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LETTER TO THE EDITOR

The cannabinoid WIN55212-2 restores rhinovirus-induced epithelial barrier disruption

To the Editor,

Bronchial epithelial cells constitute the first physical barrier with mucociliary clearance and immunologic defence capacity against environmental inhaled insults.[1] Disruption of tight junctions (TJs) and epithelial barrier dysfunction is a hallmark of chronic inflammatory airway diseases such as rhinitis, chronic rhinosinusitis with nasal polyposis or asthma.[2,3] Rhinovirus infections induce the disruption of the airway epithelial barrier and the production of pro-inflammatory cytokines.[2] Cannabinoids are lipid-derived mediators with anti-inflammatory properties in different disorders.[4] Cannabinoid receptor 1 (CB1) agonists inhibited the development of arthritis by restoring the intestinal barrier in the in the pre-phase of arthritis.[5] CB1 expression is significantly increased in atopic patients.[6] WIN55212-2 is a non-selective synthetic cannabinoid belonging to the aminoalkylindole group (Figure 1A) with anti-inflammatory properties,[4] but its capacity to modulate the bronchial epithelial barrier function during viral infections remains elusive.

Basolateral stimulation of human bronchial epithelial cells (HBEC) in air-liquid interface (ALI) cultures with 5 and 10 μM but not at 1 and 2.5 μM WIN55212-2 drastically decreased transepithelial electric resistance (TER) due to cytotoxic effects (Figure 1B). We investigated whether non-toxic concentrations of WIN55212-2 could impact human rhinovirus-16 (HRV-16)-induced epithelial barrier disruption. ALI cultures of HBEC were apically infected with HRV-16 in the absence or presence of basolateral 2.5 μM WIN55212-2. TER was slightly and equally reduced after 4 h of stimulation in all the assayed conditions (Figure 1C), likely due to potential little physical alterations induced during the application of the stimulus. After 24 h, TER values were restored in the unstimulated, 2.5 μM WIN55212-2 alone and ultraviolet (UV)-inactivated HRV-16 conditions and remained without significant changes up to 72 h. HRV-16 significantly decreased TER after 24 h, and these values continued almost unchanged up to 72 h (Figure 1C). After 24 h of HRV-16-infection in the presence of WIN55212-2, TER was also significantly decreased with values equal to those observed by HRV-16 alone, indicating that WIN55212-2 does not prevent HRV-16-induced barrier damage (Figure 1C). However, a significant increment of TER in the presence of WIN55212-2 compared to HRV-16-infection alone was observed after 48 h. Remarkably, in the presence of 2.5 μM WIN55212-2, TER values were similar to that observed for unstimulated, WIN55212-2 alone or UV-inactivated HRV-16 conditions after 72 h, demonstrating that WIN55212-2 help to restore the barrier damage caused by HRV-16 infection of ALI-cultured HBEC (Figure 1C). Supporting these data, the presence of WIN55212-2 also significantly restored the paracellular flux (Figure 1D). Interestingly, after 72 h, HRV-16-infected HBEC in the presence of WIN55212-2 showed higher occludin and ZO-1 immunofluorescence intensity compared to cells infected with HRV-16 alone and similar to that observed under unstimulated or WIN55212-2 conditions (Figure 1E). WIN55212-2 significantly increased the expression of occludin and claudin-7 mRNAs with respect to HRV-16-infected cells without significant changes observed for zonula occludens (ZO)-1, claudin-1 and claudin-4 (Figure 1F). During viral infections, epithelial cells produce pro-inflammatory cytokines and chemokines that enhance the recruitment of peripheral inflammatory cells into the airway submucosa.[1] WIN55212-2 alone or UV-inactivated HRV-16 did not induce cytokine production in HBEC. In contrast, HRV-16-infected ALI-cultured HBEC produced significantly higher levels of TNFα, IL-8, IP-10, MCP-1 and G-CSF than unstimulated cells after 24 h, which were not significantly inhibited by WIN55212-2 (Figure 2), suggesting that WIN5212-2 does not alter the innate immune responses triggered by HRV-16 infection in HBEC.

Rhinovirus infection induces severe airway epithelial barrier damage, which plays a significant role in the pathophysiology of different airway chronic inflammatory diseases.[1] Up to date, few drugs have been reported to prevent or restore rhinovirus infection-induced respiratory epithelial barrier alterations.[1] Herein, we uncover a previously unknown capacity of the synthetic cannabinoid WIN55212-2 to help to restore the integrity of the airway epithelial barrier during rhinovirus infection, which might well pave the way for the future development of potential novel therapeutic approaches for different chronic inflammatory airway diseases.

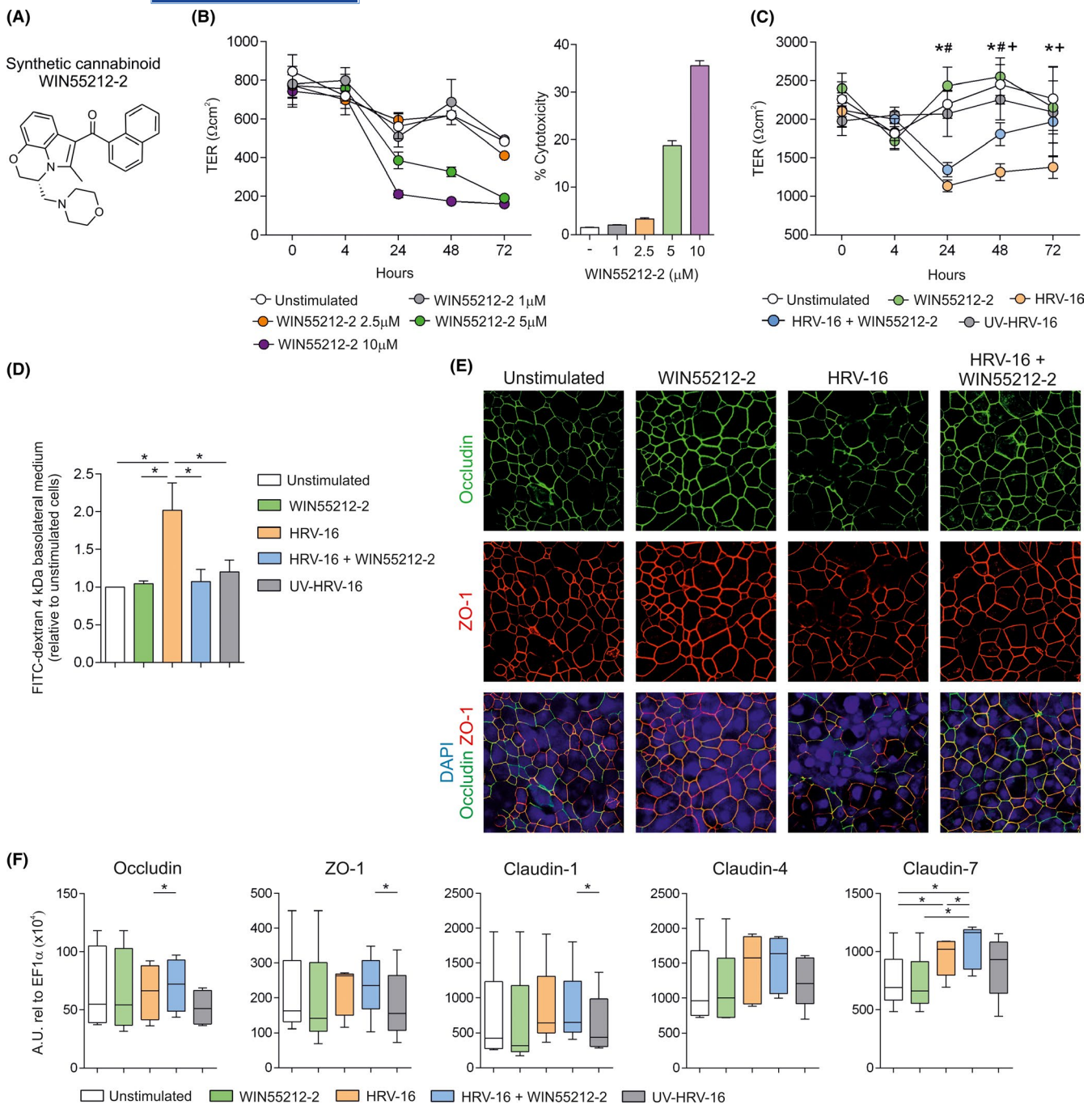

FIGURE 1 (A) Chemical structure of WIN55212-2. (B) TER values in ALI-cultured HBEC after basolateral stimulation with the indicated doses of WIN55212-2 (n = 1 representative donor out of 2 different donors). The graph at the right shows cytotoxicity after 24 hours of stimulation quantified by LDH release in the medium (n = 2 different donors). (C) TER values in ALI-cultured HBEC after apical HRV-16 infection (MOI of 50) with or without basolateral WIN55212-2 (2.5 μM), WIN55212-2 alone or UV-inactivated HRV-16 (n = 4 different donors). Each TER determination was performed by triplicate. *Unstimulated vs. HRV-16,$^{\#}$Unstimulated vs HRV-16+WIN55212-2, $^{+}$HRV-16 vs HRV-16+WIN55212-2. (D) Paracellular flux of FITC-dextran in ALI-cultured HBEC in the indicated conditions. FITC-dextran was apically added after 72 h of treatment and quantified in the basolateral compartment after 24 h (n = 4 different donors). (E) Representative immunofluorescence staining and confocal microscopy images of occludin (green) and ZO-1 (red) in ALI-cultured HBEC after 72 h of stimulation with the indicated conditions. DAPI (blue) was included for nucleus visualization. (F) mRNA expression levels of the indicated TJs genes in ALI-cultured HBEC after 24 h of stimulation with the indicated conditions as determined by qPCR. Arbitrary units (A.U.) are $2^{-(\Delta Ct)}$ values multiplied by 10^4, with ΔCt defined as the difference between the cycle threshold valued for each gene and elongation factor 1 α (EF1α) as housekeeping gene (n = 4 different donors). Data represent the mean ± SEM. Statistical significance determined using one-way ANOVA with Tukey's post hoc comparisons (ANOVA p value < .05). *p < .05

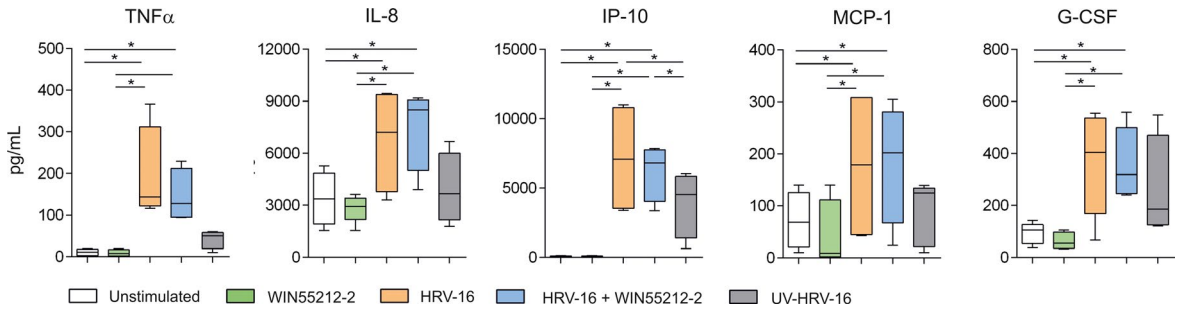

FIGURE 2 Levels of the indicated cytokines and chemokines in cell-free supernatants produced by ALI-cultured HBEC unstimulated, stimulated with WIN55212-2 (2.5 μM) alone, UV-HRV-16, infected with HRV-16 (MOI of 50) or with HRV-16 + WIN55212-2 (2.5 μM) for 24 h as determined by cytometric bead array (n = 4 different donors). Data represent the mean ± SEM. Statistical significance determined using one-way ANOVA with Tukey's post hoc comparisons (ANOVA p value < .05). *p < .05

CONFLICT OF INTEREST

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Alba Angelina[1]
Mar Martín-Fontecha[2]
Beate Rückert[3]
Paulina Wawrzyniak[3]
Mario Pérez-Diego[1]
Jacobo López-Abente[1]
Mübeccel Akdis[3]
Cezmi A. Akdis[3]
Oscar Palomares[1]

[1]*Department of Biochemistry and Molecular Biology, School of Chemistry, Complutense University of Madrid, Madrid, Spain*
[2]*Department of Organic Chemistry, School of Chemistry, Complutense University of Madrid, Madrid, Spain*
[3]*Swiss Institute of Allergy and Asthma Research (SIAF), University of Zürich, Davos, Switzerland*

Correspondence
Oscar Palomares, Department of Biochemistry and Molecular Biology, Chemistry School, Complutense University of Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain.
Email: oscar.palomares@quim.ucm.es

ORCID

Paulina Wawrzyniak https://orcid.org/0000-0001-9641-2103
Mübeccel Akdis https://orcid.org/0000-0003-0554-9943
Cezmi A. Akdis https://orcid.org/0000-0001-8020-019X
Oscar Palomares https://orcid.org/0000-0003-4516-0369

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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