

## Induction of Apoptosis by Celecoxib in Cell Culture: An Uncertain Role for Cyclooxygenase-2

### In Response:

We appreciate the letter by Schönthal stating some interesting aspects of the role of prostaglandin pathways in the liver. The data obtained by Schönthal basically confirms our experimental findings (1). In his letter, Schönthal, however, disagrees with one of our conclusions, i.e., that the observed effects are caused to a significant extent by the cyclooxygenase-2 (COX-2)-inhibitory effect of celecoxib. Schönthal focused his analyses on cell viability and the detection of (down-regulated) Mcl-1 proteins by Western blot. We have shown that the effects of celecoxib lead to the activation of the intrinsic apoptosis pathway and have linked this to Mcl-1 (1). In addition, we have established that celecoxib-induced apoptosis is also, to a significant extent, mediated by activation of death receptor signaling pathways (1). This issue is not addressed by the data of Schönthal. We have shown that celecoxib increased the cell surface expression of different death receptors and, indeed, accumulating data provide evidence that death receptors are up-regulated in several hepatocellular carcinoma cells cell lines after treatment with other COX-2 inhibitors (2, 3). Thus, using down-regulation of Mcl-1 as the only molecular readout of the proapoptotic effects of COX-2 inhibitors is certainly not appropriate.

Schönthal claims that coxib concentrations of 0.1 to 1.0  $\mu\text{mol/L}$  are sufficient to block prostaglandin  $E_2$  ( $\text{PGE}_2$ ) production (previously shown in a squamous cell carcinoma cell line) and that, therefore, concentrations of up to 100  $\mu\text{mol/L}$  as used in our study would prove that the observed effects are  $\text{PGE}_2$ -independent. In contrast, Wu et al. showed in cholangiocarcinoma cells that coxib concentrations of 40  $\mu\text{mol/L}$  were necessary to inhibit  $\text{PGE}_2$  production by 74 % (4). Thus, the coxib concentration required to inhibit  $\text{PGE}_2$  production varies in different cancer cell lines.

Furthermore, Schönthal suggests that the drug effects of celecoxib are mimicked by other COX-2 inhibitors and traditional nonsteroidal anti-inflammatory drugs. Indeed, we have previously shown that the same proapoptotic effects are generated by the use of meloxicam as well as with a slightly

lower efficiency by the classic nonsteroidal anti-inflammatory drug sulindac (5). In addition, we have shown that reduced tumor cell viability induced by celecoxib is efficiently reverted by  $\text{PGE}_2$  supplementation (5).

Although it is certainly not the central issue of our article, evidence obtained by us and other groups shows a significant COX-2 dependency of the observed proapoptotic effects of celecoxib. Of course, additional COX-2-independent effects cannot be ruled out. Schönthal discusses important issues, but his data do not disprove that the observed effects are COX-2-dependent.

**Michael A. Kern**  
**Peter Schirmacher**  
 Institute of Pathology,  
 University of Heidelberg,  
 Heidelberg, Germany

**Tobias Schilling**  
 Department of Internal Medicine I,  
 University Hospital Heidelberg,  
 Heidelberg, Germany

**Martina Müller**  
 Department of Internal Medicine IV,  
 University Hospital Heidelberg,  
 Heidelberg, Germany

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