Oral

[O25-5] O25-5: Clinical toxicology (2)

Chairs: Kei Zaitsu, Japan / Manuela Neuman, Canada
Mon. Sep 25, 2017 11:15 AM - 12:00 PM Room C1 (1F)

(Mon. Sep 25, 2017 11:15 AM - 12:00 PM Room C1)

[O25-5-3] An oxidative reactive metabolite of nevirapine activates inflammasomes leading to nevirapine-induced liver injury

Ryuji Kato[1], Yoshio Ijiri[2], Kazuhiko Tanaka[3], Tetsuya Hayashi[4], Jack Uetrecht[5] (1.Osaka University of Pharmaceutical Sciences, 2.Osaka University of Pharmaceutical Sciences, 3.Shirasagi Hospital, 4.Osaka University of Pharmaceutical Sciences, 5.University of Toronto)

Keywords: nevirapine, reactive metabolite, inflammasome, cytochromeP450

Background

There is increasing evidence that most idiosyncratic drug-induced liver injury (IDILI) is immune mediated, and in most cases reactive metabolites appear to be responsible for induction of this immune response. Reactive metabolites can cause the release of damage-associated molecular patterns (DAMPs), and inflammasomes can be activated by DAMPs. This may be a common mechanism by which DAMPs initiate an immune response. In this study, inflammasome activation by reactive metabolite of nevirapine, which is associated with a relatively high incidence of IDILI, was evaluated using 3D culture of human hepatocarcinoma functional liver cell-4 (FLC-4) cells and differentiated human THP-1 macrophages.

Methods

The FLC-4 cells were cultured with nevirapine for 7 days, and then the supernatant was added to differentiated THP-1 cells and incubated for 24 hr. The control was incubation without nevirapine, and in other hepatocyte cultures a P450 (aminobenzotriazole) or sulfotransferase (1-phenyl-1-hexanol) inhibitor was added. IL-1β concentration in the THP-1 culture medium was measured using an ELISA kit. Caspase-1 activity was also measured using the Caspase-Glo® 1 Inflammasome Assay. FLC-4 and THP-1 cells were lysed and nevirapine covalent binding was determined by western blot using rabbit anti-nevirapine primary antibody.

Results

The supernatant from the incubation of nevirapine with FLC-4 cells at therapeutic concentrations led to the activation of inflammasomes in THP-1 cells with release of IL-1β. The pattern of caspase-1 activity was similar to that of IL-1ß. Nevirapine can be bioactivated to a reactive quinone methide and a benzylic sulfate. Production of IL-1ß was inhibited by adding aminobenzotriazole to the hepatocyte culture but not by 1-phenyl-1-hexanol. This is presumably because the hepatocytes produced the quinone methide, which caused the release of DAMPs, and these DAMPs were responsible for inflammasome activation.

Conclusions

Our results are consistent with the hypothesis that the mechanism of IDILI involves oxidation to a reactive metabolite by hepatocytes resulting in the release of DAMPs, and these DAMPs activate inflammasomes leading to an immune response.