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CANNABINOID RECEPTOR AGONISTS ENHANCE SYNCYTIA FORMATION IN MT-2 CELLS INFECTED WITH CELL FREE HIV-1_{MN}

Sasha N. Noe, Susan B. Nyland, Kenneth Ugen, Herman Friedman, and Thomas W. Klein

Department of Medical Microbiology and Immunology
University of South Florida College of Medicine
Tampa, Florida 33612

ABSTRACT

Marijuana and other drugs have been suggested to act as cofactors for HIV infection. Interestingly, Δ^9-THC has been shown to upregulate NFκB, a transcription factor utilized by HIV. Therefore, it was of interest to investigate whether cannabinoids can modulate HIV infection and replication. Initially, we tested for evidence of receptor expression by examining for receptor mRNA in various cell lines used to study HIV infection and replication. Cellular RNA was isolated from SupT, and H9, $H9_{MN}$, and MT-2 cells and RT-PCR was performed. Results showed that, although all of the cell lines tested were positive for CB2 mRNA, only the MT-2 cells also expressed CB1 mRNA. Since the MT-2 cells expressed both CB1 and CB2 receptor mRNA, we next wanted to determine whether different cannabinoid receptor agonists such as CP-55,940, Δ^9-THC, WIN-55,212-2, and WIN-55,212-3 influenced infection of these cells by cell free HIV-1_{MN}. Infectivity assays were performed where MT-2 cells were incubated with drug and cell free virus for 90 min., the free virus washed off, and the cells incubated further, and checked for virus growth by syncytia formation. It was found that the drugs significantly increased syncytia formation when MT-2 cells were cultured in the presence of both drug and cell free HIV-1_{MN}. In conclusion, of the cell lines tested, only the MT-2 cells were positive for both CB1 and CB2 mRNA. In addition, since syncytia formation is an indication of virus infection and cytopathicity it was concluded that cannabimimetic drugs may enhance HIV-1 infection of susceptible cells.

INTRODUCTION

Cannabis is the generic name for various preparations which are derived from the hemp plant, *Cannabis sativa*. The primary psychoactive constituent in cannabis, delta-9-

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tetrahydrocannabinol or Δ^9-THC, is one of over 60 cannabinoids present in marijuana smoke. Two cannabinoid receptors have been identified thus far, cannabinoid receptor 1 (CB1) from rat and human brain cDNA libraries, and cannabinoid receptor 2 (CB2) from a human leukemic cell line (HL60) cDNA library (Gerard et al., 1991; Matsuda et at., 1990; Munro et al., 1993). In addition, an endogenous cannabinoid ligand, anandamide, has been described and shown to modulate immune cell function (Devane et al., 1992; Smith et al., 1994; Mechoulam et al., 1995). There have been several lines of evidence that suggest that THC has immunomodulatory effects. These include the inhibition of mitogen-induced T-lymphocyte proliferation, (Nahas et al., 1974; Pross et al., 1987; Luo et al., 1992), the inhibition of γ-interferon production (Blanchard et al., 1986), and the suppression of induction of cytolytic function of cytotoxic T cells (Klein et al., 1991). THC has also been shown to increase circulating levels of TNF-α and IL-6 in mice infected with *Legionella pneumophila* (Klein et al., 1993). In addition, it has been suggested that THC augments the secretion of IL-1 from endotoxin-stimulated macrophages (Zhu et al., 1994). Recent studies have also demonstrated that THC treatment of NKB61A2 cells increases IL-2R gene transcription by increasing the level of NFκB via CB1 (Daaka et al., 1997; Zhu et al., 1995). For many years it has been discussed that drugs of abuse may act as cofactors in HIV susceptibility. Interestingly, it has been shown that the secretion of the proinflammatory cytokines such as IL-1β, IL-6 and TNF-α are increased in HIV-infected individuals (Kinter et al., 1995). NFκB, a cellular transcription factor responsible for inducing numerous cellular gene has also been show to be important in HIV-1 replication. Because THC has been shown to impact these cytokines and transcription factors, it was of interest to investigate whether cell lines used to study HIV infection and replication express cannabinoid receptors, and whether cannabinoid agonists can affect HIV infection and replication.

MATERIALS AND METHODS

RNA Isolation

Total cellular RNA was isolated from H9, $H9_{MN}$, Sup T, and MT-2 cells using TRI REAGENT (Molecular Research Center, Inc., Cincinnati, OH) which is composed of phenol and guanidine thiocyanate in a monophase solution. All samples were then treated with DNaseI (2 U/μg of RNA in Tris-$MgCl_2$ buffer; Boehringer Mannheim, Indianapolis, IN) for 30 min at 37°C before RT-PCR.

RT-PCR

Messenger RNA was reversed transcribed followed by PCR amplification as previously described (Daaka et al., 1996). Briefly, cDNA was synthesized using random primers, RNasin, and AMV RT (Promega, Madison, WI). The resulting cDNA template was used in a PCR reaction containing deoxyribonucleoside triphosphates (0.2 mM), 5 μl of 10x PCR Buffer (KCl, 500 mM, Tris-HCl 200 mM, pH 8.4, and $MgCl_2$, 25mM), 1 U of *Thermus aquaticus* DNA polymerase (Promega, Madison, WI), and 0.5 ng of each primer. Primers for PCR were selected using the DNASIS program and are based on the reported human cDNA sequences of CB1 and CB2 (Shire et al., 1995; Munro et al., 1993). The sequence for the CB1 primers were as follows: sense 5′-TGG TGT ATG ATG TCT TTG GG-3′ and antisense 5′-ATG CTG GCT GTG TTA TTG GC-3′ and amplify a region of 324bp. The sequence for the CB2 primers were as follows: sense 5′-CAT GGA GGA ATG CTG GGT GAC-3′ and antisense 5′-GAG GAA GGC GAT GAA CAG GAG-3′ and amplify a region of

604bp. Primers for β_2-microglobulin (Ehlers et al., 1992) were amplified as an internal control for sample-to-sample variation in the amount of starting mRNA. Amplified products were then resolved on a 2% agarose-ethidium bromide gel and autoradiographed.

Infectivity Assays

MT-2 cells (4×10^5) were incubated in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (Hyclone, Logan, UT) and either Δ^9-THC (NIDA, Rockville, MD), CP-55,940 generously provided by Dr. Lawrence Melvin (Pfizer Central Research, Groton, CT), WIN-55,212-2, and WIN-55,212-3 (Research Biochemicals International, Natic, MA) at increasing concentrations for 30 min. at 37°C. Cell free HIV-1_{MN} ($TCID_{50}$ 4.1×10^3 particles/ml) was then added and incubated for 2 hr at 37°C. Negative controls included uninfected cells, cells infected with virus but no drug, and cells treated with DMSO (Sigma Chemical, St. Louis, MO) at a concentration corresponding to highest Δ^9-THC concentration. Following infection, cells were then centrifuged for 5 min. at 2000 rpm and the supernatant was removed. Following a second washing, cells were then resuspended in medium and drug and incubated at 37°C. Cultures were checked daily for syncytia by microscopy.

RESULTS AND CONCLUSIONS

RT-PCR of RNA from the various cell lines using primers for CB2 resulted in the amplification of a 604 bp band indicating that MT-2 and SupT (Fig. 1) and H9 and $H9_{MN}$ (Fig. 2) were positive for CB2 mRNA. In contrast, amplification using primers for CB1

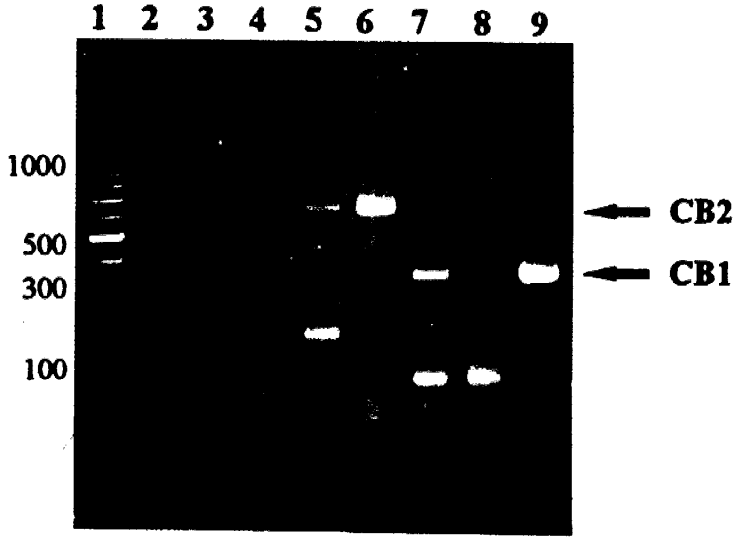

Figure 1. Ethidium bromide stained gel of RT-PCR results using CB1 and CB2 primers and RNA from MT-2 and SupT cells. RNA was isolated from cells and DNase treated. PCR was performed using primers for CB1 and CB2. The amplicon size for CB1 and CB2 are 324bp and 604bp, respectively. No amplification was observed in negative controls (data not shown) in which reactions were run without reverse transcriptase. *Lane 1*, 100 bp Molecular Marker, *Lane 2*, BMG in MT-2 cells, *Lane 3*, BMG in SupT cells, *Lane 4*, CB2 in MT-2 cells, *Lane 5*, CB2 in SupT cells, *Lane 6*, CB2 in HL60 cells as a positive control for CB2, *Lane 7*, CB1 in MT-2 cells, *Lane 8*, CB1 in Sup T cells, and *Lane 9*, CB1 from SKR6 plasmid as a positive control for CB1.

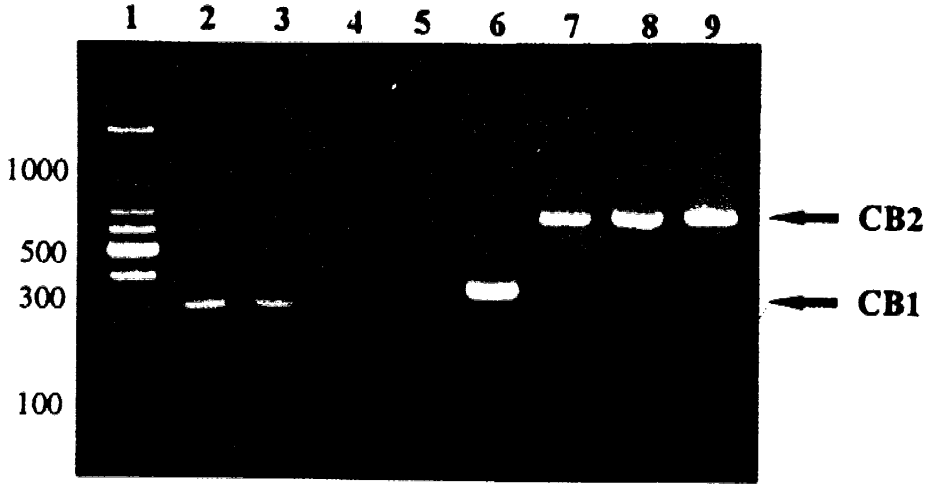

Figure 2. Ethidium bromide stained gel of RT-PCR results using CB1 and CB2 primers and RNA from H9 and $H9_{MN}$ cells. RNA was isolated from cells and DNase treated. PCR was performed using primers for CB1 and CB2. The amplicon size for CB1 and CB2 are 324bp and 604bp, respectively. No amplification was observed in negative controls (data not shown) in which reactions were run without reverse transcriptase. *Lane 1*, 100 bp Molecular Marker, *Lane 2*, BMG in H9 cells, *Lane 3*, BMG in $H9_{MN}$ cells, *Lane 4*, CB1 in H9 cells, *Lane 5*, CB1 in $H9_{MN}$ cells, *Lane 6*, CB1 from SKR6 plasmid as a positive control for CB1, *Lane 7*, CB2 in H9 cells, *Lane 8*, CB2 in $H9_{MN}$ cells, and *Lane 9*, CB2 in HL60 cells as a positive control for CB2.

resulted in the presence of a 324 bp band only in MT-2 cells and not in SupT (Fig. 1) or H9 and $H9_{MN}$ (Fig. 2). These results are interesting since the MT-2 cells are also the only cells that are HTLV-1 transformed. MT-2 cells are naive CD4+ T-cells which express various activation markers such as CD25 and CD2 (unpublished observations), and expression of these activation markers as well as both cannabinoid receptors may be due to the fact that they are virally transformed. Interestingly, amplification of a smaller fragment of approximately 100–200 bp was also observed especially when the CB2 primers were used

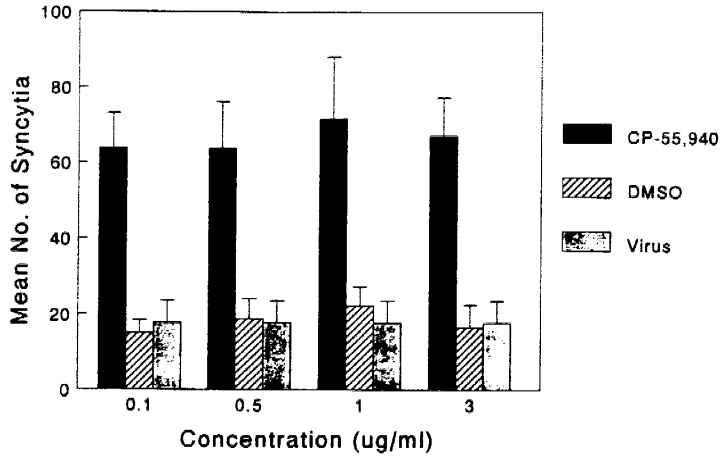

Figure 3. Effect of CP-55,940 on infection of MT-2 cells by HIV-1_{MN} as determined by syncytia formation. Cells were infected as mentioned in materials and methods. Results represent syncytia counts from day 3. Cells were treated with the indicated concentrations of drug (solid bars), DMSO (hatched bars), virus only (shaded bars).

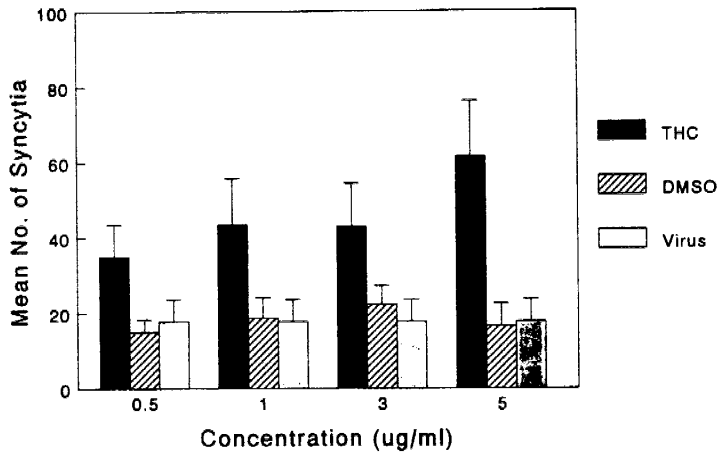

Figure 4. Effect of Δ^9-THC on infection of MT-2 cells by HIV-1_{MN} as determined by Infectivity Assay. Cells were infected as mentioned in materials and methods. Results represent syncytia counts from day 3. Cells were treated with the indicated concentrations of drug (solid bars), DMSO (hatched bars), or virus only (shaded bars).

with RNA from SupT cells and further experiments will be performed to determine the identity of this amplicon.

Based on the results that MT-2 cells expressed both CB1 and CB2 we next wanted to investigate whether cannabinoid agonists can affect HIV infection of these cells. Infectivity assays were performed using the different cannabinoid agonists, CP-55,940 (Fig. 3), Δ^9-THC (Fig. 4), WIN-55,212-2 (Fig. 5), and WIN-55,212-3 (Fig. 6) at different concentrations. The results demonstrate a significant increase in syncytia formation when cells were cultured in the presence of both drug and cell free HIV-1_{MN}. The number of syncytia formed with cells treated only with virus was not significantly different from the DMSO treated cells indicating that the enhancement of syncytia observed in these experiments was not a result of DMSO. The observed effects of these cannabinoids could be mediated

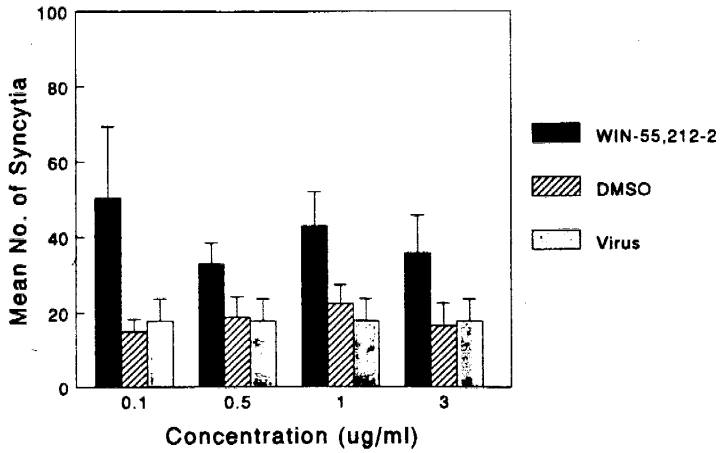

Figure 5. Effect of WIN55,212-2 on infection of MT-2 cells by HIV-1_{MN} as determined by Infectivity Assay. Cells were infected as mentioned in materials and methods. Results represent syncytia counts from day 3. Cells were treated with the indicated concentrations of drug (solid bars), DMSO (hatched bars), or virus only (shaded bars).

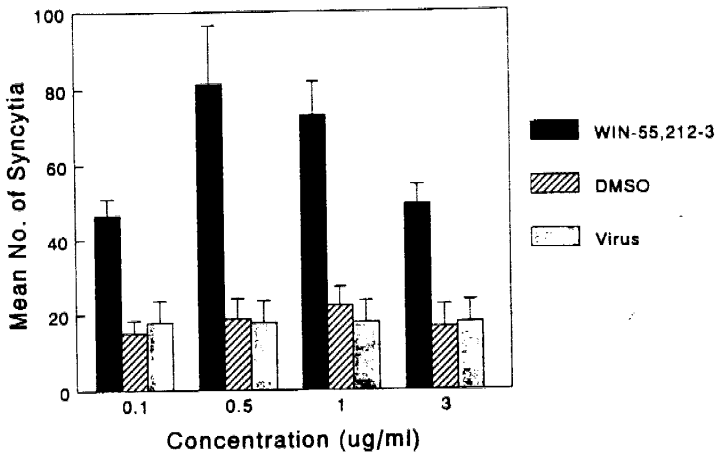

Figure 6. Effect of WIN55,212-3 on infection of MT-2 cells by HIV-1_{MN} as determined by Infectivity Assay. Cells were infected as mentioned in materials and methods. Results represent syncytia counts from day 3. Cells were treated with the indicated concentrations of drug (solid bars), DMSO (hatched bars), or virus only (shaded bars).

through the cannabinoid receptors, however, since WIN-55,212-3 was equipotent in syncytia formation with the active enantiomer WIN-55,212-2, the cannabinoids may be acting via non-receptor mechanisms. Future experiments will be performed to further investigate whether the enhancement of syncytia formation in HIV-1_{MN} infected MT-2 cells by cannabinoids is receptor mediated or non-receptor mediated. To do this other pairs of active and non-active agonists e.g., cannabinol and cannabidiol, will be used to demonstrate via pharmacological technique whether or not the effects observed are receptor mediated. A receptor antagonist for CB1, SR141716A, will also be used to demonstrate receptor specificity (Rinaldi-Carmona et al., 1994). In addition, lower drug concentrations will be used to determine whether syncytia formation can be affected in a dose responsive manner. It is possible that a primarily non-receptor mediated response is observed at the concentrations used and a receptor mediated response may be observed at lower drug concentrations. The number of syncytia observed at the lowest drug concentrations were significantly higher than that of the controls and as a result the concentration end point of the response was not observed, hence another reason why experiments will be repeated at much lower concentrations. Although we have been able to demonstrate cannabinoid receptor mRNA in the MT-2 cells, it will be necessary in the future to demonstrate the presence of receptor protein. In conclusion, all four cell lines were positive for CB2 mRNA but only the MT-2 cells were positive for both CB1 and CB2 mRNA. In addition, the cells cultured in the presence of the cannabinoid agonists and cell free HIV-1_{MN} demonstrated a significant increase in syncytia formation. Since the formation of syncytia is an indication of virus infection and cytopathicity it can be concluded from these experiments that cannabinoid drugs may enhance cell free HIV-1 infection of susceptible cells.

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