TMC120 Blocks HIV-1 Infection in Cellular and Human Cervical Tissue Models

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Background

TMC120 is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that demonstrates potent anti HIV-1 activity, a good resistance profile and efficacy in the vaginal hu-SCID mouse model of transmission. To evaluate the potential of this compound as active ingredient in a microbicide formulation, we have investigated TMC120 as a base compound using cellular and human cervical explant models. Furthermore, we have optimized its activity through rational formulation design

Methods

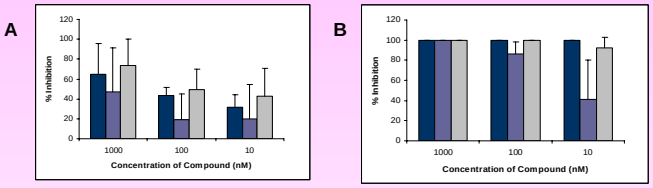
Anti HIV-1 activity of TMC120 was assessed by treatment of virus, cells, or human cervical explants with either base compound or formulated gel. Results were obtained by p24 ELISA, gp120 ELISA, quantitative PCR or reverse transcriptase assay. The effect of various concentrations of TMC120 on the viability of vaginal epithelial cell lines, cervical tissue or penile tissue was determined using MTT dye reduction assay, with Nonoxynol-9 as a control. Effects on cytokine expression following exposure of cervical tissue to TMC120 were measured using Bioluminex.

Table 1: TMC120 inhibits both X4 and R5 strains of HIV-1

	Inhibitory Concentration 50%
HIV-1 Rf	2.8 nM
HIV-1 BaL	3.5 nM

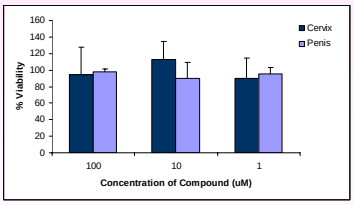
Data represents the inhibitory concentration 50% of TMC120 against HIV-1 in a cell based assay. Virus was treated with compound for 1 hour prior to subsequent culture with jurkat-tat/CCR5 cells. HIV-1 replication was determined by reverse transcriptase measurement.

Figure 1: TMC120 shows inhibition of HIV-1 infection of cervical tissue and transfer of virus by migratory cells in the presence of semen and cervical fluid simulant.



Cervical explants were exposed to HIV-1 BaL (2h,37ºC) in the presence of compound alone (■), compound and semen (■) or compound and cervical fluid simulant (■). Compound, virus and semen/cervical fluid simulant were removed by washing. Following overnight culture, the explants were transferred to fresh culture plates (A) and any cells which had migrated from the tissue were co-cultured with the T cell line PM-1 (B). Viral infection is expressed as % of control infection (p24 antigen) and data represents the mean of 3 independent experiments from separate tissue donors, using n=3 replicates.

Figure 2: TMC120 shows no toxicity at therapeutic levels



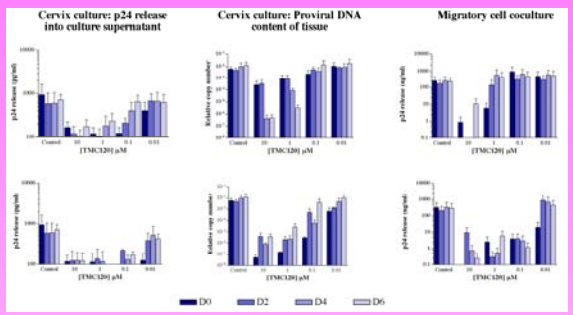
Cervical or penile tissue explants were treated with TMC120 (2hrs, 37º C) then compound removed by washing. Toxicity was assessed 24 hours post exposure by MTT dye reduction assay. Viability is expressed as a percentage of untreated control. Data shown represent the mean of 3 independent experiments.

Table 2: TMC120 does not modulate cytokine production

IL-6	IL-8	MCP-1	IL-1b
MIG	MIP-1a	IL-16	IL-1a

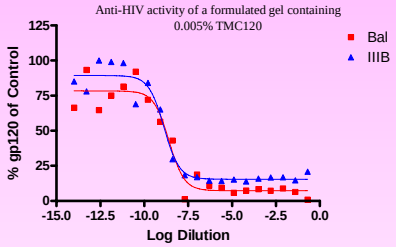
Cervical explants were exposed to 1000nM TMC120 (2h,37ºC). Following compound removal, explants were transferred to fresh tissue culture plates, and cytokine levels measured 24 hours post exposure, by Bioluminex. The cytokines listed were screened for, and no changes in expression levels were observed between treated and untreated explant supernatants.

Figure 3: TMC120 inhibits both HIV-1 BaL infection of cervical tissue and transfer of virus by migratory cells up to 6 days post drug treatment



Cervical explants were treated with TMC120 for 2 or 24 hours. Following compound removal, explants were transferred to fresh tissue culture plates. Explants were exposed to HIV-1 BaL (2h, 37ºC) on Day 0, 2, 4 or 6 post compound treatment. After virus was removed, explants were cultured overnight prior to transfer to fresh culture plates. Any cells which had migrated from the tissue were co-cultured with the T cell line PM-1. Data represents the mean of 3 independent experiments from separate tissue donors, using n=3 replicates.

Figure 4: Optimization of TMC120 formulation greatly increases activity of the compound



A clear gel containing 0.005% TMC120 was formulated and assessed for toxicity and anti-HIV activity. Toxicity of the compound was determined by the XTT-formazan method and found to indicate compatibility with T cell lines and cervical explant tissue. This formulation demonstrated a selectivity index of > $1x10^8$.

Conclusions

TMC120 exhibits potent activity against both X4 and R5 isolates in cell based assays.

TMC120 shows no toxicity at therapeutic levels in cell based assays and in cervical and penile explant models.

The presence of semen and cervical fluid simulant has little effect on the efficacy of TMC120.

TMC120 demonstrates good activity against HIV-1 BaL infection of cervical tissue, and prevents HIV-1 transfer from migratory cells to co-culture T cells. In addition, significant memory effect is shown in both cervical tissue and migratory cells, when challenged with virus up to 6 days post drug treatment.

Optimization of TMC120 formulation greatly increases activity of the compound providing a selectivity index of >1 in a billion.

There was no change in expression of listed cytokines, following exposure of cervical tissue to TMC120.

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