genicity of ORPH in experimental animals. In contrast, our previous study demonstrated that ORPH has a liver tumor-promoting effect resulting from oxidative stress due to increases in CYP2B induction, microsomal ROS production and DNA oxidation (Morita *et al.*, 2013). In the present study, the mRNA level of *Cyp1a1*, one of the phase I drug-metabolizing enzymes, significantly increased in the High ORPH and PB+ORPH groups compared with the DEN-alone group, but there was no synergistic enhancing effect in the PB+ORPH group. In contrast, the mRNA level of *Cyp2b1/2*, another phase I drug-metabolizing enzyme, significantly increased in all treated groups compared with the DEN-alone group. There was a synergistic effect in *Cyp2b1/2* induction in the PB+ORPH group in the heteroadditive model but not in the isoadditive model. These results suggest that combined administration of PB and ORPH remarkably enhanced CYP2B induction during the metabolic process in the rat liver. It has been reported that chemicals that induce CYP2B and have a hepatic tumor-promoting activity produce ROS during their metabolism and result in oxidative stress that stimulates tumor-promoting effects in rat liver (Dewa *et al.*, 2009; Morita *et al.*, 2011). In the present study, ROS production significantly increased in all treated groups, and the level of TBARS significantly increased in the High ORPH and PB+ORPH groups compared with the DEN-alone group. In the heteroadditive model, the levels of ROS and TBARS in the PB+ORPH group were significantly higher than the sum of the net value of the low dose groups. However, in the isoadditive model, there was no synergistic enhancement in the levels of ROS and TBARS in the PB+ORPH group. The synergistically increased level of TBARS in the heteroadditive model in the PB+ORPH group that indicates lipid peroxidation level in hepatocytes (Ohkawa *et al.*, 1979) suggests that ROS overproduction causes high levels of oxidative stress in liver and results in the enhanced induction of preneoplastic lesions in the livers of rats given PB and ORPH simultaneously.

Furthermore, the mRNA levels of *Gstm3*, one of the phase II drug-metabolizing enzymes and antioxidative genes, significantly increased in the Low/High PB, High ORPH and PB+ORPH groups compared with the DEN-alone group and synergistically increased in the PB+ORPH group in the hetero/isoadditive models. The net value of *Gstm3* in the PB+ORPH group was significantly higher than the sum of the net value of that in the low dose groups, and the value of *Gstm3* in the PB+ORPH group was significantly higher than the average value of that in each individual treatment at a high dose of PB and ORPH. In addition, the mRNA levels of *Gpx2*, another phase II drug-metabolizing enzyme and antioxidative gene, significantly increased in the Low/High PB, High ORPH and PB+ORPH groups compared with the DEN-alone group, and there was a synergistic increase in the PB+ORPH group in the heteroadditive model but not in the isoadditive model. The net value of the PB+ORPH group was significantly higher than the sum of the net value of the low dose groups. These antioxidative genes are regulated by Nrf2. Nrf2, which is a transcriptional factor, regulates the expression of genes for phase II drug-metabolizing enzymes by binding to antioxidant responsive elements (AREs) (Itoh *et al.*, 1997) and is activated by oxidative/electrophile stress (Baird and Dinkova-Kostova, 2011). Nrf2 is present in the cytosol in a latent complex with actin-anchored chaperone Kelch-like ECH-associated protein 1 (Keap1), a sulfhydryl-rich protein that is oxidized by oxidative/electrophile stress, thereby uncoupling the association between Nrf2 and Keap1. Subsequently, Nrf2 translocates to the nucleus and associates with small Maf protein. The heterodimers bind to the AREs of target genes including *Gpx2* and *Gstm3* (Reisman *et al.*, 2009; Thimmulappa *et al.*, 2002). Glutathione *S*-transferases (GSTs), including *Gstm3*, detoxify xenobiotics by conjugating glutathione to a range of electrophilic substrates (Thimmulappa *et al.*, 2002). *Gpx2* is a member of the glutathione peroxidase (GPX) family and reduces H_2O_2 (Naiki-Ito, 2007). Upregulation of these genes suggests that ROS was produced because of the combined administration of PB and ORPH and most likely eliminated by Nrf2 gene batteries. However, as demonstrated by the increased levels of TBARS in the PB+ORPH group, it can be concluded that excess ROS generation overcomes the functions of Nrf2-related antioxidative enzymes. Such an excess in ROS production and increased levels of TBARS strongly suggest the occurrence of "redox imbalance" in rats given PB and ORPH simultaneously.

In conclusion, we have demonstrated that combined administration of PB and ORPH causes a synergistic liver tumor-promoting effect in rats. The outcome of the present study suggests the possibility that the combined liver tumor-promoting effect of two different compounds that have similar capabilities to induce CYP2B is synergistic. In addition, our results suggest that combined administration of CYP2B inducers enhanced microsomal ROS production and "redox imbalance" and causes enhanced liver tumor-promoting effects in rats. Therefore, particular attention should be paid to the combined exposure of plural chemicals that are recognized as CYP inducers, and we must eliminate enhanced adverse effects from simultaneous exposure to plural chemicals that have the capability of inducing CYPs. As our data are limit-

ed to the combined administration of PB and ORPH only, further examination using other CYP inducers is vital.

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