

Thymidine kinase and deoxycytidine kinase in HIV-infected children

Francesca Falasca¹, Paola Maida¹, Arianna Boni¹, Stefania Bernardi², Laura Cursi², Bambina Rizzo¹, Guido Antonelli¹, Guido Castelli Gattinara², Ombretta Turriziani¹

¹Department of Experimental Medicine, Virology Section, "Sapienza" University of Rome;

²Pediatric Division "Bambino Gesù" Children Hospital, Rome, Italy

SUMMARY

It has been demonstrated that HIV infection may affect the levels of thymidine kinase (TK) and deoxycytidine kinase (dCK) in peripheral blood mononuclear cells from HIV infected adults. The aim of this study was to examine the effect of HIV infection and/or antiretroviral therapy on the activity of the above enzymes in HIV-infected children.

The results showed that an inter-individual variability in TK and dCK activities does exist in both HIV infected and uninfected children. TK and dCK levels in PBMC from HIV infected and non infected children did not significantly differ. Furthermore, the therapeutic regimen, including zidovudine, does not seem to affect TK activity.

KEY WORDS: Thymidine kinase, Deoxycytidine kinase and HIV

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Heterogeneity in the response to antiretroviral treatment for HIV infection has been observed and it is currently attributed to virological, immunological and pharmacological factors (Antonelli *et al.*, 1996; Fletcher, 1999; Yeni *et al.*, 2002; Hirsch *et al.*, 2003; Turriziani *et al.*, 2005; Boffito 2007).

From a pharmacological point of view, it is well known that the antiviral activity of the different treatment drugs strictly depends on their concentration at the site of action (Holford *et al.*, 1981). Indeed, differences in individual ability to metabolize drugs may influence systemic and intracellular drug levels, and may possibly result in variability of the response to antiretroviral therapy (Fletcher, 1999; Fletcher *et al.*, 2002; Turriziani *et al.*, 2004a). In the case of nucleoside reverse transcriptase inhibitor (NRTI) the variability could be due not only to the systemic concentration of the parent drug but also to the

activity of the enzymes involved in the dideoxynucleoside phosphorylation which depends on the activation state of the target cells. The active form of NRTI is not the parent drug but the anabolite triphosphate that is formed intracellularly by a phosphorylation process mediated by cellular kinases. Specifically, zidovudine and stavudine are converted to the 5-monophosphate level by cytosolic thymidine kinase (TK), a cell cycle-regulated enzyme (Balzarini *et al.*, 1989). The activity of this cellular enzyme fluctuates with DNA synthesis, being high in dividing cells and low in quiescent cells. In comparison, the deoxycytidine (dCyd) analogues, whose phosphorylation is catalysed by cytosolic dCyd kinase (dCK), show an antiviral activity independent from the cellular cycle and expressed both in resting and stimulated lymphocytes (Gao *et al.*, 1994; Shirasaka *et al.*, 1995; Piliero, 2004; Turriziani *et al.*, 2005).

A previous study reported that a marked range of inter-individual variability of TK and dCK activities is expressed in PBMC of HIV-infected individuals but not in healthy donors. In adults, TK expression is greater in HIV-infected individuals than in non infected volunteers (Turriziani, *et al.*, 2005). It has also been reported that patients

Corresponding author

Ombretta Turriziani

Department of Experimental Medicine,
Virology Section, "Sapienza" University of Rome,
Viale di Porta Tiburtina, 28 - 00185 Rome, Italy
Email: ombretta.turriziani@uniroma1.it

treated with zidovudine (ZDV) containing regimens showed a significant reduction in TK activity compared to naïve patients (Turriziani, *et al.*, 2004b).

Since all the above findings have been demonstrated in adults, the aim of this study was to evaluate whether HIV infection, as well as a treatment with ZDV, might affect the activity of the above specific cellular enzymes also in HIV-infected children.

Specifically, samples of peripheral blood mononuclear cells (PBMC) were isolated from 28 HIV-infected children [14 female and 14 male, median age 9.0 years (range: 2-16 years)] and 6 HIV exposed uninfected children [all male, median age 1.25 years (range: 0.25-6 years)]. On the basis of their treatment, patients were divided into three groups. Group 1: 11 patients treated with a ZDV containing regimen (median period 5 years; range: 2-7 years); group 2: 12 patients on ARV not including ZDV (median period 1.5 year; range: 1-4 years); group 3: 5 patients not treated at the time of the blood sample collection.

PBMC were isolated from venous blood and cells were pelleted and sonicated. TK and dCK activity were measured in PBMC extracts using [3 H]-thymidine (Thd) (Amersham Biosciences - Milan, Italy) and [3 H]-ZDV (Moravek Biochemical-Brea, CA) as substrate for TK and [3 H]-dCyd (Amersham Biosciences- Milan, Italy) as a substrate for dCK. The assay was performed using a method detailed elsewhere (Antonelli *et al.*, 1996). A moderate interindividual variability of TK activity, versus Thd, exists in both HIV-infected children and in uninfected children. Specifically, for HIV-infected individuals, the highest variability was detected in group 2 with a coefficient of variability (CV) of 73% and the lowest CV was detected in group 1 (38%). The inter-individual variability was also observed when ZDV was used as substrate. In this case the highest variability was detected in group 1 with a CV of 81.5% and the lowest CV was detected in seronegative children (32%). As far as dCK activity is concerned, an inter-individual variability was also observed, and the extent of variability was higher than that detected for TK. In fact, the highest CV, observed in control group, was 91.4% and the lowest was 30.4 (group 3).

TK activity as well as dCK activity did not significantly differ between infected and non infected

children, and no difference was observed between TK activity in PBMC from children treated with ZDV and children treated with other non thymidine containing ART. In the same subjects there was no correlation between the levels of enzymatic activity of both enzymes and sex, age, viral load and CD4 cells counts.

All together the data suggest that an inter-individual variability of TK and dCK does exist both in infected and uninfected children. On the contrary, in the adult population such a variability was observed only in HIV-infected individuals (Turriziani *et al.*, 2005). Furthermore, contrary to what has been recorded in adults (Turriziani *et al.*, 2005) HIV infection "status" does not seem able to up-regulate the TK activity in HIV-infected children.

It should be emphasized however that in children a "true" naïve patient does not exist. In fact the enzymatic activity of PBMC from non-infected children has been compared with that of PBMC from previously-treated HIV-infected children who were not being treated at the time of the sample collection. Then, HIV infection "status" might have been modulated by previous therapy and this could have reduced the ability of HIV to up-regulate TK activity. Additionally, the HIV non-infected group was represented by infants born from seropositive mothers and treated with ZDV at birth and it cannot be excluded that the activity of cellular enzymes might have been affected by this treatment.

It should also be mentioned that the non-infected group of children included a few subjects whose median age is 1.25 years, while in the other groups the median age was 7-9 years. However while the number could be important for draw definite conclusions, apparently the age should not be taken into account if one considers that no correlation was found between age and activity of TK and dCK.

The above finding is consistent with the observation that the levels of proliferation and other important parameters of lymphocyte activation, such as IL-2 and IFN- gamma production, by cells from HIV-infected children were indistinguishable from those by cells of control adults (McClosely *et al.*, 2004), thus suggesting that the level of examined enzymes might be similar, too. Another important difference with previous studies is that here no TK activity reduction was ob-

served in children treated with ZDV-containing regimens while in adults PBMC from zidovudine-treated patients showed a significant reduction in TK activity compared to naive patients (Turriziani *et al.*, 2004b). Although the clinical significance of these results has to be established, it can be speculated that in children the phosphorylation of ZDV may occur even in the presence of chronic treatment with a drug known to induce an enzymatic defect at level of cellular kinases. Furthermore this finding could be related to the fact that in children HIV infection does not up-regulate TK activity and contrary to what suggested for adults, the effect of the drug on infection status did not affect TK activity.

In conclusion, we consider that although our research has major limitations, the results suggest that, in children, TK and dCK activities are unaffected either by HIV infection or by treatment, which is not what is observed in adults. This emphasizes the fact that HIV infection in children is different from that in adults, not only in terms of natural history but also in terms of possible interventions.

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