Short communication

Changes in mitochondrial RNA production in cells treated with nucleoside analogues

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Introduction

The metabolic activity of mitochondria is governed by different proteins and enzymes encoded in the nuclear genome as well as in a circular DNA molecule residing in the mitochondrial (mt) matrix [1]. Human mtDNA is a double-stranded circular molecule of 16571 base pairs encoding 13 proteins, 22 transfer RNAs (tRNAs) and two ribosomal RNAs (rRNAs) [1–3]. Mitochondrial messenger RNAs (mRNAs) (that is, mtRNAs) are completely processed and translated within the organelle by dedicated machinery made of both nuclear-encoded components (for example, mtRNA polymerase) and of mt-encoded ones (for example, tRNAs). The mt genome has a different organization from the nuclear one: genes do not contain introns, they are separated by small spacers and the genome is transcribed as two large polycistronic units from both template DNA strands [heavy (H) and light (L)] [4–6]. The endonuclease that processes these large molecules gives origin to all functional mtRNA species.

Very few data exist on the effects of antiretrovirals on the functionality of mtDNA in terms of RNA transcription [7] and, to our knowledge, studies on human cells are lacking. To investigate this aspect, we have developed a new method for quantifying three different mt transcripts in human cells: ND1, encoding for the subunit 1 of the NADH-dehydrogenase complex and lying at the 5′ end of the H strand; CYTB, encoding for the cytochrome B and lying at the 3′ end of the H strand; and ND6, encoding for the subunit 6 of the NADH-dehydrogenase complex and being the only mRNA encoded by the L strand (5′ end). We have evaluated the effects of different nucleoside reverse transcriptase inhibitors (NRTIs) on mtRNA production in three human cell lines and have compared the data with those relative to the quantification of mtDNA.

Materials and methods

Cell lines and treatments

The following human cell lines were studied: U937 of monoblastic origin, and CEM and HUT78 of lymphocytic origin. Cells were kept in complete culture...
medium, that is, RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin (reagents from GIBCO Invitrogen, Carlsbad, CA, USA) at 37°C in a humidified atmosphere (5% CO₂ in air).

To analyse the effects of antiretrovirals, cells were cultured in the presence of 1–10 µM stavudine (d4T), didanosine (ddI) or zidovudine (AZT) for 7 days (all from Sigma Chemicals, St. Louis, MO, USA). Control groups consisted of the same cells cultured without treatment. After the incubation period, two aliquots of each culture were collected for the extraction of DNA or RNA. Total RNA was extracted from all samples with the RNeasy Mini Kit (Qiagen, Hilden, Germany) and 1 µg was subjected to a standard random-primed reverse transcription reaction [8,9]. DNA was obtained as described [9]. Each experiment was repeated a minimum of four times.

Results

Mtrna content in human cells

The reference DNA molecule that guarantees a fixed ratio (1:1:1:1) among amplicons is shown in Figure 1A. The control of the sequence fully confirmed the expected configuration (data not shown).

We first analysed mtRNA content in human cell lines and found that the transcription of the three genes under investigation was different (Figure 1B). HUT78 and U937 behaved similarly since ND6 was the most represented mtRNA, while this gene was expressed at a lower level in CEM cells. In all cell lines ND1 was significantly higher than CYTB.
Antiretroviral drugs alter mtRNA synthesis and mtDNA content

We investigated the capacity of therapeutic concentrations of d4T, ddI and AZT to alter mtRNA synthesis. As shown in Figure 2, 1 µM and 10 µM doses of d4T were able to decrease the synthesis of mtRNA in all cell lines. On the contrary, 1 µM (not shown) and 10 µM ddI or AZT were less efficient in provoking changes in mtRNA production. Paradoxically, in U937 cells AZT provoked a slight but significant increase in ND1 and ND6 gene transcription.

We then investigated the capacity of d4T, ddI and AZT to alter mtDNA content (Figure 2). We found that d4T, at the 10 µM dose only, had a significant effect on U937 and CEM but not on HUT78 cells; 10 µM AZT paradoxically increased mtDNA content in HUT78 cells; ddI was ineffective (not shown).

Discussion

The new method we have developed for the quantification of mtRNA revealed that NRTIs alter mtRNA production. We observed that mtRNA content has a consistent variability in the cells we have studied and that mt genes are expressed at different levels. This could be due to the fact that such cell lines have a different number of organelles and amount of mtDNA [12]. Moreover, mtRNA polymerase transcribes both template DNA strands (H and L) as large polycistronic units. Theoretically, this would lead to the transcription of an equal number of ND1 and CYTB RNAs, both being encoded on the H strand. However, the amount of each RNA is determined not only by its transcription (presumably occurring at the same rate for ND1 and CYTB genes) but also by its degradation,
which probably occurs at different rates according to the intrinsic stability of each transcript and to the cellular functional microenvironment. Additional studies are needed to further investigate this aspect. Several in vitro studies have shown that NRTIs such as d4T, ddI or AZT are able to impair mt functionality, though with different efficiency [12–14]. We studied their capability of altering mtRNA synthesis and found that d4T was significantly more effective than ddI and AZT in decreasing synthesis of mtRNA. At a high concentration, d4T was capable of altering mtDNA content in two out of the three cell lines we have investigated, while mtRNA was altered in all three cases. Changes in mt functionality caused by a low concentration of d4T were revealed only by the quantification of mtRNA and were evident in U937 cells that maintained a normal mtDNA content. This indicates that the assay we have developed can provide additional information to that given by the quantification of mtDNA only.

Alterations in mtRNA production after treatment with antiretrovirals are not only quantitative but also qualitative. One of the causes could lie in the activity of mtRNA polymerase. Though the mt genome is quite small, there is no evidence that such an enzyme is always capable of reaching the end of its templates (that is, the H and the L strands). Such failure in the polymerase activity, probably occurring at low frequencies in normal conditions, could probably increase under stress caused by, for example, an NRTI. This could result in the synthesis of a polycistrionic transcript lacking a 3′ fragment of variable size, presumably containing all, or part of, the CYTB coding part (among which is ND1 but not CYTB). Clearly, this would result in an impairment of the functionality of the organelle that would not appear if one quantified mtDNA.

A relevant and growing interest currently exists in studies on the mitochondrial toxicity of antiretroviral drugs for its consistent clinical implications [15–24]. The method we have developed can be used in a variety of situations where the analysis of mtDNA functionality, in terms of quantification of different mtRNAs, is of interest.

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