HGF receptor), which is expressed in most epithelial cells, endothelial cells and neurons, and mediates all the effects of HGF [44]. Liver HGF receptor becomes activated 30–60 min after partial hepatectomy [45]. Genetic deletion of cMet from the liver has been shown to abolish liver regeneration [46]. HGF has therefore been described as an "irreplaceable contributor" to liver regeneration [28].

Epidermal growth factor (EGF)

Experiments in rats have suggested that EGF may be involved in liver regeneration. Surgical removal of major salivary glands [47], where EGF is present in high concentrations [48], or ligation of their draining veins significantly decreases the DNA synthesis peak after partial hepatectomy and results in impaired liver regeneration [47]. Exogenous injection of EGF compensates the effects of salivary gland ablation and restores liver regeneration [47]. EGF is normally supplied by Brunner's glands of the duodenum through the portal circulation [49] to be deposited in the periportal matrix [50]. EGF is thought to have mitogenic potential in liver regeneration given its abrupt availability to hepatocytes after partial hepatectomy. Resection of ⅔ of hepatic parenchyma increases the concentration of EGF per unit liver weight by a factor of three despite only slight elevation of plasma EGF concentrations [47].

Transforming growth factor β_1 (TGF β_1)

Similarly to other cytokines involved in liver regeneration, plasma TGF β_1 levels also rise very shortly after partial hepatectomy [25]. TGF β_1 is produced predominantly by hepatic stellate cells (myofibroblasts) [51]. TGF β_1 is known to have growth inhibitory effects on liver regeneration, eg it inhibits proliferation of hepatocytes in cell culture [52], suppresses production of HGF [53] and activation of HGF receptors [54]. High doses of TGF β_1 result in delay or partial suppression of DNA synthesis after partial hepatectomy [55]. However, a decrease in the expression of TGF β_1 receptors in the first 48 hours after partial hepatectomy seems to enable the hepatocytes to resist the regeneration-inhibitory effects of TGF β_1 during this period [52, 56]. Other factors may include the high levels of norepinephrine following partial hepatectomy or enhanced production of TGF-α by regenerating hepatocytes [57]. Regardless of its potential as a regeneration terminator, TGF β_1 is thought to play an important role in the assembly of hepatic tissue towards the end of regeneration. TGF β_1 stimulates production of numerous extracellular matrix proteins in many tissues, including the liver. TGF β_1 also stimulates tubulogenesis and formation of neovascular structures in endothelial cells in collagen gels [58, 59]. Concomitant with the peak of TGF β_1 synthesis at the end of regeneration, new extracellular matrix is synthesised with the appearance of new sinusoidal capillary network. Similar events occur at the end of wound healing with a concurrent increase in TGF β_1 [28, 60].

Other growth factors with autocrine or paracrine effects

Other growth factors appear to be involved in liver regeneration, eg TGF-α, VEGF and PDGF. The complexity of interaction and overlap between their functional capacities implies that even in the absence of one of them liver regeneration is likely to continue, albeit at a slower pace.

Platelets and liver regeneration

Our interest in the impact of platelets on the liver dates back to the late nineties. We and others showed that platelets may significantly enhance hepatic reperfusion injury after cold ischaemia [61–63]. On the basis of our previous experiments we focused on the potential role of platelets in liver regeneration [64]. Platelets are non-nucleated discoid particles which are essential for haemostasis and thrombosis [65, 66]. Apart from coagulation, platelets contribute to the inflammatory reaction after many forms of tissue injury [67].

Table 1

Growth factors contained in platelets. [Adapted and modified from: Rozman P, Bolta Z. Use of platelet growth factors in treating wounds and soft tissue injuries. Acta Dermatovenerol Alp Panonica Adriat. 2007;16:156–65.]

Growth factor	Abbreviation
Epidermal growth factor	EGF
Platelet-derived growth factor	PDGF
Transforming growth factor-α	TGF-α
Transforming growth factor β_1	TGF-β_1
Keratinocyte growth factor	KGF
Fibroblast growth factor-1	FGF-1
Fibroblast growth factor-2	FGF-2
Vascular endothelial growth factor	VEGF
Connective tissue growth factor	CTGF
Granulocyte/macrophage colony-stimulating factor	GM-CSF
Insulin-like growth factor	IGF

Contribution of platelets in liver regeneration

The liver and platelets display a very intimate, albeit complex, interconnection [68]. The liver plays a critical role even during the synthesis of platelets from megakaryocytes through thrombopoetin (TPO) [68]. TPO, the most important growth factor in the regulation of megakaryocyte development and platelet production, is produced mainly in the liver and kidney [68]. Hence platelets are not expected to function properly in diseased liver states [69].

A number of proteins which induce opposing effects on liver regeneration are present in platelets. For instance, platelets harbour important growth factors for execution of liver regeneration, eg HGF [1, 70, 71]. Contrariwise, platelets contain TGF-α [72, 73] which is required for termination of liver regeneration [1]. Thus, it is plausible that platelets may participate in orchestrating liver regeneration through harmonised stimulation and inhibition of growth-related signals.

Until 2006 it was unclear whether platelets are promoters, inhibitors of, or not even active contributors to, liver regeneration. Many in vitro studies demonstrated that platelets contain several growth factors [74] (table 1) which may theoretically contribute to the process of liver regeneration [36, 75]. However, the only in vivo study on the role of platelets in liver regeneration in rats failed to identify a correlation between platelets and liver regeneration [76].

In a previous study on regeneration of liver in rats it was noted that splenectomy increases platelet counts and accelerates liver regeneration via an unclear mechanism [77]. To investigate more closely the role of platelets in liver regeneration in mice, we applied inhibitors of platelet function which remarkably reduced liver regeneration. In a second step we depleted platelets to less than 5% by applying a platelet-specific antibody. After 70% liver resection, these mice exhibited significantly impaired liver regeneration, suggesting that a factor contained in platelets may be required to induce or maintain liver regeneration [64]. In another study thrombocytotic mice exhibited increased liver regeneration while a thrombocytopenic group showed impaired regeneration [78]. Matsuo et al. reported that direct contact between platelets and hepatocytes is necessary for the proliferative effect [79]. These authors concluded that platelet-hepatocyte contact initiates signal transduction involved in growth factor activation. HGF, VEGF and insulin-like growth factor-1 were found to contribute chiefly to hepatocyte proliferation [79]. Murata et al. have recently demonstrated that platelets promote liver regeneration even under conditions of Kupffer cell depletion, by stimulating HGF and insulin-like growth factor-1 expression [80].

Serotonin: the novel participant in liver regeneration

Our studies on the role of platelets in hepatic regeneration have raised the question which of the factors stored and secreted by platelets could be responsible for platelet-mediated induction of regeneration. Exploratory experiments enabled us to identify serotonin as one of the crucial factors for liver regeneration. Serotonin (5-hydroxytryptamine, 5HT) is widely distributed in animals, fungi and plants, including fruits and vegetables [81]. The history of serotonin dates back to 1937 when enteramine, an enterochromaffin cell-derived substance, was described by Erspamer [82, 83] as causing smooth muscle contraction. In 1948, Rapport and colleagues successfully purified and isolated the same substance, which has been shown to induce vasoconstriction, from beef serum, and determined that it is 5HT. Pointing out its source (serum) and effect (enhancing vascular tone), the investigators named this compound serotonin.

Serotonin acts as a physiological mediator of gastrointestinal functions (fig. 3), eg regulation of intestinal motility. In contrast, serotonin is an important component of a variety of pathological conditions [84–87]. For instance, it is responsible for many symptoms related to carcinoid syndrome [88]. The substantial role of serotonin as a neurotransmitter in the central nervous system, as a local hormone in the peripheral vascular system and the gastrointestinal tract and as a mediator of brain-gut connection, is well established [81]. With respect to the liver, several studies have already focused attention on the remarkable impact of serotonin on hepatic microvascular perfusion, diameter of hepatic sinusoids, adhesion of platelets and leukocytes to sinusoidal endothelium [89, 90], hepatic ischaemia/reperfusion injury [91] and lodgement of tumour cells in the liver [92]. Almost 7 decades elapsed until platelet-derived serotonin was introduced by our group as a key player in liver regeneration [64].

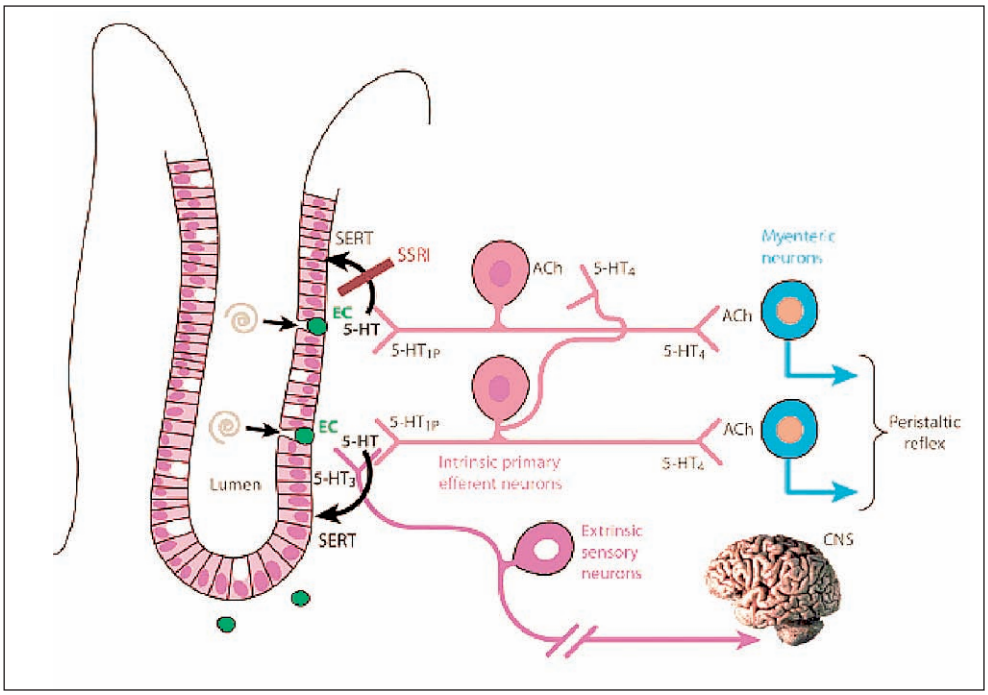

Figure 3

Action of serotonin in the bowel wall. Serotonin released from stimulated enterochromaffin cells activates submucosal intrinsic primary efferent neurons (pink) through 5-HT1P receptors. The latter spread information to the myenteric plexus (blue) allowing peristaltic reflexes. Serotonin also activates extrinsic sensory neurons (purple) which relay information to the central nervous system. Black arrows represent reuptake of selective serotonin by its transporter (SERT) which is blocked by serotonin reuptake inhibitor (SSRI) [Adapted from: Lesurtel M, et al. Role of serotonin in the hepatogastrointestinal tract: an old molecule for new perspectives. Cell Mol Life Sci. 2008;65(6):940–52, and reprinted with permission.]

Serotonin synthesis and storage

A two-step enzymatic pathway is essential for serotonin synthesis from the aromatic amino acid tryptophan. Initially, tryptophan hydroxylation is performed by two isoforms of tryptophan hydroxylase (TPH-1 and TPH-2), resulting in the formation of 5-hydroxytryptophan [81, 93]. It has been demonstrated that TPH-1 –/– mice exhibit peripheral serotonin deficiency but almost normal brain levels. Tryptophan hydroxylation by TPH-1 was found to be the rate-limiting step in peripheral serotonin synthesis [94], while the second isoform TPH-2 is responsible for serotonin synthesis in the brain. 5-hydroxytryptophan is subsequently decarboxylated by the ubiquitous enzyme amino acid decarboxylase which converts it to serotonin [81, 93]. About 95% of serotonin in the body is found in the gastrointestinal tract, of which 90% is in enterochromaffin cells and 10% in enteric neurons. The remaining serotonin (5%) is found in the brain. Serotonin does not have the ability to cross the membrane lipid bilayer; it has therefore to be bidirectionally transported [93]. The serotonin reuptake transporter (SERT) is the major protein responsible for uptake and release of serotonin [95]. SERT is widely distributed in the central and peripheral sympathetic nervous system as well as in platelets, the gastrointestinal tract and lungs [96]. Platelets are able to attract serotonin from the gut and lung. Serotonin is present in high concentration in platelets, where it accumulates from the plasma via the active transport system SERT. Thus, serotonin participates in aggregation of platelets and coagulation of blood [97, 98]. Operating as buffers, platelets maintain low levels of free circulating serotonin. As the carrier and reservoir, platelets store serotonin in dense electron-opaque granules [93]. The study of Walther and Bader on enterochromaffin cells report-ed two forms of serotonin storage; a minor portion which remains outside the secretory granules, and another which is bound to proteins and must be stored in platelets [94].

Serotonin metabolism

Serotonin in tissues can be rapidly metabolised, mainly as a result of the activity of monoamine oxidase (MAO). In the kidney and the liver the enzymes MAO and aldehyde dehydrogenase convert serotonin to 5-hydroxyindoleacetic acid, which is excreted in the urine [81]. We have recently shown that serotonin may mediate the pathology of specific liver diseases. We tested the role of serotonin in the pathogenesis of steatohepatitis wild-type versus TPH-1 –/– mice, both fed a choline-methionine-deficient diet. Despite an almost equal degree of fatty infiltration in both mouse strains, TPH-1 –/– mice displayed reduced hepatocellular injury and less severe inflammation. It is known that serotonin enhances the levels of reactive oxygen species and lipid peroxides as a result of serotonin catabolism by MAO. We concluded that serotonin promotes the progression of steatohepatitis by oxidative stress. In the mouse strain lacking peripheral serotonin hepatocellular injury was decreased, with concomitant reduction of mitochondrial damage and inflammation [99]. In the central nervous system as well as the gut, SERT plays an important role in terminating transmitter action and in maintaining transmitter homoeostasis since there is no extracellular enzyme analogous to acetylcholinesterase that could rapidly catabolise serotonin [81, 93].

Serotonin receptors

There are a wide variety of serotonin receptor isoforms distributed in the enteric neurons, enterochromaffin cells, gastrointestinal smooth muscle cells and possibly in enterocytes, immune tissues and hepatocytes [100, 101]. So far seven

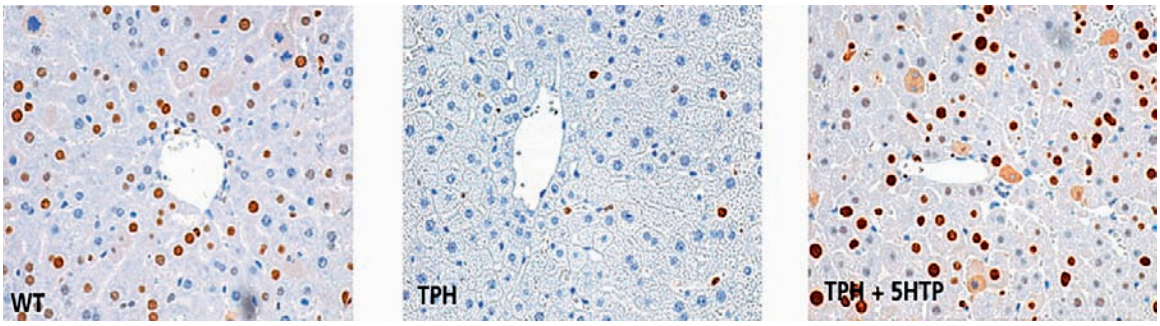

Figure 4

Representative proliferating cell nuclear antigen (PCNA) stained sections from remnant livers showing decreased hepatocyte proliferation 2 days after hepatectomy in TPH-1 knockout (TPH) compared with wild type (WT) mice and restoration of liver regeneration by serotonin reloading (TPH+5HTP). [Adapted from: Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, Gachet C, Bader M, Clavien PA. Platelet-derived serotonin mediates liver regeneration. Science. 2006;312:104–7.]

types of serotonin receptors have been recognised [102]. The seven families of serotonin receptors are designated 5-HT1 to 5-HT7. Serotonin receptors can also be classified into functional subtypes which signal through different intracellular pathways, eg cyclic adenosine monophosphate or inositol triphosphate pathways. The pharmacology of serotonin receptors is very complex. Many drugs are used clinically, the majority of which target serotonin receptors or serotonin reuptake transporters (SERT). We have previously summarised the information concerning the types and subtypes of serotonin receptors [81]. One of the end points of our studies on the impact of serotonin on liver regeneration was to identify the serotonin receptor and subtype which mediates serotonin signaling in liver regeneration. To that end, mRNA levels of various serotonin receptors after partial hepatectomy indicated that 5-HT2 is involved in liver regeneration. In a series of experiments using agonists and antagonists of serotonin receptors we were able to show that liver regeneration is predominantely mediated by 5-HT2A and 5-HTB receptors [103–105].

Interconnection among serotonin, platelets and liver regeneration

Platelets maintain a sort of mutual cooperation with serotonin. Platelets are responsible for picking up serotonin from the gut and lungs and provide the main peripheral storehouse of serotonin [93]. On the other hand, serotonin has a mitogenic influence on megakaryocytes via 5-HT 2 receptor [106]. Moreover, serotonin is essential for platelet aggregation and subsequent blood coagulation. A mitogenic circuit has been identified linking serotonin receptor 5-HT2B and the receptor of PDGF [107, 108]. Similarly, a crosstalk between SERT and PDGF receptor in smooth muscle cells has been described [109]. In 1998, a study on hepatocyte cell culture showed that serotonin causes a dose-dependent increase in (3H)-thymidine incorporation into hepatic DNA in the presence of insulin and EGF, indicating a potential role for serotonin in liver regeneration [110]. Some 25 years ago Kulinskii et al. reported that a serotonin precursor is able to increase the endogenous serotonin level in the regenerating liver and stimulate mitotic activity [111].

Role of serotonin in liver regeneration

Serotonin may be involved in liver regeneration at the cerebral level also. The relationship between the functional status of the liver and the brain has been known for centuries [81]. For example, alteration in the serotonin-brain circuits is well established in hepatic encephalopathy [112–114]. An up-regulation of the brain 5-HT2C receptor has been observed after partial hepatectomy and in hepatic neoplasia. The increased serotonin content and 5-HT2C receptor in the brainstem and cerebral cortex seems to enhance hepatocyte proliferation, possibly through the sympathetic pathway [115].

Our interest in regeneration led us to investigate platelets and factors stored by these cells. In an attempt to further identify the impact of platelets we identified serotonin as a mediator of regeneration. Experiments with serotonin antagonists convincingly demonstrated that serotonin mediated its effect via the receptor 5-HT2A and possibly to a lesser degree through 5-HT2B. In another approach we used knock-out mice of TPH-1 which display a normal phenotype except for a dramatic reduction in serotonin content in platelets. Following 70% hepatectomy, these mice failed to induce liver regeneration as measured by proliferating hepatocytes. All parameters used to estimate the regenerative potential consistently indicated a loss of proliferative activity.

Since TPH-1 –/– mice exhibit normal levels of amino acid decarboxylase, TPH-1 –/– mice were then treated with the serotonin precursor 5-HTP. This led to efficient conversion to serotonin which was easily detectable in blood. Concurrently with the restoration of serotonin levels in TPH-1 –/– mice, the regenerative potential was rescued (fig. 4) [64]. Papadimas et al. showed that 5-HT2 receptor inhibition by ketanserin arrests liver regeneration when administrated close to the G1/S transition point. This finding suggests that serotonin may be a cofactor for DNA synthesis [116]. More recently we studied the impact of platelets and serotonin in a mouse model of normothermic ischaemia/reperfusion injury of the liver [117]. Neither inhibition of platelet function nor platelet depletion led to a reduction in postischaemic tissue injury. Postischaemic inflammation, as well as liver regeneration and consequently tissue repair, were however strikingly impaired. In particular, platelet-derived serotonin

was found to mediate hepatocyte proliferation, which is an integral component of postischaemic tissue repair. Liver regeneration and repair were significantly impaired in platelet-depleted animals. Mice lacking peripheral serotonin (TPH-1 knock-out mice) show a failure of hepatocyte proliferation after ischaemia, but otherwise display normal tissue remodeling. The results suggest that platelets may not cause postischaemic liver injury, but mediate tissue repair through modulation of inflammation and release of serotonin [117].

Conclusions and perspectives

Despite the impressive number of studies on liver regeneration, the putative role of many molecules and cells in the pathophysiology of the liver and particularly liver regeneration remains only partially known. Serotonin, platelets and liver are unique examples of fruitful cooperation between molecule, corpuscle and organ. Serotonin and platelets are essential one for another, with a close cross-talk with the liver. The novel role of serotonin and platelets in liver regeneration may hopefully contribute to the understanding of pathways involved in liver regeneration. It might open the door to innovative methods to save the lives of patients in whom partial hepatectomy is indicated. However, future laboratory and clinical studies are needed to discover the precise action of serotonin and platelets in this process.

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