Role of COX2 in hepatitis

Complex roles of cyclo-oxygenase 2 in hepatitis

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There is currently insufficient evidence to support the use of COX-2 inhibitors in treating chronic hepatitis or in preventing liver fibrosis

> There is a bark of an English tree, which I have found to be a powerful astringent, very efficacious in curing agues and intermitting disorders.[1]

So wrote the Reverend Edmund Stone in 1763 in a letter to the Royal Society wherein he described his success in treating patients with fever with an extract of powdered willow bark. Although willow extract was used in many ancient cultures as an antipyretic, the active component, salicylic acid, was not identified until the 19th century. The discovery of the chemical structure of salicylate by Herman Kolbe paved the way for the synthesis of acetylsalicylic acid by Bayer in the late 19th century. The mechanism by which it exerted its anti-inflammatory effects remained a mystery until John Vane revealed in the 1970s that aspirin and the newly developed non-steroidal anti-inflammatory drugs (NSAIDs) are non-selective inhibitors of cyclo-oxygenase (COX), the enzyme that catalyses the formation of prostaglandins from arachidonic acid. The enzyme contains two active sites, a cyclo-oxygenase which converts arachidonic acid to prostaglandin G_2 (PGG_2), and a haem with peroxidase activity which reduces PGG_2 to the type-2 prostanoid precursor PGH_2, which is subsequently converted into biologically active molecules including the classical prostaglandins (PGE_2, PGD_2 and PGJ_2), prostacyclin and thromboxane-A_2.

Three COX isoenzymes have been described, COX1, COX2 and COX3, a splice variant of COX1. COX1 and COX2 display 60% homology at the amino acid level and are both membrane proteins located primarily in the endoplasmic reticulum (COX1) and perinuclear envelope (COX2). COX1 is constitutively expressed in most tissues and is responsible for many cytoprotective and physiological functions. COX2 expression is negligible in most tissues in the absence of inflammation, but is induced in the immediate/early inflammatory response by pro-inflammatory mediators. The inhibition of COX2 is responsible for the antipyretic and anti-inflammatory properties of non-selective NSAIDs. COX2 has been implicated in a variety of inflammatory diseases,[2 3] and has provided insights into the mechanisms that underlie tissue responses to injury and the link between chronic inflammation and cancer.[4] Overexpression of the COX2 gene in tumour-associated fibroblasts and macrophages is associated with the development of malignancy,[5] and selective inhibition of COX2 has been shown to reduce the size and frequency of colonic polyps in studies on familial adenomatous polyposis (FAP) in mice and humans.[5 6]

COX is upregulated in the livers of patients with chronic hepatitis and cirrhosis.[7 8] Although COX1 expression does not differ between normal and diseased livers, COX2 is markedly increased in cirrhosis and chronic hepatitis, particularly at sites of leucocyte infiltration in the portal tract and sinusoids.[9] These observations suggested that COX2 might be implicated in the development of hepatocellular carcinoma complicating cirrhosis. But this appears not to be the case because, although COX2 is increased in the non-cancerous liver, it is not expressed at high levels in the tumour itself.[8 10] This illustrates that the induction of COX2 depends on the nature, site and kinetics of the injury. The fact that COX2 is overexpressed at areas of active inflammation in chronic hepatitis suggests that it is of functional significance. Indeed, inhibition of COX2 ameliorates the severity of hepatitis in several models including a murine model of steatohepatitis.[11–13] However, COX2 overexpression can have both pro-inflammatory and anti-inflammatory consequences depending on the setting.[14] These paradoxical effects are explained by the fact that the profile of COX2-generated eicosanoids changes during the course of an inflammatory response and differs according to the site and nature of the inflammatory stimulus.[2 15] For example, leucotrienes and PGE_2 are expressed in the early phase of the inflammatory response and amplify acute inflammation, whereas the lipoxins and prostaglandins PGJ_2 and PGD_2 are produced later and antagonise local pro-inflammatory signals.[16] Thus, COX2 may be pro-inflammatory in the early phase of tissue injury, but subsequently can aid resolution by switching prostaglandin synthesis to an alternative set of anti-inflammatory eicosanoids.[17]

With this in mind, the paper by Yu *et al*[18] (***see page 991***) provides an interesting insight into the functional significance of COX2 in the liver. They generated a transgenic mouse, which, under the control of a transthyretin promoter, overexpresses the human isoform of COX2 selectively in hepatocytes. The effects of COX2 overexpression were then studied at intervals of 3 months and compared with wild-type littermate controls. Further evidence that the changes observed were a consequence of COX2 activity was obtained by treating a second cohort of transgenic mice with celecoxib, a selective COX2 inhibitor, for 4 weeks before killing. Enhanced COX2 expression in the transgenic animals led to increased PGE_2 synthesis and was associated with activation of the transcription factor, nuclear factor κB (NFκB), which regulates cellular responses to stress, injury, cytokines and infection. The authors detected increased levels of the pro-inflammatory cytokines, tumour necrosis factor α (TNFα), interleukin 1β (IL1β), IL6 and interferon γ (IFNγ), and the chemokine, CXCL2, in the transgenic animals. Interestingly, there was no increase in IL12 despite increased levels of TNFα, and, although IFNγ was increased, the IFNγ-inducible chemokines, CXCL9 and CXCL10, were not upregulated as one might expect in a hierarchical inflammatory response. The lack of IL12 is consistent with observations made in patients with breast cancer, in whom increased COX2 activity was associated with reduced local expression of IL12. However, in these patients, unlike in the transgenic mice, IFNγ and TNFα were also reduced. The transgenic animals also developed hepatitis associated with an inflammatory infiltrate of macrophages, B cells and a smaller number of CD4 T cells. Inflammatory foci developed close to transfected hepatocytes, which displayed high levels of apoptosis. Liver histology was restored to normal in animals given celecoxib.

This study suggests that COX2 is directly implicated in hepatitis. Two of the observations, in particular, warrant further examination. Firstly, the cellular infiltrate was only seen in mature mice (aged 12 months) and was preceded by a period of accelerated hepatocyte apoptosis. Hence, it is unclear whether the mononuclear infiltrate was a response to local hepatocyte apoptosis or a direct consequence of COX2-driven recruitment.

Secondly, the animals did not develop fibrosis despite the inflammatory infiltrate. There are several possible explanations. In the presence of high COX2 activity, initial hepatitis may fail to progress because of the subsequent generation of reparative eicosanoids that inhibit scarring. Alternatively, the hepatitis may itself be a reparative response to COX2-driven hepatocyte apoptosis rather than a necroinflammatory response that drives tissue damage and fibrosis. It will be interesting to see how the transgenic mice respond in hepatitis models such as the concanavalin A model. In such a model, one might predict that COX2 overexpression would be protective and promote accelerated recovery.

Although the study suggests that COX2 might play an important role in determining the outcome of clinical hepatitis, more information is required before these murine findings can be translated to human disease. For instance, COX2 activity may play different roles in fulminant hepatitis, chronic hepatitis and fibrogenesis. Mouse models of acute liver failure have demonstrated significant upregulation of COX2 in response to injury, but, interestingly, COX2-deficient mice have a higher mortality and morbidity than wild-type controls,[19] and are protected by treatment with PGI_2 or PGE_2.[20] Moreover, studies of carbon tetrachloride-induced liver injury suggest that the onset of fibrosis can be delayed when animals are preloaded with high doses of COX2 inhibitors.[21] Similarly, although expression of COX2 is upregulated in patients with chronic hepatitis B, there is no positive correlation with the levels of necroinflammatory injury seen in patients with the e antigen and in those without e antigen.[22] Taken together, these observations suggest that COX2-derived mediators have distinct functions at different times in the pathogenesis of chronic hepatitis. They may act to amplify the initial early inflammatory response, but subsequently take on a more reparative role by modulating fibrosis and repair. Attempts to determine the function of COX2 in hepatitis must take into account that the outcome of COX2 activity varies with the duration, site and nature of the inflammatory response. Thus, the generation of early pro-inflammatory prostaglandins such as PGE_2 is followed by the generation of regulatory eicosanoids such as PGJ_2, PGD_2 and lipoxin A_4 as the inflammatory response becomes chronic.[16] These late eicosanoids can change the nature of macrophage function from a pro-inflammatory state to a reparative state by modulating signalling through the peroxisome proliferator-activated receptor γ to inhibit inflammatory cytokine secretion while enhancing the ability of monocytes/macrophages to phagocytose apoptotic neutrophils.[16 23 24]

These observations emphasise that COX2-derived products do more than simply switch inflammation on or off and underline how little is understood about their role in chronic inflammation. Experience gained from the use of selective COX2 inhibitors in inflammatory diseases has illustrated that inhibiting these pathways can have diverse and sometimes unexpected effects, reflecting the complex physiological and pathological roles of COX2. The changes in eicosanoids seen during the evolution of inflammation and their opposing actions determine the shape of the inflammatory infiltrate and thereby the outcome following tissue injury. As such, the function of these molecules should not be assessed in isolation but viewed within the complex multicellular microenvironment that constitutes chronic inflammation. It follows that determining when or where to inhibit or enhance these pathways therapeutically requires careful thought based on robust experimental data.

So, do COX2 inhibitors have a role in treating chronic hepatitis or in preventing liver fibrosis? On the strength of current evidence, probably not, but by improving our understanding of the mechanisms that govern the switch from acute to persistent inflammation, we will be better placed to design targeted pharmaceutical inhibitors that can modulate the signals that drive chronic inflammation and cancer.

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REFERENCES

1 **Stone E**. An account of the success of the bark of the willow in the cure of agues. In: A letter to the Right Honourable George Earl of Macclesfield, President of R.S. from the Rev. Mr Edmund Stone, of Chipping-Norton in Oxfordshire.
2 **Park GY**, Christman JW. Involvement of cyclooxygenase-2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases. *Am J Physiol Lung Cell Mol Physiol* 2006;**290**:L797–805.
3 **Paramo JA**, Rodriguez JA, Beloqui O, *et al*. Monocyte cyclooxygenase-2 activity: a new therapeutic target for atherosclerosis? *Curr Drug Targets Cardiovasc Haematol Disord* 2005;**5**:303–11.
4 **Oshima M**, Dinchuk JE, Kargman SL, *et al*. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 1996;**87**:803–9.
5 **Vandoros GP**, Konstantinopoulos PA, Sotiropoulou-Bonikou G, *et al*. PPAR-gamma is expressed and NF-kB pathway is activated and correlates positively with COX-2 expression in stromal myofibroblasts surrounding colon adenocarcinomas. *J Cancer Res Clin Oncol* 2006;**132**:76–84.
6 **Steinbach G**, Lynch PM, Phillips RK, *et al*. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000;**342**:1946–52.
7 **Kondo M**, Yamamoto H, Nagano H, *et al*. Increased expression of COX-2 in nontumor liver tissue is associated with shorter disease-free survival in patients with hepatocellular carcinoma. *Clin Cancer Res* 1999;**5**:4005–12.
8 **Sung YK**, Hwang SY, Kim JO, *et al*. The correlation between cyclooxygenase-2 expression and hepatocellular carcinogenesis. *Mol Cells* 2004;**17**:35–8.
9 **Mohammed NA**, Abd El-Aleem SA, El Hafiz HA, *et al*. Distribution of constitutive (COX-1) and inducible (COX-2) cyclooxygenase in postviral human liver cirrhosis: a possible role for COX-2 in the pathogenesis of liver cirrhosis. *J Clin Pathol* 2004;**57**:350–4.
10 **Morinaga S**, Yamamoto Y, Noguchi Y, *et al*. Cyclooxygenase-2 mRNA is up-regulated in cirrhotic or chronic hepatitis liver adjacent to hepatocellular carcinoma. *J Gastroenterol Hepatol* 2002;**17**:1110–16.
11 **Yu J**, Ip E, Dela PA, *et al*. COX-2 induction in mice with experimental nutritional steatohepatitis: role as pro-inflammatory mediator. *Hepatology* 2006;**43**:826–36.
12 **Cheng AS**, Chan HL, To KF, *et al*. Cyclooxygenase-2 pathway correlates with vascular endothelial growth factor expression and tumor angiogenesis in hepatitis B virus-associated hepatocellular carcinoma. *Int J Oncol* 2004;**24**:853–60.
13 **Rahman MA**, Dhar DK, Yamaguchi E, *et al*. Coexpression of inducible nitric oxide synthase and COX-2 in hepatocellular carcinoma and surrounding liver: possible involvement of COX-2 in the angiogenesis of hepatitis C virus-positive cases. *Clin Cancer Res* 2001;**7**:1325–32.
14 **Serhan CN**, Brain SD, Buckley CD, *et al*. Resolution of inflammation: state of the art, definitions and terms. *FASEB J* 2007;**21**:325–32.
15 **Gilroy DW**, Colville-Nash PR, Willis D, *et al*. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* 1999;**5**:698–701.
16 **Levy BD**, Clish CB, Schmidt B, *et al*. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* 2001;**2**:612–19.
17 **Lawrence T**, Willoughby DA, Gilroy DW. Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nat Rev Immunol* 2002;**2**:787–95.
18 **Yu J**, Hui YA, Chu ES, *et al*. Expression of COX-2 transgene in murine liver causes hepatitis. *Gut* 2007;**56**:991–9.
19 **Reilly TP**, Brady JN, Marchick MR, *et al*. A protective role for cyclooxygenase-2 in drug-induced liver injury in mice. *Chem Res Toxicol* 2001;**14**:1620–8.
20 **Yin H**, Cheng L, Langenbach R, *et al*. Prostaglandin I(2) and E(2) mediate the protective effects of cyclooxygenase-2 in a mouse model of immune-mediated liver injury. *Hepatology* 2007;**45**:159–69.
21 **Planaguma A**, Claria J, Miquel R, *et al*. The selective cyclooxygenase-2 inhibitor SC-236 reduces liver fibrosis by mechanisms involving non-parenchymal cell apoptosis and PPARgamma activation. *FASEB J* 2005;**19**:1120–2.
22 **Cheng AS**, Chan HL, Leung NW, *et al*. Expression of cyclooxygenase-2 in chronic hepatitis B and the effects of anti-viral therapy. *Aliment Pharmacol Ther* 2002;**16**:251–60.
23 **Jiang C**, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998;**391**:82–6.
24 **Ricote M**, Li AC, Willson TM, *et al*. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998;**391**:79–82.