Mechanisms and genes involved in enhancement of HIV infectivity by tobacco smoke

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Abstract:

HIV infection is more common among smokers than nonsmokers, and, remarkably, HIV-infected individuals are about 3 times more likely to smoke than the uninfected general population. However, the relationship between tobacco smoking and HIV/AIDS disease progression remains controversial.

In this study, we demonstrate a potent enhancing effect of aqueous tobacco smoke extract (TSE) on HIV infectivity that is nicotine-independent. This increased infectivity is neither NF-κB mediated nor a direct result of oxidative stress, as it cannot be blocked by antioxidants. On the contrary, TSE itself was found to possess significant antioxidant potential, enabling it to protect the viability of both infected cells and HIV virions in the presence of peroxide. Assessment of TSE-induced alterations in cellular gene expression that may be involved in increasing HIV infectivity in T cells showed that TSE up-regulates some genes known to be capable of enhancing HIV and HCV infection, or protecting HIV, but down-regulates several genes involved in cellular defense and antigen presentation.

These results demonstrate that tobacco smoke can enhance HIV infectivity, possibly by a combination of direct (antioxidant) and indirect (gene-based) mechanisms. This raises the concern that smoking may thereby increase the risk of acquisition or progression of HIV infection.

Keywords: Tobacco | Smoke | HIV-1 | Infectivity

Article:

Abbreviations: FRAP, ferric reducing ability of plasma; t-BOOH, tert-butyl-hydroperoxide; Trx, thioredoxin; TSE, tobacco smoke extract

1. Introduction

The relationship between tobacco smoking and HIV/AIDS progression still remains controversial (Furber et al., 2007). Some studies suggest that tobacco smoking by HIV-1-seropositive individuals is associated with a more rapid progression to AIDS (Crothers et al., 2005, Nieman et al., 1993). In contrast, a survey that enrolled 2499 HIV-1-seropositive men for up to 9 years found that tobacco smoking does not have a major effect on the progression of HIV-1 infection to AIDS (Galai et al., 1997). However, one of the stronger conclusions presented recently is that smoking increases the risk of becoming infected with HIV (Furber et al., 2007). Evidence also suggests that AIDS patients who smoke have a higher incidence of dementia (Burns et al., 1996).

Perhaps partly because of these controversies and inconsistencies in study results, and because the life expectancies of AIDS patients have been increased dramatically since highly active antiretroviral therapy (HAART) became the standard of care, comparatively little attention has been paid to the interactions between tobacco smoking and HIV/AIDS. A decreased awareness among health care providers of current smoking behaviors of HIV-positive compared to HIV-negative veterans has been reported (Crothers et al., 2007). However, it would be unwise to ignore studies suggesting that HIV/AIDS patients who are smokers suffer more clinical complications such as pneumonia, oral candidiasis, hairy leukoplakia, vascular diseases, AIDS-related malignancies and neuropathy (Conley et al., 1996, Elzi et al., 2006). At the same time, some of the benefits provided by HAART are negated in tobacco smokers (Feldman et al., 2006). Patients with HIV infection who smoke also have poorer health-related quality of life than nonsmokers (Turner et al., 2001). During pregnancy, tobacco smoking can increase risk of maternal-child HIV transmission and complications (Kalish et al., 1998, Turner et al., 1997).

In defining the relation between tobacco smoking and HIV/AIDS, most research has been done based on epidemiological profiles; few studies have explored the possibility of direct interactions between tobacco constituents and/or smoking and actual viral replication in HIV/AIDS.

Nicotine is the single tobacco constituent demonstrated to have at least the potential to stimulate the production of HIV-1, as has been shown using *in vitro*-infected alveolar macrophages (Abbud et al., 1995). Pretreatment of microglia with nicotine (300 μM) was shown to increase HIV-1 expression (Abbud et al., 1995). However, the median concentration of nicotine in the plasma of smokers does not usually exceed about 0.23 μM (Taylor et al., 1986), and at this concentration, nicotine showed no effect on HIV infection (Rock et al., 2008).

Tobacco smoking is widely viewed as a source of oxidative stress. Significantly, oxidative stress can induce HIV replication, resulting in disease progression (Boelaert et al., 1996, Miller et al., 1997). Smoking can induce the NF-κB activation pathway in lymphocytes via intracellular formation of peroxynitrite, through a reaction between smoke-derived NO and endogenously produced superoxide (Hasnis et al., 2007). However, when samples of alveolar air from smokers and non-smokers were analyzed for ethane using mass spectrometry, they showed no evidence that cigarette smoking is related to increased n-3 lipid peroxidation (Puri et al., 2008). Similarly, blood samples from clinically stable smoking and nonsmoking men with HIV/AIDS showed no striking differences in oxidative stress or antioxidant capacity (Cole et al., 2005).

Here, an aqueous tobacco smoke extract (TSE) was used to study the direct effect of smoking on HIV infection and its effects on gene expression in human T cells. The data demonstrate that TSE can enhance HIV infectivity in a nicotine-independent manner, and, surprisingly, that TSE has significant antioxidant potential, capable of protecting cells and virions from oxidative damage. An mRNA microarray analysis of TSE-treated human T-cells suggests that a number of genes that are up-regulated can be related to HIV infection, whereas several genes involved in cell defense or redox functions are down-regulated by TSE treatment.

2. Material and methods

2.1. Preparation of aqueous tobacco smoke extract (TSE)

Into 40 mL of sterile saline (PBS) in a water pipe-like apparatus (sidearm conical flask), 5 cigarettes (Marlboro Light brand, Philip Morris, USA) were puffed through the PBS solution. The nicotine concentration in this TSE is typically around 100 μM, as measured with a standard method based on bromination of nicotine (Rai et al., 1994). This TSE is used throughout all these studies. Note that when diluted 100-fold in culture media (e.g., 2 μL TSE in 200 μL final volume of media), this would give a nicotine concentration of about 1 μM.

2.2. Cell lines

The TZM-bl cell line was obtained from the NIH AIDS Research and Reference Program (Cat # 8129). This cell line was originally generated from JC53 cells (which express CD4 and the CCR5 HIV co-receptor) by introducing separate integrated copies of the luciferase and β-galactosidase genes under control of the HIV-1 long terminal repeat (LTR) promoter/enhancer. This cell line is highly sensitive to infection with diverse isolates of HIV-1, and produces luciferase in proportion to the amount of replicating HIV; hence, it is useful for assessments of viral infectivity and relative viral titers or "pseudo viral load". Hela, 293T and Jurkat cell lines were purchased from ATCC. TZM-bl cells, 293T cells, Hela cells or Jurkat cells were cultured at 37 °C in 5% CO_2, in RPMI 1640 or DMEM medium supplemented with glutamine (2 mM) and 10% fetal bovine serum.

2.3. Cell proliferation assay

TZM-bl cells (1.2×10^4 per well) were plated in a 96-well plate and incubated with serial doses of TSE. At 48 h post-incubation, cell proliferation was measured by the MTT method.

2.4. Preparation of HIV stocks

pNL4-3 plasmid DNA was transfected into 293T cells with calcium phosphate transfection protocol (Fortin et al., 1997).

2.5. TSE oxidation/reduction potential assays

The ferric reducing power-based antioxidant capacity assay (Berker et al., 2007) and the FRAP method (Benzie and Strain, 1996) were both used as cell-free assays to measure TSE total

antioxidant activity. Identical volumes of TSE, l-ascorbic acid or Fe^{+2} stock solutions were added to assay solution, and the absorbance at 700 nm or 595 nm, respectively, was measured. The human erythrocyte hemolysis assay was performed according to the procedure by Ajila and Prasada Rao (2008). Briefly, to 200 μL of 5% (v/v) suspension of erythrocytes in PBS, serial dilutions (achieved by adding either 0, 20, 40, 80, 100 or 120 μL) of TSE or 68 mM ascorbate were added, and the mixture was adjusted with PBS to 1.77 mL of final volume. To this, 100 μL of 400 μM H_2O_2 (in PBS pH 7.4) was added, giving a total reaction volume of 1.87 mL. The reaction mixture was shaken gently while being incubated at 37 °C for 3 h. The reaction mixture was diluted with 8 mL of PBS and centrifuged at 2000 × *g* for 10 min. The absorbance of the resulting supernatant was measured at 540 nm by spectrophotometer to determine the extent of hemolysis. The percentage of hemolysis was calculated by taking hemolysis caused by a blank control (100 μL of 400 μM H_2O_2 without TSE or ascorbate) as 100%. The protective effect of TSE against oxidative damage by tert-butyl-hydroperoxide (t-BOOH) was performed on the basis of a fluorescence cytolysis assay (Nakamura et al., 2001). 10^5 Jurkat cells loaded with Calcein-AM were plated per well in a 96-well plate in DME-medium with 3% FBS and 0.04% Trypan Blue. Then t-BOOH was added to the wells at a final concentration of 4 mM. Increasing concentrations of TSE were added to generate the set of curves shown. Fluorescent density was measured with excitation at 485 nm and emission at 520 nm. To test the ability of TSE to protect HIV virions from damage by t-BOOH, identical amounts of HIV-1 viral stocks were added to 200 μL of media containing t-BOOH, t-BOOH/TSE or PBS control and maintained at room temperature for 1 h. Following these treatments, virions were collected by centrifugation at 30,000 × *g* for 100 min. Resuspended virus was used to infect TZM-bl cells to measure the infectivity.

2.6. Effect of TSE on HIV infectivity in both TZM-bl cells and Jurkat T cells

For TZM-bl cells, 1.2×10^4 cells were plated on a 96-well plate and pre-incubated with serial dilutions of TSE in 200 μL of medium. The cells were then infected with HIV-1 (MOI = 0.1); after 48 h incubation, luciferase activities were measured. For Jurkat cells, 1×10^6 cells were infected with HIV-1 (MOI = 0.1), then treated with either PBS vehicle or TSE (2 μL per 200 μL of culture medium) for 2 days. The relative HIV titers in 100 μL of the supernatants were then detected in TZM-bl cells as described above.

2.7. Vector construction and cell transfection

Based on the pHIV-lacZ expression vector (NIH AIDS Research and Reference Reagent program Cat # 151), vector pHIV-4NF was generated with four copies of the NF-κB site in the HIV-LTR region. The vector was used in studying the effect of TSE on NF-κB activation. Hela cells (8×10^4) were co-transfected with pHIV-4NF and pGL-2 (transfection efficiency control) with Lipofectamine 2000 (Invitrogen), then treated with PBS, TSE or TNF-α. At 48 h post-transfection, β-galactosidase and luciferase activities were measured in cell lysates.

2.8. mRNA microarray assay

Human Jurkat T cells were treated with a non-toxic dose of TSE overnight, then total cellular RNA was prepared and purified by standard methods and sent for RNA microarray analysis,

along with mRNA from cells treated with PBS alone as a control. The samples were run on Affymetrix HG-U133 Plus 2.0 chips at the MicroArray Core at Wake Forest University, Winston Salem, NC.

3. Results

3.1. Toxicity of TSE on TZM-BL cells

As assessed by the MTT cell viability assay, TSE shows dose dependent cellular toxicity in TZM-bl cells (Fig. 1). At dilutions as low as 0.15 μL of TSE in 200 μL of cell culture medium, TSE shows a mild proliferative effect, whereas amounts larger than 5 μL of TSE per 200 μL of culture medium show increasing toxicity to cells. Therefore, a dose of 2 μL per 200 μL microplate well (i.e., 1% TSE in culture media) was used as the standard dose for HIV stimulation in later experiments.

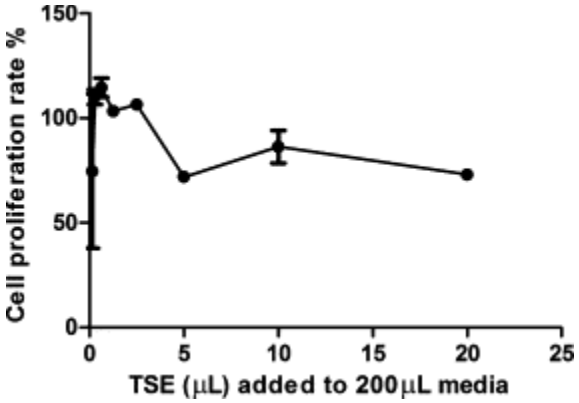

Figure 1. Effect of TSE on cell proliferation and viability. After 48 h exposure to various concentrations of TSE, cell viability of TZM-bl cells was assessed using the MTT assay. The *x*-axis corresponds to the volume of TSE (data points from 0.15 to 20 μL) added to 200 μL final volume of culture medium. At around 1 μL, there is a trend towards a slight proliferative effect (~20% increase) on cells. At 5 μL and above, there is a significant decrease in the number of viable cells. At 2 μL per 200 μL media (1% TSE) there is no significant effect, either positive or negative, on cell proliferation; this concentration was used in subsequent experiments unless otherwise stated (in this and all subsequent figures, data are from three independent experiments; error bars represent SEM).

3.2. TSE enhances HIV virion production/infectivity in both T cells and CD4+ Hela (TZM-bl) cells

As shown in Fig. 2A, TSE enhances HIV-1 infectivity in TZM-bl from doses as low as 0.15–5 μL TSE, with final dilution to 200 μL of culture medium. Based on the dose–response curve of Fig. 2A, an optimal "HIV stimulating" dose of TSE of 2 μL per 200 μL medium was used for further studies in other cell lines; at this dose, there is a negligible effect on T cell proliferation *per se* (Fig. 1). Various antioxidants (including a combination of glutathione and selenium, or vitamin C alone) were unable to block the stimulating effect of TSE on HIV infectivity. The effect of TSE on HIV production was also tested in human Jurkat T cells. As shown in Fig. 2B, when Jurkats were infected with HIV, with and without TSE, and the supernatants tested for HIV

titers in TZM-bl cells, HIV production increased over 50% following TSE stimulation ($P < 0.0004$, $n = 3$), comparable to the direct HIV-simulating effect of TSE in TZM-bl cells alone (Fig. 2A).

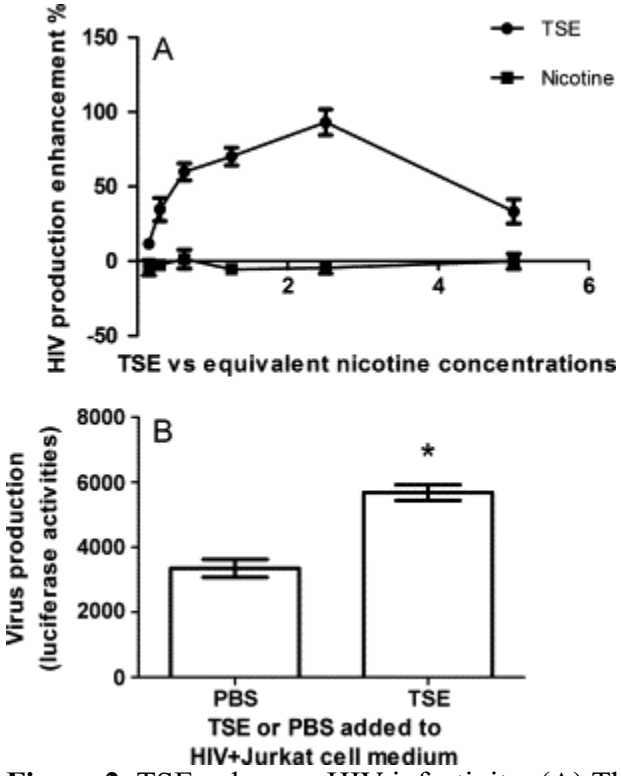

Figure 2. TSE enhances HIV infectivity. (A) The *y*-axis represents luciferase production in TSE or nicotine-treated TZM-bl cells infected with HIV-1, relative to untreated HIV-infected cells. Thus 100% enhancement represents a doubling of viral infectivity relative to the control. The *x*-axis represents volume added in μL for TSE (as in Fig. 1), and final μM concentration for nicotine. Increasing concentrations of TSE enhance HIV infectivity to a maximum at 2.5 μL TSE per 200 μL media; at higher concentrations, there is a decline, probably due to the cytotoxicity of TSE at high concentrations (Fig. 1). Nicotine shows no enhancement at concentrations from 0.075 to 5 μM. (B) In Jurkat T cells, HIV production was increased by 51% ($P < 0.0004$) following stimulation with TSE. (The *y*-axis shows light units from luciferase activity; these units are only significant in relative terms).

3.3. Nicotine alone shows no enhancement of HIV infectivity

Since TSE showed an enhancing effect on HIV infectivity, the corresponding equivalent concentrations of nicotine were tested to see if it has a similar effect. As shown in Fig. 2A, nicotine alone has no effect on HIV infectivity at concentrations of up to 5 μM (about 5 times higher than the nicotine levels in the "standard" dose of 1% TSE). This rules out the possibility that nicotine alone can contribute to the enhancing effect of TSE on HIV infectivity.

3.4. Increased HIV infectivity is not due to chemical tobacco additives: TSE from a "natural" tobacco shows a similar enhancing effect on HIV infectivity

Since Marlboro Lights may contain some "flavor" additives in addition to tobacco, TSE from American Spirit brand cigarettes, which are claimed to contain no additives, was also prepared. Both TSEs show an identical enhancing effect on HIV infectivity. Thus this effect is not due to a chemical additive, but rather, must be caused by some natural tobacco constituent(s).

3.5. TSE does not activate NF-κB or HIV LTR signaling

One possible mechanism whereby smoking might activate HIV is via stimulation of NF-κB, which can be activated by oxidant stimuli. Because smoking has been suggested to activate the NF-κB signaling pathway (Hasnis et al., 2007), we asked if TSE has the same effect on the HIV-LTR enhancer region which contains two copies of the NF-κB binding site. Based on the pHIV-lacZ expression vector pHIV-LacZ, a construct with four copies of the NF-κB site in the HIV-LTR region (pHIV-4NF) was generated (2.7). When Hela cells were transfected with this vector and treated with TSE (final conc. of 1%), the results showed that TSE has no significant effect on the HIV-LTR or NF-κB activation in this *in vitro* system ($P > 0.05$, $n = 3$, Fig. 3). The observation that this increased infectivity is not NF-κB mediated, along with the inability of antioxidants to block the enhancing effect of TSE on HIV virus production (Section 3.2), suggests that this effect is not a result of oxidative stress due to TSE components.

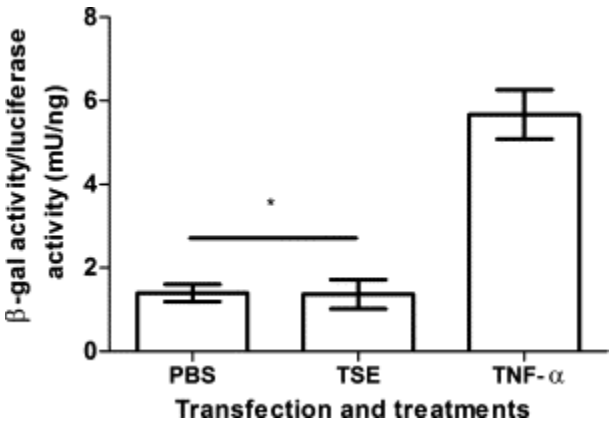

Figure 3. TSE shows no activation effect on NF-κB of HIV-LTR. Hela cells were co-transfected with pHIV-4NF and pGL-2 (transfection efficiency control), then treated with PBS, TSE or TNF-α as a positive control for NF-κB stimulation. Luciferase reporter activity was not significantly different between saline control and TSE treatments (*).

3.6. TSE oxidation/reduction potential assays

Since TSE is unable to activate NF-κB or other sites in the HIV LTR, we decided to directly test whether TSE has pro-oxidant activity, which had seemed a likely assumption. A ferric reducing power-based antioxidant capacity assay (Berker et al., 2007) and the ferric reducing ability of plasma (FRAP) method were both used in cell-free assays to measure TSE total antioxidant activity (Benzie and Strain, 1996). Surprisingly, TSE proved to have a net antioxidant rather than oxidant activity (Fig. 4A and B), and its reducing power is almost as great as a 1 mM solution of ascorbate (Fig. 4A).

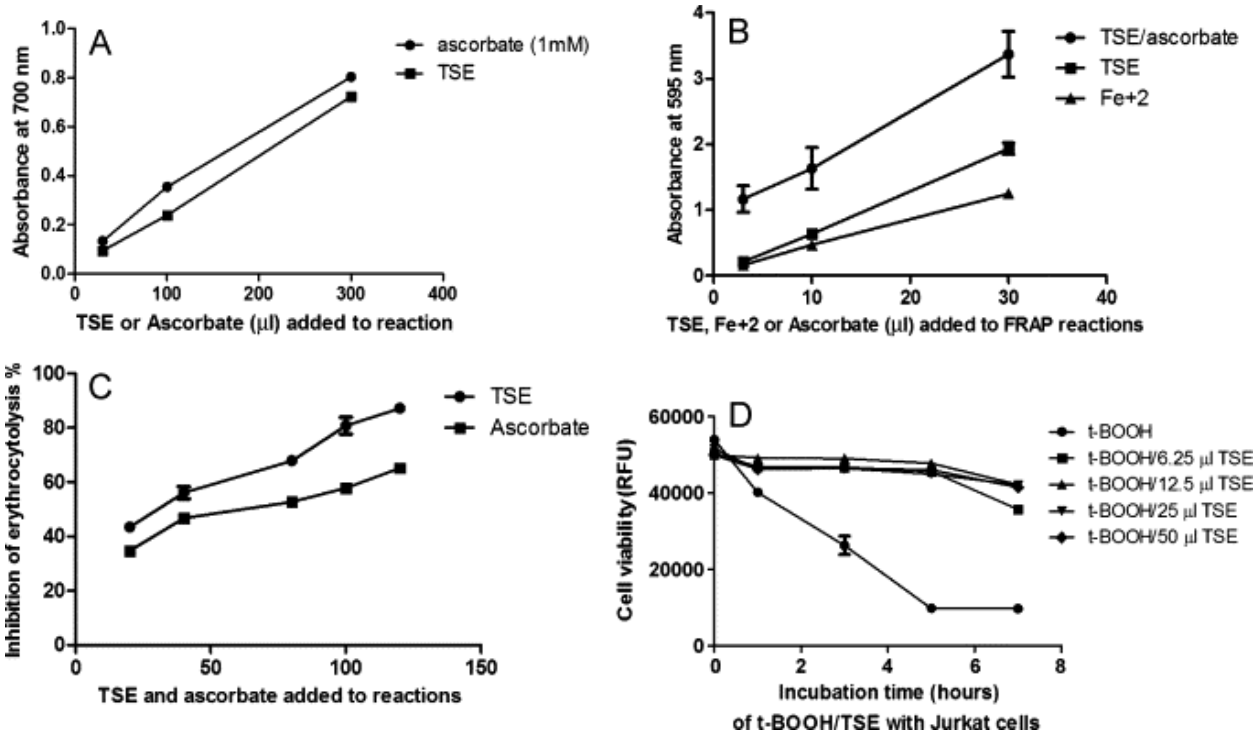

Figure 4. Antioxidant activity of TSE. (A) Cell free reducing power. 30–300 µL (*x*-axis) of TSE was added to 5 mL ferric reducing power-based antioxidant capacity assay solution, and compared to the same volume of 1 mM ascorbate alone as a positive control. The results show that the TSE stock solution is almost as potent a reducing agent as 1 mM ascorbate. (B) Cell free FRAP assay. 3–30 µL of TSE was added to 300 µL of FRAP assay solution to assess antioxidant power of TSE and its additive effect with ascorbate, with comparison to a Fe^{+2} standard curve (the *x*-axis indicates µL volume added of either stock TSE, 1 mM ascorbate, or 0.1 mM ferrous sulfate). (C) Anti H_2O_2-induced hemolysis assay. In the non-toxic range, TSE shows a strong inhibitory effect on H_2O_2-induced hemolysis. The *x*-axis shows volume in µL of TSE or 68 mM ascorbate stock solutions added to a final volume of just under 2 mL, corresponding to a final concentration range of approximately 1–6% TSE and 1–4 mM ascorbate. (D) Protective effect of TSE against oxidant challenge in Jurkat T cells. Even at the highest dilution (as low as 0.3 µL into 200 µL of culture medium, omitted from graph for clarity), up to 5 h or more after treatment, TSE has a significant protective antioxidant effect against challenge with tert-butyl hydroperoxide (t-BOOH).

3.7. Carbon monoxide is not a reductive constituent in TSE and shows no effect on HIV infection/infectivity

Carbon monoxide (CO) is a significant product of tobacco combustion, and is substantially soluble in water. Thus, it is probably present in TSE, and, being in a reduced state relative to carbon dioxide, it has the potential to act as a reducing agent, e.g., to reduce ferric iron (Bickar et al., 1984). Thus, it seemed possible that CO could be responsible for the reducing activity of TSE observed in the assays shown in Fig. 4A and B. A saturated CO solution was tested in the FRAP assay; the result shows that CO has no detectable antioxidant activity by this method. A PBS solution saturated with CO was also tested for its ability to stimulate HIV in TZM-bl; it had no detectable effect on HIV production/infectivity.

3.8. Ability of TSE to protect cells against oxidant challenge

Because TSE has antioxidant activity in the cell free FRAP assay (Fig. 4A and B), to answer whether TSE can protect cells from oxidative stress, human blood cells were used to test its antioxidant activity in a hemolysis assay. TSE showed a strong protective effect against H_2O_2 induced hemolysis (Fig. 4C). The fluorescent cytoplasmic dye Calcein can be used as cell membrane permeability marker; this is the basis of a fluorescence cytolysis assay (Nakamura et al., 2001), which was applied to study the antioxidant activity of TSE against t-BOOH-induced oxidative damage to Jurkat T-cells. The result showed that TSE can protect cells from oxidative stress (Fig. 4D); this is consistent with the antioxidant activity of TSE observed in Fig. 4A and B.

3.9. TSE protects HIV virions against tert-butyl-hydroperoxide

As shown in Fig. 5, in addition to its ability to protect cells against oxidant damage (Fig. 4C and D), TSE confers significant protection to HIV virions from exposure to a potent oxidant, t-BOOH, as the t-BOOH-exposed virus retains its ability to replicate at the same level as the control untreated virus only when TSE is present ($P < 0.001$, $n = 3$).

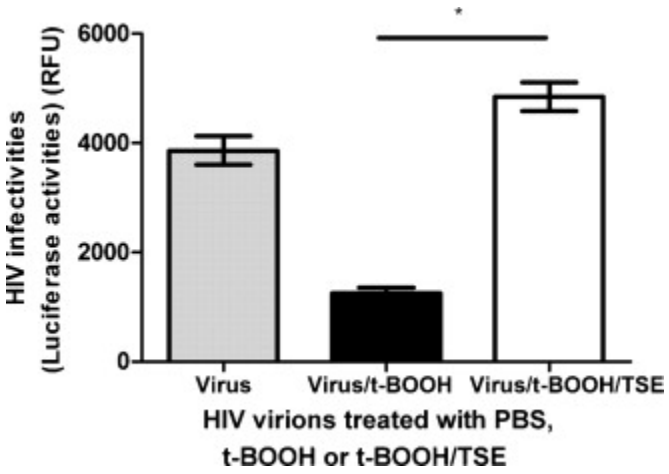

Figure 5. TSE protects HIV-1 virions from damage by tert-butyl hydroperoxide. Identical amounts of HIV-1 viral stocks were added to 200 μL of media containing 4 mM t-BOOH, 4 mM t-BOOH plus 1% TSE, or saline (left bar labeled "virus") at room temperature for 1 h. Following these treatments, virions were collected by centrifugation and resuspended virus was used to infect TZM-bl cells to measure the infectivity. 1% TSE provided complete protection against t-BOOH induced damage to viral viability/infectivity.

3.10. TSE regulates cellular gene expression

In the light of the foregoing results showing that TSE can enhance HIV infectivity in a nicotine-independent manner, and that this effect is not mediated via activation of the viral LTR, it was evident that the most effective way to proceed would be to study the effect of the active tobacco constituents on cellular gene expression, in order to better understand how TSE might be modulating the process of HIV infection. Because human T cells are a primary HIV infection target, the effect of tobacco constituents and TSE on cell gene expression in T cells, as opposed

to other cell types, ought to be more meaningful for understanding the interaction of tobacco constituents and HIV infection. Thus, human Jurkat T cells were treated with a non-toxic dose of TSE (1% relative to culture medium) and used for the microarray studies. The resulting microarray gene expression profiles (Table 1) showed that the expression of more than a dozen genes was altered 3 fold or higher by TSE treatment. One group of genes is involved in different stages of HIV infection; significantly, all these genes are up-regulated by TSE. Another group of affected genes have redox-related functions, as discussed below.

Table 1. Genes whose expression is most significantly altered by TSE exposure in Jurkat T cells.

Genes	Accession numbers	Functions	Refs.
Up-regulated genes			
CD59, protectin	NM_000611	Protects virions from complement mediated destruction	Bohana-Kashtan et al. (2004)
Calmodulin1	NM_006888	Binds HIV gp160 and mediates gp160 enhanced apoptosis	Micoli et al. (2000)
TAX1BP1	NM_006024	Indirectly binds with viral LTR	Chin et al. (2007)
Coatomer protein complex b-2	NM_004766	Golgi budding and vesicular trafficking	Benichou et al. (1994)
Sjogren syndrome antigen B	NM_003142	Enhances HCV replication	Domitrovich et al. (2005)
DDX3	NM_001356	Required for HCV replication	Ariumi et al. (2007)
HSP90 β 1	NM_003299	P-TEFβ-mediated Tat transactivation.	O'Keeffe et al. (2000)
Thioredoxin-related transmembrane protein 1	NM_030755	Involved in clathrin-mediated endocytosis and HIV mediated downregulation of CD4; a coreceptor of coxsakie B virus	Toh et al. (2005)
Down regulated genes			
Thioredoxin (Trx) interacting protein	NM_006472	Binds and inhibits the reducing activity of Trx	Maulik and Das (2008), Nakamura et al. (1996)
PHGPX	NM_002085	Inhibits NF-κB activation	Brigelius-Flohe et al. (1997)
Stearoyl-CoA desaturase	AA678241	Essential for the assembly of VLDL particles	Miyazaki et al. (2000)
MHC, HLA-E	NM_005516	When down-regulated, protects the virus from MHC I-restricted cytotoxic T-lymphocytes	Williams et al. (2002)

4. Discussion

Few studies have explored the possibility of direct interactions between non-nicotine tobacco constituents and viral replication in HIV/AIDS. The current study has identified direct interactions between tobacco constituents and HIV/AIDS infection at both the virion and cellular levels. The data show that as-yet unidentified non-nicotine smoke constituents are involved in enhancing HIV infectivity/virus production. We propose here that the mechanism involved in smoke-enhanced stimulation of HIV production involves an increase in HIV infectivity rather than direct activation of HIV replication. This follows from the key observation that TSE has absolutely no transactivating effect on the HIV LTR promoter/enhancer (Fig. 3), so the increased virus production observed is not due to a direct effect on viral mRNA transcription.

Over the past few decades, much of the emphasis of research on tobacco constituents has been placed on the formation and identification of toxic and tumorigenic agents in tobacco smoke (Hoffmann and Wynder, 1986). Our results rule out nicotine at any physiologically relevant concentration as the key activator of HIV, due to its inability to mimic TSE at concentrations up to 2.5 μM, about 10 times higher than the median concentration of nicotine in the plasma of smokers (Taylor et al., 1986).

Transforming growth factor-beta1 (TGF-β1) has been suggested to be involved in the enhanced expression of HIV-1 by nicotine (Rock et al., 2008), but in the current studies and in one other report (Maunders et al., 2007), TGF-β1 was found to be down-regulated.

Oxidative stress and the lipid peroxidation it induces are obvious candidates for potential involvement in the mechanism of the interaction between tobacco smoking and HIV/AIDS. The NF-κB signaling pathway is believed to be activated during the interaction (Hasnis et al., 2007).

Our results show that TSE has sufficient antioxidant potential to protect cells and virions from oxidative damage (Fig. 4, Fig. 5). The finding that TSE has the ability to protect against oxidative damage goes against the prevailing view in which tobacco smoke is generally considered to be a fundamentally oxidative agent. This is a highly significant result in regard to a possible cell-independent mechanism whereby TSE could increase HIV infectivity, by stabilising the virion against degradation and oxidative attack, e.g., by the "respiratory burst" of lymphocytes.

Gene expression profiles have also shown that TSE can down-regulate thioredoxin (Trx)-interacting protein (TXNIP), thus increasing Trx activity. In the current *in vitro* studies, TSE showed no activating effect on an HIV-LTR reporter expression vector which bears 4 copies (double the normal number) of the NF-κB binding site in the enhancer region.

The interactions between tobacco constituents and tobacco smoke on gene expression have been extensively studied previously by microarray on different cell types. The fact that the observed gene expression profiles are different one from another may be due to differences in the cell type used. A study in oral leukoplakia cells treated with TSE showed that genes whose expression was altered grouped into different pathways such as cell proliferation, inflammation, apoptosis, coagulation, and tumor suppression (Gumus et al., 2008). Specifically, epidermal growth factor receptor (EGFR) related networks as well as several aryl hydrocarbon receptor-dependent genes such as cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) and cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1) were induced. Notably, CYP1A1 and CYP1B1 levels were increased in the oral mucosa of smokers.

Macrophages are key inflammatory cells in chronic obstructive pulmonary disease (COPD). When monocyte derived macrophages (MDMs) from whole blood from patients with COPD were stimulated with cigarette smoke extract (CSE) for 6 h, microarray analysis revealed that IL-8 mRNA levels were up-regulated; other cytokines and chemokines were down-regulated. The expression of the NF-κB component p50 and IκBα were suppressed by CSE, while there was up-regulation of the AP-1 components c-Jun, FOSL1 and FOSL2. The effects of acute CSE exposure appear to encompass both up-regulation of chemotaxis mechanisms through IL-8, but also down-regulation of innate immunity (Kent et al., 2008).

When tracheobronchial epithelium was exposed to air or whole cigarette smoke for 1 h in a specially designed chamber, gene expression was altered in cellular processes including xenobiotic metabolism, oxidant/antioxidant balance, and DNA damage and repair. Notably, there was marked down-regulation of the transforming growth factor-β pathway (Maunders et al.,

2007). TGF-β1 is reported to be upregulated in microgial cells by nicotine pre-incubation (Rock et al., 2008).

RNA microarray results from the current study are different from the reported nicotine-altered gene expression profile of HIV infected microglial cells (Rock et al., 2008). Transforming growth factor β-1, for example, is up-regulated in nicotine-treated microglial cells. However, it is actually down-regulated in TSE treated human T-cells. The differences between the current results and those reported previously (Rock et al., 2008) may be due to the difference in cell type and the treatments, i.e., pure nicotine verses TSE (a complex mixture).

There are two groups of genes that show significantly altered expression levels (3 fold or more) in human T cells treated with TSE in the current study. One group of genes is involved in different stages of HIV infection. All these genes are up-regulated by TSE. CD59 (also called protectin) can incorporate into HIV-1 particles and protect virions from complement-mediated destruction (Bohana-Kashtan et al., 2004); Calmodulin1 binds HIV gp160 and mediates gp160 enhanced apoptosis (Micoli et al., 2000); human T-cell leukemia virus type I binding protein 1 (TAX1BP1) indirectly binds to LTR (Chin et al., 2007); Coatomer protein complex b-2 is essential for golgi budding and vesicular trafficking (Benichou et al., 1994); HSP90 beta 1 is responsible for positive transcription elongation factor b (P-TEFb)-mediated HIV Tat transactivation (O'Keeffe et al., 2000); thioredoxin-related transmembrane protein 1 is involved in HIV-mediated down-regulation of CD4 and is also a co-receptor of coxsackie B virus (Toh et al., 2005). There are two up-regulated genes related to HCV infection. DEAD (Asp–Glu–Ala–Asp) box polypeptide 3 (DDX3) is needed for HCV replication (Ariumi et al., 2007); Sjogren Syndrome antigen B (SSB) can enhance HCV replication (Domitrovich et al., 2005). HCV infection is common in HIV/AIDS, particularly in injection drug users, and also in crack smokers (Shannon et al., 2008). Apparently, from our results, tobacco smoke may be harmful especially for HIV/or HCV carriers because TSE stimulates expression of these genes and thereby may affect virus production.

Another group of affected genes is characterized by redox-related functions. Trx interacting protein (TXNIP) is down-regulated by TSE. TXNIP acts as an endogenous inhibitor of the reducing ability of Trx, by interacting directly with the catalytic center of reduced Trx (Maulik and Das, 2008). The Trx system plays a vital role in the maintenance of a reduced intracellular redox state. Trx plays a role in limiting oxidant stress through either its direct effect as an antioxidant, or through its interactions with TXNIP (Maulik and Das, 2008). Therefore, inhibition of TXNIP by smoking could potentially rescue and increase Trx reducing activity. Interestingly, HIV/AIDS patients show elevated levels of Trx in plasma (Nakamura et al., 1996) and this elevation is a significant predictor of mortality in HIV infection (Nakamura et al., 2001). Phospholipid hydroperoxide glutathione peroxidase (PHGPX) is also down-regulated by TSE. This enzyme is located on cellular membranes, where it serves to protect membrane lipids from peroxidation (Brigelius-Flohe et al., 1997). Lastly, several genes whose functions are related to very-low-density lipoprotein (VLDL) assembly (Miyazaki et al., 2000) and major histocompatibility complex I (MHC-I) antigen presentation (Williams et al., 2002) are also down-regulated in TSE treated cells. This suggests the hypothesis that although tobacco smoke may down-regulate cellular membrane PHGPX and thereby cause membrane peroxidation, at the

same time, tobacco smoke can increase the intracellular redox potential via increased Trx, which may protect virions and infected cells from oxidant damage.

In conclusion, we have revealed a novel aspect of the interaction between tobacco smoking and HIV infectivity, and identified cellular genes and mechanisms that are potentially involved. Furthermore, constituents of tobacco smoke other than nicotine and carbon monoxide are responsible for the increased HIV infectivity and virus production. Given that tobacco smoke contains more than 3900 constituents, a reasonably comprehensive study of the effects of smoking on HIV infectivity has the potential to reveal a novel viral activation mechanism, for which inhibitors could be discovered, giving a new way to inhibit the spread of HIV, and to potentially reduce pathologies in HIV-infected smokers.

Conflict of interest

The authors declare no conflict of interest with the study or preparation of the manuscript.

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