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REVIEW ARTICLE

Purinergic signalling in the liver in health and disease

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Abstract Purinergic signalling is involved in both the physiology and pathophysiology of the liver. Hepatocytes, Kupffer cells, vascular endothelial cells and smooth muscle cells, stellate cells and cholangiocytes all express purinoceptor subtypes activated by adenosine, adenosine 5′-triphosphate, adenosine diphosphate, uridine 5′-triphosphate or UDP. Purinoceptors mediate bile secretion, glycogen and lipid metabolism and indirectly release of insulin. Mechanical stress results in release of ATP from hepatocytes and Kupffer cells and ATP is also released as a cotransmitter with noradrenaline from sympathetic nerves supplying the liver. Ectonucleotidases play important roles in the signalling process. Changes in purinergic signalling occur in vascular injury, inflammation, insulin resistance, hepatic fibrosis, cirrhosis, diabetes, hepatitis, liver regeneration following injury or transplantation and cancer. Purinergic therapeutic strategies for the treatment of these pathologies are being explored.

Keywords Purinoceptors · Diabetes · Cirrhosis · Hepatitis · Cancer · Fibrosis

Synopsis

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Introduction

The purinergic signalling hypothesis, i.e. adenosine 5′-triphosphate (ATP) acting as an extracellular signalling molecule, was proposed in 1972 [32]. In 1978, separate families of receptors for adenosine (P1) and ATP (P2) were identified [33]. Throughout the 1990s, various receptors for purines and pyrimidines were cloned and characterised. Nucleotide receptors were separated into P2X ligand-gated ion channel and P2Y G protein-coupled receptors [206]. Currently, four subtypes of P1 receptors (A_1, A_{2A}, A_{2B} and A_3), seven subtypes of P2X (P2X1-7) and eight subtypes of P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$) are

recognised [34]. ATP is established as a cotransmitter with classical transmitters in most, if not all, nerves in the peripheral and central nervous systems (see [35]), and receptors are expressed on many non-neuronal, as well as neuronal cell types (see [36]).

The liver is the largest internal organ in the body. The liver is a vital organ with a diverse range of functions including crucial metabolic pathways, carbohydrate metabolism, protein synthesis and detoxification and bile secretion, among others. These functions are absolutely necessary for survival and are chiefly performed by the parenchymal cells or hepatocytes. The necessity of these functions is best appreciated in the absence of adequate liver function. Here, roles of carbohydrate and glucose metabolism, detoxification and immunologic function are lost, and hyperglycemia, hepatic encephalopathy bleeding and infection inevitably result in demise without liver transplantation. Through this, one begins to appreciate the complexity of the liver in homeostasis in health and the resulting loss in disease.

Relevant to this review is that the liver synthesises most of the nucleotides in the body. These molecular compounds are released or can leak from cells and extracellular nucleotides [e.g. ATP, adenosine diphosphate (ADP), uridine 5′-triphosphate (UTP) and uridine diphosphate (UDP)] are key signalling molecules recognised by hepatocytes impacting metabolic processes. Specifically, extracellular nucleotides bind type-2 purinergic/pyrimidinergic (P2Y G protein-coupled receptors and P2X ATP-gated cation channels), whilst the phosphohydrolytic product adenosine is recognised by P1 adenosine receptors, as detailed above. These purinergic effects are closely regulated by ecto-enzymes termed ectonucleotidases (ecto-ADPases, ecto-ATPases, etc.) that hydrolyse extracellular nucleotides, ultimately to the respective nucleosides that often exert opposing effects.

There are a growing number of papers concerned with purinergic signalling in the liver, but only a few reviews [14, 22, 89, 120, 146]. Purinergic signalling regulates important hepatic processes such as bile secretion, glycogen metabolism and, indirectly, release of insulin. Biological stress may lead to alteration of release of nucleotides or uptake of nucleosides or may decrease enzyme function of ectonucleotidases [211].

Perturbations in purinergic signalling responses result in heightened inflammation, insulin resistance, vascular injury and abnormal liver cell regeneration. We have shown that high levels of extracellular nucleotides promote injury while adenosine can be protective during acute inflammatory settings as in vascular reperfusion [14] or with acetaminophen (APAP) toxicity [130]. In the liver, like many tissues, ethanol or fructose ingestion leads to an increase in adenine nucleotide release with both CD39 and ecto-5′-nucleotidase (CD73)-dependent extracellular increases in adenosine concentration. Chronic adenosine exposure as a direct result of ethanol or fructose metabolism and two of the adenosine receptors mediate ethanol-induced fatty liver disease by direct effects on lipid metabolism. Adenosine, however, also activates those cells that cause hepatic fibrosis and participates in a final common pathway leading not only to hepatic steatosis but also to fibrosis and ultimately to cirrhosis.

Thus, the liver is of major importance for both system nucleotide homeostasis as well as for local intercellular signalling within canalicular networks and biliary systems. In addition to hepatocyte parenchymal cells, the liver contains Kupffer, vascular endothelial and smooth muscle cells, stellate cells (fat stroma) and biliary canaliculi (cholangiocytes) all of which express purinoceptors. This review will examine purinergic signalling in first health and then in disease. Following recent advances, several of these divergent elements of the purinergic response are now susceptible to intervention. Non-selective adenosine receptor antagonists are in common clinical use (e.g. caffeine and aminophylline), and the more specific targeting of the adenosine receptor subtypes involved in liver injury and cirrhosis may reduce the toxicities associated with such nonselective antagonists as caffeine, theophylline and aminophylline.

Receptor subtypes for purines and pyrimidines on hepatocytes

Evidence for two Ca^{2+}-mobilising purinoceptors on rat hepatocytes was reported [58], and ATP and 2-methylthio ATP (2-MeSATP) were claimed to act via different P2 receptors [149] probably by $P2Y_2$ and/or $P2Y_4$ receptors [219]. One is suramin-sensitive, coupled to phospholipase C (PLC) in a stimulatory manner; the other is suramin-insensitive and coupled to adenylate cyclase in an inhibitory manner [245]. 5′-[α,β-Methylene]-triphosphate potentiates oscillations in cytosolic $[Ca^{2+}]_i$ of hepatocytes induced by ATP, but not ADP, again suggest two different P2 receptors are involved [59].

ATP and UTP have similar effects on activation of glycogen phosphorylase, generation of inositol 1,4,5-triphosphate ($InsP_3$) and inhibition of glycogen synthase [152], suggesting that $P2Y_2$ or $P2Y_4$ receptors were involved. However, ADP and 2-MeSATP produced different effects from those of ATP [60], suggesting that $P2Y_1$, $P2Y_{12}$ or $P2Y_{13}$ receptors might be present. In a later paper, it was shown that functional $P2Y_1$ and $P2Y_2$ receptors were expressed on rat hepatocytes [61]. Three different receptors to purines and pyrimidines were proposed to be present on liver plasma membranes, one involving activation of PLC, another activation of phospholipase D (PLD) and a third the inhibition of adenylate cyclase [146, 172]. The $P2Y_{13}$ receptor is a key regulator of hepatocyte high-density lipoprotein (HDL) endocytosis by cultured hepatocytes as well as in situ in perfused mouse livers [137]. It was also shown that the $P2Y_{13}$ receptor antagonist, AR-

C69931MX, is a stimulator of this pathway and opens up the way for the design of new drugs able to increase HDL-cholesterol clearance, thus increasing the atheroprotective effect of the HDL [171]. ADP, acting via $P2Y_{13}$ receptors, controls insulin signalling as well as lipoprotein secretion [44].

ATP-activated cation currents via P2X receptors have also been identified in hepatocytes [40]. In more recent papers, P2X4 and P2X7 receptor mRNA and protein were detected on rat hepatocytes and functional roles established [75, 103]. In a study of P2X receptors on immune cells in the rat liver during postnatal development, it was shown that P2X6 receptors were up-regulated by 15-fold on hepatic sinusoid cells during postnatal days P1 to P30, subpopulations of Kupffer cells co-expressed P2X4 and P2X6 receptor subtypes, and dendritic cells co-expressed P2X4 and P2X7 receptors [258]. The P2X6 receptor on Kupffer cells was substantially up-regulated by exposure of animals to lipopolysaccharide, suggesting that they may be evoked by endotoxin. ATP mediates calcium-dependent killing of isolated rat hepatocytes via P2X7 receptors [269]. Table 1 summarises the expression and function of P2 receptor subtypes by cellular compartments of the liver.

There was early recognition of the effect of adenosine on hepatic cell activity [52, 212]. Stimulation of glycogenolysis and vasoconstriction by adenosine and adenosine analogues was shown in perfused rat liver [39, 227]. Adenosine produced dose-dependent stimulation of urea biosynthesis in hepatocytes [109] via all three P1 receptor subtypes, A_1, A_2 and A_3 [110], although different second messengers are involved [112]. Adenosine reverses in vivo hepatic responsiveness to insulin [178]. Evidence for adenosine A_2 subtype receptors on Kupffer cells was presented [209]. It was claimed that adenosine stimulates cyclic adenosine monophosphate (cAMP) formation and regulation of glycogenolysis and gluconeogenesis most likely through the A_{2B} receptor subtype in rat hepatocytes [265]. Adenosine A_{2A} receptor occupancy stimulates collagen expression by hepatic stellate cells (HSC; [45]).

Actions of purines and pyrimidines in liver

Early studies showed that ATP and ADP hyperpolarise guinea-pig liver cells and enhance the response to β-agonists that probably involve steps subsequent to receptor activation [138], perhaps by increasing K^+ permeability [30]. Later, this group showed that quinine and apamin greatly reduced the effect of ATP on K^+ content [31]. Extracellular ATP and ADP induce Ca^{2+} uptake into rat liver cells [131, 157, 166, 219]. Single cell measurements have shown increases in intracellular Ca^{2+} in T51B rat liver epithelial cells stimulated by ATP [26]. ATP induces intercellular Ca^{2+} waves in the Fischer 344 rat liver epithelial cell line (WB-F344) derived from normal liver [96]. Purine nucleotides increase the cellular level of $InsP_3$ [43], suggesting that P2Y receptors might be involved. On bile epithelial cells, ATP has been shown to stimulate transepithelial secretion of potassium. This effect can be inhibited by suramin and 2-aminoethoxydiphenyl borate ($InsP_3$ receptor inhibitor), suggesting that the mechanism is operative through P2Y-$InsP_3$ coupled pathways. Adenosine did not change $[Ca^{2+}]_i$, but did increase cAMP, suggesting that P1 receptors were also involved.

Evidence has been presented that P2X4 receptors are functionally important in mediating ATP control of Na^+ and Ca^{2+} transport and may be a mechanism for autocrine regulation of hepatic glycogen metabolism [75]. ATP (rather than ADP and

Table 1 Expression and function of P2 receptors and ectonucleotidases by cellular components of the liver

Cellular component	Expression	Function
Hepatocytes	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{13}$, NTPDase8 (apical membrane)	Glycogen metabolism, insulin resistance
Cholangiocytes	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, P2X2, P2X3, P2X4, P2X6	Bile (anion) secretion, nucleotide salvage, canalicular contraction, interaction with hepatocytes, adenosine resorption from bile
Endothelial cells	$P2Y_1$, $P2Y_2$, $P2Y_6$, P2X4, P2X7; NTPDase1	NO release, secretion of prostaglandins E2
Vascular smooth muscle cells	$P2Y_1$, $P2Y_2$, $P2Y_6$, P2X4, P2X7	Portal vein contraction
Hepatic stellate cells	$P2Y_2$, $P2Y_4$, $P2Y_6$, NTPDase2	Secretion of prostaglandin F2 and D2, cell contraction
Portal fibroblasts	NTPDase2	Hepatic fibrosis
Kupffer cell/macrophages	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, P2X1, P2X4, P2X7; NTPDase1	Killing of intracellular pathogens, secretion of prostaglandin E2, interleukin-6
Liver-associated lymphocytes: NK, NKT, T cells, B cells	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{14}$, P2X1, P2X2, P2X4, P2X7 NTPDase1	Modulate concanavalin A-mediated hepatitis
Neutrophils	$P2Y_2$	Chemotaxis

NK natural killer cells, *NKT* natural killer T cells.

Modified from [14].

AMP) was shown to cause hepatic cell degeneration [142] before the presence of P2X7 receptors (which are known to mediate apoptotic cell death on hepatocytes) was recognised. Hypoxic injury to hepatocytes is associated with ATP and blebbing occurs [116], another feature of P2X7 receptor activation. The role of P2X7 receptors in the control of liver homeostasis is discussed in recent reviews [81, 83].

UTP was more effective than ATP in regulating hepatocyte metabolism, ion fluxes and haemodynamics [121], suggesting that $P2Y_2$ or $P2Y_4$ receptors might be involved. Using ATPα^{35}S as a radioligand, high-affinity P2Y receptors were identified in both human and rat liver plasma membranes [150], perhaps suggesting that they might be $P2Y_{11}$ receptors.

ATP release from hepatocytes and biliary epithelium

ATP can be released by hepatocytes into different extracellular compartments via basolateral, sinusoidal or apical exocrine routes. Secretion of ATP into the bile is mediated by an increase in cholangiocyte cell volume, which stimulates nucleotide release by vesicular exocytosis [90, 99]. Movement of ATP from the intracellular compartment to the extracellular compartment in liver epithelium may occur via ATP channels or exocytosis, although it has been claimed that sustained release of ATP from liver cells is not mediated by vesicular exocytosis [66]. Exocytosis of ATP-containing vesicles can be in response to cell volume changes [91]. Hypotonicity resulted in cell-swelling triggering release of ATP from human Huh-7 hepatoma cells, followed by volume regulatory decrease [77]. Two ATP transport mechanisms were identified, one of which was vesicular exocytosis. Aside from volume changes, mechanical stress seems to cause release of ATP from both Kupffer cells and hepatocytes. It has been suggested in this scenario that ATP from hepatocytes is released from lysosomes by exocytosis [104]. The source of ATP involving various actions in the liver is likely to be by paracrine or autocrine release from hepatocytes [165, 251], but the possibility that ATP released as a cotransmitter from sympathetic nerves is another source of ATP has also been proposed [35].

Extracellular nucleotides are released into the canaliculus and modulate bile secretion. The biliary epithelium and hepatocytes constitutively release ATP into the bile [46]. In biliary epithelium, ATP is stored in vesicles and is released in response to cell swelling [99]; concentrations of canalicular adenine nucleotides in bile samples are estimated to be 5± 0.9 μM [88, 89]. Extracellular nucleotides entering the bile ducts are potent stimuli for secretion of bile fluid including anions, and canalicular contraction, which is, in part, explained by interaction and communication between hepatocytes and bile duct via local ATP release [216]. These effects are mediated and coordinated by apical $P2Y_2$ receptors and NTPDase8 [69].

Bile acid ursodeoxycholic acid stimulated secretion of ATP by isolated hepatocytes and perfusion of ATP into bile duct segments induced Ca^{2+} signalling in bile duct epithelia [187]. The authors suggest that this may be used as a strategy for the treatment of secretary disorders of the liver.

Kupffer cells

Stimulation of parenchymal cell glycogenolysis by adenosine involves release of ATP from parenchymal cells and stimulation of eicosanoid release from Kupffer cells [190]. Nucleotide receptors responsive to both ATP and UTP are present on stellate (fat storing) cells in the rat that mediate contraction of the cells [237]. ATP release from Kupffer cells stimulated after mechanical stress promotes liver regeneration [104].

Bile canaliculi and hepatic couplets

Fluid absorption and secretion across intrahepatic bile duct units (IBDUs) play a key role in modifying the volume and composition of bile. Bile formation by the liver results from the combined complementary interactions and functions of two distinct liver cell types. Secretion is initiated by hepatic parenchymal cells (about 80 % of liver mass) that actively transport bile salts and other organic solutes into the canalicular space between cells. Subsequently, canalicular bile flows into the lumen of an extensive network of intrahepatic ducts, where it undergoes dilution and alkalinisation as a result of cholangiocyte Cl^- and HCO_3^- secretion. $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and P2X4 receptor mRNA was identified in isolated, microperfused IBDUs using RT-PCR [69]. cAMP-induced secretion of bicarbonate from IBDUs involves apical release of ATP and stimulation of apical P2 receptors [180]. An interesting correlation is cystic liver disease. P2X4 receptors are expressed in liver cyst epithelia, and it was speculated that they mediate fluid secretion leading to increase in luminal pressure, causing cell proliferation and cyst expansion [64].

Contraction of bile canaliculi is ATP-dependent [154, 252]. External ATP regulates canalicular bile formation in isolated perfused rat liver, lowering bile flow, whilst inducing release of glucose and lactate [158]. ATP and UTP increase $[Ca^{2+}]_i$ in human intrahepatic biliary epithelial cell lines [255], probably via $P2Y_2$ or $P2Y_4$ receptors. Cholangiocytes, which secrete Cl^- and HCO_3^- in the intrahepatic bile ducts, are activated by purinergic receptors, which were assumed to be activated via autocrine and/or paracrine release of ATP [213]. Recent data suggests that vesicles containing ATP within the biliary epithelial cells are, in part, responsible for the initiation of purinergic signalling in the biliary system [215].

ATP breakdown and phosphohydrolysis in liver

Among all tissues, the liver has one of the highest ATPase and ADPase activities. Histochemical studies showed that most of liver ectonucleotidase activity was associated with the canalicular domain of hepatocytes [78]. Further studies revealed that NTPDase1 (CD39) is expressed by Kupffer cells and vascular endothelial cells [224], whereas NTPDase2 is produced by portal fibroblasts and activated HSC [70]. NTPDase2 is a preferential ATPase. This ectoenzyme expressed by these adventitial cells may have differential effects in inflammation and fibrogenesis that are related to generation of ADP.

More recently, cloning and biochemical characterisation of NTPDase8 in human and rat species and its identification as the hepatic canalicular ecto-ATPase/ATPDase with potential roles in nucleoside salvage from bile as well as biliary electrolyte/fluid secretion have been reported [82]. Deletion of CD39 results in hepatic insulin resistance [76]. Other ATPases, such as NTPDase3 and NTPDase5, have been found in hepatic tissue, but their functional relevance is not yet known.

Nucleotide pyrophosphatase/phosphodiesterase (NPP) has been localised on the basolateral membranes of hepatocytes and associated with hepatocellular growth [221]. NPP1 is absent during the foetal period and is decreased during liver degeneration [228]. Ecto-5′-nucleotidase (CD73) has been detected in the canalicular plasma membrane and on hepatic satellite cells [217]. Ecto-5′-nucleotidase (CD73)-mediated extracellular adenosine production plays a critical role in hepatic fibrosis [199]. Coexpression of CD73 with specific NTPDases (NTPDase 1, 2 and 8) differentially regulated adenosine formation in rat liver [84]. A recent presentation has reported the presence of ATP synthase in rat hepatocyte plasma membrane as well as in mitochondria [167].

Metabolism of glucose

One of the major functions of the liver is the storage and production of glucose. Purinergic signalling has been shown to have an impact on almost every element glucose production and storage including glycogenolysis, gluconeogenesis, and glycolysis. Glycogenolysis is mainly mediated via the actions of glucagon. Noradrenaline (NA) and ATP released via the splanchnic nerve also serve to stimulate glycogenolysis. In fact glucagon hyperpolarises the liver cell membrane, partly by inducing the release of ATP to act on P2 receptors [93]. Extracellular ATP comes not only from the splanchnic nerve but also from surrounding hepatocytes and activated platelets [24]. Activation of $P2Y_1$ receptors on rat and human hepatocytes stimulates glycogen phosphorylase [62, 63]. The mechanism involves increases in intracellular calcium as well as activating PLD, which may enhance hepatic glycogenolysis. In hepatocytes and perused livers, extracellular ATP stimulates glycogenolysis [38, 122, 147, 148, 151]. ATP, however, is rabidly degraded to adenosine, which, via the action of P1 receptors, increases both cAMP and intracellular calcium. This results in the activation of glycogen phosphorylase. Despite similar levels of activation of glycogen phosphorylase, adenosine is inferior to glucagon at increasing glucose production and even antagonises the stimulation of glycogenolysis by glucagon or cAMP [24]. Thus, whilst the net effect of ATP and adenosine in the liver is to stimulate glycogenolysis, it is unclear to what extent that plays a role in intracellular glucose concentrations. Hepatocyte heterogeneity in response to ATP in perfused rat liver has been described, including glycogenolysis to glucose predominantly in the periportal area, ATP is predominantly hydrolysed by a small hepatocyte population located at the perivenous outflow of the acinus, contractile elements (sphincters) exist near the inflow of the sinusoidal bed, and a portion of the Ca^{2+} mobilised by ATP is derived from liver cells that do not contribute to hepatocyte glucose output [122].

In isolated rat liver cells exposed to ATP (10μM), gluconeogenesis is increased, an effect that was mimicked to a lesser degree with adenosine [226]. The initial transient rise in $[Ca^{2+}]_i$ evoked by ATP plays a significant role in triggering gluconeogenesis [155]. The effect, however, may be dependent on the carbon source for gluconeogenesis or the concentration of extracellular ATP. In isolated hepatocyte cells, gluconeogenesis from pyruvate and lactate (but not glycerol or fructose) is inhibited by ATP at high extracellular concentrations (1 mM), with adenosine producing a similar effect [9].

Stimulation of glycolysis by insulin in cultured hepatocytes is attenuated by ATP via inhibition of phosphofructokinase 2 [205]. The mammalian target of rapamycin (mTOR) pathway is one pathway utilised in hepatocytes to control glucose metabolism. Purinergic regulation (mainly $P2Y_1$ and $P2Y_2$) of certain hepatocyte functions, such as glucose metabolism, may be controlled by the mTOR pathway [47]. It has been claimed that activation of A_{2B} receptors modulates glucose homeostasis and obesity [141].

Lipid metabolism and fatty acids

The effects of ATP on hepatic fatty acid metabolism have been studied and it was shown that inhibition of acetyl-CoA carboxylase activity by ATP may be mediated by elevation of $[Ca^{2+}]_i$, whereas carnitine *O*-palmitoyltransferase may be inhibited through a protein kinase C-dependent mechanism [113]. A slight decrease in intracellular ATP coincided with stimulation of fatty acid biosynthesis from glucose in rat hepatocytes, whilst further lowering of intracellular ATP led to a gradual inhibition [100]. It has been reported recently that A_1 receptors do not play a major role in the regulation of lipogenic gene expression in hepatocytes [262].

Reverse cholesterol transport has been shown to be, in part, modulated by purinergic signalling. Chronic activation of

$P2Y_{13}$ receptors decreases HDL-cholesterol levels in mice [223]. $P2Y_{13}$-deficient mice had decreased hepatic HDL cholesterol uptake. In addition to decreased HDL uptake, hepatic cholesterol transport and biliary cholesterol output (but not plasma HDL) were also decreased [79]. $P2Y_{13}$ knockout mice have also been shown to have lower faecal concentrations of sterols indicating a role for purinergic signalling in cholesterol transport to the faeces [23].

Cell volume regulation

ATP, acting via P2 receptors, is a critical determinant of membrane Cl^- permeability and cell volume regulation [90]. In response to hypotonic solution, rat hepatoma cells release ATP into the extracellular environment and rapidly activate volume-sensitive outward-rectifying Cl^- channels [246].

Vascularity of liver

Portally infused ATP leads to trans-hepatic vasodilatation via P2Y receptors on the endothelium, whilst ATP released from sympathetic nerves leads to vasoconstriction of hepatic arteries via P2X receptors on the muscle [27, 28, 203]. In the sinusoidal vascular bed, ATP impairs the flow-limited distribution of [^{3}H]water [92]. Both endothelium-dependent and endothelium-independent vasodilation of rabbit hepatic artery mediated by purines has been described [28]. In the ATP perfused liver, both P2X vasoconstrictor and P2Y vasodilator receptors [leading to release of nitric oxide (NO)] were identified in the hepatic vascular bed [177, 207]. Adenosine-induced dilation of the rabbit hepatic arterial bed is mediated by A_2 receptors [176]. ATP-magnesium chloride ($MgCl_2$) restores depressed hepatocellular function and hepatic blood flow following haemorrhage and resuscitation [249].

There is evidence for two types of P2 receptor on the longitudinal muscle wall of the portal vein [145]. ATP dilates the portal circulation via P2Y receptors on endothelial cells leading to release of NO [238]. The non-adrenergic contractile response of the guinea-pig portal vein to electrical field stimulation mimics the response to UTP, but not ATP [136], perhaps indicative of the presence of a $P2Y_2$ or $P2Y_4$ receptor on smooth muscle. P2X1 receptors have also been identified on rat portal vein myocytes [183, 196].

Purinergic signalling in liver disease

Readers are referred to a recent review entitled ‘Pathological roles of purinergic signalling in the liver’ with an emphasis on potential future clinical applications ([247]; see Fig. 1).

Inflammation, liver injury and immune regulation

Purinergic signalling is one mechanism by which the immune response is regulated in the liver. UTP and ATP stimulate thromboxane release from perfused liver [123]. Adenosine, resulting from the breakdown of ATP, inhibits the incorporation of radiolabelled leucine into proteins in isolated hepatocytes [244]. Alcohol-induced liver injury is associated with enhanced inflammatory responses and it has been reported that adenosine, acting via A_{2A} receptors, may be a useful anti-inflammatory pathway for reducing these effects [204]. In another study, it was claimed that endogenous A_1 receptor activation protects mice against acute ethanol-induced liver injury by reducing oxidative stress and decreasing lipid accumulation [264]. In cholestasis, bile flow from the liver is impaired, resulting in liver injury. In mice lacking the A_1 receptor, there was attenuation of α-naphthylisothiocyanate-induced cholestatic liver injury [263]. Prostaglandin (PG) $F_{2\alpha}$ and PGD_2 are released from rat liver cells after stimulation by ATP, but not adenosine [10]. Prostanoid secretion by rat hepatic sinusoidal endothelial cells is regulated by ATP [119].

Liver plasma membrane properties have been used for studies of nucleotide signalling. For example, phosphatidylcholine breakdown by rat liver plasma membrane was increased by guanosine triphosphate (GTP; [135]). Ca^{2+}-ATPase is located in hepatocyte plasma membranes and is involved in calcium homeostasis [57]. A functionally active ecto-F_0F_1-ATP synthase has been identified on the plasma membrane of hepatocytes that mediates extracellular formation from ADP and inorganic phosphate [173]. Membrane-bound ATP synthesis has a role in modulating the concentrations of extracellular ADP and is regulated by a plasma apoliproprotein [175]. Synergistic activation of mitogen-activated protein kinase by insulin and ATP in liver cells has been demonstrated [114]. Autophagy, i.e. autophage-lysosomal degradation, in isolated hepatocytes is inhibited by purine nucleotides and nucleosides [156]. Activation of $P2Y_2$ receptors plays a key role in endotoxin-induced acute liver injury in mice [214]. $P2Y_2$ receptors mediate neutrophil infiltration, thereby regulating immune responses associated with hepatocyte death in mice with acute liver injury, and it was suggested that $P2Y_2$ receptor antagonists might be used to treat inflammatory liver disease [11]. A review about purinergic signalling during sterile liver injury has been published recently [195].

Diadenosine triphosphate and tetraphosphate, probably acting via P2Y receptors, like ATP regulate hepatic haemodynamics and metabolism involving complex interactions between parenchymal and non-parenchymal cells [37, 74]. Adenine dinucleotide-related cytosolic free Ca^{2+} oscillations in single hepatocytes have been reported [107]. Adenine

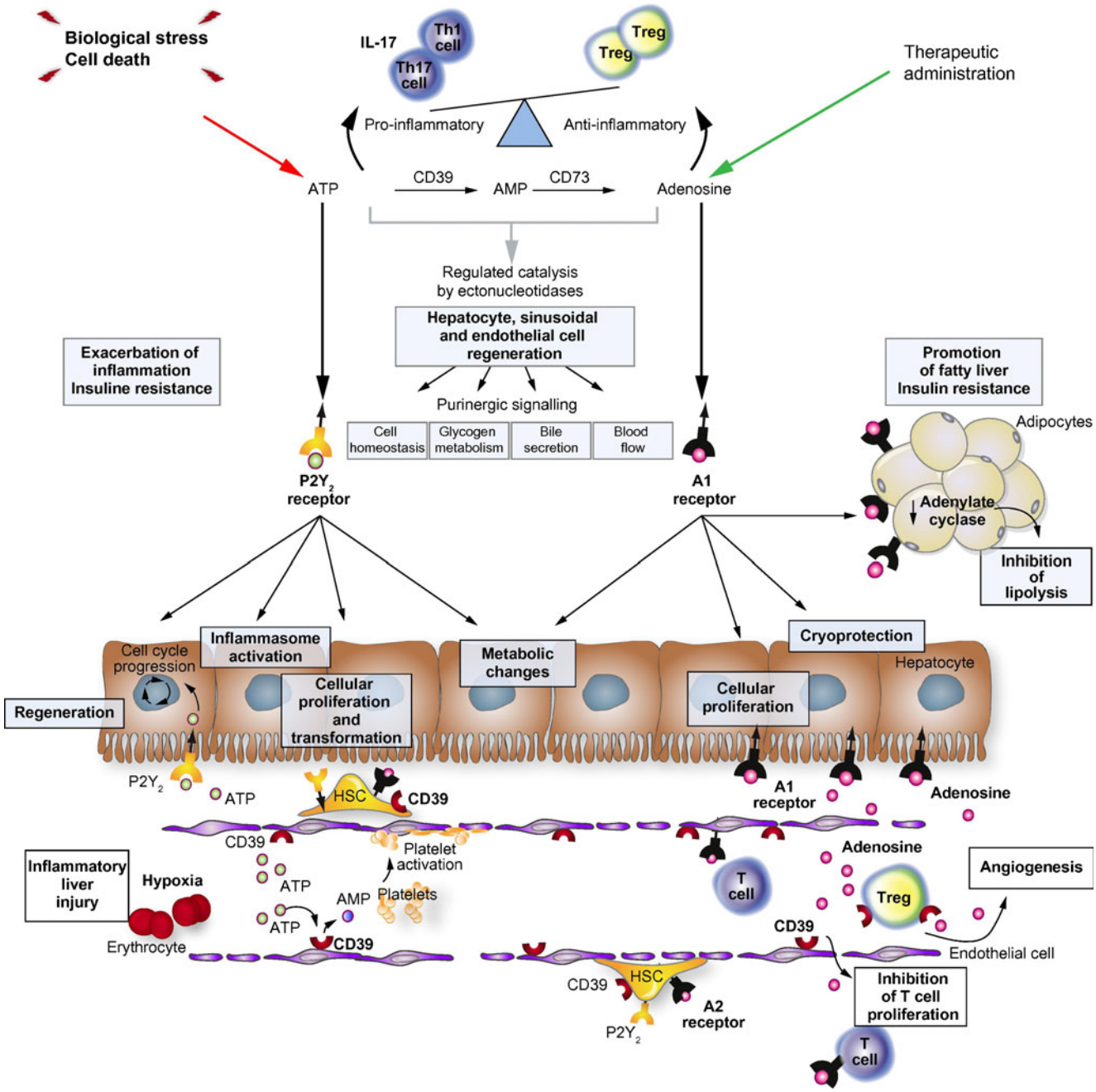

Fig. 1 Schematic overview of the complex interplay mediated between extracellular ATP and adenosine by ectonucleotidases within the liver. As illustrated, purinergic signalling influences multiple cell types in the liver and in the hepatic sinusoids to impact processes of inflammation, metabolism and parameters of insulin signalling, cellular regeneration, healing, fibrosis and cell transformation. As an example, extracellular ATP arising from biologic stress can incite hepatocytic inflammation mediated by P2X7 and the inflammasome, whilst driving parenchymal cell proliferation via $P2Y_2$. With ATP metabolized to adenosine by ectonucleotidases expressed by non-parenchymal sinusoidal cellular surfaces, the inflammatory response is down-regulated and tissue remodelling develops. Induction of CD39 expression by immune suppressive T regulatory cells as well as endothelial cells with associated alterations in sinusoidal vascular responsiveness and hepatic stellate cell (*HSC*) responses. Heightened levels of adenosine generated over a protracted time may result in increased fibrosis, as formed by myofibroblasts and activated stellate cells. These considerations and the crucially important levels of nucleotide:nucleoside balance controlled by CD39 have important implications for the development of purinergic therapies in liver transplantation and inflammatory fibrotic liver diseases. (Reproduced from [247] with permission—License number: 3172040541217; license date: 6/18/2013; Journal of Hepatology)

dinucleotides activate glycogen phosphorylase in isolated liver cells [53]. The metabolic and haemodynamic effects of extracellular nicotine adenine dinucleotide are caused mainly by interactions with purinergic receptors with a highly significant participation of its main transformation product ADP-ribose [29].

Hepatocyte lipoapoptosis induced by saturated free fatty acids contributes to hepatic inflammation in lipotoxic liver injury. It has been suggested that pannexin1 may play an important role in hepatic inflammation by mediating an increase in ATP release in lipotoxic liver injury [259]. APAP is often used to treat fever and pain, but it can cause damage to hepatocytes. In mouse models of APAP-induced inflammation, full injury after overdose required P2X7 receptor activation [4]. P2X7 is required for hepatic caspase-1 activation and migration of neutrophils into the liver. This suggests that extracellular ATP may play a pivotal role in development of the inflammasome after APAP overdose [130]. The P2X7 receptor antagonist A438079 is protective against APAP-induced liver injury, and it was claimed that it acted by inhibiting P450 isoenzymes rather than by inflammasome activation [260].

It has been suggested that ATP affects polymerisation of cytoskeletal elements [143]. Extracellular ATP induces rapid cell rounding in cultured human Chang liver cells (ATCC CCL B) [225]. Roles of natural killer (NK) T cells and relationships with stellate cells as modulated by local mediators is controversial and of great interest in both murine models and in clinical studies. Early studies suggest that T-, NK and NKT cells are exquisitely susceptible to regulation by purinergic and the adenosinergic microenvironment within inflamed tissues.

Hepatic encephalopathy is a neuropsychiatric syndrome that is a complication of either acute or chronic impaired liver function. It is the result of the inability of the liver to clear various “toxins” (often amino acids) from the portal tract. Adenosine had been postulated to have anticonvulsive effects and potentially anti-ischaemic effects in the brain [68]. Thus, it was postulated that adenosine could have a therapeutic effect [218].

Diabetes

Liver dysfunction can produce diabetic syndrome or may be secondary to it. ATP has been shown to increase insulin secretion in normal and alloxan diabetic rats and influence liver function [235]. An increase in adenosine A_1 receptor expression was claimed in the liver of streptozotocin (STZ)-induced diabetic rats [168]. A later paper showed a significant increase in A_{2A} and A_3 receptor mRNA levels, whilst A_{2B} receptor mRNA decreased and A_1 receptors were unchanged; administration of insulin for 4 days to the STZ rats led to return to control levels of P1 receptor expression [106]. In addition, CD39 knockout mice have higher plasma insulin levels following a glucose challenge. Whilst the mice do not develop obesity, they do have higher leptin levels. It is suggested that CD39 acts in the metabolic pathway directly via insulin receptor substrate-2 phosphorylation; however, it is also likely that there are indirect mechanisms through altered inflammatory responses. The end result, however, is that aberrations in the ability to process extracellular ATP lead to insulin resistance and likely is involved in the complex pathogenesis of diabetes [76]. This process may indicate an important metabolic cross-link between inflammation and insulin resistance in other disease states.

Fibrosis and HSCs

Liver fibrosis with subsequent cirrhosis is the most common cause of liver failure. Cell types implicated in hepatic fibrosis are becoming better defined. The most relevant effector cells are activated HSC, ductular epithelial cells and (portal and perivascular) fibroblasts. More recently, it has become evident that a minor proportion of fibroblastic cells originate from bone marrow-derived circulating fibrocytes. HSC appear to be the primary fibrogenic cells of the liver, and these express functional nucleotide receptors [159, 237], which mediate PLD activity [20]. Adenosine A_{2B} receptors have also been identified in human HSC, which play pro-fibrotic roles [266]. During fibrosis, HSC undergo proliferation and senescence and it has been reported that A_{2A} receptors mediate both these key processes, making A_{2A} receptor antagonists potential antifibrotics [1]. Quiescent stellate cells express $P2Y_2$ and $P2Y_4$ receptors activated by UTP and ATP, whereas activated stellate cells express $P2Y_6$ receptors responding to UDP [71]. These authors have also shown that activation of these P2Y receptors regulates procollagen-1 transcription. They suggest that these cells may be a target to intervene therapeutically to prevent liver fibrosis and preclude development of cirrhosis and chronic liver failure. The P2 receptor antagonist, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate, inhibited proliferation of HSC and prevented non-biliary liver fibrosis [72]. NTPDase2 is produced by portal fibroblasts and activated HSC [71]. NTPDase2 is a preferential ATPase that appears to have major effects in inflammation and biliary type fibrogenesis that are related to generation of ADP. Ecto-5′-nucleotidase (CD73) activity was found to be higher in the quiescent, rather than in the activated phenotype of a HSC line, suggesting that ecto-5′-nucleotidase-dependent adenosine production may play a role in the regulation of quiescent HSC functions [6]. However, it has been claimed recently that ecto-5′-nucleotidase (CD73) gene expression increased in both HSC and portal fibroblasts during myofibroblastic differentiation and represents a promising target for antifibrotic therapy [85].

A_{2A} receptors play an active role in the pathogenesis of hepatic fibrosis and it has been proposed that A_{2A} receptor antagonists will inhibit ethanol-induced fibrosis and stellate cell activation [48, 49, 234]. Fatty liver is associated with alcohol abuse and it has been reported that adenosine, generated by ethanol metabolism, plays a role in ethanol-induced hepatic steatosis via both A_1 and A_{2B} receptors and it was

suggested that targeting these adenosine receptors may be effective for the prevention of alcohol-induced fatty liver [210]. The A_{2B} receptor antagonist, MRS1754, reduces hepatic collagen deposition during fibrosis progression [229].

In a recent paper, it has been claimed that ATP released from human platelets contributes to suppression of both human HSC activation and type I collagen production in vitro [132]. It was suggested further that an adenosine-cAMP signalling pathway mediated this process. In progressive fibrosis, eicosapentaenoic acid replenishes hepatic ATP levels resulting in reduction of inflammation and steatosis [140].

Cirrhosis

ATP-$MgCl_2$ has been used to improve survival following massive hepatectomy among cirrhotic rats [127]. It has also been used to treat trauma-haemorrhage and resuscitation, which may be due, in part, to the restoration of P2 receptor binding capacity and the enhancement of receptor affinity [170]. Blockade of intrahepatic adenosine receptors improved urine excretion in cirrhotic rats induced by thioacetamide [182]. Adenosine partially reversed cirrhosis induced by carbon tetrachloride in rats [124] and reversed induced micronodular cirrhosis through enhancing collagenolytic activity and stimulating hepatocyte cell proliferation [125]. A_{2A} receptors play an active role in the pathogenesis of hepatic cirrhosis [42]. During fulminant hepatitis, A_{2A} receptors play an important role in the physiological anti-inflammatory mechanisms that limit liver damage [50]. Adenosine receptor blockade reduces splanchnic hyperaemia in cirrhotic rats [162]. Adenosine deaminase activity was elevated during tuberculous peritonitis in patients with underlying liver cirrhosis [164].

As cirrhosis progresses, the normal response to increased portal venous vascular resistance of decreasing hepatic artery vascular resistance is decreased. The process is mediated by adenosine in normal livers. In cirrhotic livers, the adenosine-mediated vasodilation of the hepatic artery is exaggerated leading to a greater vasodilator effect of the hepatic artery to adenosine [268].

Platelet dysfunction in cirrhosis may also be mediated, in part, by purinergic signalling. Patients who had variceal bleeding had platelets that displayed low levels of impaired actin polymerisation compared to both non-bleeders and controls. The authors suggest this may be related to cytosolic calcium levels [7]. Cerebral A_1 receptors have been implicated in liver cirrhosis [25]. Ectonucleotidase NTPDase 2 is selectively down-regulated in biliary cirrhosis [70].

Cancer

Liver cancer exhibits chronic inflammation, which is associated with aberrant cell proliferation, disordered metabolism and immune dysregulation. Deletion of CD39 promotes the development of both induced and spontaneous liver cancer in contrast to the effects on transplanted tumours to the liver [233]. These manifestations are comparable to what has been observed with Entpd5 studies and knockout mice [80, 208].

Calcium uptake by rat hepatoma cells is increased by ATP [13]. Nucleotide receptors mediated activation of cation, potassium and chloride currents in HTC cells from a rat liver tumour line [94]. There was increased incidence of spontaneous and induced hepatocellular carcinoma with associated metabolic perturbations in CD39 knockout mice [256].

ENTPD5/CD39L4 is a related ectoenzyme to CD39 and is a soluble endoplasmic reticulum UDPase involved in intracellular purine metabolism, which promotes glycolysis as well as proliferation in cancer cells via the PTEN signalling pathway [80]. Interestingly, there are also contrasting and somewhat overlooked roles of this ectonucleotidase in the suppression of liver cancer development [208] vs. the promotion of transplanted tumour growth in mice [80]. ATP infusions into the intraperitoneal space of a two-stage rat model of hepatocarcinogenesis displayed an increase of preneoplastic foci in the liver. In this experiment, ATP and adenosine altered the balance of apoptosis and proliferation towards malignancy [98]. In developed malignancy, however, ATP plays in important role as an early danger signal to the immune system. ATP released from necrotic cells stimulates neighbouring cells to die. CD39 is an ectonucleotidase that converts ATP to AMP. CD39 expression on endothelial cells suppresses the anti-tumour effect of ATP [87]. Swelling-induced ATP release results in activation of P2X4 receptors, which leads to modulation of volume-sensitive outwardly rectifying chloride channels in rat hepatoma cells [246]. Ecto-NTPDase2, which converts ATP to ADP, was expressed on human Huh-7 hepatoma cells and ADP then activates $P2Y_{13}$ receptors, which mediate volume regulatory decrease [77]. The generation of adenosine both inhibits T cell proliferation and promotes angiogenesis, which ultimately is permissive of the growth of malignant cells [231].

Metastatic melanoma from mouse livers that were CD39 null had more ATP and less tumour cell growth. In addition, CD39 expression on T regulatory cells plays a suppressive role on NK cell-mediated tumour suppression of metastatic tumours to the liver. Thus, it further supports the role of purinergic signalling as an important and potentially therapeutic modulator in tumour biology in the liver. High concentrations of ATP switched autophagy to apoptosis in anchored and non-anchored human hepatoma cell lines [253]. The authors suggest that this work provides evidence that explains how hepatoma cells escape from ATP-induced cytotoxicity as well as offering another clue about effective manipulation of liver cancer.

The hepatoma call line N1S1-67 has been used to study signal transduction activated by ATP via $P2Y_2$ or $P2Y_4$ subtypes [201]. Increase in intracellular calcium leads to the opening of Ca^{2+}-activated K^+ channels and membrane

hyperpolarisation. Intra-arterial injection of an inhibitor of ATP production has been proposed as a novel therapy for liver cancer [101]. Release of ATP is, at least in part, by vesicular exocytosis from HTC cells, and a Cl^- channel inhibitor has been used to specifically stimulate ATP release through exocytotic mechanisms [65]. Hepatoma cell growth inhibition by adenosine was reported [115]. The A_3 receptor agonist, CF101, caused inhibition of liver metastasis (following colon carcinoma) [12]. Human hepatocellular carcinoma HepG2 cells expressed high-affinity A_1 receptors, which mediated decreased AMP and erythropoietin production [193]. ATP, via the A_3 adenosine receptor, induced cell apoptosis of the human hepatoma cell line Li-7A [12, 254]. A_{2B} receptors were highly expressed in human hepatoma carcinoma [257].

Hepatitis

Suramin was shown to inhibit in vitro infection by duck hepatitis B virus, *Rous sarcoma* virus, and hepatitis delta virus [202]. An influence of sympathetic nerves in immune-mediated experimental hepatitis has been demonstrated [188], and ATP released as a cotransmitter might be involved. P2X7 receptors regulate NKT cells in autoimmune hepatitis [144]. In fact, while working with concanavalin A models for NKT cell-mediated inflammation used to study immune liver disease, deletion of CD39 was noted to be protective against liver injury [15]. This suggested that modulation of NKT cell activation by novel pharmacologic therapies could quell inflammation and injury. P2X7 receptor-mediated responses are needed for infection of human hepatocytes by hepatitis delta virus and hepatitis B virus [241]. Chronic hepatitis C virus (HCV) infection results in progressive liver disease including fibrosis, cirrhosis, insulin resistance and eventually hepatocellular carcinoma. The mechanism of ATP binding has been explored to facilitate targeting of the ATP-binding site for potential therapeutic development for hepatitis C [197]. It has been suggested that P2X4 receptors are a major component of the purinergic signalling complex in HCV-induced liver pathogenesis [174].

Inosine triphosphate (ITP) is broken down by ITPase (ITPA). A protective effect of ITPA gene variants against ribavirin associated anaemia has been reported [86]. ITPA deficiency results in the build-up of ITP that may alter the pharmacokinetics of ribavirin. Ribavirin has been associated with low levels of intracellular ATP which is part of the pathogenesis of anaemia. High levels of ITP, such as those from deficiency ITPA, allow ITP to substitute for GTP in the generation of AMP, which may be how high ITP levels attenuate the ribavirin-induced anaemia [128].

A_{2a} receptor activation prevents hepatocyte lipotoxicity and non-alcoholic steatohepatitis in rats [134].

Ischaemia and vascular injury

That infusion of ATP-$MgCl_2$ improved hepatic function and survival after hepatic ischaemia was recognised early [97, 126, 194]. It was also effective following reperfusion [51]. The beneficial effect of ATP-$MgCl_2$ treatment following trauma-haemorrhage may be associated with a down-regulation of the circulating levels of the inflammatory cytokines tumour necrosis factor and interleukin-6 [250]. It was also suggested that reduction of ischaemic damage by ATP-$MgCl_2$ infusion may be mediated through improvement in mitochondrial energy metabolism [139]. Treatment of ischaemia by ATP was particularly effective in old mice; aging of the liver is related to mitochondrial dysfunction [222]. During 60 min of ischaemia, there is a 90 % ATP loss from hepatocytes [108]. Hepatocyte resistance to hypoxia is promoted via $P2Y_2$ receptors by down-modulating ERK1/2-mediated signals that promote Na^+ influx through the Na^+/H^+ exchanger [41]. Vascular NTPDase activity was lost after hepatic ischaemia and reperfusion injury and deletion of NTPDase1 in mice led to increased injury and decreased survival [133]. Also deletion of CD39 in NK cells attenuated hepatic ischaemia/reperfusion injury in mice, suggesting that ATP modulates NK cell function during liver regeneration. NK cells that lack the CD39 gene had less secretion of interferon gamma in response to inflammatory mediators. This probably, in part, accounts for the decrease in tissue damage after ischaemia reperfusion injury [17]. Interestingly, vascular CD39, however, seems to have a protective role in hepatic ischaemia reperfusion injury. CD39-null and heterogeneous mice had decreased survival compared to wildtype after an induced model of ischaemia. The CD39 deficient mice that received adenosine were protected from reperfusion injury [232].

Adenosine can also play a protective role against ischaemia reperfusion injury [73, 189] probably by activation of A_2 receptors [8, 200], especially A_{2A} receptors [18, 55, 56, 160]. Administration of an adenosine A_1 receptor antagonist before ischaemia attenuated ischaemia-reperfusion injury [153, 169]. Oxidative preconditioning by ozone was mediated by A_1 receptors in a rat model of liver ischaemia reperfusion [163].

Pharmacologic preconditioning is a potential mechanism to protect against hepatic ischaemic reperfusion injury. Blockage of A_1 receptors abolished ischaemic preconditioning whilst activation of A_1 receptors decreased the effect of ischaemic reperfusion injury [2]. Hepatic ischaemic preconditioning is associated with up-regulation of CD39. This is likely mediated by transcription factor Sp1 and is a potential therapeutic target for the treatment of liver ischaemia [117]. This also has renal implications as well, as one study demonstrated that activation of renal A_1 receptors was protective for the liver as well as kidney after liver ischaemia reperfusion injury [198].

Administration of UTP before induction of ischaemia can attenuate, via $P2Y_2$ and/or $P2Y_4$ receptors, post-ischaemic hepatocyte apoptosis and thereby reverse liver damage [19]. The authors suggest that the UTP-mediated protective effect may be regulated through nuclear factor-κB inactivation. Inosine is an endogenous nucleotide that may be useful in maintaining homeostasis after tissue ischaemia. Through the action of adenosine receptors (A_3), high extracellular inosine stimulated gluconeogenesis [111].

Interestingly, ADP-dependent platelet aggregation was shown to correlate with reperfusion injury as well as thrombocytopenia and early graft survival. ADP-triggered platelet function may have a role in ischaemia reperfusion injury [220].

Metallothionein protein (MT) is induced in vivo in rat liver by P1 adenosine agonists, probably via A_2 receptors [261]. The authors suggest that adenosine via modulation of transcription of MT genes may be important in stress situations involving tissue damage, hypoxia and haemorrhage shock.

Hepato-renal syndrome

Purinergic signalling may play a role in hepato-renal syndrome. The administration of intra-hepatic caffeine into the portal vein of rats has been shown to increase urine output. This effect was not seen with intravenous caffeine or after the liver was denervated, which suggests a porto-renal effect of adenosine [181]. This effect is mediated by hepatic A_1 receptors. This presents a potentially novel therapeutic option for a difficult to treat complication of cirrhosis. In fact, selective blockade of the hepatorenal reflux with SLV329 (an A_1 receptor antagonist) resulted in a diuretic and natriuretic effect without a change of creatinine clearance in a rat model of cirrhosis [129].

Regeneration of liver

It is interesting that the sympathetic nervous system has been implicated in the regulation of liver repair (see [191]). Since ATP is now well established as a cotransmitter with NA in sympathetic nerves, it may be a source of ATP involved in liver regeneration. ATP, released from nerves, from hepatocytes or after synthesis of inorganic phosphate and ADP by the cell membrane via kinases, may participate in the transmembrane signal transduction from growth factors to the cell effector system [102]. ATP activates c-jun N-terminal kinase signalling and cell cycle progression in hepatocytes, with involvement in the initiation of regeneration, liver growth and development [242]. Gene expression profile analysis of regenerating liver using a cDNA microarray system suggests that increase in ATP metabolism is associated with rapid regeneration of liver [185]. Hepatocellular proliferation is impaired in $P2Y_2$ receptor knockout mice, establishing a trophic role for ATP in hepatocyte proliferation with implications for liver regeneration and growth after injury [243]. Regulated catalysis of extracellular nucleotides by vascular CD39 (NTPDase1) is required for both hepatocyte and endothelial cell proliferation during liver regeneration [16]. Adenosine, perhaps via A_{2B} or A_3 receptors, has been reported to accelerate the cell cycle during rat liver regeneration induced by partial hepatectomy [179]. A selective A_{2A} agonist, ATL-146e, has been claimed to prevent concanavalin A-induced acute liver injury in mice [192]. A_3 receptor activation decreases mortality and hepatic injury in murine septic peritonitis [161]. After a partial hepatectomy adenine nucleotides have been noted to undergo a rapid decrease in the remnant liver. The onset of liver regeneration occurs after seconds, possibly related to this loss of nucleotides [54]. ATP release after partial hepatectomy regulates liver regeneration in the rat [104].

A recent insight into this process is the modulation of extracellular ATP on NK cells after partial hepatectomy. Immediately after partial hepatectomy, extracellular ATP is increased and will bind to P2 receptors on NK cells that, in turn, inhibit their function. Administration of exogenous apyrase (CD39/NTPDase1) depletes extracellular ATP and allows NK cells to regulate the immune response and improves liver regeneration [105]. Liver regeneration is enhanced by the ATP-sensitive K^+ channel opener, diazoxide, after partial hepatectomy [186].

Liver transplantation

Accurate, rapid, non-invasive markers of graft viability have valuable clinical uses. A combination of liver ATP levels and serum hyaluronic acid has been recommended as a measure of graft viability [230]. Whilst the human liver has been successfully maintained under hypothermic conditions for up to 10 h using solutions with high concentrations of adenosine, organ preservation to overcome ischaemic damage is a major obstacle to liver transplantation. Infused ATP preserves sublethally injured cells by enhancing their recovery after ischaemic injury; this action is enhanced by the synergistic effect of superoxide dismutase [95]. Purinergic receptor antagonists prevent cold preservation-induced cell death [5]. Hepatocyte viability and ATP content decrease linearly over time during conventional cold storage of rat liver grafts [21].

With organ transplantation, NTPDase1 activity is lost with reperfusion or rejection and up-regulation occurs with graft survival [133]. Administration of soluble NTPDase in the bloodstream or up-regulation of CD39 post-adenoviral infection has been shown to prolong transplant graft survival. Regeneration of the donor liver after transplantation is important. ATP activates cell cycle progression and proliferation of hepatocytes in vitro and in vivo and modulates growth factor activities probably via $P2Y_2$ receptors [242]. A_{2A} receptor

activation has been claimed to have a protective effect in small-for-size liver transplantation [239]. This group have demonstrated that A_{2A} receptor activation down-regulated proinflammatory cytokines, adhesion molecules and ultimately improved liver function in small for size liver transplantation in rats [240].

Another development from purinergic signalling is the ability to monitor and predict progression of fibrosis and rejection in the post-transplant allograft. The ImmuKnow assay is measure of peripheral blood CD4+ total ATP. After living donor transplantation, the ImmuKnow assay was studied as a tool of immune response. Based on the results of the ImmuKnow assay, there was a correlation between immune response and required immune suppression with tacrolimus [184]. It was concluded that it had an excellent ability to monitor immune response especially in combination with assessment of the CYP3A5 allele. Blood from patients who had acute rejection displayed a significantly elevated level of intracellular ATP in CD4+ lymphocytes compared to those without acute rejection. This may be developed as a useful clinical tool to diagnose early rejection [67, 236].

The ImmuKnow assay has also showed potential in areas other than acute rejection. Recurrence of hepatitis C after transplant is a difficult to predict problem. In patients with HCV after orthotopic liver transplant, low CD4+ T cell ATP levels based on the ImmuKnow assay were significantly associated with progression to fibrosis. Thus, the greater suppression of cellular immunity measured by the ImmuKnow assay, the greater the risk of development of fibrosis [3].

In a separate study, in patients with HCV who had been transplanted, the ImmuKnow assay was assessed to distinguish acute cellular rejection from recurrent HCV. Recipients with recurrent HCV had a significantly lower immune response compared to those with acute cellular rejection. Interestingly, those patients with overlap features of both HCV and acute cellular rejection who had a low immune response were more often found to have HCV. Thus, the ImmuKnow assay has potential to serve as a clinical tool to distinguish recurrent HCV after transplantation or acute cellular rejection [118]. Clinical utility of the ImmuKnow assay, which determines immunosuppression levels, is limited in children with kidney transplants, but it is very valuable with serious infections [248]. The A_{2A} receptor agonist, regadenoson, increases hepatic artery flow in the recipients of small-for-size liver grafts, giving some improved outcome [267].

Conclusions and future directions

As reviewed above, there are now substantial data implicating extracellular nucleotides and nucleosides in a variety of liver functions in both health and disease. Purinergic signalling is involved in the vascular and immune responses to liver transplantation and can influence the pathophysiological responses to ischaemic injury, disordered bile flow and metabolic disorders such as insulin resistance. All of these entities are critical to optimal clinical outcomes following hepatic allografting.

In terms of other therapeutic non-transplant strategies, the expectation is for the development and study of non-toxic drugs that can modulate breakdown of ATP by ectonucleotidases, in addition to selective agonists and antagonists for purinoceptor subtypes that are orally bioavailable and stable in vivo. Such therapies might be employed for several of the more common liver diseases to not only improve hepatic steatosis but also ameliorate the progression to scarring, disordered regeneration and cirrhosis.

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