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LIVER CYTOPROTECTION BY PROSTAGLANDINS

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Abstract—During the last decade intensive work on the relationships between the liver and the arachidonic acid cascade has greatly expanded our knowledge of this area of research. The liver has emerged as the major organ participating in the degradation and elimination of arachidonate products of systemic origin. The synthesis in the liver of arachidonate products derived from the cyclooxygenase, lipoxygenase and cytochrome P450 system pathways has been demonstrated. The participation of leukotriene B₄ and cysteinyl-leukotrienes as mediators of liver damage and the possible therapeutic usefulness of prostaglandins (PGs) in acute liver injury has attracted the interest of clinicians. This article reviews the essential features regarding the role of arachidonate metabolites in liver disease and specially focuses on the cytoprotective effects on the liver displayed by PGE₂, PGE₁, PGI₂ and synthetic PG analogs in experimental models of liver damage induced by ischemia-reperfusion injury, carbon tetrachloride, bacterial lipopolysaccharide and viral hepatitis and on the possible mechanisms underlying liver cytoprotection in these experimental models. The therapeutic usefulness of PGs in clinical practice is critically analyzed on the basis of available evidence in patients with fulminant hepatic failure and primary graft nonfunction following liver transplantation.

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Abbreviations—ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCl₄, carbon tetrachloride; dmPGE₂, dimethyl, dimethyl-PGE₂; FHF, fulminant hepatic failure; HBV, hepatitis B virus; IL, interleukin; LPS, lipopolysaccharide; LT, leukotriene; LTB₄, leukotriene B₄; MHV-3, murine hepatitis virus-3; PG, prostaglandin; SFHF, subfulminant hepatic failure; TNF, tumor necrosis factor; TxA₂, thromboxane A₂.

1. BACKGROUND

Prostaglandins (PGs) are biologically active polyunsaturated fatty acids arising from arachidonic acid. In addition to their major roles as local regulatory factors and mediators of inflammation, PGs have been shown to have a unique property: that of being cytoprotective. Cytoprotection by PGs was originally described by Robert and coworkers in the 1970s (Lancaster and Robert, 1978; Robert *et al.*, 1979). These authors observed that some PGs were able to protect the gastric mucosa against a variety of noxious stimuli. These studies gave rise to a rapidly expanding field of research on the therapeutic potential of this action in several organs, including the liver.

In the last decade much has been learned regarding the relationships between the liver and the compounds of the arachidonic acid cascade such as PGs and leukotrienes (LTs) under both, normal and pathologic conditions. However, the puzzle is far from complete. The functional complexity of the liver makes the understanding of these relationships difficult. The liver synthesizes virtually all the major products of the arachidonic acid cascade and it is also the target of their biological actions. The liver, on the other hand, is the major organ accounting for the biodegradation of prostanoids and it represents the main route for the elimination of most of their final metabolites via the biliary tree (Huber and Keppler, 1990). Moreover, the normal liver contains a variety of cell subpopulations (hepatocytes and several types of nonparenchymal cells) of which most, if not all, are able to produce arachidonate derivatives. Thus, the identification of the specific metabolites synthesized by each particular cell type and the recognition of interactions between different cell subpopulations influencing the arachidonate cascade have been difficult and still remain uncompleted.

The problem becomes even more complex when the study of the damaged liver is envisaged. The inflammatory cells infiltrating the liver probably represent the cellular subpopulations with the highest capacity for eicosanoid synthesis. In addition, the interactions between products of the arachidonate cascade and the host of messengers released following liver injury (vasoactive agents, hormones, lymphokines, acute phase reactants and others) are only partially known (Brouwer *et al.*, 1990; Lewis, 1990). Finally, since the liver is crucial in the biodegradation and elimination of nonhepatic arachidonate derivatives (Huber and Keppler, 1990), liver diseases will influence to some extent systemic arachidonate metabolism (Huber *et al.*, 1989).

The evidence that a given specific compound, as in the case of some arachidonate metabolites, may act as a regulating agent under normal conditions but also as a mediator of tissue damage after liver injury adds difficulty to the understanding of the role of each particular eicosanoid.

Despite these difficulties, experimental models using liver homogenates, isolated liver cells, the isolated and perfused liver and the intact animal have been useful in establishing some basic concepts regarding the relationships between the liver, arachidonate derivatives and hepatoprotection.

(a) The liver synthesizes all the major metabolites of the arachidonate cascade, including PGs.

(b) PGs and arachidonate derivatives other than PGs are involved in physiological processes in the liver (Table 1).

(c) Some of these substances act as mediators of liver damage due to chemicals, ischemia, infection and others.

(d) Some endogenous PGs, have been found to be hepatoprotective in conditions of liver damage by modulating the extent of immunologic or inflammatory local responses.

(e) Exogenous PGs have been found to be beneficial in several experimental models of liver damage, both *in vivo* and *in vitro*.

(f) Results of preliminary clinical trials using PGs as therapeutic agents in patients with severe acute liver injury have been encouraging. These studies require further confirmation before PGs are accepted as a standardized treatment in some forms of liver injury. Furthermore, much must be still learned about the mechanisms by which PGs behave as hepatoprotective agents. A better knowledge of the intimate mechanisms responsible for the hepatoprotection will improve the therapeutic use of these compounds.

This article summarizes the essentials in regard to the cytoprotective effect of PGs in the liver, the mechanisms potentially involved in this action and the role of these compounds in clinical

practice. Reference to arachidonic acid derivatives other than PGs such as thromboxane A₂ (TxA₂), leukotriene B₄ (LTB₄) and cysteinyl-LTs are forcibly included because of their intimate relationships with PGs and their relevant role in liver damage.

2. ARACHIDONIC ACID DERIVATIVES IN THE LIVER

2.1. BIOSYNTHESIS OF PGs AND OTHER ARACHIDONATE DERIVATIVES

Arachidonic acid is a polyunsaturated fatty acid, which is integrated into the phospholipids of the plasma membrane of all cells in the body. Free arachidonic acid is released from its membrane stores by the action of phospholipase A₂ and phospholipase C which are activated by specific stimuli. Under abnormal conditions phospholipases may be activated by unspecific stimuli (i.e. uncontrolled calcium entry into cells). Phospholipases are negatively regulated by lipomodulin, a glucocorticoid-inducible protein (Flower and Blackwell, 1979; Hirata *et al.*, 1980; Lapetina *et al.*, 1981; Dennis, 1987). The availability of free arachidonic acid is the rate limiting step in the synthesis of its derivatives. This precursor is converted to biologically active products by three enzymatic pathways: the cyclooxygenase, the lipoxygenase (there are several lipoxygenases acting at specific sites of the arachidonic acid) and the P450 system (Fig. 1).

The cyclooxygenase pathway yields as final active metabolites the classic PGs such as PGE₂, PGF_{2α} and PGD₂, prostacyclin (PGI₂) and TxA₂ (Fig. 2). Cyclooxygenase activity is inhibited by nonsteroidal antiinflammatory drugs (Higgs and Vane, 1983).

The major lipoxygenase products in the liver are LTB₄ and the cysteinyl-LTs LTC₄, LTD₄ and LTE₄, which are 5-lipoxygenase derivatives (Huber and Keppler, 1990; Haggmann *et al.*, 1991) (Fig. 3). No metabolites generated by the lipoxygenases acting at positions 12 or 15 of arachidonate have been demonstrated to be produced by cells present in the normal liver. Lipoxygenase activity is inhibited by some fatty acids (ETYA) and by several agents bearing antioxidant activity

TABLE 1. *Arachidonic Derivatives in the Liver*

Major cellular source		Major biological actions
Cyclooxygenase pathway		
PGI ₂	Endothelial cells	Vasodilation, platelet aggregation (+)
PGD ₂	Kupffer cells	Vasoconstriction, glycogenolysis (+)
PGE ₂	Kupffer cells (activated) Endothelial cells	Platelet aggregation (-) IL-2 Production (-) T and B cell proliferation (-) LTB ₄ release (-) Vasodilation Fever (+) Macrophage TNFα gene expression (-) Self-modulation upon activated cells IL-1 production (-) TNFα production (-)
PGF _{2α}	Kupffer cells endothelial cells	Vasoconstriction
TXA ₂	Kupffer cells	Vasoconstriction Platelet aggregation (+)
Lipoxygenase pathway		
LTB ₄	Kupffer cells Hepatic mast cells Inflammatory cells	Chemotaxis (+) Leukocyte adherence (+)
LTC ₄	Hepatic mast cells	Mediation of shock
LTD ₄	Kupffer cells	anaphylaxis
LTE ₄	Endothelial cells	tissue injury Plasma extravasation Mucus secretion (+) Smooth muscle contraction

(-), inhibition; (+), stimulation.

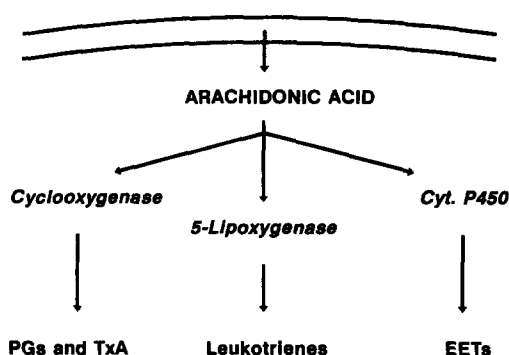


FIG. 1. Biotransformation of free arachidonic acid in the liver. Cyt. P450, cytochrome P450 pathway; PG, prostaglandins; TxA, thromboxane A₂; EETs, epoxyeicosatrienoic acids. Cyclooxygenase (prostaglandin endoperoxide synthase): E.C. 1.14.99.1; 5-lipoxygenase (arachidonate 5-lipoxygenase): E.C. 1.13.11.34.

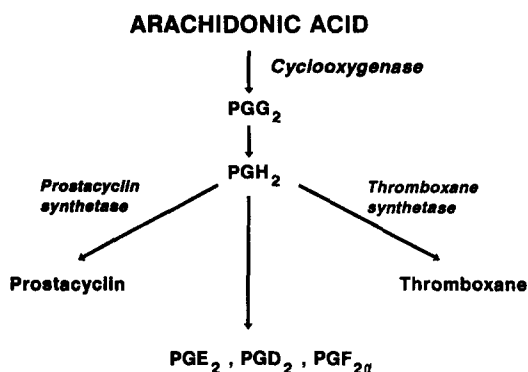


FIG. 2. The cyclooxygenase pathway. PGG₂, hydroperoxyendoperoxide; PGH₂, hydroxyendoperoxide; PG, prostaglandin. PGE₂, PGD₂ and PGF_{2α} may be formed from PGH₂ either nonenzymatically or enzymatically by the action of prostaglandin E synthase (E.C. 5.3.99.3), prostaglandin D synthase (E.C. 5.3.99.2) and prostaglandin F synthase (E.C. 1.1.1.188). Cyclooxygenase (prostaglandin endoperoxide synthase), E.C. 1.14.99.1; prostacyclin synthase (prostaglandin I synthase), E.C. 5.3.99.4; thromboxane synthetase (thromboxane A synthase), E.C. 5.3.99.5.

(BW755c, nordihydroguayaretic acid) (Piper, 1984). LT receptor-antagonists and more specific inhibitors of the lipoxygenase pathway have been developed (Ford-Hutchinson, 1990).

In recent years newer compounds arising from arachidonic acid by the action of the cytochrome P-450 system (epoxyeicosatrienoic acids), which were initially isolated from kidney tissue (Capdevila *et al.*, 1982), have been found also to be produced in other organs including the liver (Fitzpatrick and Murphy, 1988).

2.2. PRODUCTION OF ARACHIDONIC ACID DERIVATIVES IN THE LIVER

The liver contains several kinds of cells, namely, hepatocytes (parenchymal cells) and a variety of nonparenchymal cells including endothelial cells, Kupffer cells (specialized macrophages), tissue

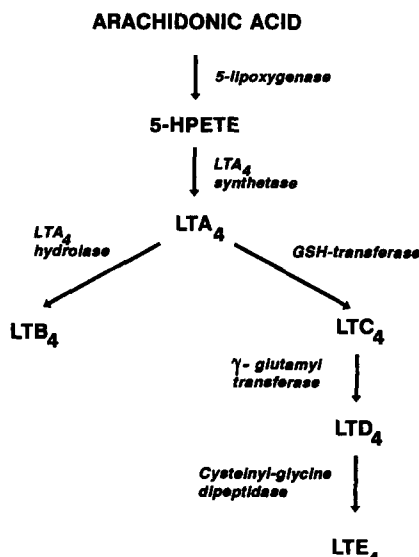


FIG. 3. The 5-lipoxygenase pathway. 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; LT, leukotriene; 5-lipoxygenase (arachidonate 5-lipoxygenase), E.C. 1.13.11.34; LTA₄ hydrolase, E.C. 3.3.2.; GSH-transferase (LTC₄ synthase), E.C. 2.5.1.; gamma-glutamyl-transferase, E.C. 2.3.2.2.; cysteinyl-glycine dipeptidase, E.C. 3.4.13.6.

macrophages, Ito cells (also called lipocytes or fat-storing cells), typical fibroblasts and myofibroblasts (specialized contractile fibroblasts, derived from Ito cells). The so-called pit cells are large granular lymphocytes located inside hepatic sinusoids. The normal liver contains no significant number of polymorphonuclear leukocytes, lymphocytes, plasma cells or eosinophils, but large numbers of one or more of these cell types invade the liver in inflammatory diseases of this organ (Le Bail *et al.*, 1992).

Most, if not all, of these cells are able to synthesize arachidonic acid derivatives through one or more of its metabolic pathways. The specific metabolites produced by each kind of liver cells remain to be definitively established. The most extensively studied have been Kupffer cells, endothelial cells and hepatocytes.

2.2.1. Kupffer Cells

Kupffer cells are able to produce PGs and LTs including cysteinyl-LTs. The major arachidonate metabolites from this type of cells are PGD₂, PGE₂, TxA₂, LTB₄ and LTC₄ (Brouwer *et al.*, 1988; Sakagami *et al.*, 1988). Under basal conditions Kupffer cells release small amounts of eicosanoids, PGD₂ being the major metabolite (Kuiper *et al.*, 1988). Stimuli such as bacterial endotoxin (lipopolysaccharide, LPS), muramyl dipeptide, zymosan, IgG-bearing particles or antigen challenge following prior sensitization, elicit a marked increase in the synthesis of PGs and/or LTs by these cells (Birmelin and Decker, 1984; Aderem *et al.*, 1986; Rieder *et al.*, 1988b). The release of PGE₂ by Kupffer cells acts as a self-modulatory signal when this cellular population becomes activated and is likely to have a direct or indirect cytoprotective role upon hepatocytes. It should be emphasized that Kupffer cells which represent less than 10% of total liver cells, account for at least 70% of all the cells belonging to the macrophage-phagocytic system in the whole body (Munthe-Kaas *et al.*, 1975). This fact reflects the important role that Kupffer cells play in the clearance from the blood of foreign (i.e. endotoxins, bacteria, viruses) and endogenous (i.e. circulating tumoral cells) noxious materials. Its anatomical location, lining the hepatic sinusoids on one side and in the neighborhood of the hepatocyte plates on the other, is functionally relevant. Kupffer cells constitute the ultimate barrier avoiding pathogenic materials of intestinal origin to pass into the systemic blood. In fact, minor amounts of bacterial endotoxin which are usually present in portal blood are cleared up by Kupffer cells as blood passes through the liver sinusoids. Activation of Kupffer cells results in the initiation of phagocytic or inflammatory responses. Phagocytosis is accompanied by the release of oxygen free radicals, while several cytokines such as a variety of ILs, interferon (IFN)-gamma, tumor necrosis factor (TNF)- α and other messengers are released as mediators of the inflammatory response (Mieli-Vergani *et al.*, 1992). Recently, IL-6 release by Kupffer cells has been demonstrated (Busam *et al.*, 1990), and, it corresponds to a Kupffer cell-derived factor described by Bauer *et al.* (1984). In turn, some of these mediators trigger the release of acute phase reactant proteins largely from hepatocytes (Fey and Gauldie, 1990) but also from Ito cells (α 2-macroglobulin) (Andus *et al.*, 1987). Activated Kupffer cells modify its usual quantitative pattern of synthesis of cyclooxygenase derivatives so that PGE₂ instead of PGD₂ becomes the predominant metabolite. The release of PGE₂ is triggered by virtually all stimuli eliciting Kupffer cells phagocytic activity or inflammatory response (Rieder *et al.*, 1988a,b). Stimulation by LPS induces a marked and sustained increase in the synthesis of PGE₂ by these cells (Karck *et al.*, 1988). The intimate mechanism by which the cyclooxygenase pathway is shifted towards PGE₂ synthesis is unsolved, but it is likely to involve a calcium- and calmodulin-dependent phospholipase A₂ activation (Bhatnagar *et al.*, 1982), which appears to require Na⁺/H⁺ exchanger operation (Decker and Dieter, 1987). This intense release of PGE₂ is important in the modulation of the inflammatory response and, hence, in the preservation of the integrity of the liver since most of the messengers released along with PGE₂ are toxic for hepatocytes and may promote endothelial damage and the initiation of intravascular coagulation in the sinusoids (Yamada *et al.*, 1989). In fact, both the endotoxic shock and the massive hepatic necrosis seen in animals exposed to LPS following prior sensitization with inactivated bacteria are cytokine-mediated (see 'experimental liver injury' in this article). It has been shown that the ability of hepatocytes to take up and degrade PGE₂ is virtually nonsaturable (Tran-Thi *et al.*, 1987). Thus, the sustained release of PGE₂ by activated Kupffer cells might be directed to maintain a local PGE₂ concentration at a level sufficient

to exert a negative feed-back upon cytokine release. Kupffer cells are negatively self-modulated by PGE₂ which inhibits, among other functions, antigen expression, tumor cytotoxicity and the release of IL-1 (Shirahama *et al.*, 1988), IFN, colony stimulating factor and LPS-induced TNF α release, which is inhibited by PGE₂ in a dose-dependent fashion (Karck *et al.*, 1988). In the later case, PGE₂ has been shown to modulate macrophage-derived TNF α gene expression (Kunkel *et al.*, 1988). These inhibitory activities are not shared by PGD₂ nor PGF_{2 α} , even when tested *in vitro* at very high concentrations (Karck *et al.*, 1988). Unlike other cytokines, LPS-induced IL-6 release by Kupffer cells was not inhibited by PGE₂ at concentrations that virtually abolished TNF α production (100 nM). Dexamethasone markedly inhibited LPS-induced IL-6 production by Kupffer cells *in vitro* but the concentration of the glucocorticoid (1 μ M) was higher than that usually required to obtain a similar inhibition of TNF α release (0.1 μ M) (Busam *et al.*, 1990). In the same study, IL-1 or TNF α were as effective as LPS in stimulating IL-6 release by Kupffer cells. This may be functionally relevant regarding the autocrine or paracrine (endothelial cells synthesize IL-1) control of Kupffer cells activation. Of interest, the most powerful stimulus releasing IL-6 was the infection of Kupffer cells with Newcastle Disease Virus (Busam *et al.*, 1990).

The immunomodulatory role of PGE₂ is not restricted to Kupffer cells but also includes actions on neutrophils, the attenuation of the local changes in vascular permeability induced by some inflammatory mediators, the suppression of B cell and T cell proliferation and the inhibition of IL-2 production (Ham *et al.*, 1983; Tate *et al.*, 1988; Fantone *et al.*, 1980; Rappaport and Dodge, 1982). Indomethacin, as it could be expected from its inhibitory action upon PGE₂ synthesis, up regulates some of these immune activities (Eisenthal, 1990).

2.2.2. Hepatocytes

From a quantitative point of view hepatocytes are PG-metabolizing rather than PG-synthesizing cells. These cells produce low amounts of PGI₂, PGE₂, PGF_{2 α} and TxA₂, which probably act as autocrine modulators or participate in cell-to-cell communications between contiguous hepatocytes (Tran-Thi *et al.*, 1987). The true functional relevance of PGs in hepatocytes is unknown, although PGs actions upon plasma membrane fluidity and membrane receptors (Dave and Knacek, 1980), glucose metabolism (Brass and Garrity, 1990; Gómez-Foix *et al.*, 1991; Okumura and Saito, 1990) and bile flow (Kaminski and Deshpande, 1980) have been described. Bile salts increase the synthesis of PGI₂, PGF_{2 α} and TXB₂ in isolated rat hepatocytes whereas PGE₂ production is reduced (unpublished data). Since it has been suggested that PGE₂ may decrease Na⁺/K⁺-ATPase activity (Verna, 1984), the negative modulation of PGE₂ synthesis during bile salt uptake (which is dependent upon the sodium gradient across the plasma membrane) may have functional significance.

Hepatocytes were thought to produce only cyclooxygenase compounds; however, hepatocytes are also able to convert LTA₄ to cysteinyl-LTs *in vitro* (Medina *et al.*, 1988). A chemotactic factor released by ethanol-stimulated hepatocytes has been tentatively identified as LTB₄ (Hultcrantz *et al.*, 1991), suggesting the possible presence of lipoxygenase in hepatocytes. Results obtained in our laboratory using lipoxygenase inhibitors in suspensions of isolated rat hepatocytes lend indirect support to this hypothesis (Quiroga *et al.*, 1991a). More recently, we have observed in the isolated and perfused rat liver that sodium taurocholate (a physiologic bile salt) enhances 5-lipoxygenase mRNA expression in liver tissue, which is accompanied by a rise in the hepatic synthesis of cysteinyl-LTs (Rodríguez-Ortigosa *et al.*, 1992). Since the only hepatic cells bearing bile salt receptors are the hepatocytes, these results suggest that bile salts may act as an enhancer of 5-lipoxygenase activity in parenchymal liver cells, although alternative explanations cannot be definitively excluded.

At least two major metabolites of arachidonic acid resulting from the action of the P450 system have also been found to be produced by hepatocytes (Capdevila *et al.*, 1984; Fitzpatrick and Murphy, 1988). In the kidney P450 system derivatives of arachidonic acid appear to influence renal tubular Na⁺/K⁺-ATPase activity, however, the physiologic or pathophysiologic relevance of these compounds in the liver is still under study.

2.2.3. Endothelial Cells

Liver endothelial cells produce PGI₂ as the predominant metabolite, but also minor amounts of PGE₂ and TxA₂. LPS induce a time- and dose-dependent stimulation of prostanoid synthesis (Rieder *et al.*, 1990). The production of PGI₂ is relevant since it is a protective agent in liver inflammation. PGI₂ counteracts the activity of locally-released substances promoting vasoconstriction, platelet aggregation and leucocyte adherence (Needleman *et al.*, 1986), such as cysteinyl-LTs, TxA₂ and other vasoactive agents. If these alterations are not inhibited soon after their initiation, the compromise of liver microcirculation will markedly amplify parenchymal liver damage.

It is likely that endothelial cells which are able to release cysteinyl-LTs and cytokines such as IL-1 participate in a network of paracrine influences involving Kupffer cells, hepatocytes, endothelial cells, resident mast cells and perhaps Ito cells. These cell-to-cell influences may be important in the modulation of immune or inflammatory responses (Lysz *et al.*, 1990). Indeed, very recently it has been demonstrated that during anaphylactic challenge, resident mast cells are the main initiators of LTs production in the liver (Hagmann *et al.*, 1992).

2.2.4. Inflammatory Cells

The recruitment of extrahepatic inflammatory cells due to the local release of chemotactic messengers during the initial phase of the inflammatory response, localizes into the liver a large population of cells with very high ability for eicosanoid synthesis. Thus in liver inflammation the bulk of eicosanoid production is accounted for by these invading cells together with Kupffer cells, with a minor contribution from the remaining cell types. This overproduction leads to an increase in the local concentration of some arachidonic acid derivatives. It must be borne in mind that some arachidonate derivatives have been demonstrated to exert opposite actions upon the same biologic function at low and high concentrations (Rodríguez-Ortigosa *et al.*, 1992). This is an important issue to be considered in relation to the cytoprotective effect of PGs, since pharmacological concentrations may produce effects quite different from those that are thought to be characteristic of the compound.

The synthesis of lipoxygenase derivatives and its role in liver disease has been recently reviewed (Huber and Keppler, 1990). The major functions of these compounds are summarized in Table 1.

3. CYTOPROTECTION BY PROSTAGLANDINS IN EXPERIMENTAL LIVER DAMAGE

The relationships between arachidonic acid derivatives and liver disease have been evaluated in a variety of experimental models of liver damage.

3.1. CYTOPROTECTIVE EFFECTS OF PGs IN ACUTE LIVER INJURY

Acute liver damage induced by carbon tetrachloride (Stachura *et al.*, 1981a; Guarner *et al.*, 1983, 1985; Rush *et al.*, 1986; Mibas *et al.*, 1991), D-galactosamine (Stachura *et al.*, 1980, 1981b; Gove *et al.*, 1990), acetaminophen (Guarner *et al.*, 1988; Ben-Zvi *et al.*, 1990), ethanol (Stachura *et al.*, 1981b; Buko and Zavodnik, 1990), aflatoxin (Rush *et al.*, 1989), α -naphthylisothiocyanate (Ruwart *et al.*, 1984), bromobenzene (Funk-Brentano *et al.*, 1984), 5-fluorouracyl plus hyperthermia (Miyazaki *et al.*, 1983), normothermic and hypothermic ischemia (Sikujara *et al.*, 1983; Mora *et al.*, 1987), transient ischemia followed by carbon tetrachloride (Alph and Hickman, 1987), murine hepatitis virus-3 (MHV-3) (Abecassis *et al.*, 1987), LPS (endotoxin) alone, or associated with, either, galactosamine or heat inactivated *Propionibacterium acnes* (Wendel *et al.*, 1987; Mizoguchi *et al.*, 1987) has been observed to be totally or partially prevented by one or more of the following compounds: PGE₁, PGE₂, dimethyl, dimethyl-PGE₂ (dmPGE₂) (a synthetic analog of PGE₂), prostacyclin, iloprost (a synthetic analog of prostacyclin) and PGF_{2 α} . Successful cytoprotection has been a rather constant feature with all of these PGs, with the only exception of PGF_{2 α} which, although it was partially effective in the ischemia plus carbon tetrachloride model, failed to prevent liver damage induced by MHV-3 virus and paracetamol (see Ballet, 1989, for a review).

Cytoprotection by PGs has been demonstrated in experiments using the intact animal, the isolated and perfused liver and isolated hepatocytes in incubation or culture, pointing to the fact that PGs may exert beneficial effects at several levels.

No clear cytoprotective effect has been observed with the use of cyclooxygenase inhibitors. TxA_2 synthase inhibitors, antagonists of either TxA_2 or LTD_4 and dual cyclooxygenase and lipoxygenase inhibitors have prevented, at least in part, liver damage in some experimental models, suggesting that LTs and TxA_2 may act as mediators of liver injury (Nagai *et al.*, 1989a,b; Hagmann *et al.*, 1984, 1987; Tiegs and Wendel, 1988).

3.2. MECHANISMS OF THE CYTOPROTECTIVE EFFECTS OF PGs

Although the precise mechanisms underlying the cytoprotective effects of PGs in acute liver injury remain to be precisely defined, some suggestions have been obtained in experimental studies using the best characterized models of acute liver damage.

3.2.1. Ischemia-reperfusion Liver Injury

Since liver transplantation has become the mainstay of therapy for irreversible acute and chronic liver diseases the mechanisms of liver damage induced by hypoxia reperfusion have been a focus of major attention for hepatologists. Ischemia-reperfusion liver injury is the main mechanism responsible for the occurrence of primary graft nonfunction, a complication leading to patient death if retransplantation is not undertaken.

Although the mechanisms of liver damage induced by ischemia-reperfusion injury need to be clarified, it is believed that on re-entry of blood into the liver graft, overproduction of toxic oxygen radicals followed by endothelial cell damage and Kupffer cell activation occurs causing disturbances in liver microcirculation. Widespread alterations in the oxygenation of the organ with secondary parenchymal damage ensues (Caldwell-Kenkel *et al.*, 1991; Goto, 1992). Oxidative stress is thought to be a key event in the production of tissue damage following ischemia-reperfusion (McCord, 1985). Koo *et al.* (1991) demonstrated that the occurrence of a delayed non-reflow phenomenon some minutes after reperfusion is crucial in compromising the microcirculation in this type of hepatic injury. The severity of the non-reflow phenomenon (and secondary liver damage) was markedly attenuated in rats pretreated 1 hr before ischemia with a long-acting form of superoxide dismutase, thus demonstrating that superoxide anion mediates, at least in part, the occlusion of liver microcirculation (Koo *et al.*, 1991). Therefore, in ischemia-reperfusion liver injury, following Kupffer cells activation, endothelial cells, rather than hepatocytes, are the most likely primary target of oxidative stress. Then, plugging of capillaries by neutrophils leads to derangements in microcirculation and hence to parenchymal liver damage (Takei *et al.*, 1991).

Several relationships between arachidonate derivatives and ischemia-reperfusion liver injury have been reported, including some beneficial effects of exogenous PGs. It has been suggested that a chemokinetic factor released during reperfusion might be an eicosanoid (Naqpal *et al.*, 1992), probably LTB_4 . The hepatic synthesis of arachidonic acid derivatives is modified as a result of ischemia-reperfusion liver injury. In isolated Kupffer cells, warm hypoxia-reoxygenation induced no changes in PGs synthesis but markedly increased the production of LTB_4 and LTC_4 (Combis *et al.*, 1991). In contrast, reoxygenation following 4 hr of cold hypoxia reduced the release of PGE_2 , $\text{PGF}_{2\alpha}$ and prostacyclin by Kupffer cells by 29%, 33% and 42%, respectively, whereas the synthesis of TxA_2 and lipoxygenase derivatives was unchanged. $\text{TNF}\alpha$ release from Kupffer cells increased in both, warm and cold ischemia-reoxygenation experiments (Combis *et al.*, 1991). Thus, in both cases the PGs/LTs ratio and the $\text{PGI}_2/\text{TxA}_2$ ratio were both reduced in face of increased $\text{TNF}\alpha$ levels. The fall of the $\text{PGI}_2/\text{TxA}_2$ ratio may have a role in promoting local adhesion of leucocytes and platelet aggregation. It is well known that both the vasoconstriction and the platelet aggregation induced by TxA_2 are efficiently counteracted by endothelial PGI_2 , provided that endothelial integrity is preserved. However, in ischemia-reperfusion liver injury endothelial cells are damaged early following reperfusion (Sutto *et al.*, 1991), thus a decreasing $\text{PGI}_2/\text{TxA}_2$ ratio in the endothelial environment may be pathogenically important. Reinforcing this concept, lowering of the $\text{PGI}_2/\text{TxA}_2$ ratio was also observed in rat livers following 90 min of warm ischemia (Besse *et al.*,

1989). In this study, imidazole, an inhibitor of TxA_2 -synthase, significantly improved the survival of animals, which was further ameliorated in rats receiving imidazole and the stable PGI_2 analog, iloprost (Besse *et al.*, 1991). Therefore, the abrogation of TxA_2 overproduction together with the supply of PGI_2 -like activity favorably modified the outcome of the transplanted animals. The relevance of a predominant influence of PGI_2 activity has been highlighted by other studies. Treatment with indomethacin plus iloprost added to the solution flushing the graft and infused i.v. in the recipient animals markedly improved early survival in liver transplanted pigs (Seelos *et al.*, 1991). In these animals, indomethacin fully inhibited the synthesis of PGs and TxA_2 , so that the effect of the PGI_2 analog predominated. On the other hand, PGI_2 improved the function of liver grafts transplanted to pigs after 24 hr of cold ischemia (Mora *et al.*, 1987) and of the isolated and perfused cat liver subjected to hypoxia (Araki and Lefer, 1980).

LTC_4 and LTD_4 -induced vasoconstriction and tissue edema, can also compromise tissue oxygenation and increase liver damage in ischemia-reperfusion injury. LTC_4 , in addition, has an increased damaging potential under conditions of tissue hypoxia. In fact, 95% of isolated rat hepatocytes preincubated for 24 hr under hypoxia were killed by the addition of 10 μg LTC_4 (4.6 μM), which had no detrimental effect on hepatocytes preincubated under normoxia (Trudell *et al.*, 1984). The LTC_4 -induced death of hypoxic cells might be related with its activity as calcium ionophore (Serhan *et al.*, 1982). In the same study, the survival of hypoxic hepatocytes was reduced by the calcium ionophore A-23187 (1 μM) which killed 95% of these cells and only 5% of normoxic hepatocytes. Thus, hypoxic cells are deficient in overcoming altered calcium fluxes. In this way, the calcium channel blocker, verapamil, had a beneficial effect on the survival of pig liver grafts transplanted after 24 hr of cold preservation (Mora *et al.*, 1987), reinforcing the potential of calcium channel blockers as hepatoprotective agents (Deakin *et al.*, 1991).

The inflammatory response of Kupffer cells is likely to be amplified if its PGE_2 synthesis is reduced, as suggested by data from Combis *et al.* (1991). As previously noted, PGE_2 released by Kupffer cells self-modulates the intensity of its inflammatory response. Thus, a low production of PGE_2 , might be associated with an increased release of $\text{TNF}\alpha$ and other cytokines contributing to the widespread tissue damage and massive hepatic necrosis which constitutes the end result of severe ischemia-reperfusion liver injury.

Therefore from a hepatoprotective point of view, efforts should be directed not only to protect parenchymal liver cells but mainly to prevent at the earliest possible step, the damage of endothelial cells.

Recently it has been reported that bile duct epithelial cells which are less vulnerable than hepatocytes to hypoxia, are, however, very sensitive to reoxygenation-induced damage (Noack *et al.*, 1992). Whether this hitherto unrecognized reoxygenation-induced damage may contribute to early graft malfunction should be investigated.

3.2.2. Carbon Tetrachloride-induced Liver Damage

Carbon tetrachloride (CCl_4) is a toxic compound which has been widely used in experimental models of acute liver injury and also in chronic administration to induce liver cirrhosis.

Stachura *et al.* (1981a) reported for the first time the hepatoprotective effect of dm- PGE_2 against CCl_4 -induced liver damage. CCl_4 (6670 mg/kg, s.c.) in non-treated rats produced fatty degeneration 2 hr after toxic challenge and severe centrilobular necrosis 24–48 hr later together with a marked increase in serum aminotransferases. Rats treated with dm- PGE_2 (5 $\mu\text{g}/\text{kg}$, 30 min before and 8 and 24 hr after CCl_4 administration) showed fatty degeneration but necrosis was absent and the increase of serum aminotransferases was transient and 20–25 times lower than in control rats. Ultrastructurally, prominent proliferation and ballooning of the endoplasmic reticulum were seen in control rats whereas no abnormality was observed in dm- PGE_2 -treated rats. CCl_4 is converted by the liver P450 system to trichloromethyl radicals which are highly reactive species and cause lipid peroxidation of polyunsaturated fatty acids in cell membranes, leading to membrane disruption and cell death (Farber, 1987). Stachura *et al.* (1981a) hypothesized that the hepatoprotective mechanism of dm- PGE_2 in this model was 'stabilization' of membranes of the endoplasmic reticulum. Subsequent studies confirmed the cytoprotective action of dm- PGE_2 against CCl_4 -induced liver damage (Ruwart *et al.*, 1981) and ruled out that interference with CCl_4 absorption or metaboliza-

tion or scavenging of trichloromethyl radicals were significant mechanisms underlying dmPGE₂ cytoprotection (Rush *et al.*, 1986). Studies performed in our laboratory showed that PGE₂ was similar to dmPGE₂ in protecting the liver against this toxin and demonstrated that this property was shared by PGI₂. Lipid peroxidation, as evaluated by malondialdehyde generation, was demonstrated to occur upon exposure of isolated hepatocytes to CCl₄. In addition, these studies provided initial insight regarding the involvement of lipoxygenase derivatives in mediating liver damage since the dual cyclooxygenase and lipoxygenase inhibitor BW755c protected isolated rat hepatocytes against CCl₄ injury whereas indomethacin did not (Guarner *et al.*, 1983, 1985). Indomethacin increased CCl₄-induced damage in isolated rat hepatocytes when added to the cell suspension early after the toxin, while preincubation of liver cells with low-dose ethanol (which effectively stimulated PGs synthesis) attenuated the toxic effect, suggesting that endogenous PGs might afford some protection against CCl₄ (Guarner *et al.*, 1985).

Calcium dependence of CCl₄-induced hepatotoxicity has been demonstrated in cultured hepatocytes (Casini and Farber, 1981). It was proposed that a calcium-dependent activation of membrane phospholipases takes place after CCl₄ exposure (Lamb and Schwartz, 1982). Uncontrolled membrane phospholipase activation causes structural degradation of membrane systems in the cell leading to cellular breakdown. Phospholipases activation is inhibited by high intracellular levels of cAMP (Lapetina *et al.*, 1981). Since PGI₂ and PGE₂ stimulate adenylate cyclase after binding to plasma membrane receptors (Samuelsson *et al.*, 1978), it was believed that phospholipase inhibition would be a mechanism underlying PGs hepatoprotection. Although calcium entry into the hepatocyte may be an early event in toxic liver injury (Lauterburg, 1987), a secondary generalized activation of phospholipases likely represents a near final step in irreversible cell damage which is common to most hepatotoxins affecting cell membrane systems (Schanne *et al.*, 1979). Thus, prevention of phospholipases activation may attenuate secondary damaging mechanisms but it has no influence on the oxidative stress due to trichloromethyl radicals. Since lipid peroxidation is demonstrated to occur following CCl₄ poisoning, two points need to be considered: (a) lipid peroxidation leads to functional changes before the structural damage of the cell membrane systems becomes apparent and, (b) it must be remembered that oxidative stress not only causes lipid peroxidation, but also affects nonlipidic cellular targets such as proteins, DNA and carbohydrates (Tribble *et al.*, 1987). In fact oxidative hepatocellular injury is due to the inactivation of specific membrane transport proteins such as the plasma membrane and endoplasmic reticular Ca²⁺ ATPases which in association with the inability of the mitochondria to retain calcium, leads to an increase in cytosolic free calcium (Bellomo *et al.*, 1985). It is thought that sustained increases in cytosolic calcium result in disruption of cytoskeletal elements and in activation of phospholipases and proteases leading to cell death (Tribble *et al.*, 1987).

The first point relates to the fact that lipid peroxidation increases microviscosity (a reduction in fluidity) of cell membranes (Ohyashiki *et al.*, 1986). This change in the microenvironment of the cell membranes milieu can markedly modify the function of membrane-associated systems such as the activity of Na⁺/K⁺ ATPase, adenylate cyclase and other membrane-bound enzymes and the number and mobility of membrane carriers (Schachter, 1984). A number of hepatotoxic agents are believed to alter hepatocyte function through increasing membrane microviscosity (i.e. monohydroxy bile acids, 17-alkylated steroids, chlorpromazine).

The compound tert-butyl hydroperoxide causes cell damage and death causally related with its ability to produce lipid peroxidation and is useful in studies evaluating this alteration (Schnellmann, 1988). In a recent study, Masaki *et al.* (1992) have shown that, in addition to PGE₂, dmPGE₂ and PGI₂, PGE₁ is protective against liver injury induced by CCl₄ (and D-galactosamine). These authors used tert-butyl hydroperoxide to evaluate the effect of PGE₁ and PGE₂ on lipid peroxidation-dependent killing of isolated rat hepatocytes in culture. Both, PGE₁ and dmPGE₂ significantly reduced cell killing despite their inability to prevent lipid peroxidation, as evaluated by malondialdehyde generation. Since these PGs increase cAMP through the activation of adenylate cyclase (Samuelsson *et al.*, 1978) and cAMP is thought to 'stabilize' the plasma membrane, the effect of di-butyl cAMP on tert-butyl hydroperoxide-dependent cell killing was tested. This compound neither inhibited lipid peroxidation nor reduced cell death, thus ruling out cAMP as mediator of the hepatoprotection exerted by PGs in this model. Serial measurements of plasma membrane microviscosity in hepatocytes incubated with tert-butyl hydroperoxide demonstrated a progressive,

time-dependent increase of membrane microviscosity, reflecting the effect of lipid peroxidation on the physicochemical characteristics of the plasma membrane. However, the addition of either PGE₁ or dmPGE₂, simultaneously with the peroxidant agent resulted in a decrease of membrane microviscosity despite ongoing lipid peroxidation (Masaki *et al.*, 1992). This finding may explain, at least in part, the 'unspecific' cytoprotective effect of PGs, since most agents damaging the liver increase cell membrane microviscosity as a primary or secondary event. This property is shared by PGI₂ (Knazek *et al.*, 1981; Dave and Knazek, 1980), thus providing a common mechanism by which all three most characterized cytoprotective PGs may protect the liver against a variety of toxic agents.

3.2.3. Endotoxin-induced Liver Injury

Several experimental models of liver injury are based on the injection of bacterial endotoxin (LPS). The most representative of these models in relation to the role of arachidonate derivatives and PGs cytoprotection are summarized.

3.2.3.1. *Endotoxin-induced cholestasis.* LPS stimulates the cells of the macrophage-phagocytic system to produce several inflammatory mediators such as LTs and several cytokines. When small amounts of LPS are injected in normal animals no or little liver cell necrosis is seen. However, in some models, acute reversible cholestasis occurs after LPS injection. The pathogenesis of this form of cholestasis, which is clinically represented by the cholestasis associated with sepsis, severe infection or acute severe inflammatory disease, is unknown (Quiroga, 1992). It has been attributed, at least in part, to an excessive production of cysteinyl-LTs (Keppler *et al.*, 1988), since the injection of high doses of LTC₄ to the guinea pig induces cholestasis, which also develops after the simultaneous administration of pharmacological doses of both, LTD₄ and PGE₂, to the rat (Keppler *et al.*, 1985). The cholestatic effect of high doses of LTC₄ has been confirmed in the isolated and perfused rat liver (Rodríguez-Ortigosa *et al.*, 1992), in which LTC₄ induced significant decreases both, in biliary bile salts output and bile flow.

On the other hand, Whiting *et al.* (1991) have observed that TNF α , a major mediator of endotoxin actions, markedly inhibited the uptake of taurocholate by cultured hepatocytes. In intact animals, endotoxin injection (7.5 mg/kg) decreased basal bile flow by 41% and bile-salt stimulated bile flow by 38%. Treatment of the animals with monoclonal anti-TNF α antibody before endotoxin injection prevented the development of cholestasis (Whiting *et al.*, 1991). Very recently, these authors have shown that IL-6 produces the same inhibitory effect in a dose- and time-dependent fashion. Neither TNF α nor endotoxin, added to the culture media of IL-6 treated hepatocytes were able to augment the inhibition induced by IL-6. The concentration of IL-6 (20–200 U/mL) which inhibited bile salt uptake is comparable to the serum levels of this cytokine observed in acute inflammation (Green *et al.*, 1992). These results represent some insight in the mechanisms of endotoxin-induced cholestasis. The use of PGE₂, lipoxygenase inhibitors, LTs antagonists, anti-cytokine antibodies, when available, or other agents blocking the inflammatory response to endotoxin might be of therapeutic interest.

3.2.3.2. *Massive hepatic necrosis induced by endotoxin in pre-sensitized animals.* Endotoxin causes a dose-dependent activation of Kupffer cells promoting an inflammatory response, which, if intense, is able to kill liver cells and to induce endotoxic shock and intravascular coagulation (Mizoguchi *et al.*, 1987). The most interesting experimental models of endotoxin-induced liver injury are those in which the experimental animal is sensitized some days before the endotoxin challenge by the i.v. injection of killed bacteria such as *Corynebacterium parvum*, *Lactobacillus casei* or *P. acnes* (Tsutsui *et al.*, 1986; Hashimoto *et al.*, 1985). In these animals, the i.v. injection of LPS at a dose that has no or little effect in non-presensitized animals, induces massive liver necrosis. The i.v. injection into mice of heat-killed *P. acnes*, a Gram-positive anaerobe, followed 1 week later by a small amount of LPS given i.v., caused within 24 hr massive hepatic necrosis and death in a majority of animals (Mizoguchi *et al.*, 1987). In this study, hepatic necrosis was attributed to a protein-like hepatocytotoxic factor released by liver macrophages (Mizoguchi *et al.*, 1987). As compared with control animals, mice given PGE₁ (30 μ g/kg) simultaneously with LPS, showed a

dramatic reduction in a liver damage 24 hr after LPS injection. As compared with control animals, PGE₁ reduced mice mortality from 86 to 10% and lowered alanine amino transferase release 100-fold. Liver stains showed widespread parenchymal necrosis in control mice while no liver cell necrosis but only lobular permeation by macrophages, lymphocytes and plasma cells was observed in PGE₁-treated animals. Although the intimate mechanisms underlying this hepatoprotective effect are obscure, at least two actions of PGE₁ appear to participate.

These authors demonstrated that cytotoxicity on isolated hepatocytes mediated by the culture supernatant of LPS-stimulated liver adherent cells prepared from mice injected with *P. acnes* was virtually inhibited by the addition of PGE₁ to the hepatocyte suspension, as evaluated by both trypan blue exclusion and protein synthesis by the target hepatocytes (Mizoguchi *et al.*, 1987). Furthermore, addition of PGE₁ together with LPS to the adherent cells culture resulted in a marked loss of cytotoxic ability of the culture supernatant when it was tested on hepatocyte suspensions. These data seem to indicate that PGE₁ inhibited the release of the cytotoxic factor from activated adherent cells but also protected hepatocytes against the damaging effect of that factor.

LPS is a well known stimulus for the release of soluble 'cytotoxic activity' from Kupffer cells (Karck *et al.*, 1988). The hepatocytotoxic factor reported by Mizoguchi *et al.* (1987) might correspond to one or more substances since LPS induces Kupffer cells to release several mediators, including cytokines, such as IL-1, TNF α , IFN-gamma and IL-6, superoxide radicals, LTs and PGs, mainly PGE₂, as previously noted (see Section 2.2). The self-modulatory effect of PGE₂ upon Kupffer cells inhibits the release of cytokines, excluding that of IL-6 (Busam *et al.*, 1990). Thus, attenuation of LPS-induced cytokine release from Kupffer cells appears to be a congruent mechanism of PGE₂ hepatoprotection in this model. In fact, Mahl *et al.* (1992) showed that exogenous PGE₂ inhibits TNF α production by Kupffer cells and prevents endotoxin-induced mortality in mice.

In the same experimental model, increases in the liver content of PGs and LTs and in the biliary output of cysteinyl-LTs precede hepatic necrosis, signaling the activation of both major branches of the arachidonate cascade (Kawada *et al.*, 1990). In addition to the hepatoprotective role of PGE₂, the potential damaging role of TxA₂ must also be noted. The hepatic necrosis induced by the injection of LPS in *C. parvum*-treated mice, is prevented by the selective TxA₂ synthase inhibitor, OKY-046 (50 mg/kg) and also by the TxA₂ receptor antagonist ONO-3708 (0.5–2.0 mg/kg). In these animals, PGI₂ attenuated liver necrosis (Nagai *et al.*, 1989), in agreement with the protective ability of some PGs against TxB₂-induced cellular damage (Horton and Wood, 1990).

3.2.3.3. Fulminant hepatitis induced by endotoxin injection in D-galactosamine treated animals. D-Galactosamine by itself causes no or minor hepatic damage. The associated injection of LPS determines the occurrence of fulminant hepatitis which, in mice, has been demonstrated to be mediated by lymphoreticular cells (Chojkier and Fierer, 1985). Additional studies have identified the subset of cells involved and some of the mechanisms leading to liver damage. In mice treated with D-galactosamine, the injection of LPS-activated autologous spleen cells produced severe hepatic necrosis (Shiratori *et al.*, 1990). In the same study, LPS-activated spleen cells were placed in dishes and the subpopulation of adherent cells (rich in activated macrophages) was recovered and injected into D-galactosamine-treated mice. This resulted in a marked augmentation of liver necrosis, coupled with an increased release of oxygen free radicals and malondialdehyde generation. Pretreatment of adherent cells before injection into mice with the lipoxigenase inhibitor azelastine, or the calcium channel blocker, verapamil, resulted in complete abrogation of free radical generation and marked attenuation of liver damage. Less effective was the pretreatment of adherent cells with the lipoxigenase inhibitors ketotifen or AA861 (Shiratori *et al.*, 1990). In addition, LTD₄/LTE₄ receptor antagonists (FPL55712) prevented D-galactosamine plus endotoxin-induced fulminant hepatitis (Keppler *et al.*, 1985), whereas exogenous LTD₄ associated to D-galactosamine causes a morphologically similar fulminant hepatitis (Tiegs and Wendel, 1988). It is suspected that LTD₄-induced vasoconstriction leading to liver hypoxia and subsequent reperfusion injury may be the underlying mechanism (Iwai and Jungermann, 1988).

3.2.4. Experimental Viral Hepatitis

MVH-3 is a single-stranded RNA virus of the coronavirus family and produces a strain-dependent spectrum of liver disease in inbred strains of mice. Mice of the A strain are fully resistant whereas BALB/cj mice are fully susceptible to acute infection and die of fulminant hepatitis. Both a direct cytopathic effect of MVH-3 and immunologic factors participate in liver damage (Abecassis *et al.*, 1987). In the fully susceptible mice, MHV-3 infection determined an increase in serum aminotransferases beginning 48 hr after the inoculus, which continued to rise until the death of the animals occurring on average 96 hr after inoculation. Mice treated with a single dose of dmPGE₂ (2 µg/kg, i.p.) given either 30 min, 24 hr or 48 hr after MHV-3 inoculation did not show elevation of aminotransferases. However, no protection was seen in rats treated 72 hr after inoculation. Confluent liver necrosis in nontreated mice and only a few parenchymal necrotic foci in those given dmPGE₂ were observed 96 hr after the infection. However, mice survival was unchanged by dmPGE₂. In mice treated with dmPGF_{2α}, neither cytoprotective effects nor changes in survival could be observed. Significant cytoprotection by dmPGE₂ (10⁻⁴ M) was observed in hepatocytes in culture infected with MHV-3, as manifested by a marked reduction in the syncytial transformation of the hepatocytes monolayers and in cell death. Macrophage procoagulant activity which was demonstrated in nontreated mice, was undetectable in dmPGE₂ treated animals (Abecassis *et al.*, 1987).

The lack of effect of dmPGE₂ on mice survival was attributed to extrahepatic viral replication and tissue damage including the brain. The dmPGE₂ cytoprotection against MVH-3-induced liver damage probably includes several mechanisms. The abrogation by dmPGE₂ of macrophage procoagulant activity suggests that secondary damage due to intravascular coagulation was prevented.

Since macrophage procoagulant activity depends on the release of cytokines, resulting from macrophage activation, an attenuation of macrophage cytotoxic activity by dmPGE₂ could contribute to cytoprotection in treated mice.

The direct damage induced by MHV-3 on cultured hepatocytes implies a cytopathic effect. The cytoprotective activity of dmPGE₂ in this *in vitro* model may be due to either an antiviral activity or to the reduction of the cell sensitivity to the cytopathic effect.

In rats, the hepatitis induced by frog virus 3 is associated with an enhanced production of cysteinyl-LTs (Hagmann *et al.*, 1987). Treatment with lipoxygenase inhibitors induced a 50% reduction in serum aminotransferase levels, suggesting that cysteinyl-LTs are relevant in the mediation of liver damage in this model.

3.2.5. Arachidonate Derivatives in Other Experimental Models of Liver Injury

(a) The mechanisms of ethanol-induced liver damage are still partially unknown. While rats fed saturated fat develop no alcoholic liver damage, rats fed corn oil and ethanol develop fatty degeneration, necrosis, inflammation and fibrosis of the liver (Nanji *et al.*, 1992a,b). These authors measured several potential mediators of liver injury during one month in plasma and in supernatants of suspensions of nonparenchymal liver cells (mainly Kupffer cells) in rats fed alcohol with either saturated fat or corn oil. The main findings were the progressive rise in the levels of plasma endotoxin and in the levels of TxB₂ in plasma and Kupffer cells supernatant (the stable metabolite of TxA₂), which were observed only in corn oil fed rats. In addition these rats showed a tendency towards increases in plasma LTB₄, whereas rats fed saturated fat showed higher levels of PGE₂. In corn oil fed rats, the histopathological severity of liver disease was directly correlated with the plasma levels of endotoxin, LTB₄ and TxB₂, but inversely with those of PGE₂ (Nanji, 1992a,b). Although preliminary, these data appear to reinforce the relevance of TxA₂ as a damaging agent. The mechanism by which endotoxin levels increase in rats fed corn oil and its causal relationship with the severity of liver disease are important points to be investigated.

(b) A potentially relevant mechanism by which PGE₂ may improve liver damage irrespectively of its cause is the ability of this PG to stimulate liver regeneration (McNeil *et al.*, 1985). It was shown that the synthesis of PGE₂ by Kupffer cells sharply increases some hours following partial hepatectomy apparently preceding the onset of DNA synthesis (Callery *et al.*, 1991). As liver

regenerates and hepatic mass returns to its original value, the overproduction of PGE₂ by Kupffer cells subsides slowly, suggesting that PGE₂ may be a factor modulating the rate of hepatic regeneration. Although the relationship between changes in PGE₂ production by Kupffer cells and liver regenerative activity might be merely coincidental, additional data suggest a causal relationship. PGE₂ stimulates mitogenesis in hepatocytes (Miura and Fukui, 1979; Andreis *et al.*, 1981) and is increased in homogenates from regenerating liver (MacManus and Braceland, 1976).

In cirrhotic rats treated with dmPGE₂ that underwent partial hepatectomy the mitotic index 30 hr after the procedure was significantly higher than in nontreated rats. Indomethacin given 6 hr before hepatectomy induced a fall of the mitotic index below control values, which was partially restored by administration of dmPGE₂ (Urakawa *et al.*, 1990). Such a negative influence of cyclooxygenase inhibitors (which block PGE₂ synthesis) upon the rate of hepatic regeneration has been reported by other authors (MacManus and Braceland, 1976; Kwon *et al.*, 1990).

In addition, it is well known that PGs increase intracellular cAMP levels and that cAMP is an intracellular signal for cell proliferation (Pastan *et al.*, 1975).

Thus, a PGE₂-stimulated enhancement of the regeneration rate of the liver may add to the initial PGE₂-mediated cytoprotection to minimize the deterioration of liver function following liver injury.

(c) Another potential beneficial effect of PGs in liver injury which has been sporadically reported is its possible antifibrogenic activity. The administration of dmPGE₂ delayed collagen formation and deposition in the liver of rats subjected to a choline-deficient diet to induce liver damage (Ruwart *et al.*, 1988). On the other hand, reduction of markers of liver cirrhosis in rats receiving CCl₄ on a chronic basis were observed in a group of animals treated with the dual cyclooxygenase and lipoxygenase inhibitor BW755c (Mourelle *et al.*, 1987).

The relevance of these findings in the long term evolution of chronic liver disease is still unknown.

4. CLINICAL USE OF PROSTAGLANDINS IN LIVER DISEASE

4.1. FULMINANT OR SUBFULMINANT HEPATIC FAILURE

The evidence of a beneficial effect of a variety of PGs in experimental acute liver damage has justified their use in the treatment of fulminant (FHF) or subfulminant hepatic failure (SFHF). FHF or SFHF are defined, respectively, as the development of hepatic encephalopathy within 2 weeks or between 2 weeks and 3 months after the initiation of jaundice resulting from acute liver injury. Hepatitis viruses (A, B, C, D and E), drugs (i.e. paracetamol, isoniazid, halothane), toxins (i.e. amanitotoxin, CCl₄) and a variety of miscellaneous conditions (Wilson's disease, acute fatty liver of pregnancy, liver ischemia) are the commonest causes of FHF and SFHF (Schalm *et al.*, 1992). FHF carries a bad prognosis with mortality rates ranging from 50 to 100% in series of patients published before the liver transplant era (De Knegt and Schalm, 1991). No specific treatment is available for these conditions excluding the cases due to paracetamol overdose (*N*-acetylcysteine, which provides substrate for glutathione synthesis) (Prescott *et al.*, 1979) and to *Amanita phalloides* poisoning (penicillin G and silirimarín, which inhibit the uptake of amanitotoxin into hepatocytes).

In 1989, Sinclair *et al.* published the first study using PGs in FHF. These authors reported on 17 consecutive patients having FHF or SFHF attributed to acute hepatitis due to hepatitis A virus (HAV) (3 cases), hepatitis B virus (HBV) (6 cases), or the Non-A Non-B type (NANB) (8 cases) who received intravenous PGE₁ infused initially at a dose of 0.2 µg/kg/hr subsequently increased to 0.6 µg/kg/hr. The therapy was maintained for 28 days and then oral PGE₂ was started at doses ranging from 2–8 mg/day. In patients in whom liver function tests deteriorated after PGE₁ withdrawal, the drug was restarted. Only five patients died (three of cerebral edema and two after liver transplantation), all of them with grade III or IV hepatic encephalopathy at presentation. The remaining 12 patients survived without need of liver transplantation. All survivors showed grade I or II hepatic encephalopathy at presentation but progression to grades III or IV was observed in all but in two cases when PGE₁ was started. Relapses were observed after withdrawal of PGE₁, only in three patients with FHF attributed to NANB hepatitis which responded well to one or more PGE₁ courses of variable duration. The clinical course of these three patients is represented in Fig. 4.

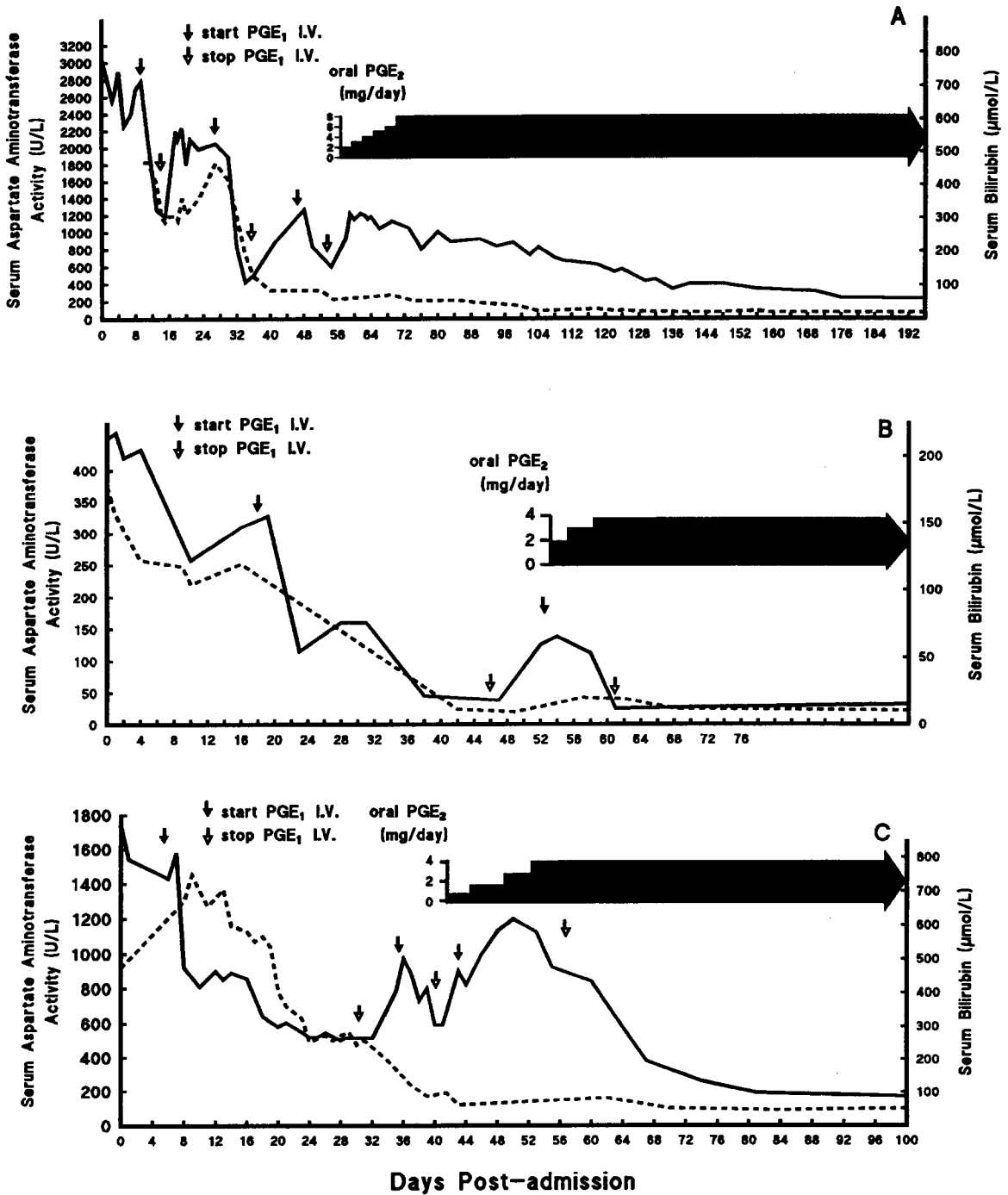


FIG. 4. Treatment of fulminant hepatic failure with prostaglandins. Effect of intravenous prostaglandin E₁ (PGE₁) and oral prostaglandin E₂ (PGE₂) on biochemical parameters in patients with fulminant NANB hepatitis. Aspartate aminotransferase (AST) and bilirubin were measured in patients A, B and C prior to and following administration of PGE₁ and PGE₂. (Patients A, B and C are patients 10, 11 and 12 in the series of 17 patients reported by Sinclair *et al.* (1989).) Reprinted from Sinclair *et al.* (1989), with permission of the authors and the copyright holder, Rockefeller University Press, New York.

The mechanisms underlying the beneficial effects on PGE₁ on FHF are uncertain. The authors of the study included antiviral, immunosuppressive and metabolic activities as possible mechanisms. The 71% survival rate in FHF patients under medical therapy, obtained in this series, is an impressive figure. However, this study was unblinded and uncontrolled. Thiele (1990) commenting

on the above results from Sinclair *et al.* (1989) remembered that initial studies in FHF patients using corticosteroids, exchange transfusions, hyperbaric oxygen, 'coenzyme infusion', crosscirculation with human beings or animals and charcoal hemoperfusion also yielded impressive survival rates when compared with historical controls. However none of these treatments 'survived' the first randomized controlled trial. Thus, caution is needed in considering this otherwise potentially life-saving therapy for patients with FHF or SFHF. Thus, Bernuau *et al.* (1990a) reported that only 3 out of 22 patients treated with intravenous PGE₁ (0.5–0.6 µg/kg/hr) recovered from FHF due to HBV hepatitis without the need of liver transplantation. This survival rate was similar to that observed by this group in the period 1980–1987 (15–20%) in patients with HBV-induced FHF. Indeed, the three transplant-free survivors in the study had no hepatic encephalopathy, suggesting severe acute hepatitis rather than a true FHF (Bernuau *et al.*, 1990a).

The same authors (Bernuau *et al.*, 1990b) reported another series of 22 patients with FHF due to drugs (10 patients) or of undetermined etiology (12 patients) which were given PGE₁ (0.5–0.6 µg/kg/hr) until recovery, emergency liver transplantation or death. In parallel with the findings in the HBV patients, only 3 out of 22 patients recovered with medical treatment alone and again all 3 belonged to the subgroup of patients exhibiting no encephalopathy at admission.

In sharp contrast with the results of Bernuau *et al.* (1990a,b), O'Brien *et al.* (1992b) recently reported on 22 patients with either viral (HAV = 5, HBV = 3) or toxic (paracetamol = 8, paracetamol plus alcohol = 3, carbamazepine = 1, sodium diclofenac = 1) FHF which received intravenous PGE₁ (30 µg/hr) for an average of 5.4 days. Ten patients had grades III or IV hepatic encephalopathy and 12 patients had grades I or II. Sixteen of the 22 patients survived without need of liver transplantation. These authors identified that a small liver volume (less than 1020 cc as measured by CT scan) was a significant predictor of death or need for transplantation, whereas an interval between onset of disease and initiation of treatment less than 7 days predicted a full recovery with medical treatment (O'Brien *et al.*, 1992b).

These same authors (O'Brien *et al.*, 1992a) independently reported a series of 11 patients with paracetamol-induced acute hepatic failure of whom four patients had grades III or IV hepatic encephalopathy, being grade I or II in the remaining seven. All patients were referred for liver transplantation and presented at admission alanine aminotransferase and aspartate aminotransferase (AST) values of $14,970 \pm 4300$ and $11,630 \pm 5830$ IU/mL, respectively, mean bilirubin levels exceeding 5 mg/100 mL and mean prothrombin time prolonged more than twice control values. Only two of these patients had received *N*-acetyl-cysteine within 16 hr of the ingestion of paracetamol (this drug minimizes paracetamol hepatotoxicity if given within that period of time) but these also presented severe hepatic failure. It must be considered that these characteristics at admission in patients with paracetamol overdose, have been reported to be associated with a mortality rate greater than 30%. The patients in the study of O'Brien *et al.* received intravenous PGE₁ (30 µg/hr), starting after 2.6 ± 1.1 days of paracetamol intake. PGE₁ was maintained for an average of 5.8 days. All the 11 patients survived without the need for a liver transplant. Indeed, no patient required dialysis because of renal failure, a common complication in paracetamol-induced FHF.

Differences in the effectiveness of PGE₁ in FHF such as those described here (see Table 2) might be explained by differences in one or more of the following points: the criteria of inclusion of the patients in the trials, the criteria for defining and grading hepatic encephalopathy, the requirements for the indication of liver transplantation or alternatively by true differences in the severity of the illness when therapy was initiated. The doses of PGE₁ were similar in all studies and the supportive care given to the patients should be assumed to be correct since these studies were performed in liver units experienced in the management of patients with FHF. Therefore, blind, controlled studies are required to establish the true efficacy of PGE₁ in FHF and to obtain predictive parameters allowing an earlier indication of liver transplantation in those with high risk of nonresponse under medical therapy alone.

Very recently the first randomized controlled trial evaluating the effectiveness of PGs in FHF has been reported in abstract form (Sheener *et al.*, 1992). Unfortunately the trial was stopped after 3 years with only 41 patients enrolled (the previously calculated requirement of patients to obtain statistical significance with a decrease in mortality from 80 to 40% had been 52 patients). Thus,

TABLE 2. Clinical use of PGE₁ or PGE₂ in Fulminant Hepatic Failure (F.H.F.)

Reference	Number of patients	Cause of F.H.F.	Treatment	Survival*
Sinclair <i>et al.</i> (1989)	17	HAV (3) HBV (6) NANB (8)	PGE ₁ i.v., 0.6 µg/kg/hr PGE ₂ p.o., 2–8 mg/day	12/17 (71%)
Bernuau <i>et al.</i> (1990a)	22	HBV (22)	PGE ₁ i.v., 0.5–0.6 µg/kg/hr	3/22 (13%)
Bernuau <i>et al.</i> (1990b)	22	Drugs (10) Undefined (12)	PGE ₁ i.v., 0.5–0.6 µg/kg/hr	3/22 (13%)
O'Brien <i>et al.</i> (1992b)	22	HAV (5) HBV (3) Toxic (14)	PGE ₁ i.v., 30 µg/hr	16/22 (72%)
O'Brien <i>et al.</i> (1992a)	20	Paracetamol (11)	PGE ₁ i.v., 30 µg/hr	11/11 (100%)
Sheener <i>et al.</i> (1992)	41		PGE ₂ i.v., 10–40 µg/hr	8/20 (40%)
			Placebo	8/21 (38%)

*Survival with medical treatment (additional patients survived with liver transplantation).

data from this trial are of limited value. It was found that from 20 patients which were given PGE₂ (10–40 µg/hr, i.v.), only 8 survived (40%) whereas in the control group survivors were 8 out of 21 (38%). Survival was worse in viral (PGE₂: 22%, placebo: 28%) than in drug- or toxic-induced hepatitis (PGE₂: 54.5%, placebo: 37.5%). The most encouraging data is that patients which were given PGE₂ within 10 days of the onset of symptoms, 8 out of 11 (73%) survived. These recent results emphasize the need for an extensive trial validating the use of PGs in FHF.

4.2. PRIMARY GRAFT NONFUNCTION AFTER LIVER TRANSPLANTATION

As previously noted, primary graft nonfunction remains as a major source of morbidity and mortality in patients undergoing liver transplantation (Quiroga *et al.*, 1991b). Primary graft nonfunction is an unspecific term that does not clarify the precise etiology of graft failure. It is thought that ischemia reperfusion graft injury is the main mechanism in most cases. As a rule, early graft failure is diagnosed within 48 hr following the operation. Characteristic manifestations of primary graft nonfunction include failure of the graft to make bile, very high and rapidly rising levels of serum aminotransferases and severe coagulopathy requiring repeated administration of blood products. Most of these patients show no postoperative recovery of consciousness and require maintenance of mechanical ventilation. Invariably, hepatic encephalopathy with coma ensues. Frequently this catastrophic picture is compounded by continuous bleeding in the surgical bed, renal failure and hemodynamic instability. In this setting the only alternative to death is liver retransplantation which offers to these patients a 45% 6-month survival rate with minimal attrition occurring thereafter.

Greig *et al.* (1989) reported on the beneficial effect of the i.v. infusion of PGE₁ on the outcome of patients with primary graft nonfunction. The first report includes 13 patients, selected between October 1985 and July 1988. Three patients showed AST levels that plateaued at over 1000 U/L for the first three days postoperatively, severe coagulopathy and production of low amounts of poor quality bile. Ten patients had rapidly rising AST (> 1000 U/L) with scant bile production and marked coagulopathy. When primary graft nonfunction was diagnosed, five patients were listed for retransplantation (non-treated group) and eight patients (treated group) were started on PGE₁ infusion (initially at 10 µg/hr with subsequent increase of 10 µg/hr until a maximum of 40 µg/hr was achieved or side effects occurred). Three patients from the non-treated group were retransplanted within 48 hr of the initial transplant and two survived, whereas no organ was available for the remaining two patients who died respectively 6 and 8 days after transplantation

(40% survival rate in the non-treated group). Seven of the eight patients receiving PGE₁ (0.5–7 days on treatment) recovered and were alive at 2–18 months post-transplant (87.5% survival rate). The only patient who died in this group received PGE₁ for less than 12 hr and was retransplanted and eventually died.

At first glance these results are brilliant and seem to demonstrate that PGE₁ is an effective therapy for primary graft nonfunction. However this preliminary study had some limitations and the authors themselves state “although the data are intriguing before one can unequivocally conclude a role for PGE₁ in treatment of primary graft nonfunction, a randomized, controlled trial must be undertaken”.

The number of patients included in the study is sparse. AST levels at the time of diagnosis of primary graft nonfunction were nonsignificantly different between groups. However, only one from five non-treated patients showed AST values below 5000 U/L, while only two from eight PGE₁-treated patients had AST values above that limit. The rise of AST levels as expressed in U/L × hr⁻¹ was significantly higher in the non-treated (397 ± 95) than in the PGE₁-treated patients (161 ± 35). Primary graft nonfunction was diagnosed significantly earlier in the non-treated patients (12 ± 4 hr after they were admitted to the Intensive Care Unit following the operation) than in those receiving PGE₁ (28 ± 4 hr). These differences might suggest that non-treated patients had deteriorated faster and to a greater extent than treated-ones. Clotting test, which were deteriorated to a similar extent in both groups have a limited value since one should assume that all patients received substitution therapy with fresh frozen plasma and cryoprecipitate. The intraoperative requirements for blood and blood products and the operative time which are well recognized parameters influencing graft and patient survival are not given in the study. Finally, some important clinical parameters that could contribute to a better knowledge of the status of the patients are not given in the paper. Despite these limitations the results obtained with PGE₁ infusion are remarkable and deserve further comments. Twelve hours after PGE₁ was started the seven treated patients (excluding the patient which was retransplanted) exhibited a drop in AST values ranging from 86 to 189 U/L × hr⁻¹ with a mean decrease in AST from 4813 ± 466 to 3195 ± 501 U/L. Even more impressive were the changes in clotting factors. Factor V changed from 27 ± 8% to 100 ± 13% and Factor VII from 10 ± 5% to 61 ± 6%. The improvement in prothrombin time (from 22 ± 3 sec to 12 ± 5 sec) and in partial thromboplastin time (from 44 ± 3 sec to 33 ± 1 sec) was dramatic too. It is very unlikely that the improvement of these seven patients occurring just 12 hr following the onset of PGE₁ infusion had been not related with PGE₁ treatment. PGE₁ appears to have two effects, on one hand it reduces hepatocellular necrosis and on the other improves liver function to the extent of virtually normalizing clotting tests. These observations suggest that, at least transitorily, the initial microcirculatory alteration responsible for hepatocellular damage is reversible.

Greig *et al.* (1989) consider that the mechanism that is more likely responsible for primary graft nonfunction in the transplant patients included in the study was ischemia-reperfusion damage. The primary target of this kind of injury is the liver sinusoidal cell which is more sensitive than hepatocytes to hypoxia and oxidative damage. The authors of the study hypothesize that vasodilation induced by PGE₁ may account in part for its beneficial effect. However, PGs of the E series can also accelerate liver regeneration and tissue repair and this effect can be relevant in the treatment of primary graft nonfunction (Skouteris *et al.*, 1988).

According to experimental data a factor critical for the success of the therapeutic intervention in ischemia-reperfusion liver injury is the timing of initiation of the PGs infusion. Thus, it seems reasonable that in the presence of signs of poor graft function after graft reperfusion (absence of bile formation, progressive hemostatic deterioration) PGE₁ or other cytoprotective compounds should be started intraoperatively. Since endothelial adherence of leukocytes and platelets appears to be one of the earlier steps leading to irreversible liver damage, the early use after graft reperfusion of compounds with antiaggregatory and vasodilatory properties, seems to be advisable. Drugs stimulating prostacyclin generation (Ferrero *et al.*, 1989), prostacyclin (Mora *et al.*, 1987), or its analog iloprost (Besse *et al.*, 1989) have proved to be useful in experimental models of ischemia-reperfusion liver injury. Early administration of these compounds either alone or in combination with other PGs (Olthoff *et al.*, 1991) or antioxidant drugs (McCord, 1985) might minimize graft injury due to ischemia-reperfusion.

4.3. OTHER POTENTIAL CLINICAL USES OF PGs OR PGs SYNTHESIS INHIBITORS IN LIVER DISEASES

4.3.1. *Acute Cellular Rejection Following Liver Transplantation*

As far as we know PGs have not been tested for the prevention of acute rejection in liver transplant patients. The PGE₁ analog misoprostol (200 µg q.i.d, p.o. for 12 weeks) has been recently reported to significantly reduce the incidence of acute rejection as compared with placebo (26% vs 51% of the patients, $p < 0.02$) in renal transplant recipients immunosuppressed with cyclosporine and prednisone (Moran, 1990). It is well known that PGs of the E and I series exhibit immunosuppressive properties. However, misoprostol lacks immunosuppressant activity by itself at the doses used in this study (Goodwin and Clay, 1986). Synergy between eicosanoids and other immunosuppressants has been demonstrated, thus the addition of misoprostol to cyclosporine and prednisone could be responsible for the reduction of renal allograft rejection observed by Moran *et al.* (1990). In experimental cardiac transplantation misoprostol has been found to reduce the need for cyclosporine and to improve graft survival (Wiederkehr *et al.*, 1990). Therefore, a possible contribution of misoprostol in preventing acute rejection in liver transplant recipients cannot be ruled out.

4.3.2. *Treatment of Chronic Viral Hepatitis with Cyclooxygenase Inhibitors*

IFN- α is effective for the treatment of chronic active hepatitis due to HCV or HBV infection. Successful treatment of HBV chronic active hepatitis is achieved in only 20–30% of the patients receiving this drug (Hoofnagle, 1990; Thomas, 1992). The therapeutic efficacy in patients with chronic HBV infection depends upon the induction by IFN- α of an immune response vigorous enough to eliminate all infected hepatocytes and to inhibit HBV replication (Camps *et al.*, 1992). An important part of the immune response against HBV is the IFN- α -induced enhanced synthesis of a series of naturally occurring antiviral proteins such as 2'5'-oligoadenylate synthetase (Peters, 1989). Since a defective immune response underlies chronification of HBV infection, no immunosuppressant activity seems to be desirable at the time when HBV has to be eliminated.

It has been recently shown that in cultured liver biopsies from patients with HBV chronic active hepatitis, the addition of IFN- α markedly stimulates PGE₂ synthesis (Andreone *et al.*, 1992a). PGE₂, as previously noted, has well known immunosuppressant properties. These authors hypothesized that the augmented synthesis of PGE₂ resulting from IFN- α administration could have negative effects in regard to HBV clearance. On the other hand, inhibition of PGs synthesis was shown to increase endogenous IFN- α release and 2',5'-oligoadenylate synthetase production (Andreone *et al.*, 1992a). In a preliminary clinical study, nine HbsAg-positive patients with chronic active hepatitis, were given IFN- α (3 MU, s.c.) either alone and after pretreatment with indomethacin (100 mg, p.o.). As compared with no pretreatment, pretreatment with indomethacin was associated with a significantly 4- to 5-fold higher serum levels of 2',5'-oligoadenylate synthetase, both before and after IFN- α administration. Ten hours after IFN- α injection, 2',5'-oligoadenylate synthetase increased from 25 ± 8 to 41 ± 10 pmol/dl in the absence of indomethacin and from 120 ± 39 to 178 ± 97 pmol/dl when this drug was given (Andreone *et al.*, 1992b).

These results are surprising since the stimulatory effect of indomethacin by itself on serum 2',5'-oligoadenylate synthetase seems to be superior to that of IFN- α . Therefore if such a stimulation is maintained during chronic administration it would be worthy to test whether the association of inhibitors of PGs synthesis (NSAID) to IFN- α could improve the antiviral response and the effectiveness of IFN- α treatment.

5. FUTURE PERSPECTIVES

In the coming years a further clarification of the metabolism of arachidonate in the liver is expected. The knowledge of the roles of its derivatives in liver physiology will improve substantially. The relevance of arachidonate derivatives by the P-450 pathway need to be investigated since these compounds may have important functions as it has been shown in the kidney. The exciting relationships which are just emerging among PGs, LTs, several cytokines, growth factors and

antiviral proteins, need to be investigated since all these and other factors modulate functions which will determine ultimately the evolution of acute liver diseases towards recovery or chronicity.

Newer compounds for therapeutic use are expected in the next years. Both synthetic analogs of cytoprotective PGs and specific inhibitors or antagonists of the noxious members of the arachidonate cascade have been developed. At present, some compounds of the first group are already available. Inhibitors of LTs synthesis and cysteinyl-LT antagonists, although highly effective in experimental models, still possess toxicity enough to avoid their clinical use.

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