HIV protease inhibitors: antiretroviral agents with anti-inflammatory, anti-angiogenic and anti-tumour activity

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Despite mild toxicity and adverse effects, human immunodeficiency virus (HIV) protease inhibitors (PIs), used in combination with reverse transcriptase nucleoside inhibitors (NRTIs), have turned AIDS into a chronic, manageable disease. Such combination therapy, known as highly active antiretroviral therapy (HAART), efficiently suppresses HIV replication leading to immune restoration in HIV-infected patients. HIV PIs act by blocking the HIV aspartyl protease, a viral enzyme that cleaves the HIV gag and gag-pol polyprotein backbone at nine specific cleavage sites to produce shorter, functional proteins. Three of these cleavage reactions occur between a phenylalanine or a tyrosine and a proline. Strikingly, none of the known mammalian endopeptidases cleaves before a proline; for this reason, most HIV PIs have been designed to mimic the phenylalanine–proline peptide bond. This confers a remarkable specificity of action to HIV PIs and, with short-term treatment, they show only mild side-effects and a tolerable toxicity.1

Unexpectedly, however, the long-term treatment of responders patients with PI-containing HAART has been shown to be associated with several unpredicted effects, such as hyperbilirubinaemia, insulin resistance, hyper- or hypo-lipidaemia, fat body redistribution, osteopenia and osteoporosis.2 A reduced incidence and an increased regression of AIDS-associated tumours including Kaposi’s sarcoma (KS) and some types of non-Hodgkin lymphomas (NHLs), namely cerebral and immunoblastic lymphomas, have been described since the introduction of PI-HAART compared with the pre-HAART era.3,7 The exact mechanism(s) of these effects, which appear to involve metabolic pathways, tissue remodelling processes and immunological responses, is unclear. However, efforts aimed at identifying specific actions of the most widely used HIV PIs (i.e. ritonavir, saquinavir, indinavir and nelfinavir) have led to the identification of non-retroviral activities of these drugs, which may explain the effects observed in patients. In particular, HIV PIs have been shown to affect enzymes and pathways that are involved in HAART side-effects. These include the glucose transporter Glut4, bilirubin UDP-glucuronosyltransferase, apolipoprotein B degradation and secretion, and enzymes involved in adipocyte, osteoclast or osteoblast function and differentiation.8,11 However, HIV PIs also exert unpredicted actions, which may have beneficial effects. For example, they can inhibit or stimulate peripheral blood mononuclear cells, T-cell or endothelial cell survival and activation.12–14 In addition, they inhibit inflammatory cytokine production and modulate antigen presentation and T-cell responses.14–16 In particular, ritonavir has recently been shown to inhibit the expression of adhesion molecules and the production or release of inflammatory cytokines (ICs) or chemokines, including tumour necrosis factor (TNF)-α, interleukin (IL)-6 or IL-8 by endothelial cells.14 HIV PIs may even affect T-cell priming, owing to their capability of inhibiting dendritic cell (DC) maturation and function.17 Finally, they have been shown to exert inhibitory effects on fungal aspartyl proteases.18 Many of these effects, which have been proven in HIV-free models, may actually increase the therapeutic efficacy of HAART by directly reducing immune activation, inflammation, T-cell apoptosis and opportunistic infections. However, their exact role in the reduced incidence or regression of KS or NHLs upon HAART remains to be determined, and the capability of HIV PIs of directly inhibiting KS burden or development should be investigated further.

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KS is an angioproliferative disease characterized by inflammatory cell infiltration, intense and aberrant angiogenesis, oedema and growth of spindle cells of endothelial or monocytic cell origin (KS cells).\textsuperscript{19,20} KS develops with a low incidence in elderly people of Mediterranean origin or in transplant patients, but its incidence and aggressiveness are dramatically increased in the setting of HIV infection. This is due to the actions of ICs, particularly interferon (IFN)-γ, IL-1β and TNF-α, which are increased in HIV-infected subjects (as well as other individuals at risk of KS).\textsuperscript{19,20} These ICs reactivate infection by human herpesvirus 8 (HHV8), a virus associated with KS development, leading to a high HHV8 load and virus dissemination to blood and tissue cells, including KS cells. This, in turn, induces immune responses against the virus that, in individuals at risk of KS, are ineffective and, paradoxically, exacerbate the reactive process and the production of ICs.\textsuperscript{20} The same ICs also activate vessels, induce production of angiogenic factors (see below) and upregulate the expression of the αβ3 and αβ5 integrins. In this context, the HIV Tat protein, released by acutely infected cells, acts as a progression factor by binding to the same integrins and increasing the effects of angiogenic factors.\textsuperscript{19,20} This leads to an increased aggressiveness of KS in patients infected by HIV.\textsuperscript{19,20}

Most notably, ICs induce the production of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). These are two potent angiogenic factors that are highly expressed in KS lesions by KS cells, endothelial cells and infiltrating cells in response to the ICs, but also by most solid tumours.\textsuperscript{4,19,20} Data from several \textit{in vitro} and \textit{in vivo} studies indicate that bFGF and VEGF are the factors promoting KS lesion formation. In particular, they promote, in a synergic fashion, the development of angioproliferative KS-like lesions induced by the inoculation in nude mice of cultured KS cells derived from human lesions. Such KS-like lesions are of mouse cell origin, are induced by the factors produced by KS cells, are highly vascularized and resemble very closely early KS lesions in humans.\textsuperscript{4,19,20} Notably, this animal model of KS, which is also an established model for pre-clinical efficacy evaluation of anti-KS therapies, lacks T-cells and HIV infection. Moreover, although KS cells are infected by HHV8 \textit{in vivo}, they loose the virus early upon culture.\textsuperscript{20} Thus, KS cells injected in mice are no longer infected by HHV8. These data indicate that bFGF and VEGF can induce KS lesion development even in the absence of T-cell responses and viral infections. In fact, KS-like lesions are triggered in nude mice also by the direct inoculation of bFGF, a process that is increased synergically by VEGF or Tat, or by the direct inoculation of ICs, which are known to induce both bFGF and VEGF.\textsuperscript{19,20} These data may explain why KS regression upon HAART is, in some cases, unrelated to HIV suppression or immune reconstitution.\textsuperscript{4}

Owing to their anti-inflammatory, immunomodulatory and antiretroviral actions, HIV PIs may affect KS in several ways. These may include the inhibition of HIV replication and the consequent production and release of the HIV-1 Tat protein, a KS progression factor.\textsuperscript{19,20} HIV PIs may also block the production of ICs by HIV-1-infected or activated T-cells, thereby blocking both angiogenic factor production and HHV8 reactivation, which are both triggered by ICs.\textsuperscript{19,20} In addition, although no direct inhibitory effects of PIs have been detected on HHV8 replication or gene expression (Kedes & Ganem\textsuperscript{21} and data not shown), treatment with PIs may clear HHV8 from tissues and circulation by restoring protective immune responses against the virus.\textsuperscript{20,22} Furthermore, a recent study reported that nuclear factor xB (NF-xB) transcriptional activation induced in an immortalized KS cell line by TNF-α, Tat or HHV8 was inhibited by ritonavir, resulting in cell apoptosis.\textsuperscript{14}

To avoid immunological and virological confoundings, and to identify direct anti-tumour effects of HIV PIs involved in KS regression, we treated nude mice by intragastric gavage with indinavir or saquinavir, and injected them with cultured primary KS cells derived from human lesions. Strikingly, both indinavir and saquinavir, used at doses similar to those administered to treated patients, significantly reduced the number and size of macroscopic KS-like angioproliferative lesions present at the injection site and in the absence of toxicity.\textsuperscript{4} In particular, both PIs promoted the formation of a large central necrotic area and a marked reduction of neo-formed vessels, oedema and spindle cell infiltration compared with mice treated with saline. These data indicated a block by HIV PIs of angiogenesis induced by bFGF and VEGF released by KS cells. This was confirmed by blocking, with indinavir or saquinavir, the formation of angiogenic lesions induced in nude mice by the direct inoculation of recombinant bFGF, or VEGF and bFGF combined.\textsuperscript{4} Moreover, bFGF- or VEGF-induced angiogenesis was also efficiently inhibited by both PIs in the chicken chorioallantoic membrane, which is a well known \textit{in vivo} assay to measure angiogenesis.\textsuperscript{4}

Angiogenesis requires sequential steps, including invasion, migration and proliferation of endothelial cells and accessory cells, such as smooth muscle cells. These steps, in turn, depend on the concurrent degradation of the basement membrane and the extracellular matrix (ECM) by matrix metalloproteases (MMPs).\textsuperscript{23} In particular, MMP-2 plays a major role in both basement membrane and ECM degradation and, in fact, is highly expressed in all forms of KS and solid tumours and is induced by bFGF.\textsuperscript{24} Moreover, invasion, migration and proliferation of tumour cells, and ECM degradation by MMPs, are also required for the growth, infiltration and metastasis of all tumours.\textsuperscript{23}

We found that indinavir or saquinavir had no effect on bFGF-promoted proliferation, basal growth or survival of macrovascular and microvascular endothelial cells, smooth
muscle cells or primary KS cells. However, they totally blocked bFGF-induced invasion of a reconstituted basement membrane by all these cell types.4 Strikingly, this was due to a block by both PIs of the proteolytic conversion of latent MMP-2 into its active form, a process that is mediated by membrane type 1 (MT1)-MMP, another MMP that acts as the major activator of MMP-2.4

Furthermore, preliminary data indicate that PIs are also effective in inhibiting tumour growth, tumour-associated angiogenesis and tumour cell invasion in other xenograft human tumour models, including lung and breast adenocarcinoma, colon carcinoma and tumours of haematopoietic cell origin such as myelomonocytic or T-cell leukaemia and Burkitt lymphoma (B. Ensoli, unpublished data).

These data are of great relevance for the management of HIV-infected individuals at high risk of KS or NHLs, particularly in view of the current trend to substitute PIs with non-nucleoside reverse transcriptase inhibitors in HAART. Furthermore, in light of their anti-tumour actions, HIV PIs should also be evaluated for the therapy of KS and other tumours in seronegative individuals. On the basis of these data, controlled clinical trials are due to start in Italy to determine whether HIV PIs alone or in combination with cytotoxic drugs may have a favourable therapeutic index in HIV-negative or seropositive patients with KS or other tumours.

The studies aimed at explaining the unpredicted effects of HAART have expanded enormously the number of HIV PI targets and the complexity of their actions, rather than group them into a few understandable mechanisms. Nevertheless, two major mechanisms of action can be delineated on the basis of the available data. One is based on the finding that HIV PIs can modulate the activity of the cell proteasome.9,15 This complex multi-subunit protease is involved in many regulatory pathways including protein turnover and degradation, antigen processing, cell cycle and apoptosis.25 In particular, by modulating the NF-κB pathway, the cell proteasome regulates inflammatory and immune responses as well as cell survival and proliferation, particularly of tumour cells.25 Effects on the cell proteasome have been invoked to explain many of the immune, immunomodulatory and anti-tumour effects of HIV PIs, and also the reduced degradation of apolipoprotein B, which may be associated with HAART-induced hyperlipidaemia.9,14,15 However, only ritonavir acts as a potent modulator of isolated proteasomes and, in living cells, this effect requires drug concentrations that are well above those reached in sera from treated patients.9,15 Moreover, although indinavir and saquinavir have only little or no effect on the proteasome activity, they have profound effects on T-cell survival and activation, IC production and tumours.5,13,16 In this regard, a recent study has indicated that proteasome modulation at therapeutic drug concentrations is effective only when PIs are used in combination with NRTIs.26 Thus, although this model may be used to explain several effects of HAART, it fails to explain many of the effects that most HIV PIs proved to exert in controlled experimental models.

Conversely, our data indicate that HIV PIs, used at therapeutic concentrations, affect pathways involved in cell invasion and MMP activity, particularly MMP-2 proteolytic activation.4 In this regard, although MMPs are the targets of anti-angiogenic and anti-tumour therapies that are being evaluated in current clinical trials, they are also known to be involved in several immune and immunomodulatory functions. In particular, MMPs are required for leucocyte transmigration and tissue infiltration by inflammatory cells. In this context, ritonavir has been shown to abolish cytotoxic T lymphocyte (CTL)-dependent inflammatory responses in a murine model of lymphocytic choriomeningitis virus infection.15 However, this effect is hardly explained by the modulation of CTL-epitope processing by the cell proteasome, as only some, but not all, epitopes are affected upon ritonavir treatment.27 Consequently, it has been suggested that ritonavir may also act by blocking enzymes required for the migration of inflammatory cells, i.e. conceivably, MMPs. Moreover, MMPs act as potent modulators of local inflammation by activating or degrading ICs and chemokines present at the cell membrane, particularly TNF-α, II-1β and IL-8.23 Evidence also indicates that MMPs may participate in antigen processing by trimming antigen epitopes.28 Furthermore, recent work has shown that the direct injection of recombinant MMPs in mice can induce DC maturation and trafficking.29 These data may explain the block of cell activation, IC production and inflammation, and the modulation of DC maturation and T-cell responses by HIV PIs. Strikingly, a single genetic deficiency in MMP-2 or MT1-MMP has been shown to lead to osteopenia and osteolysis syndromes,30 which have also been associated with PI-containing HAART.21 Finally, MMPs are known to be involved in cell apoptosis,23 thereby explaining part of the effects of HIV PIs on cell survival.

Recent work has indicated that the cell proteasome may regulate cell invasion by modulating MMP expression and/or activation.31 This suggests a connection between two major pathways targeted by HIV PIs, which may turn out to be part of an integrated network. Hopefully, the study of the effects of PIs will lead to a better understanding of the cross-talk of these pathways and will unveil the mechanisms underlying the unpredicted effects of HAART and HIV PIs. Finally, the anti-angiogenic, anti-tumour and anti-inflammatory effects of PIs, their relatively low toxicity and the large body of data on their pharmacokinetics and tissue distribution, not only opens new avenues for a rapid clinical evaluation of these drugs alone or combined with other molecules in tumour patients, but also new fields in drug discovery aimed at the synthesis of more specific drugs with a high therapeutic index.
References


Leading article

