Human relevance of hepatic PPARα-mediated events in the mode of action of propaquizafop

Christian Strupp, Werner H. Bomann, François Spézia, Frédéric Gervais, Roy Forster, Lysiane Richert and Pramila Singh
INTRODUCTION

We investigated the human relevance of peroxisome proliferator-activated receptor α (PPARα) mediated key events in the hepatocarcinogenic mode of action (MOA) of propaquizafop in rats. PPARα can lead to stimulation or inhibition of gene expression by binding with various of endogenous ligands, or with peroxisome proliferators. PPARα is highly present in the liver in rodents. It has been postulated that the MOA via activation of PPARα is rodent-specific, and experience with PPARα-induced pharmaceuticals indicates a lack of human relevance.

In rodents, peroxisome proliferators are considered as non-genotoxic hepatocarcinogens while in humans they have much lower levels of induction/promoter activity and have not been associated with increased cancer risk (IARC Technical Report No.24, Lyon, 1995).

An MOA Human Relevance Framework (HRF) has been developed by international regulatory and health-research scientists to evaluate available data from various species and MOA data consisting of measurable and reproducible key events leading to a given adverse effect observed in animal studies. The hypothesized MOA for PPARα-induced hepatocarcinogenicity in rodents was recently reviewed in detail and considered for human relevance using the MOA/HRF (Corton et al., 2014), and the expert panel determined that the collective data support the temporal and dose-response relationships of key events linked with many activators of PPARα.

We present the results of our MOA investigation following 2-week dietary administration of propaquizafop in wildtype (WT) and PPARα-knockout (KO) rats to confirm the dependency on PPARα of key events finally leading to adenosoma formation.

METHODS

Male Sprague Dawley rats: Wt (RjHan:SD; 10-11 weeks old) were obtained from Janvier (La Genest-Saint-Isle, France) and KO (PPARα tm1sage (bi-allelic deletion within the PPARα gene); 11-12 weeks old) were obtained from SAGE Labs (B borrowed PA, USA).

Propaquizafop (propaquizafop technical, 93.19% purity, batch No. 191) was provided by ADAMA Agan Ltd. (Ashdod, Israel).

Three groups of 18 WT and 18 KO male rats received propaquizafop in diet for 2 weeks at 75, 500 or 1000 ppm (dose levels selected in order to avoid overt toxicity while exceeding the tumorigenic threshold) for 2 weeks. Two control groups of 18 WT and 18 KO male rats received standard (untreated) diet under the same experimental conditions. Two positive control groups of WT and KO rats received WY-14643 (PPARα agonist and fibrate drug) by the oral route (gavage) at 50 mg/kg/day.

BrdU was injected (i.p.) on days 3, 11 and 14 for cell proliferation evaluation. Liver slices were processed and the frequency of positive nuclei was determined.

Unfixed liver slices were homogenized and centrifuged in three steps. Microsomal fractions were prepared for evaluation of total protein and total cytochrome P450 content. CYP content was determined by absorbance reading after CYP oxidation.

CYP isoenzyme activities were measured before analysis by UV-visible light spectrophotometry, UV fluorescence spectrophotometry, mass spectrometry and micro-capillary electrophoresis.

RESULTS

In WT rats, propaquizafop and the positive control, WY-14643, induced marked increases in relative liver weights (+88% and 89%) that correlated with liver enlargement and hepatic hypertrophy, along with increased peroxisomal lauric acid hydrolase (CYP4A) activity (2.4 and 1.5 fold) and acyl-CoA oxidase activity (10.0-23.3 fold) versus control, while in KO rats only increased relative weight (24%) was observed without any of the other effects, thus confirming the PPARα-dependency of the changes.

BrdU labeling resulted in higher numbers and density of positive hepatocytes on day 15 compared to the untreated control group, indicating increased mitotic activity and cell proliferation.

CONCLUSION

We evaluated the data according to the MOA Human Relevance Framework and our findings support the conclusion that liver tumors observed in rodents after dietary propaquizafop administration do not pose a relevant health risk to humans.

BIBLIOGRAPHY


Figure 1: BrdU-positive hepatic cell nuclei in rats: A: WT control. B: WT propaquizafop-treated. C: KO control. D: KO propaquizafop-treated. Marked increase of positive nuclei in the propaquizafop-treated wildtype rats only (arrows show BrdU-positive hepatic cell nuclei).
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