The mechanism of flutamide induced liver injury via inflammasome activation Tanaka S, Noda T, Urashima K, Kohda Y, Kato R Osaka Medical and Pharmaceutical University

Background: Flutamide (flu) is a non-steroidal anti-androgen agent, which is mainly used for the treatment of prostate cancer. Flu is known to cause idiosyncratic liver injury as severe adverse events. However, the detail is unclear. On the other hand, we have reported that reactive metabolite induces the release of damage-associated molecular patterns（DAMPs）that activate immune cells, which is causes the idiosyncratic drug-induced serious adverse effects. Flu is known to be partially hydrolyzed into an arylamine metabolite, 5-amino-2-nitrobenzotrifluoride by carboxylesterase. Arylamines are activated by cytochrome P450, which is reactive metabolite. In this study, we evaluated whether flu or its reactive metabolites lead to inflammasome activation with the human hepatocarcinoma functional liver cell-4 (FLC-4) cells and the human macrophage cell line (THP-1 cell) to test flu or its reactive metabolite activate immune cells.

Methods: FLC4-cells were cultured with 0.3-3.0 μM flu for 7 days. The supernatant from the FLC-4 cells was added to THP-1 cells, which was incubated for 24 hr. These supernatant and cells were used for measurement of IL-1β production and caspase-1 activity. 1-aminobenzotriazole (ABT, 1 mM) was used to inhibit cytochromes P450 activities, bis-p-nitrophenyl phosphate (BNPP, 100 μM) was used to inhibit carboxylesterase, and Ac-YVAD-cmk (YVAD, 1 μM) was used to inhibit caspase-1 activity. Moreover, we tested the effects of dexamethasone. The protein of HMGB1、heat shock protein（HSP）32、HSP40、HSP60、HSP70、HSP90、S100A8、S100A9 were measured by the western blotting.

Results: The supernatant from an incubation of FLC-4 cells with flu increased the IL-1β production and caspse-1 activity of THP-1 cells. In addition, the production of IL-1β and caspase-1 activation were inhibited by adding the ABT, BNTT, YVAD, or dexamethasone. HSP60 was significantly increased in the supernatants from FLC-4 cells incubated with flu.

Conclusions: These results support that the reactive metabolite of flu can cause the release of HSP60 and activate inflammasomes, which can cause immune-related adverse events. Moreover, steroid use might be one of the treatment options to decrease the risk of flutamide induced liver injury.

Keyword: flutamide, reactive metabolite, DAMPs, inflammasome