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GUEST EDITORIAL

Liver cell proliferation and tumor promotion by phenobarbital: relevance for humans?

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The anticolvulsant phenobarbital (PB) and other activators of the constitutive androstane receptor (CAR) act as transient inducers of hepatocyte proliferation and as potent nongenotoxic carcinogens in rodent liver. Epidemiological studies, however, did not provide sufficient evidence for liver tumor induction by PB in humans whilst also not entirely ruling out this possibility (Whysner et al. 1996; Holsapple et al. 2006). The IARC has classified PB as a class 2B carcinogen (IARC 2001). Mechanistically, it has been proposed that species differences in the induction of hepatocellular proliferation by PB might underlie the observed discrepancy in tumor induction (Elcombe et al. 2014). The proliferative response of rodent hepatocytes following PB treatment in vitro is not observed in comparable cultures of their human counterparts (Parzefall et al. 1991). An inseparable correlation of PB-induced proliferation and tumor growth, however, cannot be assumed with absolute certainty; in mice, PB exclusively promotes the outgrowth of a very distinct population of tumors, which carry activating mutations in the β-catenin gene *Ctnnb1* (Aydinlik et al. 2001). Accordingly, tumor promotion by PB is absent from mice with hepatocyte-specific knockout of *Ctnnb1* (Rignall et al. 2011), which, however, still exhibit a proliferative response (Braeuning et al. 2011).

Studying tumor promotion by PB in rodents has contributed to our understanding of the mechanisms of nongenotoxic carcinogenesis, but has not been able to definitely answer the question of human relevance of these findings. Recently, humanized mice, which express the human versions of certain xeno-sensing nuclear receptors, have become available, providing new opportunities for the analysis of potential species differences (Scheer et al. 2008; Scheer and Wolf 2013). The impact of CAR humanization on hepatic effects of PB has been addressed in different publications; after short-term PB treatment, Huang et al. (2005) show DNA synthesis in livers of humanized CAR mice (hCAR; in some of the experiments cited in the following, mice with humanized CAR and PXR were used, but are referred to as hCAR in the following for the sake of clarity) after treatment with PB. By contrast, results from another study indicate that hCAR does not support the hyperplastic response (Ross et al. 2010) and a slight but significant increase in hepatocellular proliferation was observed in wild type (WT) but not hCAR mice after administration of sulfoxaflor (LeBaron et al. 2013). The reason for these discrepant findings is still unclear. Thus, when considered together, these publications do not clarify the question whether hCAR has the potential to induce hepatocellular proliferation. Due to their short-term study design, the question of tumorigenesis is not addressed by these studies.

Very recently, the proliferative and tumor-promoting effects of PB in hCAR mice have been addressed in two comprehensive studies; the first publication deals with the effects of PB for up to 90 days of exposure (Luisier et al. 2014), while the second paper contains the results of a long-term tumor initiation/promotion carcinogenesis experiment (Braeuning et al. 2014). In the study by Luisier et al. (2014), a broad-spectrum transcriptomic approach was used to detect possible differences between hCAR and WT mice in their response to PB. In essence, no fundamental genotype differences regarding the transcriptomic fingerprint of CAR activation is reported and the similar regulation of characteristic gene expression fingerprints related to cell division and proliferation indicates an induction of hepatocellular

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proliferation by PB independent of the CAR genotype of the animals (Luisier et al. 2014). This is in accordance with the study by Huang et al. (2005), but at variance with the data by Ross et al. (2010). In the second study, a diethylnitrosamine (DEN)/PB protocol for chemical tumor induction was followed. The results of this experiment on the one hand demonstrate that tumor promotion by PB does in fact occur in hCAR mice, whereas, on the other hand, it becomes evident that tumor promotion in the hCAR group is significantly less pronounced than in WT mice treated according to the same experimental schedule. The particular phenotype of the tumors observed in DEN/PB-treated hCAR mice, i.e., eosinophilic, *Ctnnb1*-mutated, and glutamine synthetase-positive, exactly matches the observations in DEN/PB-treated WT animals (Braeuning et al. 2014), whereas this tumor phenotype is almost never seen in DEN-induced tumors not promoted by PB (Aydinlik et al. 2001). This observation further substantiates that tumor promotion has occurred in the hCAR model. In summary, the newly available data conclusively show that PB acts as an inducer of hepatocellular proliferation and as a tumor promoter via the human CAR protein, even if the magnitude of the tumorigenic response differs between mCAR and hCAR mice.

These new data challenge the viewpoint that PB-mediated tumor promotion is not relevant to humans. PB cannot be absolved from being possibly carcinogenic to humans, since hCAR, in principle, is able to exert tumor-promotional effects when activated by PB. This view is in accordance with the evaluation of PB by the IARC (2001). However, caution has to be applied when interpreting the hCAR studies, since humanized mice express a human protein within a murine background. The behavior of hCAR in a mouse hepatocyte might not reflect the biological consequences the activated receptor will exert in a fully human background. A deeper understanding of the mechanisms of nongenotoxic carcinogenesis and of the connection of hepatocyte proliferation and tumor promotion might help to experimentally solve this issue.

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