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Rapid Report

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Activation of Inflammasomes by Agents Causing Idiosyncratic Skin Reactions: A Possible Biomarker

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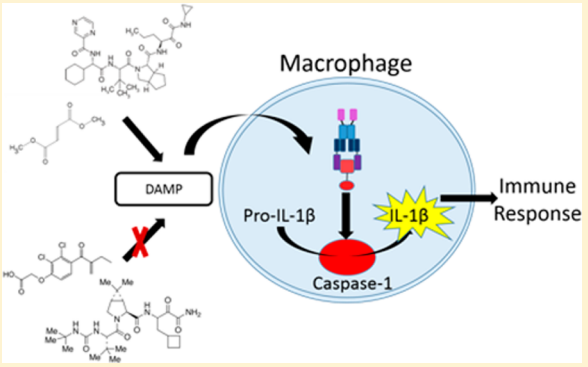
ABSTRACT: Chemically reactive drugs and drugs that form reactive metabolites often cause idiosyncratic drug reactions (IDRs); however, not all such drugs are associated with IDRs. Most IDRs appear to be immune mediated; therefore, the ability of a drug to induce an immune response may be the determinant of which drugs will cause IDRs. Inflammasome activation plays an important role in the initiation of an immune response. In this study, we studied two pairs of similar chemically reactive drugs, telaprevir/boceprevir and dimethyl fumarate/ethacrynic acid. In both pairs, the drug associated with skin reactions activated inflammasomes in THP-1 cells, and the drug not associated with skin reactions did not.



Most idiosyncratic drug reactions (IDRs) appear to be immune mediated.[1] This is especially true of skin rashes. We developed an animal model of nevirapine induced skin rash that is immune mediated.[2] It is caused by the chemically reactive sulfate formed in the skin from a benzylic alcohol metabolite formed in the liver.[3] This raises the question of how the reactive benzylic sulfate induces an immune response. Chemically reactive chemicals applied to the skin often cause contact hypersensitivity, and it is known that animals deficient in components of the inflammasome are resistant to contact sensitizers.[4] Inflammasome activation, especially NALP3, is an important mechanism for immune system activation.[5] It is believed that inflammasome activation involves damage associated molecular patterns (DAMPs, table of contents figure), but exactly how contact sensitizers produce DAMPs is unknown.[6] Inflammasomes have several components, but the basic mechanism involves conversion of pro-caspase 1 into its active form, and this active caspase, in turn, converts an interleukin-1β (IL-1β) precursor into its active form. Caspase 1 can also convert a precursor of IL-18 into its active form. These inflammatory cytokines are an essential component of immune activation. We have preliminary evidence that nevirapine induced skin rash involves activation of inflammasomes.[7] This suggests that inflammasome activation may be an important step in the mechanism of IDRs.

In the present study, we compared two pairs of similar chemically reactive compounds, one of which is associated with a serious idiosyncratic reaction or immune response, and the other is not. One pair is telaprevir and boceprevir (MedChem Express, 11 Deer Park Drive, Suite 102D, Monmouth Junction, NJ 08852; Figure 1). Both are similar α-ketoamide protease inhibitors, which form a covalent bond to their target and are used for the treatment of hepatitis C.[8] Although this covalent

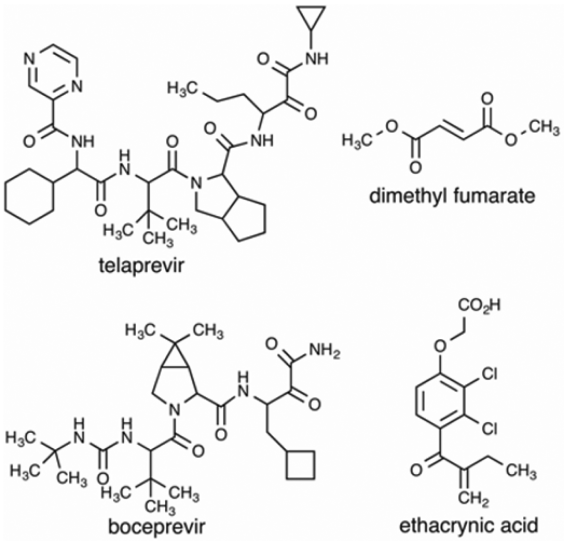


Figure 1. Chemical structures of telaprevir, boceprevir, dimethyl fumarate, and ethacrynic acid.

bond is reversible, it is possible that after reaction with some proteins, a rearrangement would lead to an irreversible interaction. Telaprevir has been linked to cases of toxic epidermal necrolysis and carries a black box warning for this serious skin rash, while boceprevir does not carry the same warning.[9]

Telaprevir or boceprevir was dissolved in 1:1 DMSO and RPMI 1640 supplemented with 10% fetal bovine serum (FBS)

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and incubated with THP-1 human peripheral blood monocyte cells. The THP-1 cells (ATCC − 30-2021; Manassas, VA, USA) were cultured in 24-well plates at 4×10^5 cells/mL in a volume of 1 mL. These monocytes were differentiated over 48 h to macrophages by the addition of 25 ng/mL phorbol myristate acetate. Cells were washed with PBS and treated in RPMI 1640 supplemented with 10% fetal calf serum and cultured at 37 °C, 5% CO_2 for 18 h. IL-1β was quantified by ELISA (Invitrogen, 81 Wyman Street Waltham, MA 02454). Telaprevir clearly activated inflammasomes in THP-1 cells with the production of IL-1β, and this production is inhibited by the caspase inhibitor, benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl-ketone (ZVAD, 10 μg/mL), while activation by boceprevir was not significant (Figure 2).

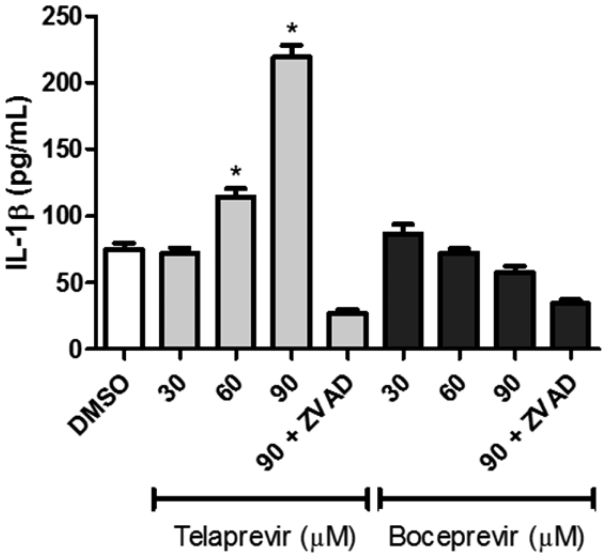

Figure 2. Levels of IL-1β secreted by THP-1 derived macrophages in response to 18 h of treatment with increasing concentrations of telaprevir or boceprevir in DMSO (0.25%), with and without a caspase inhibitor, ZVAD, as measured by ELISA; *$p < 0.05$, $n = 6$.

The other pair of drugs was dimethyl fumarate and ethacrynic acid (Sigma, Oakville, ON); both are reactive Michael acceptors (Figure 1). Dimethyl fumarate was used as a biocide in furniture, but it was banned because it causes contact hypersensitivity.[10] It has immunomodulatory effects and is being studied for the treatment of psoriasis and multiple sclerosis.[11] In contrast, the diuretic, ethacrynic acid, clearly covalently binds to proteins, but it has a remarkable safety record with respect to IDRs.[12] The ability of these two drugs to activate inflammasomes was tested using the same procedure as that for telaprevir and boceprevir. Dimethyl fumarate also activated inflammasomes in THP-1 cells with the production of IL-1β, which was prevented by ZVAD, while ethacrynic acid did not (Figure 3).

Drugs that covalently bind to their target are becoming more common; however, there is a legitimate fear that such drugs will be associated with an unacceptable risk of IDRs.[13] The results of this study suggest that it may be possible to determine which drugs with chemically reactive "warheads" will be associated with IDRs. In addition, most drugs that cause IDRs form reactive metabolites, but not all drugs that form reactive metabolites are associated with a significant incidence of IDRs. Therefore, the ability of a reactive metabolite to activate

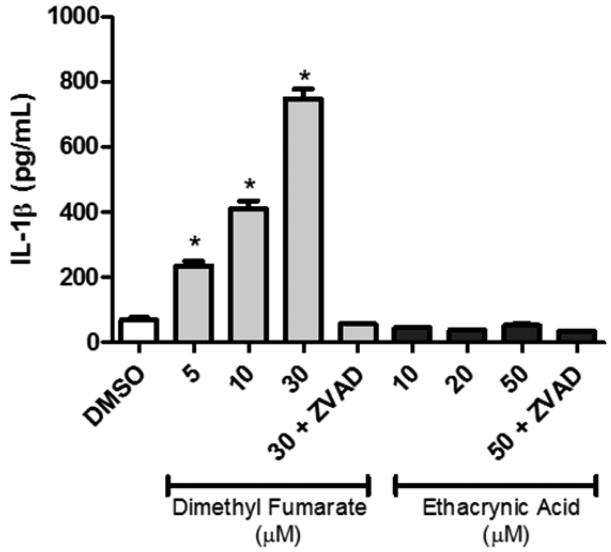

Figure 3. Levels of IL-1β secreted by THP-1 derived macrophages in response to 18 h of treatment with increasing concentrations of dimethyl fumarate or ethacrynic acid in DMSO (0.25%), with and without the caspase inhibitor, ZVAD, as measured by ELISA; *$p < 0.05$, $n = 6$.

inflammasomes may be an important determinant of which reactive metabolites are likely to cause IDRs. It is more difficult to test drugs that require bioactivation to form reactive metabolites, but preliminary results suggest that this may also be possible. Although this study is limited by only testing two pairs of drugs, it provides a clue to how reactive drugs and metabolites cause IDRs, and it may lead to a biomarker combined with covalent binding studies to more accurately predict IDR risk.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

DMSO, dimethyl sulfoxide; IDR, idiosyncratic drug reaction; DAMP, damage associated molecular pattern

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