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Inhibition of *Catechol-O-methyl Transferase (COMT)* by Tolcapone Restores Reductions in *Microtubule-associated Protein 2 (MAP2)* and *Synaptophysin (SYP)* Following Exposure of Neuronal Cells to Neurotropic HIV

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Abstract

This investigation aimed to assess whether inhibition of catechol-O-methyl transferase (COMT) by Tolcapone could provide neuroprotection against HIV-associated neurodegenerative effects. This study was conducted based on previous work, which showed that a single nucleotide polymorphism (SNP) at position 158 (val158met) in COMT, resulted in 40% lower COMT activity. Importantly, this reduction confers a protective effect against HIV-associated neurocognitive disorders (HAND), which have been linked to HIV-associated brain changes. SH-SY5Y differentiated neurons were exposed to macrophage-propagated HIV (neurotropic MACS2-Br strain) in the presence or absence of Tolcapone for 6 days. RNA was extracted and qPCR was performed using Qiagen RT2-custom-array consisting of genes for neuronal and synaptic integrity, *COMT* and pro-inflammatory markers. Immunofluorescence was conducted to validate the gene expression changes at the protein level. Our findings demonstrated that HIV significantly increased the mRNA expression of *COMT* while reduced the expression of microtubule-associated protein 2 (*MAP2*) ($p=0.0015$) and synaptophysin (*SYP*) ($p=0.012$) compared to control. A concomitant exposure of Tolcapone ameliorated the perturbed expression of *MAP2* ($p=0.009$) and *COMT* ($p=0.024$) associated with HIV. Immunofluorescence revealed a trend reduction of *SYP* and *MAP2* with exposure to HIV, and that concomitant exposure of Tolcapone increased *SYP*

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The authors TL conducted the tissue culture experiment and data analysis; GC, PG, MC and IE contributed in the methodological design of the experiment; AE was responsible for the macrophages culture and generating viral supernatant; CB contributed in the statistical analyses; LG contributed in experimental troubleshooting; all authors were involved in editing of manuscript drafts and approved the final manuscript.

Conflicts of Interest

No conflicts of interest were declared for all authors.

($p=0.016$) compared to HIV alone. Our findings demonstrated *in vitro* that inhibition of COMT can ameliorate HIV-associated neurodegenerative changes that resulted in the decreased expression of the structural and synaptic components MAP2 and SYP. As HIV-associated dendritic and synaptic damage are contributors to HAND, inhibition of COMT may represent a potential strategy for attenuating or preventing some of the symptoms of HAND.

Keywords

COMT protein, human; HIV; Tolcapone; synaptophysin; MAP2 microtubule-associated protein 2

Introduction

Cognitive impairment remains prevalent among individuals living with HIV despite the effectiveness of combination antiretroviral therapy (cART) in reducing systemic viral burden. Although antiretrovirals (ARVs) suppress HIV-1 RNA levels, detectable cerebrospinal fluid (CSF) viral load still persists in some patients (Garvey *et al*, 2009; Mellgren *et al*, 2005; Spudich *et al*, 2005; van Lelyveld *et al*, 2010). While some evidence suggests that ARVs could potentially improve cognitive performance (Guevara-Silva, 2014), others have found deterioration of cognitive function (Kaul, 2009; Marra *et al*, 2009). These inconsistencies have been attributed to the varying degrees of drug toxicity and blood brain barrier (BBB) penetrance (Marra *et al*, 2009; Rawson *et al*, 2012). However, neurobiological heterogeneity, particularly in biological pathways relevant to cognition, is also likely to explain the current inconsistencies in the literature.

On such pathway is dopamine biosynthesis and regulation, which play an essential role in modulating prefrontal cortex (PFC) cognitive functions, especially high level executive functioning (Gupta *et al*, 2011; Tunbridge *et al*, 2006). Goldman-Rakic *et al*. (Goldman-Rakic *et al*, 2000) described an inverted-U-shaped relationship between levels of cortical dopamine/ dopamine (D1) receptors activation and PFC cognitive performance. An intermediate level of cortical dopamine was described as being optimal; with sub- or supra-optimal levels of dopamine causing a decrease in PFC associated cognitive performance. Thus, mechanisms involved in regulating levels of dopamine within synapses are of particular importance.

One of the main enzymes responsible for the degradation of dopamine is catechol-O-methyl transferase (COMT). COMT is responsible for the majority of dopamine degradation in the PFC due to the low expression of dopamine transporter (DAT) in this region, which has 1000-fold higher affinity for dopamine and is responsible for the clearing of dopamine from synapses in other brain regions (Lewis *et al*, 2001). Of particular interest, a functional polymorphism of COMT involving a substitution of Valine (*Val*) with Methionine (*Met*) at codon 158 for membrane bound COMT (equivalent to codon 108 for soluble COMT) has been associated with 40% lower COMT activity, and enhanced PFC related cognitive performance among individuals with *Met/Met* compared to *Val/Val* genotype (Chen *et al*, 2004; Egan *et al*, 2001). Several studies have supported these initial findings in healthy individuals, showing that *Met/Met* genotype carriers perform better than individuals with

Val/Val in various PFC related functional tasks, including the Wisconsin Card Sorting Test and multi-source interference task (Barnett *et al*, 2007; Egan *et al*, 2001; Shehzad *et al*, 2012). However, recent work suggests that this relationship is context dependent. Amongst individuals with HIV, *Met/Met* carriers were found to have better cognitive function compared to *Val/Val* carriers, similar to that observed in healthy controls; however, in the presence of methamphetamine use, the protective effect of *Met/Met* was attenuated, presumably due to supra-optimal levels of extracellular dopamine (Bousman *et al*, 2010).

The difference in COMT enzymatic activity has also been replicated in an animal model of COMT *Val158Met*, where cognitive deficit was observed using T-maze (working memory task) in mice with *Val/Val* alleles (Risbrough *et al*, 2014). Interestingly, treatment with Tolcapone, a central nervous system (CNS) penetrant COMT inhibitor that is approved for the treatment of Parkinson's Disease (Marsala *et al*, 2012), significantly reversed the deficits observed in *Val/Val* mice (Risbrough *et al*, 2014). Various studies have also reported a genotype dependent effect of Tolcapone where cognitive performance was improved in *Val/Val* but not *Met/Met* individuals, further strengthening the notion of the inverted-U-shaped relation of the optimal dopamine level and cognitive performance (Apud *et al*, 2007; Bitsios and Roussos, 2011; Farrell *et al*, 2012).

Previously, we have also shown at the cellular level that individuals with HIV associated neurocognitive disorder (HAND) have synaptic and dendritic loss in the frontal cortex (Everall *et al*, 1999; Everall *et al*, 1991; Everall *et al*, 1993). This has further been characterised by our findings of a loss of calbindin interneurons in layers II and III of the frontal cortex of individuals with HAND (Chana *et al*, 2006; Masliah *et al*, 1995). Masliah *et al*. (Masliah *et al*, 1997) also reported a strong relationship between the severity of HIV associated neurocognitive impairment and neurodegeneration, as quantified by immunohistochemistry signal of microtubule-associated protein 2 (MAP2). This neuronal and synaptic loss is believed to have contributed to cerebral atrophy in individuals living with HIV, leading to the disruption of connectivity that may play a significant role in cognitive impairment (Steinbrink *et al*, 2013).

The current study aimed to investigate the effect of decreasing COMT activity on HIV-associated neurotoxicity. Based on past evidence, we hypothesised that inhibiting COMT activity using Tolcapone would have a protective effect against HIV-associated neurotoxicity.

METHODS

SH-SY5Y neuronal model

Commercially available SH-SY5Y neuroblastoma cells (ATCC, USA) were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) (Life Technologies, USA) containing fetal bovine serum (FBS) [10%] (Sigma, AUS), L-Glutamine [2 mM] (Life Technologies, USA), penicillin [100 U/ml] and streptomycin [0.1 mg/ml] (Life Technologies, USA). For neuronal differentiation, SH-SY5Y cells were differentiated in complete media with retinoic acid [10µM] (Sigma, USA) for 7 days, followed by a serum-free media containing brain derived neurotrophic factor (BDNF) [50

ng/ml] (Prospec Bio, USA) for 5 days. This double differentiated protocol for SH-SY5Y cells has been shown to increase the expression of neurofilament, MAP2 and β -III tubulin, as markers indicative of a more mature neuronal phenotype (Agholme *et al*, 2010; Encinas *et al*, 2000).

Macrophages Cultures

Peripheral blood mononuclear cells (PBMCs) were purified from buffy coat from 4 donors (Australian Red Cross Blood Service, AUS) by Histopaque (Sigma, USA) density gradient centrifugation. Monocytes were isolated from PBMCs by plastic adherence. Following 2 hours incubation, the culture flask with PBMCs was gently agitated and washed with Isocove's Modified Dulbecco's Medium (IMDM) (Life Technologies) with 10% heat inactivated FBS to isolate monocytes, which stay adhered to the flask. Monocytes derived macrophages (MDM) were cultured in IMDM with 10% heat inactivated human serum (Australian Red Cross Blood Service, AUS), human macrophage colony-stimulating factor (M-CSF) [12.5ng/ml] (Bio-Scientific, Australia), penicillin [100 U/ml] and Streptomycin [0.1 mg/ml]. MDM were cultured for 6 days to mature with media changed completely twice a week.

HIV culture & harvest

Mature MDMs (6 days old) were inoculated with a neurotropic HIV strain (MACS2-Br) isolated from autopsy brain of a subject who died from AIDS and had HIV-associated dementia (HAD) as well as HIV encephalitis (Gorry *et al*, 2001). MDMs were incubated at 37°C and 5% CO₂ overnight in viral supernatant before a fresh media change. Cultures were given three quarter media changes twice weekly with harvested supernatant filtered through a 0.45µm filter and stored at -80°C. Cultures were terminated when cytopathic effect (ie the presence of multinucleated giant cells) was evident in most cells. A p24 ELISA assay (Leidos Biomedical Research, USA) was used to determine the virus concentration.

Tolcapone LDH toxicity assay

Tolcapone was dissolved in dimethyl sulfoxide (DMSO) and its toxicity was measured using CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega, USA), which is a colorimetric assay measuring the extracellular lactate dehydrogenase (LDH) released by damaged cells. Various concentrations of Tolcapone were assessed for toxicity including 0.01nM, 0.1nM, 1nM, 10nM (IC₅₀) and 100nM.

Experimental conditions

The technical triplicates of double differentiated SH-SY5Y cells were cultured in 6-well plates for RNA extraction and quantitative real-time PCR (qRT-PCR), as well as in 12-well-plates containing 16mm coverslips to be processed for immunofluorescence. Cells were exposed to MACS2-Br HIV supernatant [500 pg/ml of p24] and Tolcapone [10nM based on IC₅₀] (Sigma, USA) or vehicle (DMSO) for 6 days as described in Table 1.

qRT-PCR

RNA samples were extracted using Purelink RNA Minikit (Life Technologies, USA) and quality examined on 2100 Bioanalyzer Instrument (Agilent, USA), with cDNA synthesised using the RT² HT First Strand Kit (Qiagen, USA) and qPCR performed using RT² custom array (SABiosciences, AUS) with SYBR Green mastermix (SABiosciences, AUS). The arrays consisted of 32 genes, including neuronal and synaptic integrity markers, *COMT*, and pro-inflammatory markers that have previously been implicated or dysregulated in HIV-associated neurodegeneration or cognitive impairment, as well as 3 reference genes (*GUSB*, *GAPDH*, *PPIA*) and 3 internal controls (*HGDC*, *RTC*, *PPC*) (Supplemental Table 1.). The qPCR reaction mix was prepared as per manufacturer's protocol and pipetted onto the array plates using EpMotion 5070 (Eppendorf, USA) to maintain high consistency. The qPCR reaction and data collection were performed on ViiA™ 7 Real Time PCR System (Life Technologies, USA) and exported to Microsoft Excel (Microsoft, USA) for further analysis. The raw data was formatted in Microsoft Excel (Microsoft, USA) template and uploaded to the Qiagen web-based RT² Profiler PCR Array Data Analysis V3.5 (SABiosciences, AUS) for fold change calculation using the arithmetic mean of the three housekeeping genes and relative to controls using 2^{-Ct} . Differences in fold changes between groups were assessed within the web based software, which is powered by the Student's t-test.

Immunofluorescence

Double differentiated SH-SY5Y neuronal cells exposed to experimental conditions were washed quickly in phosphate buffered saline [0.1M] (PBS) (Sigma Aldrich, USA) followed by fixation in 4% paraformaldehyde (PFA) (Sigma Aldrich, USA) in PBS for 15 minutes. Cells were washed in PBS twice for 10 minutes to remove excess PFA and then permeabilised in PBS containing 0.25% Triton X-100 (Sigma Aldrich, USA). Following this, sections were blocked for non-specific binding with 3% normal goat serum (NGS) in 0.1% Triton X-100 in PBS (PBST) for 1 hour at room temperature (RT) with agitation. Cells were then incubated with a mouse monoclonal antibody to MAP2 [1:100] (M4403, Sigma Aldrich, USA) and a rabbit polyclonal antibody to synaptophysin (SYP) [1:100] (RM-9111, Thermofisher, USA) simultaneously overnight at 4°C with agitation. Following that, they were incubated simultaneously in the Alexa Fluor 488 goat anti-mouse antibody [1:1000] (Life Technologies, USA) and the Alexa Fluor 594 goat anti-rabbit antibody [1:1000] (Life Technologies, USA) for 2 hours covered in foil at RT with agitation. Finally, the cells were counterstained with 4',6-diamidino-2phenylindole (DAPI) [500 nM] (Sigma Aldrich, USA) for 3 minutes at RT with agitation and mounted on microscope slides with polyvinyl alcohol mounting medium with DABCO® (PVA-DABCO) (Sigma Aldrich, USA).

Pilot Study for Integrated Optical Density (IOD) Acquisition

A pilot study was conducted to optimise the parameters of IOD acquisition. Slide-mounted coverslips were visualised using fluorescence microscopy. A contour defining our area of interest (AOI) was traced in StereoInvestigator 10 (MBF Biosciences, USA) to include the maximum square within the round coverslip. The fractionator probe was used to generate approximately 50 systematic random sampling (SRS) fields within the AOI. Numbers of cells were counted and monochrome images were taken across 3 channels (DAPI, Alexa

488, Alexa 594) for each field. Sampling fields with no cells were excluded and sampling parameters such as exposure time, UV intensity and frame size were kept consistent across all sampling sites and conditions. The sampled images were then imported into Image Pro Plus (Media Cybernetics, USA) where IOD measurements were carried out and data exported to Microsoft Excel (Microsoft, USA). IOD measurements were calculated based on multiplying the area (μM^2) by the intensity (mapped to a black and white scale where 0 = black and 255 = white). The acquisition returned with 33 fields satisfying the inclusion criteria. A correlation of 95.1% reflects that DAPI IOD serves as a suitable parameter to correct for cell number variation in each field of interest (Supplemental Table 2.). The cumulative mean intensity of DAPI, MAP2 as well as SYP were plotted against the number of sampling fields, and revealed that 25 sampling fields were required to generate reliable IOD measurements.

Measurement of IOD

Slide-mounted coverslips were visualised using fluorescence microscopy. The acquisition of IOD measurements were carried out as optimised in the pilot study. The intensity of fluorescence signal for MAP2 and SYP were calculated as a ratio of the IOD of DAPI to account for variation in cell counts and degradation of signal across time. The corrected data was analysed using a Student's t- test in IBM SPSS Statistics 21 (IBM Corp., USA).

RESULTS

Tolcapone toxicity via LDH assay

LDH assay revealed no significant toxicity in SY5Y neurons exposed to different concentrations of Tolcapone [0.01 nM – 100 nM] for 30 minutes and 24 hours (Supplemental Figure 1). Therefore, the concentration of 10 nM (IC_{50}) was used in the experiment.

Gene expression changes by qRT-PCR

The current study examined the mRNA expression of candidate genes (Supplemental Table 2) in SH-SY5Y neurons exposed to HIV and/or Tolcapone. All RNA samples have RNA integrity numbers (RIN) of 9.6 and above. A oneway-ANOVA comparison between groups revealed no difference in the arithmetic mean of the expressions of the 3 reference genes ($F=0.285$, $p=0.758$) and that they are suitable for calculation of fold change (Supplemental Figure 2). HIV significantly increased the expression of COMT (fold change =1.075; $t=3.206$, $p=0.015$; Cohen's $d=0.665$, effect size $r=0.316$) while reducing the expression of Dopamine receptor D4 (*DRD4*) (fold change=0.536; $t=6.430$, $p=0.0004$; $d=4.777$, $r=0.922$), suggesting the involvement of dopaminergic pathway. We observed significant reductions in SYP (fold change=0.742; $t=3.344$, $p=0.012$; $d=2.466$, $r=0.777$) and MAP2 (fold change=0.764; $t=5.050$, $p=0.0015$; $d=4.251$, $r=0.905$) compared to control SH-SY5Y neurons, suggesting a HIV-induced synaptic and dendritic damage. The reduced MAP2 expression was ameliorated with concomitant exposure of Tolcapone [10 nM], with the expression normalised relative to controls and increased compared to HIV alone (fold change=0.940; $t=4.690$, $p=0.009$; $d=3.837$, $r=0.887$). Tolcapone also normalised the expression of COMT and DRD4 relative to controls, significantly decreased COMT versus

HIV alone (fold change=0.915; $t=3.529$, $p=0.024$; $d=2.882$, $r=0.822$). The expression of *DRD4* was also significantly increased versus HIV alone (fold change=0.819, $t=4.715$, $p=0.0009$; $d=3.850$, $r=0.887$). Finally, Tolcapone restored the level of SYP, however, this did not reach statistical significance (fold change=0.901, $t=1.344$, $p=0.250$; $d=1.097$, $r=0.481$) (Figure 1).

Protein expression changes by Immunofluorescence

Immunofluorescence was performed in attempt to validate the gene expression changes observed for *SYP* and *MAP2* at the protein level. We found a trend of HIV decreasing the intensity of SYP ($t=1.192$, $p=0.299$; $d=0.973$, $r=0.438$) and MAP2 ($t=0.951$, $p=0.396$; $d=0.776$, $r=0.362$) compared to control SY5Y neurons. Concomitant exposure of Tolcapone ameliorated the reduced intensity of SYP ($t=4.519$, $p=0.011$; $d=3.690$, $r=0.879$) and MAP2 ($t=1.458$, $p=0.219$; $d=1.191$, $r=0.512$) compared to HIV alone. Interestingly, concomitant exposure to Tolcapone and HIV significantly increased the intensity of SYP compared to control ($t=2.691$, $p=0.055$; $d=2.198$, $r=0.740$) (Figure 2). These results suggest that the disturbance in mRNA expression was present at the protein level too.

DISCUSSION

Taken together, our results suggest that Tolcapone, a known inhibitor of COMT confers a protective effect against HIV-associated dendritic and synaptic damage. Consistent with previous findings (Masliah *et al*, 2004), we observed synaptic and cytoskeletal damage associated with HIV, as conferred by the reduction of the expressions of *SYP* and *MAP2*. However, this effect was not observed when COMT activity was inhibited with a concomitant exposure of Tolcapone at both the gene and protein expression levels. Nevertheless, our results are the first to demonstrate that Tolcapone may be protective against HIV-associated neurodegeneration through the inhibition of COMT. Restriction of COMT enzymatic activity would act to preserve the more optimal downstream cortical dopamine level needed for normal cognitive performance. We induced HIV-mediated neurodegeneration *in vitro* in our SH-SY5Y differentiated neurons as shown by the decreased mRNA expression of neuronal markers *SYP* and *MAP2* in cultures exposed to a brain-derived, neurotropic HIV strain. The mRNA expression of *COMT* was also upregulated while *DRD4* was downregulated following HIV exposure, indicating possible involvement of dopaminergic system. Based on the inverted-U curve model, inhibiting COMT in our SH-SY5Y model potentially altered the level of dopamine, therefore affecting the expression of this dopamine receptor. Specifically for MAP2 expression changes following the inhibition of COMT, it was previously reported that D1-like dopamine receptor may alter the phosphorylation state of MAP2 protein, therefore changing the stability of dendritic cytoskeleton. Our findings suggest a possible involvement of DRD4 (D2-like dopamine receptor) in regulating MAP2 in an opposite manner compared to D1-like receptors.

We further examined the protein expression of SYP and MAP2 in order to validate the findings of altered mRNA expression of the neuronal markers in SY5Y neuronal culture. Quantitative immunofluorescence staining intensity revealed changes of SYP and MAP2

protein expression consistent with that of mRNA expression when exposed to HIV as well as HIV and Tolcapone concurrently. We considered that the fragmentation of DNA may have influenced the DAPI signal correction for HIV cultures versus controls, however the observed increase in SYP and MAP2 is highly consistent with the qPCR results, therefore supporting its reliability. These findings indicate that COMT dysregulation may be a possible mechanism that leads to HIV-associated cognitive deficit as portrayed in the inverted-U shaped curve (Goldman-Rakic *et al*, 2000). Furthermore, HIV-associated neurodegeneration induced by dysregulation of COMT may be restored via the treatment of Tolcapone, and appears to occur at the mRNA and protein levels. It is noteworthy for our experiments that the neurotoxic effects we observed for HIV as shown by reduced expression levels of *SYP* and *MAP-2* in our cultures may have been elicited via the release of soluble factors from MDMs exposed to the whole virus, such as pro-inflammatory cytokines or alternatively by free viral proteins present in the supernatant. Likewise, our observed neuroprotective effect of Tolcapone on HIV associated neurotoxicity may have also been achieved through attenuating effects of cytokines or viral proteins in the supernatants. Therefore, further mechanistic work is warranted to assess Tolcapone's ability to prevent neurotoxicity elicited by individual HIV viral proteins, including gp120, gp41, Tat, Nef and Vpr (Adamson *et al*, 1996; Brenneman *et al*, 1988; Jones *et al*, 2007; Kaul *et al*, 2001; King *et al*, 2006; Mordelet *et al*, 2004; Tunbridge *et al*, 2006).

Tolcapone is a US Food and Drug Administration (FDA) approved compound and is widely used in the treatment of Parkinson's Disease (PD). Given its already known pharmacokinetics and availability on the market since 1997, Tolcapone may be favoured compared to other COMT inhibitors and substances in the potential therapeutic development for HAND (Mocchetti *et al*, 2012). Despite its potential liver toxicity, Tolcapone has been found to be efficient in the treatment of PD due to its ability to cross the blood brain barrier (BBB) and reach the brain while liver injury could be minimised via liver function monitoring (Gottwald *et al*, 1997). This is also important in the context of targeting HIV-associated neurodegeneration in the CNS, which may be a significant contributor to HIV associated neurocognitive impairment.

Further study needs to be conducted to validate the potential therapeutic effects of Tolcapone against different strains of HIV, and in primary neuronal cultures, which closer represent the microenvironment in the brain as compared to the current model of SH-SY5Y neurons. Moreover, it is important to conduct a larger scale experiment focusing on these synaptic and dendritic markers in order to overcome the sample size limitation in the current study. More importantly, it will be crucial to investigate the effect of other COMT inhibitors such as Entacapone in order to validate the current findings and determine if Tolcapone is a suitable adjunctive therapy with ARVs in the treatment of HIV and HIV-associated neurodegeneration.

Tolcapone is known to exhibit different outcomes in PFC function dependent on COMT genotype. While *Val/Val* individuals who are believed to have suboptimal level of cortical dopamine may benefit from the treatment of Tolcapone, *Met/Met* individuals who presumably have near optimal baseline level of cortical dopamine are found to deteriorate with Tolcapone (Bitsios and Roussos, 2011). While the current study did not investigate the

genotype of SH-SY5Y, it is important for future studies to determine the effect of Tolcapone treatment towards neurotoxicity across different genotype variants *in vitro*. This could be achieved by transfecting SH-SY5Y neurons with *Met/Met* or *Val/Val* COMT variant in order to generate 2 distinct populations of cultures reflecting the two genotypes via novel and elegant genome editing techniques such as TALEN or CRISPR.

In conclusion, our findings demonstrated that *in vitro*, inhibition of COMT ameliorates HIV-associated neurodegenerative changes that resulted in the decreased expression of the structural and synaptic components MAP2 and SYP. As HIV-associated neurodegeneration is a likely contributor to HAND, inhibition of COMT may represent a potential strategy for attenuating or preventing some of the symptoms of HAND.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

mRNA expression fold changes of (a) SYP, (b) MAP2 (c) COMT and (d) DRD4 across Control ($FC_{SYP}=1.006$, $FC_{MAP2}=1.003$, $FC_{COMT}=1.001$, $FC_{DRD4}=1.005$) HIV ($FC_{SYP}=0.742$, $FC_{MAP2}=0.764$, $FC_{COMT}=1.075$, $FC_{DRD4}=0.536$) and HIV + Tolcapone ($FC_{SYP}=0.901$, $FC_{MAP2}=0.940$, $FC_{COMT}=0.915$, $FC_{DRD4}=0.819$) groups, calculated using the Qiagen web-based RT² Profiler PCR Array Data Analysis V3.5 using the arithmetic mean of the three housekeeping genes and relative to controls using 2^{-Ct} . HIV increased the mRNA expression of COMT ($p=0.015$) while reducing that of DRD4 ($p=0.0004$), MAP2 ($p=0.0015$) and SYP ($p=0.012$) compared to control SH-SY5Y neurons. The disturbance was ameliorated with a concomitant exposure of Tolcapone where the expression of COMT ($p=0.024$), DRD4 ($p=0.0009$) and MAP2 ($p=0.009$) were restored.

Figure 2.

Immunofluorescence staining intensity of (a) SYP & (b) MAP2 across control, HIV, and HIV + Tolcapone groups. Images of double immunofluorescence staining of MAP2 [1:100] (red) and SYP [1:100] (green) with DAPI counterstain [500 nM] (blue) in (c) Control, (d) HIV and (e) HIV + Tolcapone. Intensity of fluorescence signal varies from site to site, the quantitative intensity measurement revealed a trend of decrease in SYP ($p=0.299$) and MAP2 ($p=0.396$) signal with exposure to HIV [500 pg/ml of p24], and the reductions were

restored in SYP ($p=0.011$) and MAP2 ($p=0.219$) with concomitant exposure to Tolcapone [10 nM].

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Table 1

Experimental conditions

Group	HIV MACS2-Br [500 pg/ml of p24]	Tolcapone [10nM]
Control	–	–
HIV	+	–
HIV + Tolcapone	+	+