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Review

The involvement of the substance P/neurokinin 1 receptor system in viral infection: focus on the gp120 fusion protein and homologous dipeptide domains

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Summary. – The human immunodeficiency virus (HIV) envelope, via a key extracellular amino acid sequence, may simulate the functionality of native undecapeptide substance P (SP) acting through the host's neurokinin 1 (SP preferring) receptor (NK-1R). Human monocytes and macrophages express both NK-1Rs and SP. In HIV/AIDS the NK-1R may function as a chemokine-like G-protein coupled co-receptor that: 1) fuses to the outer envelope of HIV; 2) enables intracellular entry of the envelope-capsid-NK-1R complex; 3) co-opts immune defence via its physiological interaction with the SP-like envelope; 4) may contribute to resistance of CD4/chemokine entry inhibitor type drugs; 5) relaxes the blood-brain barrier to support entry of the HIV into the central nervous system, and 6) mediates most of the common clinical sequelae of HIV/AIDS (encephalopathy and AIDS dementia complex). The data support the idea that NK-1R antagonists could be useful to treat HIV/AIDS.

Keywords: human immunodeficiency virus; NK-1 receptor; NK-1 receptor antagonist; aprepitant; fusion protein; virus

Contents:

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Abbreviations: BBB = blood-brain barrier; EMCV = encephalomyocarditis virus; FP = fusion protein; HRSV = human respiratory syncytial virus; HIV = human immunodeficiency virus; MV = measles virus; NK-1R = neurokinin 1 receptor; NK-1RAs = NK-1R antagonists; SP = substance P

1. Introduction

Substance P (SP) is an undecapeptide (Fig. 1) belonging to the tachykinin family of peptides. It is derived from the pre-protachykinin-A gene and is present in most human organ systems including the immune system. In the nervous system, SP mainly acts as a neurotransmitter/neuromodulator. The biological actions of tachykinins are mediated by three neurokinin (NK)-1, NK-2 and NK-3 receptors (Rupniak and Kramer, 2017). SP, the natural ligand of the

NK-1 receptor (NK-1R), shows the highest affinity for this receptor. The C-terminus sequence of SP is essential for affinity and the minimum SP fragment retaining good affinity for the NK-1R is SP_{6-11} (Gln-Phe-Phe-Gly-Leu-Met). Moreover, like SP, the undecapeptide hemokinin-1 (HK-1) has a similar affinity for the NK-1R and both peptides have the same 6–11 C-terminus (Gln-Phe-Phe-Gly-Leu-Met). In a concentration-dependent manner, SP amplifies, modulates or induces pain, increases blood-brain barrier (BBB) permeability and is involved in inflammation, neurodegeneration, psychological distress and virus infection (Elsawa *et al.*, 2003; Harrowe *et al.*, 1990; Ho *et al.*, 2002; Kramer *et al.*, 1998; Robinson *et al.*, 2009; Rodríguez *et al.*, 2014; Zimmer *et al.*, 2003). Conversely, in a concentration-dependent manner, NK-1R antagonists (NK-1RAs) mitigate *in vitro* and *in vivo* the above-mentioned pathophysiological actions mediated by SP.

Many studies have confirmed the involvement of the SP/NK-1R system in human immunodeficiency virus (HIV) infection (Azzari *et al.*, 1992; Douglas *et al.*, 2001; Ho *et al.*, 1996, 2002; Li *et al.*, 2001; Wang *et al.*, 2008). The gp120 protein is located in the outer HIV envelope and has been involved in the attachment/entry of HIV into cells. This mechanism is dependent on CCR5/CXCR4 chemokine co-receptors (Briz *et al.*, 2006). However, many authors have suggested that the attachment/entry of HIV is not exclusively provided through this mechanism (Livingstone *et al.*, 1996; Moss *et al.*, 2014; Stins *et al.*, 2003; Wilen *et al.*, 2012). Here, we update the involvement of the SP/NK-1R system in HIV infection. Moreover, according to the homology of the amino acid sequences between SP and gp120, we also suggest that, in addition to CCR5/CXCR4 chemokine co-receptors, the NK-1R ligation of SP-like gp120 amino acid sequences is crucial for the entry of HIV into cells.

SP
Arg-Pro-Lys-Pro-**Gln-Gln-Phe-Phe-Gly-Leu-Met**-NH2 (RPKP**QQFFGLM**)

gp120 HIV
1 vpvwrdadtt lfcasdaksh vteahnvwat hacvptdpnp qeihlenvte nfnmwknnmv
61 eqmqedvisl weqslkpcvk ltplcvtlnc tnanltnanl tnannitnve nitdevrncs
121 fnvttdlrdk qqkvhalfyr nstxvkaxge ssdyrlincn tsvikqacpk isfdpipihy
181 ctpagyailk cndknfngtg pcknvssvqc thgikpvvst qlllngslae eeiiirsenl
241 tnnvktiivh lnksveinct rpsnntrtsi tigpgqvfyr tgdiigdirk vscelngtkw
301 nevlkqvkek lkehfnknis fqppsggdle itmhhfscrg effycnttql fnntysngti
361 tlpckikqii nmwqgvgqam yappisgrin clsnitgll trdgnngtne tfrpgggnik
421 dnwrselykc kvvqieplgi aptrakrrvv erekk

gp120 HIV V3 to V5
1 csrpgnntrk svrigpgrys vfyatrriig dirqahcnis radwnktlqr vgkeladrfn
61 ktiifaphsg gdleitthsf ncrgeffycn taqlfnstym lngtyrtegnssvitlpcri
121 kqiinmwqev gramyappis gnitcnstit gllltrdggi enitngtdtf rpe

Viral Fusion Protein Inhibitor
Z-D-**Phe-Phe-Gly**-OH

Fig. 1

Substance P, HIV gp120 and viral fusion protein inhibitor amino acid sequences
SP (in black, dipeptides of the amino- and carboxy-termini), gp120 HIV (in black, SP homologous dipeptides of the amino- and carboxy-termini), gp120 HIV (V3 to V5) (in black, SP homologous dipeptides of the amino- and carboxy-termini) and viral fusion protein inhibitor (in black, SP homologous dipeptides of the carboxy-terminus) sequences.

gp41 HIV
1 avgalgamfl gflgaagstm gaasxtltvq arlllsgiaq qqnnmlraie aqqhllqltv
61 wgikqlqari laverylkdq qllgiwgcsg klicttavpw ntswsgsxnl sqiwdnmtwm
121 eweraidnht dtiyrliees qnqqekneqe lleldkwasl wswfditqwl wyixifimiv
181 ggliglrivf avfxlvnrvr qgysplslqt hfpgprgpdr pegigeeggd rdkggstrlv
241 hgflalvwdd lrslclfsyh rlrdltliva rivellgrrg wtalkywwnl lkywxqelkn
301 savslxnata iavaegtdri iexaqrif

Fig. 2

HIV gp 41 amino acid sequence
gp41 HIV protein sequence (in black, SP homologous dipeptides of the amino- and carboxy-termini).

2. Potential SP-like affinity of gp120 for the NK-1R

According to the amino acid sequence of SP (e.g., its C-terminus sequence is crucial for the affinity to the NK-1R) (Fig. 1), the presence of SP homologous dipeptide domains (which are able to bind to the NK-1R) can be detected in gp120 and other attachment/entry viral proteins (Figs. 1–7). The gp120 contains 455 aa (Fig. 1) (Phan *et al.*, 2000) and is named according to its molecular weight (120 kDa). gp120 contains three mimetic dipeptides of the SP C-terminus sequence: Gln-Gln (131–132), Phe-Phe (342–343) and Gly-Leu (397–398) (Fig. 1). Moreover, gp120 contains five mimetic peptides of the SP N-terminus sequence: Arg-Pro (261–262; 413–414); Lys-Pro (76–77; 215–216) and Pro-Lys (169–170) (Fig. 1). gp120 is characterized by a notable genetic variability inferred from the expressed variants of reverse transcriptase during replication of the virus. This hyper-variability originates in the non-conserved V1 to V5 extracellular regions of gp120 (Starcich *et al.*, 1986); among them are sequences which indispensably bind the envelope to the host cell. These sequences have been implicated in host immune escape (Cenci *et al.*, 2014) and in resistance to attachment-entry inhibitory drugs (Briz *et al.*, 2006). Importantly, variants of gp120 (V3 to V5) (Fig. 1) (Phan *et al.*, 2000) contain two mimetic dipeptides (Phe-Phe (86–87); Gly-Leu (151–152)) of the SP C-terminus sequence. These sequences, though short, are homologous to those found in SP and it is known that they contribute to binding of native SP to the NK-1R. It seems that this region of gp120, involved in host immune escape (Cenci *et al.*, 2014), is necessary for HIV and mimics the SP function (due to the presence of Phe-Phe and Gly-Leu SP homologous domain sequences).

gp160 HIV

1 mrvretqmnw qhlwrwgt**lm** l**gl**viicsas dxlwvtvyyg vpawedadtt lfcasdakay
61 steshnvwat hacvptdpnp qeislenvte nfnmwknnmv eqmxediisl wdesl**kp**cvk
121 ltplcvtldc anitnnvtxd nstxvkaxge lkncsfnitt elrdrksqey aifykldivq
181 idksndstnn yrlincnvst vkqac**pk**vsf dpipihycap agfailkcrd kxfngtgpck
241 nvstvqcthg i**kp**vvstqll lngsiaeeev mirsenitns akniiv**qf**nk tveiict**rp**n
301 nntrrsitlg pgqafyatga iignirqahc nvsetawrer mkevkakleg ifnnksiifn
361 sssggdieit shsfncrge**f f**ycnts**gl**fn nsmlnstdng titlpckikq ivrmwqrvgq
421 amyappiagn itcksnit**gl** lltrdggntn ssetf**rp**sgg dmrdnwrsel ykykvvki**kp**
481 lgiaptkarr rvvqrekrav **gl**gavllgfl gtagstmgaa sitltvqxrq llsgiv**qqq**n
541 nllraiea**qq** hllqltvwgi kqlqarvlai erylrd**qq**ll giwgcsgkli cttnvpwnxs
601 wsnktydeiw dnmtwiqwer eisnyt**qq**iy slieesqn**qq** ekneqdllal dkwtslwswf
661 ditnwlwyik ifimivg**gl**i **gl**rivfavls ivnrvrqgys plsfqtlthh qrepd**rp**eri
721 eegggeqdkd rsirlvsgfl alawddlrsl clfsyhrlrd filiaartve llghsslk**gl**
781 rlgwe**gl**kyl gnllsywgre lknxainlld tlaiatanwt drvieigqra craflnvprr
841 irqgferall

Fig. 3

HIV gp 160 amino acid sequence
gp160 HIV protein sequence (in black, SP homologous dipeptides of the amino- and carboxy-termini).

Human respiratory syncytial virus
F protein

1 mellilkana ittiltavtf cfasgqnite efyqstcsav skgylsalrt gwytsvitie
61 lsnikknkcn gtdakvklik qeldkyknav telql**lm**qst qatnnrarre lprfmnytln
121 nakktnvtls kkrkrrflgf llgvgsaias gvavskvlhl egevnkiksa llstnkavvs
181 lsngvsvlts kvldlknyid kqllpivnkq scsisnietv ief**qq**knnrl leitrefsvn
241 agvttpvsty mltnsellsl indmpitndq kk**lm**snnvqi vr**qq**sysims iikeevlayv
301 vqlplygvid tpcwklhtsp lcttntkegs nicltrtdrg wycdnagsvs **ffpq**aetckv
361 qsnrvfcdtm nsltlpsevn lcnvdifn**pk** ydckimtskt dvsssvitsl gaivscygkt
421 kctasnknrg iiktfsngcd yvsnkgvdtv svgntlyyvn kqegkslyvk gepiinfydp
481 lvfpsdefda sisqvnekin qslafirksd ellhnvnagk sttnimitti iiviivills
541 liav**gl**llyc karstpvtls kdqlsginni afsn

G protein

1 msknkdqrta ktlertwdtl nhllfisscl yklnlksvaq itlsilamii stsliiaaii
61 fiasanhkvt pttaiiqdat sqiknttpty ltqn**pq**lgis psnpseitsq ittilasttp
121 gvkstlqstt vktknttttq tqps**kp**ttkq rqn**kp**p**sk**pn ndfhfevfnf vpcsicsnnp
181 tcwaickrip nk**kp**gkkttt **kp**tk**kp**tlkt tkkd**pkpq**tt kskevptt**kp** teeptinttk
241 tniittllts nttgnpelts qmetfhstss egnpspsqvs ttseypsqps sppntprq

Fig. 4

F/G protein of respiratory syncytial virus amino acid sequence
F/G protein (respiratory syncytial virus) sequences (in black, SP homologous dipeptides of the amino- and carboxy-termini).

The fusion domain of HIV-1 gp41 transmembrane portion of viral envelope spikes exhibits seven mimetic dipeptides of SP: N-terminus (Gln-Gln (40–41), Gln-Gln (41–42), Gln-Gln (52–53), Gln-Gln (80–81), Gln-Gln (143–144)) and C-terminus (Gly-Leu (182–183), Gly-Leu (185–186)) (Fig. 2). Accordingly, both the extracellular docking heads of envelope spikes (gp120) and the gp41 transmembrane portion as previously suggested, may enhance HIV entry through a SP/NK-1R mechanism (Ho and Douglas, 2004). The presence of SP mimetic dipeptides, in both gp120 and gp41, could explain this mechanism. The HIV-1 envelope

Hemagglutinin measles virus

1 ms**pq**rdrina fykdnph**pk**g srivinrehl mid**rp**yvlla vlfvmflsli **gl**laiagirl
61 hraaiytaei hkslstnldv tnsiehqvkd vltplfkiig dev**gl**rt**pq**r ftdlvkfisd
121 kikflnpdre ydfrdltwci npperikldy dqycadvaae e**lm**nalvnst lletrttn**qf**
181 lavskgncsg pttirg**qf**sn mslsllldlyl srgynvssiv tmtsqgmygg tylve**kp**nls
241 skgselsqls myrvfevgvi rnp**gl**gapvf hmtnyfeqpv sndlsncmva lgelklaalc
301 hggdsitipy qgsgkgvsfq lvklgvwksp tdmqswvpls tddpvidrly lsshrgviad
361 nqakwavptt rtddklrmet cf**qq**ackgki qalcenpewa plkdnripsy gvlsvdlslt
421 velkikiasg **fg**plithgsg mdlyksnhnn vywltippmk nlalgvintl ewiprfkvsp
481 ylftvpikea gedchaptyl paevdgdvkl ssnlvilpgq dlqyvlatyd tsrvehavvy
541 yvyspsrsfs yfypfrlpik gipielqvec ftwdqklwcr hfcvladses gghithsgmv
601 gmgvsctvtr edgtnsr

Fig. 5

Hemagglutinin of measles virus amino acid sequence
Hemagglutinin (measles virus) sequence (in black, SP homologous dipeptides of the amino- and carboxy-termini).

FP measles virus

1 msim**gl**kvnv saifnavllt lqtptgqihw gnlskigvvg igsasykvmt rsshqslvik
61 **lm**pnitllnn ctrveiaeyr rllrtvlepi rdalnamtqn i**rp**vqsvass rrhkrfagvv
121 lagaalgvat aaqitagial hqsmlnsqai dnlraslett nqaieairqa gqemilavqg
181 vqdyinneli psmnqlscdl igqkl**gl**kll ryyteilsl**f g**pslrdpisa eisiqalsya
241 lggdinkvle klgysggdll gilesrgika rithvdtesy fivlsiaypt lseikgvivh
301 rlegvsynig sqewyttv**pk** yvatqgylis nfdessctfm pegtvcsqna lypmspllqe
361 clrgstksca rtlvsgs**fg**n rfilsqgnli ancasilckc yttgtiinqd pdkiltyiaa
421 dhcpvvevng vtiqvgsrry pdavylhrid lgppislerl dvgtnlgnai akledakell
481 essdqilrsm k**gl**sstsivy iliavc**lgl** igipaliccc rgrcnkkgeq vgms**rpglkp**
541 dltgtsksyv rsl

Fig. 6

Fusion protein of measles virus amino acid sequence
Fusion protein (measles virus) sequence (in black, SP homologous dipeptides of the amino- and carboxy-termini).

VP1 encephalomyocarditis virus

1 gvtedtdata dfvaqpvylp enqtkva**ff**y drsspigaft vksgslesgf gpfsnetcpn
61 sviltpg**pqf** dpaydql**rpq** rlteiwgngn eetskvfplk skqdysfclf spfvyykcdl
121 evtlsphtsg nh**gl**lvrwcp tgtpa**kp**ttq vlhevsslse grt**pq**vysag pgvsnqisfv
181 vpydsplsvl pavwynghkr fdntgslgia pnsd**fg**t**ff** agt**kp**dikft vylryknmrv
241 fcp**rp**tv**ff**

Fig. 7

VP1 protein of encephalomyocarditis virus amino acid sequence
VP1 protein (encephalomyocarditis virus) sequence (in black, SP homologous dipeptides of the amino- and carboxy-termini).

glycoprotein is synthesized as a precursor glycoprotein (gp160) and is then processed into gp120 and gp41 (Salminen *et al.*, 1997). gp160 contains 24 SP homologous dipeptides (Fig. 3), gp120, 8 (Fig. 1) and gp41, 6 (Fig. 2). It seems

that the greater number of mimetic peptides of the virus fusion protein (FP), the greater NK-1R binding.

It has been reported that some oligopeptides inhibit virus infectivity/cell fusion and hemolysis induced by the paramyxoviruses (Richardson *et al.*, 1980). In this sense, it has been reported that the viral FP antagonist Z-D-Phe-Phe-Gly (Fig. 1) (shows the same active site as SP) blocked the SP function, i.e. inhibiting viral fusion and replication (Richardson *et al.*, 1980). Moreover, it is known that the tripeptide Z-D-Phe-L-Phe-L-(NO_2)-Arg (Z designates the carbobenzoxy group) inhibited the replication of measles virus (MV) (Richardson *et al.*, 1980). Z-D-Phe-Phe-Gly and Z-L-Phe-L-Phe mimic the N-terminus of the F1 polypeptide (N-terminal region of the paramyxovirus F polypeptide or the myxovirus HA2 polypeptide): the first is 240 times more effective than the second (Z-D-Phe-Phe-Gly: IC_{50} 0.2 μM; Z-L-Phe-L-Phe: IC_{50} 42 μM) (Richarson *et al.*, 1980). The specific inhibition of infectivity by oligopeptides resembling a region of a viral polypeptide could be a strategy for the chemical inhibition of viral replication. In addition, the data reported in this section also suggest a potential SP-like affinity of gp120 for the NK-1R.

Moreover, it is important to note that other attachment/entry viral proteins also contain SP homologous dipeptides (Figs. 4–7). For example, in human respiratory syncytial virus (HRSV), F (fusion) and G (attachment) proteins are the only ones targeting cell membranes (Le Nouën *et al.*, 2014). F protein contains 574 aa and eight SP homologous dipeptides (Fig. 4): Phe-Phe (351–352), Gly-Leu (545–546), Gln-Gln (224–225; 283-284), Leu-Met (96–97; 273–274), Pro-Gln (353–354) and Pro-Lys (389–390). The G protein (298 aa) contains ten SP homologous dipeptides (Fig. 4): Lys-Pro (145–146; 154–155; 158–159; 193–194; 201–202; 205–206; 229–230), Pro-Lys (215–216) and Pro-Gln (95–96; 217–218). In MV, the hemagglutinin protein (617 aa) (Parks *et al.*, 2001) and the fusion protein (553 aa) (Komase *et al.*, 1990) are involved in the binding of the virus to the cells. The hemagglutinin contains the following thirteen SP homologous dipeptides (Fig. 5): Gly-Leu (51–52; 104–105; 264–265), Gln-Gln (383–384), Leu-Met (162–163), Gln-Phe (179–180; 197-198), Phe-Gly (431–432), Pro-Gln (3–4; 108–109), Pro-Lys (18–19), Arg-Pro (34–35) and Lys-Pro (236–237), whereas the fusion protein contains twelve SP homologous dipeptides (Fig. 6): Gly-Leu (5–6; 206–207; 492–493; 509–510; 537–538), Leu-Met (61–62), Phe-Gly (220–221; 378–379), Arg-Pro (102–103; 535–536), Pro-Lys (319–320) and Lys-Pro (539–540). In the encephalomyocarditis virus (EMCV), the VP1 protein (249 aa) is involved in viral infection (Meng *et al.*, 2016). This protein contains thirteen SP homologous dipeptides (Fig. 7): Phe-Phe (28–29; 219–220; 248–249), Gly-Leu (133–134), Gln-Phe (69–70), Phe-Gly (215–216), Pro-Gln (68–69; 79–80; 164–165), Arg-Pro (78–79; 244–245) and Lys-Pro (146–147; 224–225). In sum, the presence of SP homologous dipeptides in HIV, HRSV, MV and EMCV attachment/entry viral proteins are a common feature and this suggests that viruses mimic SP sequences, included in the sequence of viral attachment/entry proteins, for entry to the host cell via the NK-1R (see below).

3. The role of SP in the immunopathogenesis of HIV and other viral infections

The general contribution of the SP/NK-1R system to HIV infection is persuasive and bidirectional. 1) SP enhances the infection of macrophages by HIV-1 and the peptide is involved in the entry of HIV into monocyte-derived macrophages (Ho *et al.*, 1996, 2002); 2) HIV-1 infection of human immune cells increases the expression of SP by these cells (Ho *et al.*, 2002); 3) SP activates HIV-1 replication in latently infected immune cells (Li *et al.*, 2001); 4) In HIV-infected men and women, SP plasma concentrations are significantly increased (Douglas *et al.*, 2001); 5) HIV-infected children show higher plasma concentrations of SP than HIV-seronegative children (born from HIV-positive mothers) or than healthy control children (Azzari *et al.*, 1992); 6) The NK-1RA, CP-96,345, inhibited HIV infection of monocyte-derived macrophages by down-regulating the expression of the chemokine receptor CCR5 (Lai *et al*, 2001). Aprepitant (SP antagonist) suppressed *in vitro* the HIV-1 infection of microglia/macrophages (Wang *et al.*, 2008) and, in a clinical trial with patients suffering from AIDS, the drug decreased the percentage of $CD4^+$ T cells expressing PD-1 (Tebas *et al.*, 2015); 7) SP and NK-1R are located in both cytoplasm and nucleus of macrophages; 8) In murine EMCV-infection, SP levels increased 61-fold but SP-precursor knockout mice are completely protected from EMCV mortality, cardiomegaly, cardiac inflammation, necrosis, cardiomyocyte apoptosis and hypertrophy (Robinson *et al.*, 2009). Moreover, the NK-1RA aprepitant reduced mortality, heart and cardiomyocyte size, and cardiac viral RNA levels. Pre-treatment with aprepitant improved heart functions; it significantly decreased end systolic diameter, improved fractional shortening, and increased peak aortic flow velocity (Robinson *et al.*, 2015), and 9) Respiratory syncytial virus (RSV)-infection increased the level of mRNA encoding the NK-1R (increased four-fold in RSV-infected lungs) (King *et al.*, 2001). In sum, the previously mentioned data show that the SP/NK-1R system contributes to HIV and other viral infections (Ho and Douglas, 2004).

Additional data support the involvement of the SP/NK-1R system in viral infection. For example, the sequences of SP and that of the FP domain of MV show homologies (Fig. 1) and for this reason the NK-1R expressed in immune cells may facilitate the fusion step of MV infection (viral entry into cells) (Harrowe *et al.*, 1990). The oligopeptide Z-D-Phe-Phe-

Gly (shows the same active site as SP) inhibited MV fusion with target cells (Harrowe *et al.*, 1990). Moreover, during viral fusion, anti-SP antisera inhibited the cell-to-cell spread of MV and the MV-NK-1R interaction (Harrowe *et al.*, 1990). This means that both MV and SP bind to the NK-1R and hence MV may use the NK-1R during a specific phase of the infectious cycle. Finally, the SP-NK-1R system has been also implicated in the pathophysiology of other viral infections (King *et al.*, 2001; Piedimonte, 2001; Robinson *et al.*, 2009; Svensson *et al.*, 2005; Zimmer *et al.*, 2003).

SP plays a critical role in HIV gp120-induced increase in permeability of rat brain endothelium cultures. SP enhances inflammatory cytokine production by immune cells and these cytokines modulate the HIV infection of immune cells (Ho and Douglas, 2004). Moreover, in HIV infection, the gp120 coupled to chemokine receptors (e.g., CCR5/CXCR4) is linked to pain, increase in BBB permeability (and central nervous system infectivity), neuronal death, and HIV encephalopathy (i.e., altered consciousness with variable neurological signs) (Annunziata *et al.*, 1998; Bachis *et al.*, 2012; Hao, 2013; Louboutin *et al.*, 2012; Toneatto *et al.*, 1999). All previous mechanisms are induced by SP and mitigated by NK-1RAs (Annunziata *et al.*, 1998; Martinez *et al.*, 2017; Wang *et al.*, 2008). Both spantide (a peptide NK-1RA) and anti-SP antibodies completely blocked the dose-dependent gp120-induced increase in albumin permeability in rat brain endothelial cells (Annunziata *et al.*, 1998). This is consistent with the notion that gp120 ligates and activates NK-1R signaling. It is also known that NK-1R signaling promotes neuroinflammation and BBB dysfunction (Rodríguez *et al.*, 2014): SP induces the activation of brain microvascular endothelial cells (HBMECs), leading to secretion of tumor necrosis factor-alpha and angiopoietin-2 from HBMECs, inducing changes in localization/distribution of tight junction protein zonula occludins-1 and claudin-5 and increasing HBMECs permeability. By contrast, *in vivo*, spantide inhibited changes in BBB permeability (Rodríguez *et al.*, 2014). Thus, NK-1RAs exert a neuroprotective effect (diminished neuroinflammation and increased repair of the BBB) (Annunziata *et al.*, 1998; Martinez *et al.*, 2017; Wang *et al.*, 2008). Altogether, the current data confirm that the NK-1R is a general viral intracellular gateway.

4. An alternate attachment/entry of HIV to host cells

Among the attachment molecules on the outer HIV envelope is the well-characterized gp120. The mechanism of intracellular transport of HIV-1 is elucidated from studies in $CD4^+$ cells through which the viral exterior envelope glycoprotein, gp120, is re-conformed by the CD4 antigen to fuse HIV with surface chemokine co-receptors such as CCR5/CXCR4 (Briz *et al.*, 2006). Despite wide consensus on this mechanism, it is the broad tropism of HIV which nevertheless prompts some authors to suggest that attachment and entry of HIV might not be wholly dependent on CCR5/CXCR4 chemokine co-receptors (Livingstone *et al.*, 1996; Moss *et al.*, 2014; Stins *et al.*, 2003; Wilen *et al.*, 2012) or the CD4 antigen (Sakaida *et al.*, 1998).

According to the data mentioned in the previous sections the following mechanism for HIV attachment and entry that supplements the gp120-CD4-chemokine model can be suggested: the attachment spikes on the outer HIV envelope (specifically on the gp120 V3 to V5) (Yuan *et al.*, 2013) are composed of dipeptides that are homologous to native SP, and may be capable of binding directly with NK-1Rs on host cells (human monocytes and macrophages express NK-1Rs). Thus, HIV, acting as SP-like, may either enter cells through classic ligated G protein-coupled receptor internalization, i.e. as an HIV-NK-1R complex or through fusion. Attached NK-1Rs generally migrate as vesicles from the cell surface to endosomes or onto the nuclear membrane. In addition, NK-1Rs (full or truncated), if directly activated by HIV, could simultaneously induce the mitogenic activity which is required for viral replication. This idea coincides with data indicating that the SP/NK-1R system is relevant to the immuno-pathogenesis of HIV/AIDS infection (Ho *et al.*, 2002). Additionally, as highlighted below, many of the chronic clinical manifestations of HIV/AIDS are putatively attributable to NK-1R activation. About 30% of patients develop the AIDS dementia complex (multifocal encephalitis, cognitive disturbances, fatigue, insomnia, depressive episodes, anxiety, variable neurological signs) as well as chronic neuropathic and rheumatic pain and finally neuronal apoptosis (González-Scarano and Martín-García, 2005).

5. The potential therapeutic role of NK-1RAs in HIV/AIDS and other viral infections

NK-1RAs may act against HIV infection by a four-fold mechanism: 1) by blocking the entry and attachment of HIV to the host cell through: a) decreasing the expression of immune cell cytokine/chemokine HIV co-receptors (Lai *et al*, 2001); b) blocking alternative HIV cell entry via variant gp120 binding to the NK-1R; or c) decreasing the number of $CD4^+$ PD-1-positive cells (Tebas *et al.*, 2015); 2) decelerating HIV replication by inhibiting NK-1R induced host cell mitosis (Ho *et al.*, 2002); 3) by reducing HIV in the central nervous system infectivity by blocking HIV access to the central nervous system through mitigating NK-1R-induced loosening of junctional complexes at the BBB (Annunziata *et al.*, 1998); 4) by mitigating encephalopathy and AIDS dementia complex/other sequelae (Martinez *et al.*, 2017; Wang *et al.*, 2008).

In stark contrast to the original "D amino-acid substituted" peptide-based NK-1R inhibitors, the scores of new non-peptide NK-1RAs are highly selective, lipid soluble, cross the BBB, and are distinguished pre-clinically and clinically by extraordinarily wide therapeutic indices (safety) (Muñoz and Coveñas, 2013). Some of them might be available for licensing for developing an anti-HIV indication. Of the many non-peptide NK-1RAs discovered, only three of them (aprepitant, fosaprepitant, rolapitant) are currently used in clinical practice. Aprepitant is available in a clinically approved form as an antiemetic (orally: <125 mg/d for 3 days). It is important to remark that in humans aprepitant has been dosed as high as > 300 mg daily for up to 8 weeks and 1,200 mg (daily) in normal volunteers without safety issues (Kramer *et al*., 1998). Moreover, the administration of aprepitant (375 mg, daily) in HIV patients for 2 weeks was safe and well tolerated (Tebas *et al*., 2015).

In vitro studies have reported that non-peptide NK-1RAs (e.g., CP-96,345, L-733,060, aprepitant) might be useful for the treatment of HIV (Ho and Douglas, 2004). However, in a human clinical study, it has been reported that aprepitant (125–250 mg/day) showed some biological activity, but no significant antiviral activity (Tebas *et al*., 2011). This apparent lack of activity could be probably linked to the low doses used. Greater SP plasma concentrations are observed in HIV/AIDS patients than in normal control, suggesting that the up-regulated SP/NK-1R system contributes to an intense neurogenic inflammatory response. Because in HIV, NK1-Rs are overexpressed (Tebas *et al*., 2015), doses/exposures should be higher (> 250 mg/day, ~ 2 μM) than those currently administered clinically to prevent emesis (125 mg, ~1 μM). However, there is a need to test efficacy. In HIV-1-infected adults, it has been reported that aprepitant (375 mg/day) decreased the number of $CD4^+$ PD-1-positive cells and the plasma level of SP (Tebas *et al*., 2015).

In an *in vivo* model of toxic hepatitis NK-1RAs (CP-96,345, L-733,060), at doses of 10 mg/kg and 20 mg/kg, respectively exerted an anti-inflammatory action (Bang *et al*., 2004). Moreover, in the rhesus model of Lyme neuroborreliosis the effects of the NK-1R antagonist aprepitant has been tested (Martínez *et al*., 2017). Drug treatment (28 ± 6 mg/kg per day) started two days before the inoculation of *Borrelia burgdorferi*; it is known that neural pathologies appear eight weeks after the infection. Two-four weeks after the infection, aprepitant attenuated *Borrelia burgdorferi*-induced elevations of CCL2, CXCL13, IL-17A and IL-6 gene expression in dorsal root ganglia, spinal cord and/or cerebrospinal fluid. Aprepitant also prevented the increase of the NK-1R expression and decreased the expression of glial fibrillary acidic protein (an astrocyte marker) (Martínez *et al*., 2017). In humans with inflammatory bowel disease, NK-1Rs are massively up-regulated, as are those in an animal model (inflammatory bowel disease induced by *Cryptosporidium parvum*) (Sonea *et al*., 2002). Pre-clinically, doses of 30 mg/kg of LY-303,870 (a subnM selective NK-1RA) were required for reversal of the pathology (Sonea *et al*., 2002).

On a pharmacokinetic basis alone, it has been suggested that a minimum dose of about 1,000 mg/day of aprepitant is required to treat HIV infection. The critical relationship between adequate human dosing and therapeutic efficacy of the mechanism has been further amplified in NK-1RA clinical antidepressant research. The sustained under-exposure of a new formulation of aprepitant tested in a massive phase III trial without benefit of its dose-efficacy response, led to the failure to replicate the antidepressant activity of the mechanism which had been replicated previously in ~ 1,500 patients and multiple independent investigator groups. Approximately 100% human receptor occupancy appears to be required for that indication (Rupniak and Kramer, 2017).

6. Conclusion

Voluminous data presented herein and elsewhere conceptually support that NK-1R antagonism is an HIV antiviral mechanism that must still be tested clinically at adequate NK-1RA exposure. We also suggest that the NK-1R binding of SP-like gp120 variable region sequences could be crucial for the entry of HIV into cells. On that basis, non-peptide NK-1RAs could be useful for the treatment of HIV and HIV resistant mutant infections. Future pre-clinical studies are needed to investigate 1) potential interactions between HIV, SP, CD4, NK-1R, CCR5/CXCR4 on a variety of human cells; 2) the influence of NK-1RAs on cell to cell spread of HIV; 3) passage of HIV across the BBB and the influence of NK-1RAs in preventing of breaching the BBB, and 4) the prevention of encephalopathy and AIDS dementia complex after using NK-1RAs. Also, clinical investigations of NK-1R antagonism system in viral infections other than HIV may be warranted. Finally, as an important cautionary note: the required dose of NK-1 receptor antagonists should be higher than previously clinically tested in order to have both biological and anti-HIV effects and thus to be able to eradicate HIV.

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