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Uridine pharmacokinetics of mitocnol, a sugar cane extract

In-vitro and limited in-vivo data suggest that uridine may be beneficial in preventing and treating the mitochondrial toxicity of pyrimidine nucleoside analogue reverse transcriptase inhibitors (NRTI). For example, the supplementation of uridine to hepatocytes exposed to pyrimidine analogues has been shown to prevent and treat mitochondrial DNA depletion and all its consequences on mitochondrial metabolism, cell survival and function [1]. Similarly, uridine also completely abrogated the lipoatrophic phenotype of adipocytes exposed to stavudine by preventing apoptosis, the loss of lipids, mtDNA depletion and mitochondrial depolarization [2]. Uridine rescued neuronal cells exposed to zalcitabine [3], and was also beneficial in both anaemia [4,5] and lipoatrophy related to zidovudine [2]. The competition of uridine or its metabolites with NRTI, either at gamma-polymerase or at enzymes responsible for NRTI activation and transport is the most plausible mechanism of action [1]. Depending on the system studied, uridine was effective at concentrations of 50–200 μM [1,4].

We have recently described an HIV patient with stavudine-related hyperlactatemia, steatohepatitis, and symptomatic elevation of creatine kinase, who rapidly improved under treatment with NucleomaxX [6]. NucleomaxX is a food supplement that contains mitocnol, a sugar cane extract with a high content (17%) of nucleosides (www.nucleomaxx.com). Because the exact effects of mitocnol consumption on the serum levels of uridine are not known in humans, we conducted a 24 h pharmacokinetic study.

After ethics committee approval and informed written consent, healthy, fasting, adult human probands (four men and four women) consumed NucleomaxX by drinking 200 ml orange juice, in which one sachet (36 g) of the dietary supplement was dissolved. Baseline serum levels of uridine were measured by high-pressure liquid chromatography [7] before drinking NucleomaxX, and during the following 24 h (Fig. 1).

Mean (± SD) uridine serum levels at baseline were 5.6 ± 1.1 μM (men 5.8 μM, women 5.4 μM). After NucleomaxX consumption, uridine serum levels rose sharply and peaked after 1.3 h. The mean maximal uridine serum concentration (Cmax) was 152.0 μM (± 29.2 μM). The Cmax range was 116.0–212.0 μM. The mean Cmax in women was slightly but nonsignificantly higher (165.4 ± 35.8 μM) compared with men (138.6 ± 15.2 μM), possibly because of the lower body weight and body surface area of the former, (mean body weight and body surface area of women: 62.3 kg and 1.71 m²) compared with the latter (men: 77.5 kg and 1.96 m², respectively). Uridine was eliminated from the serum with an initial half-life of 2 h and a terminal half-life of 11.1 h. After 8 and 24 h, the mean uridine serum levels were 19.3 μM (± 4.7 μM) and 7.5 μM (± 1.6 μM), respectively. The mean area under the curve calculated using the linear trapezoidal rule between the timepoints in the time data range was 736 μM h (± 95 μM h) and was identical between the sexes. Adverse events were not observed.

We concluded that mitocnol effectively increases uridine levels in human serum. Randomized, placebo-controlled trials are currently testing NucleomaxX in order to evaluate its efficacy and safety in the prevention of NRTI-induced lipoatrophy in HIV patients.

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Iatrogenic Cushing’s syndrome in an HIV-infected patient treated with ritonavir and inhaled fluticasone

A 27-year-old woman was diagnosed as HIV positive in 1995. She was seen 10 weeks post-partum complaining of weight gain. Her medical history included asthma (treated with inhaled fluticasone and salmeterol) and infection with hepatitis B and hepatitis C. She was a previous user of intravenous drugs and was on a maintenance programme of methadone and benzodiazepines. She had previously been poorly compliant with antiretroviral therapy, and was resistant to nucleoside and non-nucleoside reverse transcriptase inhibitors because of K65R, M184V and K103 reverse transcriptase mutations.

During her pregnancy she had become more compliant with therapy, and as a result her HIV-RNA level was undetectable and the CD4 cell count was 225 cells/dl. Her therapy at presentation to the clinic included combination lopinavir and ritonavir (total daily dose of 800 and 200 mg, respectively), saquinavir 1 g twice a day, seretide 250/25 inhaler (a combination of fluticasone and salmeterol giving a total daily dose of fluticasone of 1000 µg a day) and maintenance methadone.

Along with the history of recent weight gain, the clinical findings (Fig. 1) were consistent with hypercortisolism, and included mild proximal myopathy, central adiposity (body mass index 27 kg/m²), the presence of a dorsocervical fat pad, and violaceous abdominal and axillary striae. There was biochemical evidence of suppression of the hypothalamic–pituitary–adrenal axis, with an undetectable morning plasma cortisol level (< 30 nmol/l, reference range 200–600), suppressed plasma adrenocorticotropic hormone (0.8 pmol/l, reference range 2–10), and an inadequate response to synacthen with a plasma cortisol level of 144 nmol/l 30 min after intramuscular injection (normal response > 550 nmol/l).

Fluticasone was stopped, and low-dose physiological replacement oral glucocorticoid therapy was instituted until her endogenous adrenal function recovered.

Ritonavir is a potent inhibitor of cytochrome P450-3A4 (CYP450-3A4), and may increase the bioavailability of

Fig. 1. The central adiposity and prominent striae in an HIV-infected patient with iatrogenic Cushing’s syndrome secondary to ritonavir and inhaled fluticasone.

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drugs metabolized by this pathway [6]. This effect is beneficially exploited using low-dose ritonavir combined with other protease inhibitors (PI) to improve plasma levels (e.g. lopinavir/ritonavir). Fluticasone, a potent glucocorticoid for the control of asthma and allergic rhinitis, has low oral bioavailability (< 1%) and is metabolized by CYP450-3A4 [7]. Compared with other inhaled glucocorticoids, fluticasone is highly lipophilic, resulting in a large volume of distribution and greater elimination half-life at steady state. The addition of ritonavir to fluticasone may thus increase the bioavailability and lead to systemic complications.

In this patient the clinical features of Cushing’s syndrome only developed post-partum. Improved compliance with medical therapy during and after pregnancy was probably a contributing factor. More importantly, recent pharmacokinetic data have shown reduced plasma levels of ritonavir during pregnancy, with increased levels by 6 weeks post-partum [8]. Therefore, it is likely that despite her improved compliance, the altered pharmacokinetics of ritonavir resulting from the normal physiological changes of pregnancy prevented a significant interaction with fluticasone at that time. It was only post-partum, when ritonavir levels have been documented to increase (presumably accompanied by more potent CYP450-3A4 inhibition), that she developed the clinical features of Cushing’s syndrome and the biochemical findings of hypothalamic–pituitary–adrenal axis suppression.

This case not only highlights the great potential for drug interactions in patients with HIV, but also emphasizes the added complexity of physiological changes in pregnancy. As an increasing number of HIV-infected patients are given low-dose ritonavir-enhanced PI-containing regimes, physicians must be aware of the potentially severe interactions between antiretroviral therapy and other drugs. If long-term administration of glucocorticoids is required to control asthma in patients treated with PI, then less systemically available glucocorticoids may be preferable to fluticasone.

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Response to ‘Single phylogenetic reconstruction method is insufficient to clarify relationships between patient isolates in HIV-1 transmission case’ by Jenwitheesuk and Liu

Jenwitheesuk and Liu criticized the procedure we used for phylogenetic tree construction to analyse true relationships between patient HIV-1 isolates [1]. Although they agree with our statement that the HIV-1 pol gene (protease and first 239 amino acids of the reverse transcriptase) is insufficient to clarify true relationships, they recommended using at least two different methods for phylogenetic analysis, as done in other studies [2–6]. In addition, they claimed that our conclusion would have been different if other methods were used.

The only study published about phylogenetic analysis in forensic investigations of HIV-1 transmission cases in Germany used only one phylogenetic method, and the results were accepted by the German court [7,8]. We therefore performed the same procedure to analyse our data [1]. However, this does not mean that we insist on using only one phylogenetic method for analysing transmission events if there is sufficient evidence for the false classification of samples.

As suggested by Jenwitheesuk and Liu, we reanalysed our data using the following different phylogenetic methods with different seed numbers: neighbour-joining, minimum evolution, and unweighted pair group method using arithmetic averages with both the Kimura-2-parameter and the Jukes–Kantor substitution module,
and maximum parsimony implemented in the Mega 2 software [9]. In addition, we used DNAML from the PHYLIP package version 3.62 [10] for a maximum likelihood analysis.

We did not find any significant differences in the phylogenetic trees when using these different methods for phylogenetic analysis of the pol and env (C2V3 region) genes. The two sample pairs linked by the confirmed transmission event clustered in all trees with significant bootstrap values, and the sample pair with the unlikely transmission event clustered only in the pol gene analysis with significant bootstrap values. The analysis of the C2V3 region confirmed the initial results [1]. Figure 1 shows the phylogenetic tree of the C2V3 region using the maximum likelihood analysis. The sample pair R004/R016 clustered with a bootstrap value of 53, which does not support a likely transmission. None of the other C2V3 trees grouped this sample pair with significant bootstrap values; in the maximum parsimony and

Fig. 1. Phylogenetic tree of the C2V3 region. GenBank isolates are indicated by their subtype followed by accession number, local control group patient isolates are indicated by ‘R’ and consecutive numbering, and the two isolates 02-30434/02-35144 with a known transmission event (GenBank accession numbers AY878685–AY878692) are shown. Trees were generated by a bootstrap test with 100 replications based on the maximum likelihood method implemented in the PHYLIP package (DNAML). Bootstrap values are shown on the nodes.
unweighted pair group method using arithmetic averages analysis this sample pair did not cluster at all (data not shown).

The additional analysis of our data using several phylogenetic methods presented here does not alter our initial results. Furthermore, several published studies using more than one method showed that analysing possible transmission events with different phylogenetic methods resulted in the same classification of significant clusters [2–6]. In summary, we do not agree that using a single phylogenetic method is insufficient for the reconstruction of HIV-1 transmission events. However, we accept that using two methods instead of only one might yield phylogenetic analyses with greater significance.

Data deposition: The sequences reported in this response and in the paper discussed here have been deposited in the GenBank database (accession nos.AY878662–AY878692).

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Single phylogenetic reconstruction method is insufficient to clarify relationships between patient isolates in HIV-1 transmission case

The accuracy of the molecular phylogenies has important implications for the understanding of HIV-1 evolution and transmission. Most studies to date on HIV-1 transmission using phylogenetic analysis have relied on the V3 loop region of the envelope, and to a lesser degree on fragments of the gag gene. The polymerase gene has recently been considered to hold sufficient genetic variability to allow the useful study of potential routes of transmission [1].

Stürmer and colleagues [2] demonstrated the relationship of HIV-1 strains derived from two patients using a phylogenetic reconstruction technique. Two phylogenetic trees were constructed based on the polymerase (entire protease and first 293 amino acids of reverse transcriptase) and the envelope (C2V3 regions) gene sequences using the neighbor-joining method with the Kimura-2-parameter substitution module. They compared the distribution patterns of the references and the sample sequences between the two trees, and came up with the conclusion that the polymerase gene sequence could not be used on its own to provide the relationship information between patient isolates. We agree that the polymerase gene is insufficient to clarify this relationship. However, we would like to raise a few issues with the computational protocols used that may affect the interpretation of their results.

The appropriate method for phylogenetic analysis in an HIV-1 transmission case is a crucial issue that may or may not provide plausible routes of infection. We have learned from the forensic investigation of previous transmission cases that at least two phylogenetic reconstruction methods are usually utilized and are accepted by court in a pretrial hearing as meeting the criteria for the admissibility of evidence [3,4]. Several studies have suggested that many phylogenetic analysis methods, including Fitch–Margoliash, neighbor-joining, minimum evolution, maximum likelihood, maximum parsimony, an unweighted pair group method using arithmetic averages and a Fitch–Margoliash method

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assuming a molecular clock (KITSCH) should be considered to reach persuasive conclusions [3–7].

Therefore the conclusion reached by Stürmer et al. [2] using the neighbor-joining method alone is not absolutely correct, although there is no flaw in their phylogenetic reconstruction protocols. The conclusion of that study would be different if the sequences were analysed by other methods, i.e. maximum likelihood or maximum parsimony. In addition, we propose that tree reconstruction should be repeated with different seed numbers. An identical distribution pattern should be obtained regardless of the seed numbers chosen or the reconstruction method used. The consensus tree generated from different methods and seed numbers would provide stronger evidence than that derived from a single method with a single seed number.

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Identifying deaths from AIDS in South Africa: an update

On the basis of a 15% sample of death notification forms for the period 1997–2001 processed by the national statistical office, which gave the underlying cause of death as HIV for approximately 8.7% of all deaths [1], Groenewald et al. [2] suggested that the official mortality statistics for South Africa underreport HIV/AIDS deaths, with only 39% of HIV/AIDS deaths being reported as such. The national statistical office recently published a new mortality report based on all registered deaths between 1997 and 2003 [3]. In this report, deaths with the underlying cause recorded or coded as HIV account for a much smaller proportion (2.1%) of the total deaths than was the case with the 15% sample.

This discrepancy is probably mainly attributable to a change in the coding system, from one which allowed for some interpretation of the details provided on the death notification forms to a strict automated mechanistic coding practice using Automated Classification of Medical Entities software (ACME 2004.02) developed by the United States National Center for Health Statistics [4]. The strict coding practice results in AIDS cases certified with synonyms or euphemisms such as ‘retroviral disease’ or ‘acquired immunosuppression’, being classified as ‘other viral diseases’ or ‘certain disorders of the immune system’, instead of AIDS if there is no other immediate cause recorded. If the immediate cause of death (e.g. tuberculosis) is specified with these synonyms or euphemisms, this will be identified as the underlying cause, in favour of a less well specified condition.

The impact of the change in coding practice can be seen by comparing the leading causes of death from the sample data for 1997–2001 with those from the complete data for 1997–2003 (Fig. 1). The trend in HIV disease for the period 1997–2001 in the sample data is upward, increasing from approximately 4.2 to 8.5% over the period. However, according to the new coding of all death notification forms, the proportion of deaths caused by HIV remains virtually constant at 2% over the same period. In contrast, the proportion of deaths caused by tuberculosis, influenza and pneumonia and intestinal infectious diseases increase over this period, and account for a larger proportion of the deaths in the full data than in the sample. In addition, deaths caused by ‘certain disorders of the immune mechanism’ rise from 1% to approximately 2.5% over the years according to the new report, whereas there were very few (< 0.02%) in the sample data. Clearly, with the new coding system, a larger proportion of what would previously have been coded as HIV deaths have been coded to the immediate cause of death (tuberculosis, pneumonia and intestinal infectious diseases) or to an ill-defined immune disorder than was the case with the sample data. A rough estimate, without access to the detailed data, suggests that only approximately 8% of all AIDS deaths in 2002 were coded as such in the new report.

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Statistics South Africa are to be congratulated on producing a very useful report on the causes of death between 1997 and 2003, which clearly states the causes of death that are recorded on death notifications in South Africa and describes how the underlying cause of death has been coded. South Africa is among a few countries in sub-Saharan Africa that produce cause-of-death statistics. This report has fuelled the debate about the extent of mortality as a result of AIDS in South Africa, a debate that at times has overshadowed the most important finding in the report, namely, the huge increase in mortality of 57% over the period 1997–2002, concentrated in young adults, especially women, between the ages of 20 and 44 years and children less than 5 years. Only a small proportion of this increase (approximately 10%) can be explained by population growth and increased completeness of death registration (approximately 5%). Given that the number of deaths as a result of unnatural causes decreased slightly between 1997 and 2003, the increase in deaths is attributable to natural causes. The cause-of-death pattern between 1997 and 2003 demonstrates marked increases in AIDS indicator conditions, which together with evidence from the antenatal seroprevalence surveys that HIV seroprevalence has been rising steadily during the 1990s [5] strongly suggests that AIDS is responsible for this increase.

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