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HIV E TUBERCULOSE: INTERACÇÕES FARMACOLÓGICAS ENTRE NEVIRAPINA (OU EFAVIRENZ) E RIFAMPICINA/ISONIAZIDA EM INDIVÍDUOS INFECTADOS PELO HIV E COM TUBERCULOSE ACTIVA (MAPUTO, MOÇAMBIQUE, ENTRE 2007 e 2011)

RIO DE JANEIRO

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RESUMO

O presente trabalho se constitui de dois estudos clínicos realizados em Maputo, Moçambique, dos quais participaram 570 pacientes com coinfecção pelo HIV e tuberculose. Seus objetivos principais foram: a) determinar os parâmetros farmacocinéticos da rifampicina e da isoniazida na ausência e na presença da terapia antirretroviral (TARV) e b) comparar as concentrações plasmáticas da nevirapina e do efavirenz em vigência do tratamento para a tuberculose e após a sua descontinuação. Os dois estudos foram parte do ensaio clínico ANRS12146-CARINEMO, cujo objectivo foi comparar a eficácia e tolerância da terapia antiretroviral com base em nevirapina ou efavirenz quando coadministrada com a terapia padrão antituberculose. O tratamento para tuberculose foi composto por doses diárias de rifampicina e isoniazida, durante 6 meses, associadas a pirazinamida e etambutol nos dois primeiros meses. A TARV foi iniciada entre 4 e 6 semanas do tratamento para a tuberculose e os pacientes foram randomizados para receber nevirapina sem dose escalonada (200mg duas vezes ao dia) ou efavirenz (600 mg dose única diária), ambos combinados com lamivudina e estavudina. Para os estudos da farmacocinética da rifampicina e da isoniazida, as amostras de sangue foram colhidas em intervalos regulares, após a administração matinal de uma dose fixa combinada de medicamentos antituberculose. Para a avaliação da concentração pré-dose de nevirapina e da concentração 13.5 horas do efavirenz, as amostras foram colhidas na semana 12 (na presença de tratamento antituberculose) e nas semanas 36 e 48 (ao final do tratamento antituberculose), após o início da TARV, em 526 pacientes. Num subgrupo de 62 pacientes, os níveis de nevirapina e efavirenz também foram determinados nas semanas 1, 2, 3 e 4 da TARV. As doses plasmáticas de medicamentos antituberculose e antirretrovirais foram analisadas por técnicas validadas. Observou-se que os parâmetros farmacocinéticos da rifampicina e isoniazida não foram alterados de forma clinicamente significativa quando combinados com nevirapina ou efavirenz em pacientes com imunodepressão severa associada ao HIV. A evolução clínica dos pacientes foi favorável, como uma possível consequência do tratamento para a tuberculose e do aumento das contagem de células T CD4+, devido à TARV. Adicionalmente, a não utilização da dose escalonada permitiu obter níveis elevados da nevirapina no início do tratamento; no entanto, a diminuição subsequente da sua concentração foi preocupante. Por outro lado, a concentração do efavirenz foi menor após a descontinuação do tratamento da tuberculose. Nos pacientes com coinfecção HIV e TB, o regime da TARV contendo efavirenz (600 mg por dia) foi menos comprometido pelo uso concomitante da rifampicina do que o regime contendo a nevirapina (400 mg por dia, sem a dose escalonada). A baixa exposição aos medicamentos, o sexo masculino e a presença de HBsAg mostraram-se importantes fatores preditivos de falência do tratamento em pacientes que iniciam a TARV com o regime baseado em nevirapina.

Palavras-chave: HIV/AIDS, tuberculose, farmacocinética, nevirapina, efavirenz, rifampicina, isoniazida, interacção medicamentosa

ABSTRACT

This work constitutes part of two clinical studies conducted in Maputo, Mozambique, in which 570 HIV/TB coinfection patients participated. Its main objectives were: a) to determine the pharmacokinetic parameters of rifampicin and isoniazid in the presence and absence of antiretroviral therapy (ART) and b) to compare plasma concentrations of nevirapine and efavirenz in patients on treatment for tuberculosis and after its discontinuation. The two studies were substudies of ANRS12146-CARINEMO trial whose aim was to compare the efficacy and tolerability of antiretroviral therapy based on nevirapine or efavirenz when co-administered with the standard antituberculous therapy. Tuberculosis treatment was composed by daily doses of rifampicin and isoniazid for six months associated with pyrazinamide and ethambutol in the first two months. Antiretroviral therapy was initiated between 4 and 6 weeks of tuberculosis treatment and patients were randomized to receive nevirapine without lead-in dose (200mg twice daily) or efavirenz (600 mg once daily), both combined with lamivudine and stavudine. For pharmacokinetics studies of rifampicin and isoniazid, blood samples were taken at regular intervals after the morning administration of fixed dose combination antituberculosis drugs. For evaluation of pre-dose nevirapine concentration and 13.5h efavirenz concentration, samples were drawn at week 12 (on-tuberculosis treatment) and at weeks 36 and 48 (off-tuberculosis treatment) after the initiation of ART, in 526 patients. In a subgroup of 62 patients, the levels of efavirenz and nevirapine were determined at weeks 1, 2, 3 and 4 of ART. Plasma levels of antituberculosis and antiretroviral drugs were analyzed by validated techniques. We observed that rifampicin and isoniazid pharmacokinetic parameters have not been altered to a clinically significant extent, when combined with nevirapine or efavirenz in these severely immunosuppressed HIV infected patients. Clinical outcome of patients was good, possibly due to tuberculosis therapy and to the increase in CD4+T cells count with HIV treatment. In addition, omitting the leading dose allowed high nevirapine concentration at initiation of treatment; however the decrease in concentration thereafter is of concern. Conversely, efavirenz concentration was lower after discontinuation of tuberculosis treatment. In patients with concurrent HIV-1 infection and TB, antiretroviral therapy regimens containing efavirenz (600 mg per day) were less compromised by concomitant use of rifampicin than were those that contained nevirapine (400 mg per day, without lead-in dose). Low drug exposure, male sex and reactive HBsAg are important predictive factors for treatment failure in patients starting antiretroviral treatment with nevirapine based regimen.

Key words: HIV/AIDS, tuberculosis, pharmacokinetics, nevirapine, efavirenz, rifampicin, isoniazid, drug-drug interaction

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LISTA DE ABREVIATURAS

AUC	Área sob a curva de concentração plasmática em relação ao tempo
AIDS	Síndrome de Imunodeficiência Adquirida
C12h	Concentração 12 horas após a tomada de medicação
CARINEMO	Coadministração de rifampicina e nevirapina em Moçambique
Cmax	Concentração máxima
Cmin	Concentração minima
Cpd	Concentração pré-dose
CYP3A4	Citocromo P450, isoenzima 3A4
DFC	Dose fixa combinada
EFV	Efavirenz
HBsAg	Antígeno de superfície de hepatite B
HBV	Vírus de hepatite B
HIV	Vírus de Imunodeficiência Humana
INNTR	Inibidores da transcriptase reversa não nucleosídeos
INSIDA	Inquérito Nacional sobre o SIDA
INTR	Inibidores da transcriptase reversa nucleosídeos
NAT2	N-acetiltransferase tipo 2 hepática
NVP	Nevirapina
OATP1B1	Inibidor do influxo do polipeptídio transportador de ânion orgânico 1B1
OMS	Organização Mundial de Saúde
PVHS	Pessoas vivendo com HIV/SIDA
RNA	Ácido ribonucléico
TARV	Terapia antiretroviral
TB	Tuberculose
UNAIDS	*Joint United Nations Programme on HIV/AIDS*

SUMÁRIO

1.INTRODUÇÃO

1.1 HIV e tuberculose em Moçambique

Moçambique é um país situado na costa sudeste do continente africano, no Oceano Índico, com 802 mil km^2 de superfície terrestre e com uma população, em 2011, de 23.9 milhões de habitantes (World Bank, 2011). Colonizado pelos portugueses desde o século XVI, conquistou sua independência em 1975, conclusão da luta pela independência iniciada em 1964. Mesmo após a independência, outros conflitos políticos civis internos só alcançaram seu fim com o Acordo Geral de Paz, em Roma, a 4 de outubro de 1992.

Sabe-se que é um dos países com maior prevalência de Vírus de Imunodeficiência Humana (HIV) no mundo (UNAIDS, 2011). De acordo com o primeiro e único inquérito de base comunitária realizado em 2009, a prevalência de infecção por HIV encontrada na população entre os 15-49 anos foi de 11.5% (INSIDA, 2009). Em 2011, 250.508 indivíduos estavam em uso de tratamento antiretroviral (TARV), no País - representando uma cobertura de 51.7% do total 484.404 indivíduos que necessitavam da TARV - e foram reportadas cerca de 100.000 mortes relacionadas à AIDS (UNAIDS, 2011). Concomitantemente, Moçambique faz parte do grupo dos 22 países com as mais elevadas taxas mundiais de tuberculose (TB), com uma prevalência estimada de 490/100.000 habitantes, e com uma incidência de TB (todas as formas) estimada em 548/100.000 habitantes, em 2011. A prevalência de HIV em casos incidentes de TB vem aumentando ao longo dos anos (63% em 2011), mas apenas 29% dessas pessoas iniciaram a TARV. A mortalidade relacionada à TB continua a ser elevada, com uma taxa estimada de 47/100.000 habitantes, em 2011 (1).

Desde a disponibilização da TARV, em 2003, na rede pública, o regime da primeira linha usado para o tratamento do HIV foi composto pela dose fixa combinada (DFC), contendo lamivudina, estavudina e nevirapina. A estavudina foi definitivamente retirada como droga de primeira linha em junho de 2010, devido aos seus efeitos secundários, razão pela qual foi substituída pela zidovudina. Nos pacientes com coinfecção HIV/TB, as normas nacionais sempre recomendaram o início da TARV com o regime contendo o efavirenz. Em abril de 2013, Moçambique adoptou parcialmente as novas recomendações da Organização Mundial de Saúde (OMS) para o tratamento do HIV e, como consequência, a nevirapina passou a ser alternativa de primeira linha para a TARV no adulto. A actual primeira linha para o tratamento do HIV é composta pela DFC, contendo lamivudina, tenofovir DF e efavirenz. Como regra, a TARV é iniciada tanto nos pacientes com sintomas associados ao HIV, independentemente da contagem do CD4, quanto nos pacientes HIV+ assintomáticos com contagem de CD4 abaixo de 350 células/mm^3. Antes de 2010, as diretrizes nacionais para o tratamento do HIV recomendavam o início da TARV para os indivíduos nos estádios clínicos I e II quando o CD4 fosse menor que 200 células/mm^3, e nos estádios III e IV, quando menor que 350 células/mm^3. Em 2010, essas diretrizes nacionais foram revistas e adoptaram-se as recomendações da OMS: a TARV iniciava-se nos estádios clínicos I, II e III caso o CD4 fosse menor que 350, e no estádio clínico IV, independentemente da contagem de CD4.

Actualmente, como já foi dito, nos pacientes com coinfecção HIV/TB, a TARV é iniciada logo após as duas primeiras semanas de tratamento da TB, desde que seja bem tolerada. As normas nacionais recomendam seis meses de tratamento antituberculose em casos novos: nos primeiros dois meses, esse tratamento consiste na administração da DFC contendo rifampicina, isoniazida, pirazinamida e etambutol, seguidos de quatro meses com rifampicina e isoniazida em DFC. Para casos de

retratamento, o tempo de uso dos medicamentos antituberculose é de oito meses: dois meses com estreptomicina associada a rifampicina, isoniazida, pirazinamida e etambutol seguidos de um mês com rifampicina, isoniazida, pirazinamida e etambutol, e mais cinco meses de rifampicina, isoniazida e etambutol.

O presente trabalho faz parte de um ensaio clínico CARINEMO-ANRS 12146 (item 1.2.)(2). De novembro de 2007 a fevereiro de 2011 foi realizado este ensaio clínico que teve como objectivo comparar a eficácia e a segurança entre o regime TARV contendo efavirenz e o regime TARV contendo nevirapina em pacientes com coinfecção HIV/TB, na cidade de Maputo (ANRS-CARINEMO 12146). A seguir, nos itens 1.3. e 1.4., introduzimos o leitor aos temas dos dois capítulos do nosso estudo propriamente dito: "A influência dos medicamentos antituberculose nas concentrações plasmáticas de nevirapina e efavirenz em pacientes com coinfecção HIV/TB" (item 1.3.) e "A influência da nevirapina e do efavirenz nas concentrações plasmáticas da rifampicina e da isoniazida em pacientes com coinfecção HIV/TB" (item 1.4).

1.2. Ensaio Clínico CARINEMO-ANRS 12146

O ensaio CARINEMO, de fase III, randomizado, de não-inferioridade, foi realizado em três unidades sanitárias, localizadas em três diferentes bairros da cidade de Maputo: o Centro de Saúde de Mavalane, o Centro de Saúde de Alto Maé e o Centro de Saúde de José Macamo. Foram incluídos 570 indivíduos com coinfecção HIV/TB, com idade maior ou igual a 18 anos, virgens de TARV, em tratamento da TB por, no mínimo, 4 semanas. Para essa inclusão, era necessário que apresentassem CD4 menor que 250/mm^3, índice de Karnofsky acima de 60%, teste de gravidez negativo, níveis de alanina aminotransferase (ALT) e bilirrubina total <5 LSN (menor que grau III) e ausência de eventos adversos clínicos e laboratoriais de grau IV.

Todos os pacientes incluídos neste estudo receberam tratamento da TB de acordo com as normas nacionais, já apresentadas. Os pacientes foram randomizados entre 4 e 6 semanas de tratamento da TB para receberem TARV contendo o efavirenz (600mg) ou nevirapina (400mg, sem a dose escalonada), ambos associados a lamivudina e estavudina. Cada indivíduo incluído foi seguido durante 48 semanas com avaliação clínica e laboratorial regular. A carga viral de HIV-1 e os níveis plasmáticos da nevirapina e efavirenz foram avaliados nas semanas 12, 24, 36 e 48; a contagem de células CD4 foi determinada antes de início da TARV e nas semanas 24 e 48 de seguimento.

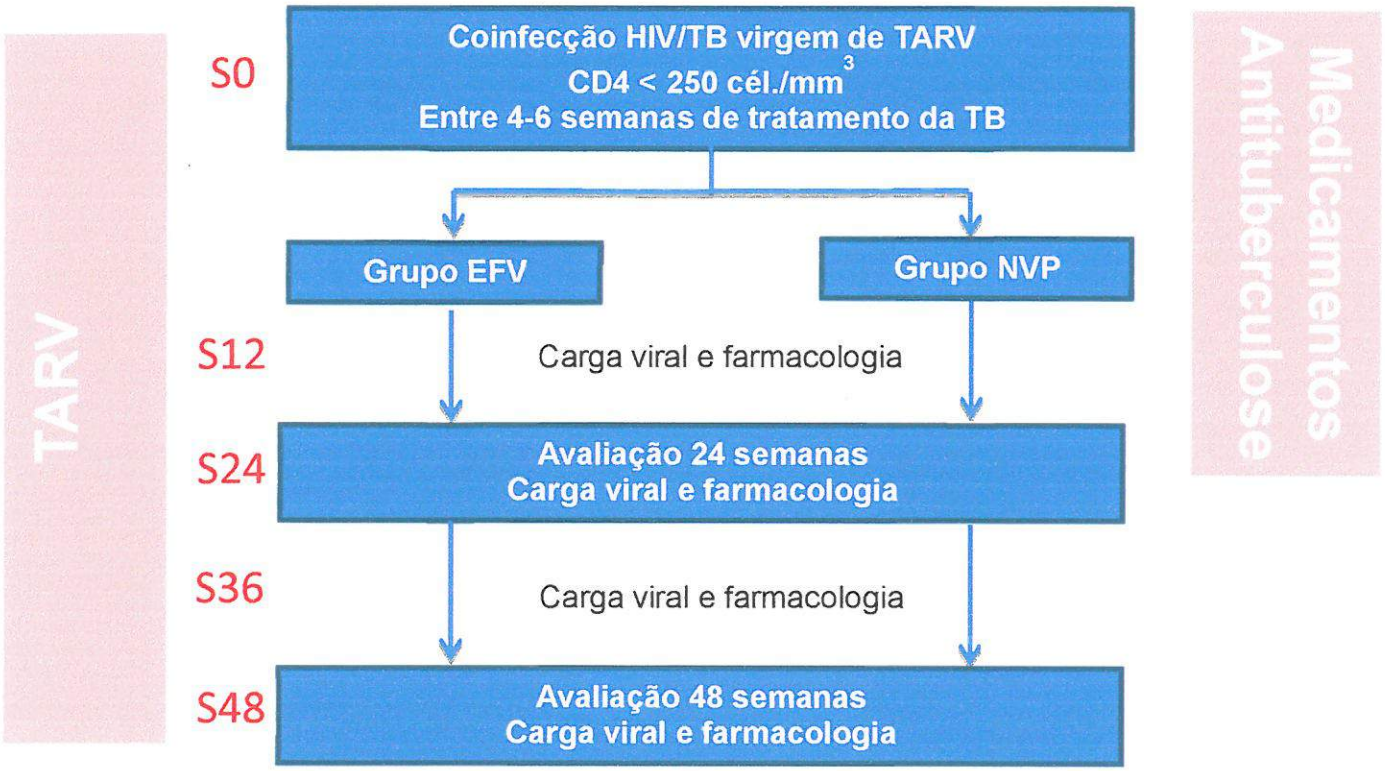

Figura 1. Desenho do ensaio clínico CARINEMO-ANRS 12146

Dos 285 pacientes incluídos em cada braço terapêutico, 242 (84.9%) do braço nevirapina e 233 (81.7%) do braço efavirenz completaram as 48 semanas de seguimento no estudo. Respectivamente, 43.9% e 40.0% dos pacientes nos braços nevirapina e efavirenz eram do sexo feminino. A idade mediana foi de 33 anos nos dois braços terapêuticos. Foi diagnosticada TB pulmonar em 80.0% (35% com baciloscopia positiva) e 77.0% (42% com baciloscopia positiva) dos casos nos braços da nevirapina e efavirenz, respectivamente. As medianas de CD4 e de carga viral (RNA HIV-1) foram, respectivamente, de 92 células/mm^3 e 5.7 log no braço da nevirapina e 86 células/mm^3 e 5.5 log no braço de efavirenz. O antígeno de superfície (HBsAg) do Vírus da Hepatite B (HBV) foi detectado em 21% e 22.0% dos pacientes nos braços nevirapina e efavirenz, respectivamente. Na tabela 1 são apresentados os resultados primários e secundários.

O ensaio CARINEMO evidenciou que a eficácia da nevirapina é inferior à do efavirenz para o tratamento de indivíduos coinfectados por HIV/TB, em tratamento antituberculose com regime contendo a rifampicina. No entanto, ainda ficaram algumas lacunas relativas a essa diferença encontrada na eficácia da TARV, que seriam respondidas com o estudo de uma potencial interação entre antirretrovirais e tuberculostáticos na mesma população. Assim, acreditamos que o nosso trabalho venha a responder a questões como o impacto do tratamento da tuberculose na eficácia terapêutica dos dois regimes de ARVs comummente usados e a eventual variação nas concentrações plasmáticas do próprio tratamento da tuberculose sob a influência dos ARVs em questão (nevirapina e efavirenz).

Tabela 1. Resultados primários e secundários do ensaio clínico CARINEMO

	NVP			EFV			Δ		
	n/N	%	IC 95%	n/N	%	IC 95%	%	Unilateral 95%CI	Valor P
Análise primária									
ITT-análise principal	184/285	64.6	58.7-70.1	199/285	69.8	64.1-75.1	5.3	11.7	
ITT-mudança=falência	171/285	60	54.1-65.7	195/285	68.4	62.7-73.8	8.4	15.0	
PP- análise principal	170/243	70	63.8-75.7	194/246	78.9	73.2-83.8	8.9	15.4	
Análise secundária									
Óbito	18/285	6.3	3.8-9.8	16/285	5.6	3.2-9.0			0.72
HIV1-RNA< 50cp/mL*	170/221	76.9	70.8-82.3	194/222	87.4	82.3-91.4			0.004
HIV1-RNA< 400cp/mL	189/285	66.3	60.5-71.8	203/285	71.2	65.6-76.4			0.21
Ganho CD4 ≥ 20%	223/285	78.2	73.0-82.9	213/285	74.7	69.3-79.7			0.32
Eventos definidores de SIDA	19/285	6.7	4.1-10.2	22/285	7.7	4.9-11.4			0.63

* População PP com exclusão de óbitos e abandonos

A taxa de sucesso de tratamento da TB (curados + tratamento completo) foi de 91.2% (260/285) e 90.3% (260/288) nos braços nevirapina e efavirenz, respectivamente. Observaram-se eventos adversos sérios em 18 (6.3%) pacientes do braço nevirapina e 17 (5.9%) pacientes do braço efavirenz. Houve diagnóstico laboratorial de hepatite severa em 20 (7%) e 17 (5.9%) pacientes dos braços NVP e EFV, respectivamente. Foi evidenciado *rash* cutâneo severo (grau≥3) em três pacientes no braço nevirapina e em um paciente no braço de efavirenz.

A não inferioridade do regime TARV contendo nevirapina em termos da eficácia terapêutica não foi demonstrada quando comparada ao regime TARV contendo efavirenz nos pacientes com coinfecção HIV/TB em terapia com rifampicina. O uso da nevirapina sem a dose escalonada foi bem tolerado. Com base nestes dados, não se recomenda o uso sistemático do regime TARV contendo nevirapina nos pacientes com coinfecção VIH/TB em terapia com rifampicina. No entanto, devido à eficácia relativamente boa e à boa tolerância ao regime TARV contendo nevirapina, o uso da nevirapina sem dose escalonada pode ser uma alternativa segura nos pacientes com intolerância ou com contraindicação ao efavirenz.

1.3 A influência dos medicamentos antituberculose nas concentrações plasmáticas de nevirapina e efavirenz em pacientes com coinfecção HIV/TB

A TB é a infecção oportunista mais frequente e a principal causa de mortalidade em pacientes que vivem com HIV a nível mundial. Em 2011, entre 8.7 milhões de casos incidentes de TB no contexto mundial, 1.1 milhão foram notificados em indivíduos com HIV, e 1.4 milhão de mortes foram atribuídas à TB, entre as quais, 430.000 mortes em indivíduos com HIV (3, 4). África subsaariana continua a apresentar o maior peso de pessoas que vivem com o HIV e TB, com 79% dos casos de coinfecção HIV/TB reportados em 2011. Além disso, o HIV é um dos factores de risco mais importantes para o desenvolvimento de TB activa; em alguns países cerca 82% de indivíduos com TB apresentam coinfecção pelo HIV (1).

A taxa de letalidade global estimada de tuberculose em indivíduos infectados pelo HIV é de cerca de 40%, e pode ser acima de 50% em muitos países em desenvolvimento (1, 5). Nos pacientes com coinfecção HIV/TB, as mortes ocorridas nos

primeiros meses de tratamento da TB são atribuíveis à TB, e as mortes que ocorrem mais tarde, ainda durante o tratamento da TB, são atribuíveis à progressão da doença de base (6-8), no caso, a AIDS. O TARV reduz em 65% a incidência da TB dos pacientes infectados pelo HIV, independentemente do valor de CD4+ (9), e reduz a mortalidade dos pacientes coinfectados por HIV/TB (5). Em 2011, no mundo, 48% dos indivíduos com TB activa iniciaram TARV e 1.3 milhões de mortes foram prevenidas entre 2005 e 2011 através da implementação de actividades colaborativas entre profissionais dos sectores da TB e HIV (4).

Em Moçambique, a primeira linha de TARV consiste em dois inibidores da transcriptase reversa nucleosídeos (INTR) associados a um inibidor da transcriptase reversa não nucleosídeo (INNTR), seja este, efavirenz ou nevirapina. Muitos países com recursos limitados usam as combinações de DFC: genéricos de baixo custo contendo efavirenz ou nevirapina. Em países da África subsaariana, aproximadamente entre 50-80% dos pacientes com tuberculose pulmonar com baciloscopia positiva são coinfectados pelo HIV (10). A OMS recomenda o início da TARV em todos os indivíduos infectados pelo HIV com tuberculose activa, independentemente da contagem de células CD4+. Para esses pacientes, o início do tratamento da TB deve ser anterior ao início da TARV (cujo regime deve conter como INNTR preferido o efevirenz). Em pacientes que iniciam a TARV durante o tratamento da TB, a OMS recomenda ainda que a TARV seja iniciada logo após as duas primeiras semanas (e não mais de 8 semanas, como até 2012) de tratamento da TB, desde que o tratamento da TB seja bem tolerado (11).

A rifampicina é um potente indutor de várias isoenzimas hepáticas do citocromo P450, principalmente a isoenzima 3A4 (CYP3A4). A rifampicina altera o metabolismo da

nevirapina e do efavirenz, levando à redução da exposição dos indivíduos aos INNTR. No entanto, os estudos clínicos demonstraram que o efavirenz pode ser usado em doentes que recebem 600 mg de rifampicina sem que haja o compromisso da sua eficácia terapêutica (12-15). A indução do metabolismo hepático pela rifampicina reduz as concentrações plasmáticas do efavirenz em 22-26%, e da nevirapina em 10-68% (16, 17). A indução hepática das várias isoenzimas do citocromo P450 (CYP2B6 e CYP3A4) acelera a metabolização da nevirapina, levando a níveis subterapêuticos dessa droga (18).

Os estudos farmacocinéticos da nevirapina indicaram uma redução na área sob a curva de concentração plasmática em relação ao tempo (AUC) e na sua concentração mínima (C_{min}), entre 30-50% e 20-68%, respectivamente (19-21). Vários estudos relataram que de 30% a 60% dos pacientes apresentavam C_{min} da nevirapina abaixo da concentração mínima terapêutica recomendada (3,5 mg/L), quando coadministrada com a rifampicina (17, 20-23). A redução na concentração plasmática da nevirapina é mais acentuada durante as primeiras duas semanas da TARV, quando a nevirapina é prescrita na dose escalonada (200mg/dia), que é utilizada para a prevenção da hipersensibilidade a essa droga (17, 23). Este facto foi demonstrado no estudo realizado em Malawi, no qual 59% dos pacientes com coinfecção HIV/TB apresentaram níveis sub-terapêuticos da nevirapina após 2 semanas de TARV, apesar da boa adesão, quando comparados com somente 14% dos pacientes nas semanas 4 e 8 da TARV. Neste estudo não houve comprometimento na eficácia da TARV após 6 meses de tratamento (17).

Já foram realizados ensaios clínicos e estudos de coorte, entretanto, que produziram resultados discordantes em relação ao impacto na eficácia da TARV

quando da coadministração da rifampicina e nevirapina. Na Tailândia, um pequeno ensaio terapêutico (N = 140) randomizado, que comparou a TARV com base em nevirapina *versus* efavirenz nos pacientes com coinfecção HIV/TB e CD4 <350/mm^3, não mostrou diferenças significativas na resposta virológica (*cut-off* de 50 cópias/mL) entre os dois braços (nevirapina 73,2 *versus* efavirenz 71,8; p> 0,99) ao fim de 48 semanas do início da TARV. Neste estudo, as baixas concentrações plasmáticas de INNTR medidas 12h após a administração e o baixo peso corporal basal foram fatores associados à falência da TARV (24). No entanto, um estudo aberto, randomizado, de não inferioridade, realizado na Índia, chama a atenção. Esse estudo comparou a NVP 400mg uma vez ao dia (com dose escalonada nas primeiras duas semanas) ao EFV 600mg, em pacientes coinfectados por HIV/TB e que iniciaram a TARV após a conclusão da fase intensiva do tratamento da TB. A causa da interrupção foi a evidência de que a proporção de pacientes com RNA HIV-1<400 cópias/mL era significativamente menor no grupo da nevirapina do que no grupo de efavirenz (25).

Outros estudos de coorte realizados até a presente data também apresentaram resultados discordantes. Na África do Sul, um estudo prospectivo fez seguimento de 2035 indivíduos que iniciaram a TARV com efavirenz (dos quais 1074 com tuberculose concomitante) e 1935 indivíduos que iniciaram a TARV com nevirapina (dos quais 209 com tuberculose concomitante). Os pacientes coinfectados que iniciaram a TARV com o regime de nevirapina apresentaram um maior risco de falência virológica nosseis primeiros meses (16.3; 95 CI, 10.6-23.5) do que os pacientes sem TB (8.3; 95 CI, 6.7-10.0). Neste estudo, não houve diferenças significativas na eficácia do tratamento dos pacientes com e sem TB que iniciaram a TARV com regime contendo efavirenz (26). Em Botswana, realizou-se um estudo retrospectivo no qual 155 pacientes coinfectados com HIV/TB que iniciaram TARV com regime contendo nevirapina ou efavirenz 600 mg

foram comparados com 155 pacientes com HIV sem TB. Após 12 meses de TARV, não houve diferenças na resposta virológica, mesmo quando a análise foi estratificada pelo regime de TARV (27).

Na Índia, um estudo de coorte prospectivo comparou a resposta imunológica das 48 semanas após o início da TARV entre 63 pacientes coinfectados por HIV/TB (NVP iniciada entre 2 e 8 semanas após o início do tratamento da TB) e 51 pacientes HIV+ sem TB, e não mostrou diferenças entre os dois grupos. Dos pacientes coinfectados com HIV/TB que completaram as 48 semanas de seguimento, 87,3% apresentaram HIV-1 RNA<400 cópias/mL. No entanto, a média da C_{min} aos 14 dias foi baixa (2,19 mg/L $\pm$ 1,49) (28). Em todos estes estudos clínicos, a nevirapina foi usada em dose escalonada (nas duas primeiras semanas, 200mg/dia). Por outro lado, a fim de evitar uma redução acentuada da concentração de plasmática da nevirapina, alguns autores sugerem abandonar o uso da dose escalonada da nevirapina nesses pacientes coinfectados quando em tratamento com a rifampicina (16, 17).

Neste sentido, um estudo realizado em Malawi por *Lamorde et al.* sugeriu o não uso da dose escalonada nos pacientes com coinfecção HIV/TB em tratamento com tuberculostáticos. Nesse estudo, os autores compararam a dose da nevirapina (200mg com 400mg) para o início do tratamento e demonstraram que a média da concentração plasmática mínima da nevirapina aos 7 dias após o início da TARV era inferior nos pacientes que iniciaram o TARV com dose escalonada da nevirapina (1504 ng/mL e 3148 ng/mL, P <0.01), *i.e.* com dose de 200mg/dia nas primeiras duas semanas do TARV (21). Um outro estudo também realizado em Malawi demonstrou que as concentrações plasmáticas da nevirapina são inferiores nas primeiras duas semanas após o início da TARV quando comparadas às encontradas nas semanas 4 e 8 após o

início da TARV nos pacientes com coinfecção HIV/TB. Como consequência, a proporção dos pacientes com níveis subterapêuticos da nevirapina encontrados neste estudo nas semanas 1 e 2 foi de 59% e de 14% nas semanas 4 e 8 após o início do TARV (17). Sendo assim, para que haja um sucesso na TARV, é necessário manter as concentrações plasmáticas da nevirapina dentro do limites terapêuticos usando a dose plena de nevirapina desde o início da terapia.

Por outro lado, o efavirenz é primariamente metabolizado pela isoenzima CYP450 2B6. Diversos polimorfismos genéticos CYP2B6 aumentam de forma significativa a exposição farmacocinética do efavirenz (29-32). Alelos CYP2B6 de metabolização lenta se encontram presentes em todas as populações em diferentes frequências, com o polimorfismo 516 G->T encontrado em africanos e asiáticos, 983 T->C encontrado em Africanos, e 15582C->T encontrado em asiáticos e europeus (33-36). Adicionalmente, pacientes que fazem terapia multidroga para TB também recebem a isoniazida, um inibidor do CYP2A6 e de outras isoenzimas que potencialmente aumentam a concentração plasmática do efavirenz e da rifampicina. CYP2A6 é uma forma alternativa para a eliminação do efavirenz, o que pode ser de importância relevante nos indivíduos com fenótipo de metabolização lenta do efavirenz (36).

Ainda na mesma linha, um estudo recentemente publicado (STRIDE) avaliou as concentrações plasmáticas mínimas do efavirenz em indivíduos com coinfecção HIV/TB, durante e após o tratamento antituberculose. Este trabalho demonstrou uma tendência ao aumento da concentração plasmática do efavirenz na presença do tratamento anti-TB. A mediana C_{min} do efavirenz com e sem o tratamento de TB foi 1.96ug/mL e 1.80ug/mL, respectivamente (p=0.06). No entanto, a concentração plasmática do efavirenz esteve significativamente aumentada durante a

coadministração entre as pessoas de cor negra (2.08ug/mL *versus* 1.75ug/mL, p=0.005). Neste estudo, o peso basal maior (≥60Kg comparado a <60Kg) esteve associado a reduzidas concentrações plasmáticas de efavirenz (respectivamente 1.68ug/mL *versus* 2.02ug/mL, p=0.021) e a uma maior eficácia no TARV (81.9% *versus* 73.8%, p=0.023) como resultado da doença HIV/TB menos avançada e ao melhor estado nutricional dos pacientes, factores estes que estão directamente ligados ao melhor resultado clínico do TARV (15).

1.4 A influência da nevirapina e do efavirenz nas concentrações plasmáticas da rifampicina e da isoniazida em pacientes com coinfecção HIV/tuberculose

A rifampicina, administrada durante 6 meses, faz parte de regime de tratamento da TB, e sua actividade bactericida é dependente da concentração (37). A rifampicina tem uma vida média curta (2 horas) e induz o seu próprio metabolismo (autoindução), reduzindo, deste modo, a sua concentração plasmática de 93%, após a primeira dose oral, para 68%, após 3 semanas de terapia – seja a rifampicina administrada por via oral ou endovenosa (38). A rifampicina é também um substrato e um potente inibidor do influxo do polipeptídio transportador de ânion orgânico 1B1 (OATP1B1), que regula a entrada de compostos endógenos e de drogas dentro do hepatócito (39). Por outro lado, a isoniazida, que também faz parte dos 6 meses de tratamento da tuberculose, tem uma excelente actividade bactericida precoce (40). A isoniazida é metabolizada principalmente pela N-acetiltransferase tipo 2 hepática (NAT2) e em menor grau pelo CYP2E1 (41). O papel dos transportadores na disposição da isoniazida ainda não é muito bem conhecido. Apesar de a farmacocinética dos medicamentos da tuberculose se alterar na presença da infecção pelo HIV (42-45), o papel da nevirapina ou do

efavirenz na importância da alteração da farmacocinética da rifampicina ou da isoniazida não é bem conhecido.

A rifampicina e a isoniazida são amplamente reconhecidas como a espinha dorsal do tratamento antituberculose, cuja prescrição é baseada no peso corporal dos pacientes. Como acontece para outros fármacos anti-infecciosos, a eficácia da rifampicina e da isoniazida é dependente do seu tempo de exposição, e um certo número de estudos demonstrou que as concentrações variam de forma importante nos pacientes com tuberculose pulmonar (46, 47). Além disso, a redução da biodisponibilidade de medicamentos antituberculose em pacientes infectados pelo HIV também já foi descrita previamente (42, 48-50). Portanto, o estabelecimento de parâmetros farmacocinéticos da rifampicina e da isoniazida em pacientes infectados pelo HIV severamente imunodeprimidos é de crucial importância. Recentemente, num estudo realizado em África de Sul, foi determinado o impacto do efavirenz nas concentrações plasmáticas das drogas antituberculose usadas na primeira linha (44). A nevirapina e o efavirenz têm enzimas ou transportadores com propriedades indutoras, e, pelo que se conhece, o efeito da biotranformação da rifampicina e da isoniazida nunca foi estudado.

Porém vários outros factores podem influenciar as concentrações plasmáticas de medicamentos antituberculose. Num estudo de farmacocinética realizado em 20 pacientes com TB activa em Tanzânia, a concentração plasmática dos medicamentos antituberculose esteve reduzida quando esta foi administrada com os alimentos (51). Em Paquistão, *Shaheem et al.* reportaram que a baixa qualidade dos medicamentos antituberculose usados nos serviços de saúde foi uma importante causa da reduzida concentração plasmática da rifampicina nos indivíduos com TB pulmonar em tratamento. Neste estudo, a concentração plasmática 2 horas após a toma (C2) da

rifampicina esteve abaixo de 4mg/L em 65% dos pacientes com TB pulmonar (N=20) (52). Um estudo realizado em Peru demonstrou que o sexo feminino ($p<0.001$) e o tempo para atingir a concentração plasmática máxima no soro (*Tmax*) no momento C2 ($p=0.012$) são factores independentemente associados à elevada exposição à rifampicina (53). O grau de imunossupressão nos indivíduos com coinfecção HIV/TB é um factor determinante para o sucesso de tratamento antituberculose. A concentração plasmática máxima (Cmax) da rifampicina em pacientes com CD4<200 é inferior quando comparada aos pacientes com CD4>200 ($p<0.01$) (49). Adicionalmente, a má absorção dos medicamentos antituberculose é frequente nos indivíduos com SIDA que apresentam diarreia e infecção por *Criptosporidium parvum* (42).

2. INVESTIGAÇÃO ACTUAL

2.1 Objectivo geral

- Descrever nos pacientes com coinfecção HIV/TB em Maputo, Moçambique, as interacções farmacológicas entre os medicamentos antirretrovirais usados na primeira linha para o tratamento do HIV e os medicamentos usados para o tratamento da tuberculose.

2.2 Objectivos específicos

- Determinar os parâmetros farmacocinéticos da rifampicina e da isoniazida antes e após o início da TARV com regime contendo nevirapina ou efavirenz em pacientes HIV positivos coinfectados com TB.

- Determinar os parâmetros farmacocinéticos da nevirapina quatro semanas após o início da TARV e quatro semanas após o fim de tratamento específico da tuberculose nos pacientes com coinfecção HIV/TB.

- Determinar, em pacientes com coinfecção HIV/TB, as concentrações plasmáticas mínimas da nevirapina e C13.5 horas do efavirenz nas semanas 12, 24, 36 e 48 após o início do TARV, assim como num subgrupo de pacientes, nas semanas 1, 2, 3 e 4 após o início da TARV

3. ARTIGOS

3.1 Artigo 1 - Pharmacokinetics of Rifampicin and Isoniazid in Tuberculosis-HIV Co-infected Patients Receiving Nevirapine or Efavirenz Based Antiretroviral Treatment (ANRS 12214 study)

Title:

Pharmacokinetics of Rifampicin and Isoniazid in Tuberculosis-HIV Co-infected Patients Receiving Nevirapine or Efavirenz Based Antiretroviral Treatment (ANRS 12214 study)

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Short/running title: Rifampicin and isoniazid pharmacokinetics

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Abstract

The pharmacokinetics of rifampicin and isoniazid were assessed in HIV/tuberculosis co-infected patients when administered alone and with non-nucleoside reverse transcriptase inhibitors based antiretroviral therapy. Thirty-eight patients (57.9% males, with a median age of 33 years-old, weight of 51.9 kg, CD4+T cells of 104 cells/µL and HIV-1 RNA of 5.5 log) on rifampicin and isoniazid based antituberculosis therapy and enrolled in ANRS 12146-CARINEMO trial were studied. Rifampicin and isoniazid daily doses were 10 and 5 mg/kg of body weight, respectively. Twenty-one patients received nevirapine 200mg bid and 17 patients received efavirenz 600mg qd in combination with lamivudine and stavudine from day 1 until the end of the study. Blood samples were collected at regular time intervals during a dosing interval after morning administration of a fixed dose combination of rifampicin and isoniazid. When administered alone, median rifampicin C_{max} and AUC at steady state were 6.59mg/L and 30.55mg.h/L, respectively. Concentrations remained unchanged whether coadministered with nevirapine or efavirenz. When administered alone, median isoniazid C_{max} and AUC at steady state were 5.14mg/L and 20.94mg.h/L, respectively. Concentrations remained unchanged when coadministered with nevirapine. A 29% decrease in isoniazid AUC was observed when combined with efavirenz. Rifampicin and isoniazid pharmacokinetic parameters were not altered to a clinically significant extent, when combined with nevirapine or efavirenz in these severely immunosuppressed HIV infected patients. Clinical outcome of the patients was good as a possible consequence of multi tuberculosis therapy and increase in CD4+T cells count with HIV treatment.

Key words: rifampicin, isoniazid, pharmacokinetics, nevirapine, efavirenz

Introduction

Tuberculosis is a leading cause of mortality among Human Immunodeficiency Virus (HIV) infected individuals, and is especially common in sub-Saharan Africa where the high burden of HIV infection further increases tuberculosis incidence. In 2011, 8.7 million incident cases of tuberculosis were notified worldwide of which 1.1 million were among HIV positive (3). Based on the evidence that the concomitant therapy reduces mortality associated to tuberculosis among HIV infected patients, the co-administration of antituberculosis drugs and antiretroviral therapy is common practice in high burden tuberculosis and HIV countries (45).

For the first line antituberculosis treatment, World Health Organization (WHO) recommends a six-month rifampicin-based regimen (46). The efficacy of this treatment regimen for curing tuberculosis in adults is >95% (47-49), but can be lower as 53.4% in untreated HIV infected individuals (50, 51). The effectiveness of antituberculosis treatment is dependent on drug exposure, and several factors such as patients´ age, gender, severe illness, malnutrition, drug formulation drug-drug interaction, comorbid disease and HIV infection itself can negatively affect antituberculosis drug pharmacokinetics and lead to prolonged infectiousness, poor treatment outcome with increased risk of relapse, development of multidrug resistant strains of *Mycobacterium tuberculosis* and death (33, 37, 40, 52).

In many resource limited countries, the first line antiretroviral treatment regimen consists of non-nucleoside reverse transcriptase inhibitor, nevirapine or efavirenz combined with two nucleosides reverse transcriptase inhibitors. Due

to the risk of sub-therapeutic nevirapine plasma concentrations in HIV/tuberculosis co-infected patients receiving concomitantly rifampicin, efavirenz based antiretroviral treatment is the preferred drug regimen during the course of tuberculosis treatment (53). However, nevirapine may still be an alternative to efavirenz in patients with contra-indication to efavirenz or who do not tolerate it. Nevirapine and efavirenz are substrates of cytochromes P-450 (CYPs), mainly CYP2B6 and have drug metabolizing enzymes or transporters inducing and inhibiting properties (54, 55), and to our knowledge, data of their effect on rifampicin and isoniazid are scarce and only documented recently for efavirenz in the African population (35).

Rifampicin is part of the 6-month tuberculosis therapy with a concentration dependant bactericidal activity (28). It has a short half-life (around 2h) and auto-induces its liver and presystemic metabolism lowering its plasma concentrations compared to those reached after a single oral dose (29). Additionally, rifampicin is a substrate and a potent blocker of the influx transporter organic anion transporting polypeptide 1B1 (OATP1B1), which regulates the uptake of endogenous compounds and drugs into hepatocytes (30). Isoniazid is part of the 6-month tuberculosis therapy with an excellent early bactericidal activity (31). Isoniazid is metabolized mainly by hepatic type 2 N-acetyltransferase (NAT2) and CYP 2E1 to a lesser extent (32). Whether transporters could be involved in isoniazid disposition is currently unknown. Although the pharmacokinetics of antituberculosis drugs has been reported to be affected by HIV infection (34-36, 56), whether efavirenz or nevirapine could impair differentially rifampicin or isoniazid pharmacokinetics, is not documented.

This study aimed to document the effect of nevirapine and efavirenz on the disposition of both rifampicin and isoniazid by comparing the pharmacokinetic

parameters of rifampicin and isoniazid with and without coadministration of nevirapine or efavirenz-based antiretroviral therapy. This is a sub-study of the ANRS 12146-CARINEMO, which compared the efficacy and safety of nevirapine and efavirenz based antiretroviral based regimen in HIV/tuberculosis co-infected patients (57).

Material and Methods

Patients

All patients included in this study gave written informed consent to participate in this ANRS12214- pharmacokinetic study, a sub study of the ANRS 12146-CARINEMO clinical trial, which was conducted in Mozambique and was approved by the Mozambican National Bioethical Committee and Ethical Review Board of Médecins Sans Frontières - Switzerland. Patients with HIV/tuberculosis co-infection were randomized to receive nevirapine- or efavirenz- based antiretroviral therapy in addition to their antituberculosis treatment. Detailed participants' characteristics and study procedures are described elsewhere (57). Patients' eligibility criteria for the trial were: new case of active tuberculosis (bacteriologically confirmed and not confirmed pulmonary tuberculosis and extrapulmonary tuberculosis), CD4+T cells count<250 cell/μL, naïve treatment HIV-infected patients, Karnofsky score ≥ 60, no significant hepatic dysfunction (levels of transaminase and total bilirubin <5x upper normal limit [UNL]), absence of severe grade 4 clinical or laboratory signs and willingness to provide informed consent. After 4 to 6 weeks of antituberculosis treatment, the trial participants were randomised to receive the fixed dose combination (FDC) of nevirapine 200mg- lamivudine 150mg- stavudine 30mg twice daily (Cipla, India) or efavirenz 600mg (Aurobindo, India) once daily

combined with the FDC of lamivudine150 mg- stavudine 30mg (Cipla, India) twice daily. The antituberculosis drugs were administered orally and the daily dose calculated per body weight: rifampicin (10 mg/kg), isoniazid (5 mg/kg), pyrazinamide (25mg/kg) and ethambutol (15mg/kg). The four FDC antituberculosis drugs were administered for two months followed by the FDC containing rifampicin-isoniazid for the remaining four months (Lupin, India). All antiretroviral therapy and antituberculosis drugs used in the trial were WHO prequalified. Patients' adherence to antituberculosis drugs and antiretroviral therapy was monitored by study staff using pill count, control of the regularity and promptness to scheduled clinical visits and detection of isoniazid metabolites in urine while on tuberculosis treatment with BBL Taxo TM isoniazid test strips (Becton Dickinson and Company, USA). Patients had regular visits in the trial to monitor clinical evolution, liver function test, treatment adherence and to detect potential adverse events. In addition, HIV-1 RNA viral load was monitored at antiretroviral treatment initiation and every 12 weeks until 48 weeks. CD4+T cells count was measured at antiretroviral treatment initiation and after 24 and 48 weeks (57). The last 172 patients enrolled in ANRS12146-CARINEMO trial had an extended follow-up until 96 weeks to identify potential tuberculosis recurrent cases. Thirty-nine patients enrolled consecutively in the ANRS12146-CARINEMO trial were included in the pharmacokinetic sub-study (ANRS 12214) if they met the following additional criteria: to be on rifampicin and isoniazid as part of tuberculosis treatment regimen, to be naïve of antiretroviral therapy at the time of enrolment, and to be willing to participate in the additional blood sampling by providing signed informed consent.

Study design

Each patient was requested to come fasting to the clinic in the morning, having had the last meal on the previous evening. At the clinic, patients were asked to recall the timing of the last antituberculosis drugs and other concomitant medication taken on the previous day. Blood samples were collected at steady-state at two occasions: within a week before antiretroviral therapy initiation to allow estimation of pharmacokinetic parameters of rifampicin and isoniazid alone, and four weeks after antiretroviral therapy initiation when patients were also receiving nevirapine or efavirenz. During these two occasions, blood samples were drawn before morning antituberculosis drug intake (time 0) and 0.5 hour, 1 hour, 1.5 hours, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours and 12 hours after antituberculosis drugs intake. The antituberculosis drugs were administered with 100 mL of water to each patient under supervision of a study nurse. Within 30 minutes after each collection, blood samples were centrifuged (800 x g for 20 min) at room temperature. To improve stability of rifampicin, each plasma sample of exactly 0.5 mL was stored in a polypropylene tube containing 0.5mL of ascorbic acid solution (200mg/L). Blood samples were also drawn to measure predose ($C_{through}$) nevirapine or mid-dose efavirenz plasma concentrations at a weekly base during first month of antiretroviral treatment. All plasma samples were kept at -80°C in the study site until shipment for analysis to the pharmacology laboratory at Bicêtre Hospital (Paris, France).

Drug assays

Plasma concentrations of rifampicin and isoniazid were measured using validated reverse-phase high performance liquid chromatography methods with UV detection according to validated assays (58, 59). In brief, rifampicin was

assayed in plasma after protein precipitation by acetonitrile and injection onto a Lichrospher® 100 RP18 column and detection at 342 nm. The mobile phase consisted in a 10/40/50 v/v mixture of methanol-acetonitrile-citrate buffer (pH = 4.3). Isoniazid was assayed in plasma after protein precipitation by 10% trichloracetic acid in water and injection onto an Atlantis T3 column and detection at 265 nm. The mobile phase consisted of a mixture of 93.5% phosphate buffer 30mM pH 7.1, methanol 5% and acetonitrile 1.5%. The lower limit of quantification of plasma rifampicin and isoniazid concentrations was 0.1 mg/L. Three quality control concentrations were included in each analytical run. Day-to-day variability of the quality controls was less than 8% for rifampicin and less than 12% for isoniazid

Nevirapine and efavirenz concentrations were assayed in plasma by HPLC with a limit of quantification of 25ng/mL and 50ng/mL, respectively (60).

Pharmacokinetic analysis

The pharmacokinetic parameters for rifampicin and isoniazid were estimated by the non-compartmental method (WinNonlin software, Pharsight Corporation, Mountain View, California, USA). The areas under the concentration-versus-time curves during a 24-hour dosing interval at steady state (AUC) were calculated using the linear up-log down trapezoidal method, up to the time of the last measurable concentration and extrapolated to 24h whenever half-life could be calculated. Most isoniazid half-lives were difficult to calculate, consequently, only AUC during the 12h post dosing were estimated. Drug concentrations below the lower limit of quantification (LLQ) were assigned a value of LLQ/2 if they occurred before the maximal plasma concentration (C_{max}). After C_{max}, concentrations below the LLQ were treated as missing data. Plasma

trough level ($C_{through}$), concentrations 2 hours post-dosing (C2), C_{max}, and time to C_{max} (T_{max}) were obtained visually from the plasma concentration–time curve. Rifampicin and isoniazid pharmacokinetic parameters were estimated in the absence and presence of nevirapine or efavirenz. Plasma concentrations, including concentrations measured 2h-post dosing were compared to the previously defined therapeutic ranges which are for rifampicin between 8-24mg/L and for isoniazid between 3-5mg/L (39).

Statistical analysis

This observational pilot pharmacokinetic study assessed plasma concentrations of rifampicin and isoniazid off and on nevirapine or efavirenz based-antitretroviral therapy. This study started after initiation of the main ANRS12146-CARINEMO trial and was proposed to all included patients expecting to enrol 20 patients in each group to detect major differences in rifampicin and isoniazid disposition.

The pharmacokinetic parameters for rifampicin and isoniazid were summarized by using descriptive statistics and unless otherwise indicated are presented as median and range. The values of $C_{through}$, C_{max} and AUCs were log transformed. C_{trough} concentrations below the limit of quantification were treated as zero. Two-sided 90% confidence intervals (CIs) were constructed for the ratios of the geometric mean values (with non-nucleosides reverse transcriptase inhibitors *vs* alone) of AUCs, C_{trough} and C_{max} for rifampicin and isoniazid which was compared to the 0.80-1.25 bioequivalence range. $C_{through}$ of nevirapine and median mid-dose of efavirenz at 3 and 4 weeks, which correspond to the period of the second pharmacokinetic sampling for rifampicin and isoniazid were also presented. The antituberculosis treatment outcomes at the end of treatment (24

weeks), the proportion of tuberculosis recurrent cases and the proportion of patients with hepatic toxicity were described. Tuberculosis treatment outcomes definitions were based on WHO guidelines (46). All statistical analysis was conducted with StataSE™ software (StataCorp. 2005. Stata Statistical Software: Release 10.0. College Station, TX: StataCorp, USA).

Results

Patient characteristics. Out of 570 patients randomised in the main trial, 39 were enrolled in the pharmacokinetic sub-study, from which 22 patients were randomised in nevirapine arm and 17 in efavirenz arm. One patient was non-adherent to antiretroviral therapy in which nevirapine concentrations were below limit of quantification at weeks 2, 3 and 4, and thus excluded from the pharmacokinetic analysis (Figure 1). The baseline characteristics of the 38 patients are summarised in table 1. The median age was 33 years and median body weight was 51.9 kg. Among 38 patients, extra-pulmonary tuberculosis was present in 10 (26.3%) patients (eight, pleural tuberculosis and two, disseminated tuberculosis). Patients were severely immunosuppressed with median CD4+T cells count of 104 cells/µL and median plasma HIV-1 RNA of 5.5 log copies/mL. The median time between tuberculosis treatment initiation and first pharmacokinetic sampling was 30 days.

At the first pharmacokinetic sampling, all patients were receiving the four antituberculosis drugs in fixed dose combination. At the second sampling, 73.7% (28/38) patients were receiving rifampicin-isoniazid in fixed drug combination and 26.3% (10/38) were still under the four antituberculosis drugs combination. Patients' body weight remained unchanged throughout the study.

Non-nucleosides reverse transcriptase inhibitors concentrations. At four weeks of antiretroviral therapy, the median C_{trough} of nevirapine was 4 113 ng/mL (range, 1 982 to 12 719 ng/mL) and median mid-dose concentration of efavirenz was 3 310 ng/mL (range, 1 513 to 24 673 ng/mL), with a median sampling time of 13 h (range, 10.9 to 14.4 h) post-dosing.

Rifampicin pharmacokinetics. Plots of rifampicin concentrations *vs* time for nevirapine and efavirenz groups are shown in figure 2A and 2B respectively. A trend for higher rifampicin peak concentration when combined with nevirapine, but not efavirenz, was noted. One patient had undetectable levels of rifampicin when rifampicin was coadministered with efavirenz and thus no pharmacokinetic parameter was estimated. In this patient, rifampicin C_{max} when administered alone was 3.92 mg/L. The pharmacokinetic parameters of rifampicin are listed in table 2. Concentrations of rifampicin before dosing were undetectable in all patients, except for three patients who had low rifampicin concentrations, below 1 mg/L. A small non-significant increase in C_{max} and AUC was observed when rifampicin was administered with nevirapine but not with efavirenz. It should be pointed out that the inter-individual drug plasma concentration was highly variable in this population. Figure 3 shows rifampicin C2 before and after introduction of antiretroviral therapy. Of note, rifampicin C2 was <8mg/L in 16 patients (76.2%) before starting nevirapine but only in 10 patients (47.6%) after the addition of nevirapine. In contrast, the number of patients presenting rifampicin C2<8mg/L was comparable before (13 patients) and after (12 patients) addition of efavirenz.

Isoniazid pharmacokinetics. Plots of isoniazid concentrations *vs* time for nevirapine and efavirenz patients group are shown in figure 2C and 2D, respectively. Time for C_{max} of isoniazid occurred sooner when combined

antiretroviral therapy, indicating a more rapid absorption. Temporal declines in isoniazid concentrations remained unchanged when isoniazid was combined with nevirapine or efavirenz, indicating that the rate of elimination is unchanged. Pharmacokinetic parameters of isoniazid are listed in table 3. A higher isoniazid C_{max} was observed when isoniazid was combined with nevirapine, although AUC remained unchanged, as a likely faster absorption. To note, a 29% lower AUC when isoniazid was combined with efavirenz, with unchanged C_{max}, although with high variability. Interestingly, the patient who had undetectable concentration of rifampicin when on efavirenz had detectable concentrations of isoniazid, although in the lower range (C_{trough} 0.54 mg/L, C_{max} 1.05 mg/L and AUC 5.05 mg.h/L). Before antiretroviral treatment initiation, isoniazid C2 <3mg/L was found in 4 patients (19.0%) of the nevirapine group and 5 patients (29.4%) of the efavirenz group. After starting antiretroviral therapy, isoniazid C2 <3mg/L was found in 10 patients (47.6%) of the nevirapine group and 9 patients (52.9%) of the efavirenz group as shown in figure 3. None of the included patients had isoniazid C2 below the minimum inhibitory concentration (MIC =0.05 mg/L).

Clinical outcome and safety

Thirty-seven patients (97.4%) successfully completed the tuberculosis treatment. One patient (2.6%), whom rifampicin plasma concentrations were below the lower limit of quantification in all-time points during the second pharmacokinetic period, died ten weeks after starting antiretroviral treatment due to wasting syndrome associated with advanced HIV infection. Defaulter or treatment failure was not observed in the study. From 38 patients initially enrolled in the pharmacokinetic study, 33 (86.8%) were followed during 96 weeks. From these 33 patients, one developed tuberculosis recurrence at week 92, with lymph nodes involvement. In this patient, rifampicin C_{max} before and

after antiretroviral therapy initiation were 8.86 mg/L and 11.63 mg/L, respectively; and isoniazid C_{max} before and after antiretroviral therapy initiation were 6.07 mg/L and 8.92 mg/L, respectively.

Five weeks after antiretroviral initiation, two patients had an increase in ALT≥grade 2 with levels of 163.9 UI/L (grade 3) and 588.0 UI/L (grade 4) and one patient had total bilirubin≥grade 2 with a level of 8.1 mg/dl (grade 3). To note, ALT grade 4 and total bilirubin grade 3 were found in a HBV carrier patient who had rifampicin and isoniazid C_{max} close to the median (6.91 mg/L and 6.11 mg/L, respectively). Both patients with increased ALT levels had C_{max} of rifampicin (3.23 mg/L and 6.91 mg/L) and isoniazid (3.29 mg/L and 6.11 mg/L) within the range described for the whole studied population. The decrease in plasma HIV-1 RNA from baseline after 12 weeks of antiretroviral therapy was at least 1 log in all enrolled patients. The proportion of patients with HIV-1 RNA<50 copies/mL at weeks 24 and 48 were 86.1% and 85.7%, respectively.

Discussion

Efavirenz is currently the antiretroviral backbone recommended in HIV/tuberculosis co-infected patients, but in the absence of an alternative to efavirenz in patients who could not receive it, nevirapine is still prescribed in some HIV/tuberculosis co-infected patients. This is the first study comparing pharmacokinetic parameters of rifampicin and isoniazid when prescribed alone and with nevirapine (without leading dose) in HIV/tuberculosis co-infected patients; and to our knowledge, our data are also contributing to the limited data on pharmacokinetics of antituberculosis drugs in HIV infected patients treated with efavirenz. Our main finding is that rifampicin exposure was not altered to a clinically significant extent when combined with either nevirapine or efavirenz. A

30% significant decrease in isoniazid exposure (AUC) was demonstrated when coadministered with efavirenz but not nevirapine. Such reduction probably has no consequence in tuberculosis treatment outcome as the efficacy parameter is the AUC/MIC ratio (61) and all AUCs are well above the half maximal effective concentration (EC50) which was estimated at 1 mg.h/L (62). The mechanism of such interaction is unclear. Induction of isoniazid CYP mediated pathway is unlikely as during the duration of the study isoniazid is combined with rifampicin, a very potent inducer of drug metabolizing enzymes. Concomitant decrease in C_{max} suggests that isoniazid absorption could be decreased. Whether this interaction could be efflux or uptake transporter-mediated, remains to be demonstrated.

In relation to the WHO recommendation to systematically start antiretroviral treatment in any HIV/tuberculosis co-infected patients, regardless CD4+ T cell levels, the information that introduction of non-nucleosides reverse transcriptase inhibitors-based antiretroviral treatment will not impair antituberculosis drug exposure is of utmost importance. Concentrations of rifampicin and isoniazid measured in our population of HIV/tuberculosis co-infected patients are close to those reported in previous studies (35, 40, 63-65). Tuberculosis and HIV-infection were found to alter absorption and consequently decrease antituberculosis drug concentrations (33, 34, 56, 66). Interestingly, Barroso *et al.*, (67) compared rifampicin and isoniazid C_{max} in healthy volunteers and patients with susceptible tuberculosis. C_{max} of rifampicin was higher in healthy controls (5.7 mg/L) than in tuberculosis infected patients (2.11 mg/L), whereas C_{max} of isoniazid were similar in healthy controls and tuberculosis patients (3.26 mg/L and 2.85 mg/L respectively). In addition, the same study demonstrated that 82% of the tuberculosis patients had rifampicin C_{max} below the 8 mg/L

threshold compared to 50% of the healthy subjects, whereas there was no difference for isoniazid concentrations below the 3mg/L threshold (39.3% and 46.7% for tuberculosis patients and healthy subjects, respectively). Reduced intestinal permeability of patients compared with healthy subjects was found to be dependent on co-factors such as alcoholism, smoking, body mass index, levels of hemoglobin and albumin. Such patients' characteristics can also be identified in severely immunosuppressed HIV-infected patients. In a study conducted in Botswana by Chideya *et al*, among 255 tuberculosis patients 84% had a low rifampicin C_{max} and 69% of them were HIV infected. Rifampicin C_{max} was significantly lower in patients with CD4+ T cells count <200 cells/ μL than in those with CD4+T cells count >200 cells/μL pointing out the role of functional immune system in drugs absorption (40). Interestingly, despite inclusion of patients with advanced immune suppression, concentrations measured 2h post dose and after starting antiretroviral therapy compared favorably to those obtained in healthy volunteers'. Of note, we found undetectable or low concentrations of rifampicin and isoniazid in one of the included patients, who died from wasting syndrome shortly after sampling for drug assay. Several factors could be associated with relatively good rifampicin and isoniazid exposure. On one hand, patients received support to enhance adherence to treatment and bioavailability was optimized with prescription of WHO pre-qualified FDC anti-tuberculosis drugs, using small body band weight ranges (43, 51, 68-71). Indeed, we used 5 kg body band weight range to adjust tuberculosis treatment; this diverged from current recommendations of national tuberculosis program which uses 10 kg body band weight range. On the other hand, early initiation of potent and efficacious antiretroviral therapy according to recent guidelines (9), may have improved patients' health status.

Our study has a number of limitations. First the sequential design of the study cannot discriminate between a drug-drug interaction and improvement of the patient with initiation of antiretroviral therapy. However, in the 4-week time period between the two sampling periods, the average patients weight remained unchanged indicating that a sequence effect is unlikely. Second, the included patients were not genotyped for drug metabolism enzymes or transporters polymorphisms. The genetically polymorphic *NAT2* is responsible for isoniazid metabolism (72), but disease progression in HIV infection and AIDS may alter expression of the *NAT2* gene (73). Rifampicin is a substrate and inhibitor of the uptake transporter OATP1B1 encoded by the *SLCO1B1* gene for which a genetic polymorphism has been demonstrated (74). Such genetic polymorphisms explain at least in part the large interindividual variability in the pharmacokinetic parameters of isoniazid and rifampicin observed and as previously reported (31, 75), but is unlikely to interfere with drug-drug interaction. Third, only rifampicin and isoniazid concentrations were measured, but they are the backbone of antituberculosis therapy of susceptible strains. Finally, no attempt was made to relate the measured concentrations to antituberculous efficacy which could be defined only on the clinical response especially for patients with smear-negative pulmonary and extra-pulmonary tuberculosis. Indeed, this study was not designed to investigate drug exposure-effect relationships, but sought to compare drug exposure on and off antiretroviral therapy, therefore our sample size was limited. Even though rifampicin and isoniazid concentrations were highly variable, peak serum concentrations remained well above the MICs for *Mycobacterium tuberculosis* (ie., $\leq$0.25 mg/L for rifampicin and $\leq$0.05 mg/L for isoniazid) in all patients, which

could be the reason for good tuberculosis treatment outcome despite advanced HIV infection.

In conclusion, our data show that neither rifampicin nor isoniazid pharmacokinetic parameters are altered to a clinically significant extent when combined with nevirapine or efavirenz, even though the leading dose of nevirapine was omitted. Although drug exposure could not be related to clinical outcome, the high treatment success rate is reassuring.

ANRS12146 - CARINEMO clinical trial study group

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Conflict of interest

The authors have no conflict of interest.

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Figure

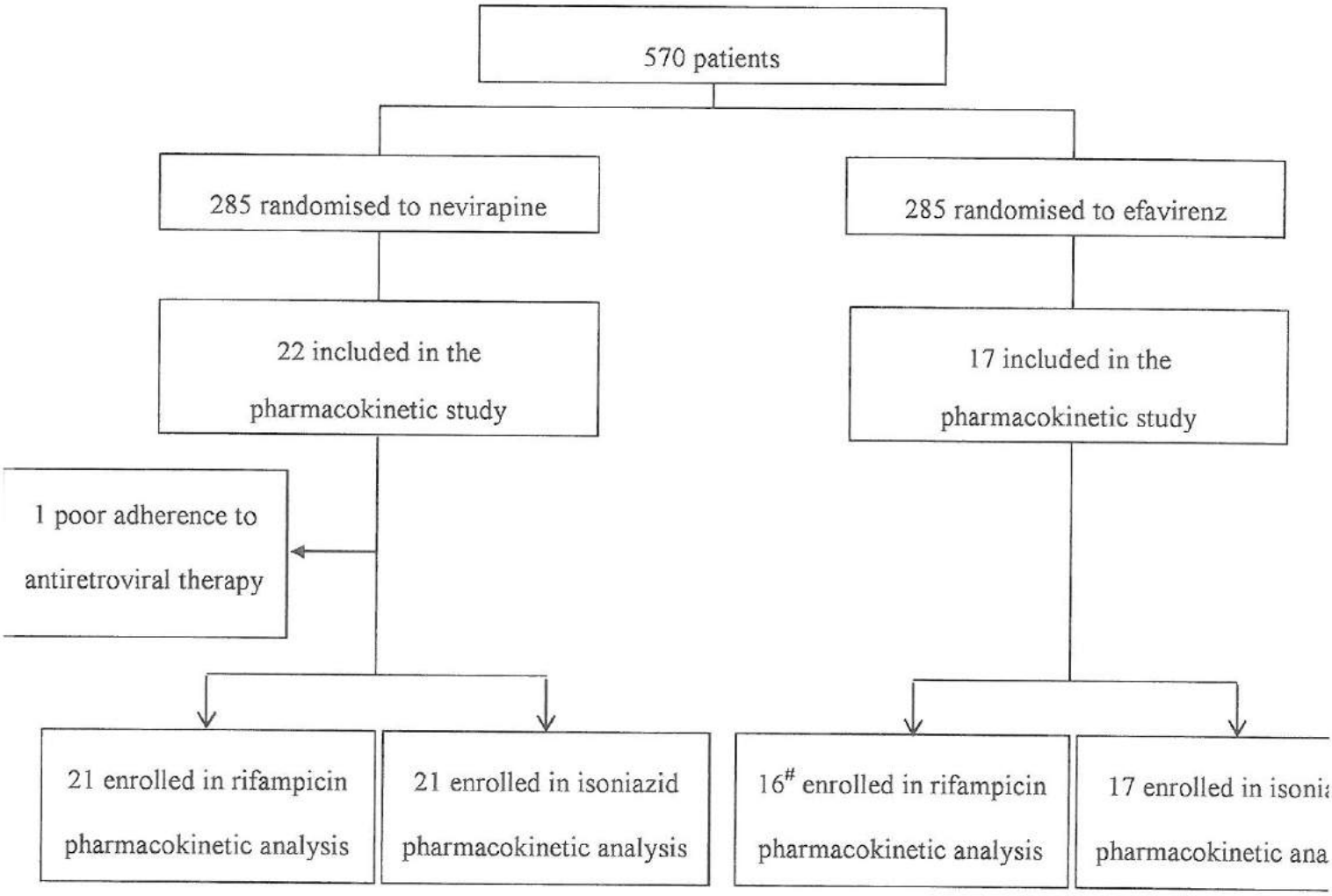
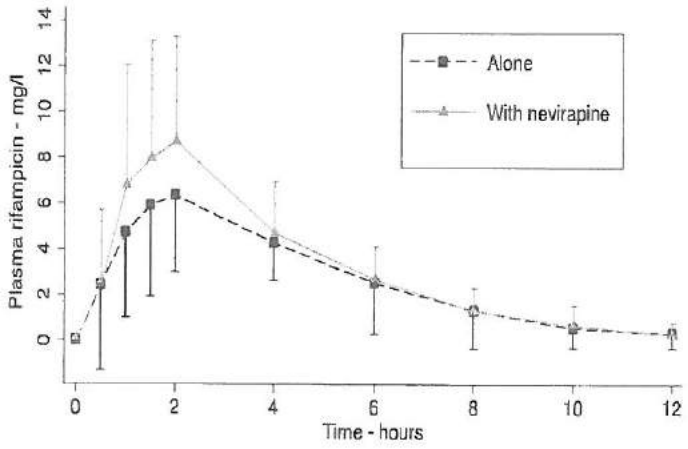
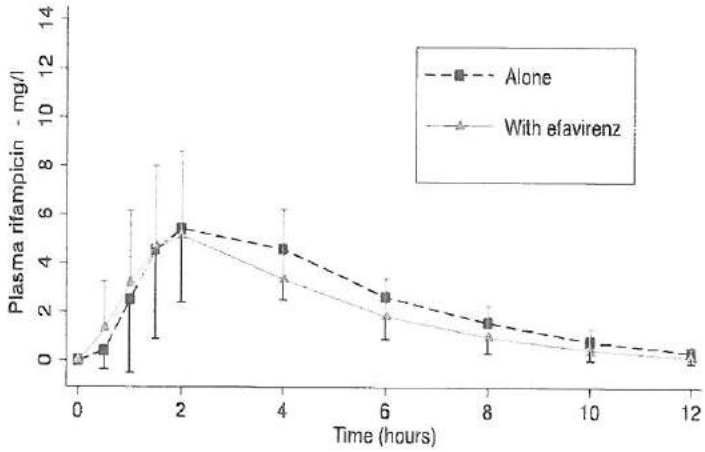


Figure 1: Study profile.

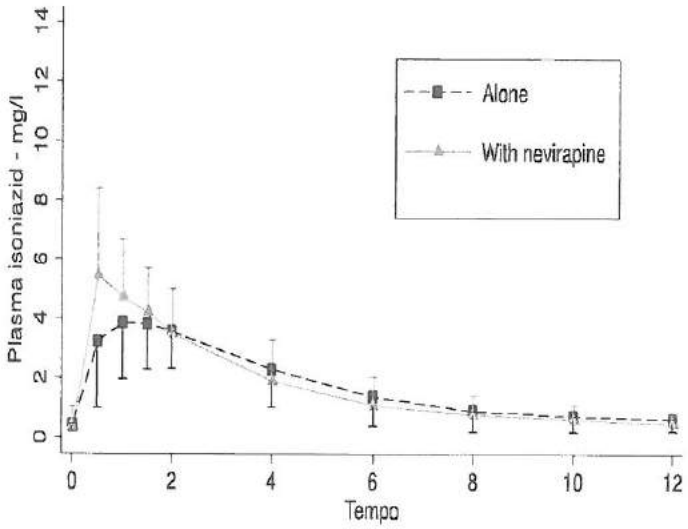
A (n=21)



B (n=16)



C (n=21)



D (n=17)

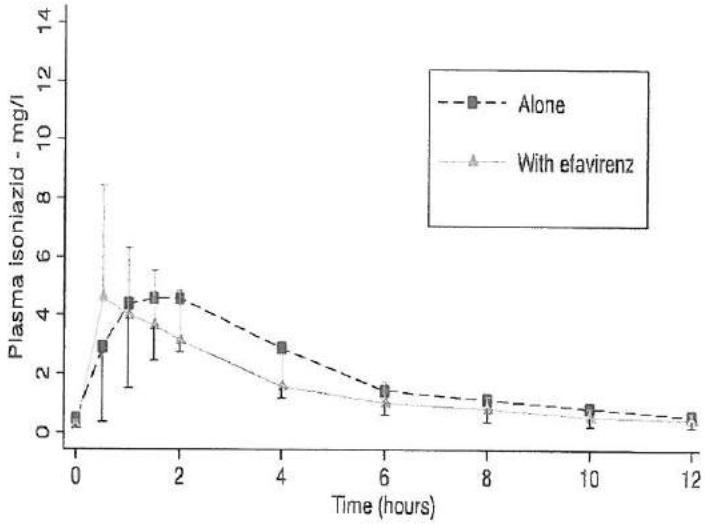


Figure 2: Mean ± standard deviation of rifampicin (panels A and B) and isoniazid (panels C and D) plasma concentrations-time profiles during the first 12h of the 24h-dosing interval, in the absence (closed squares and dotted lines) or the presence (closed triangles and solid lines) of nevirapine (panels A and C) or efavirenz (panels B and D).

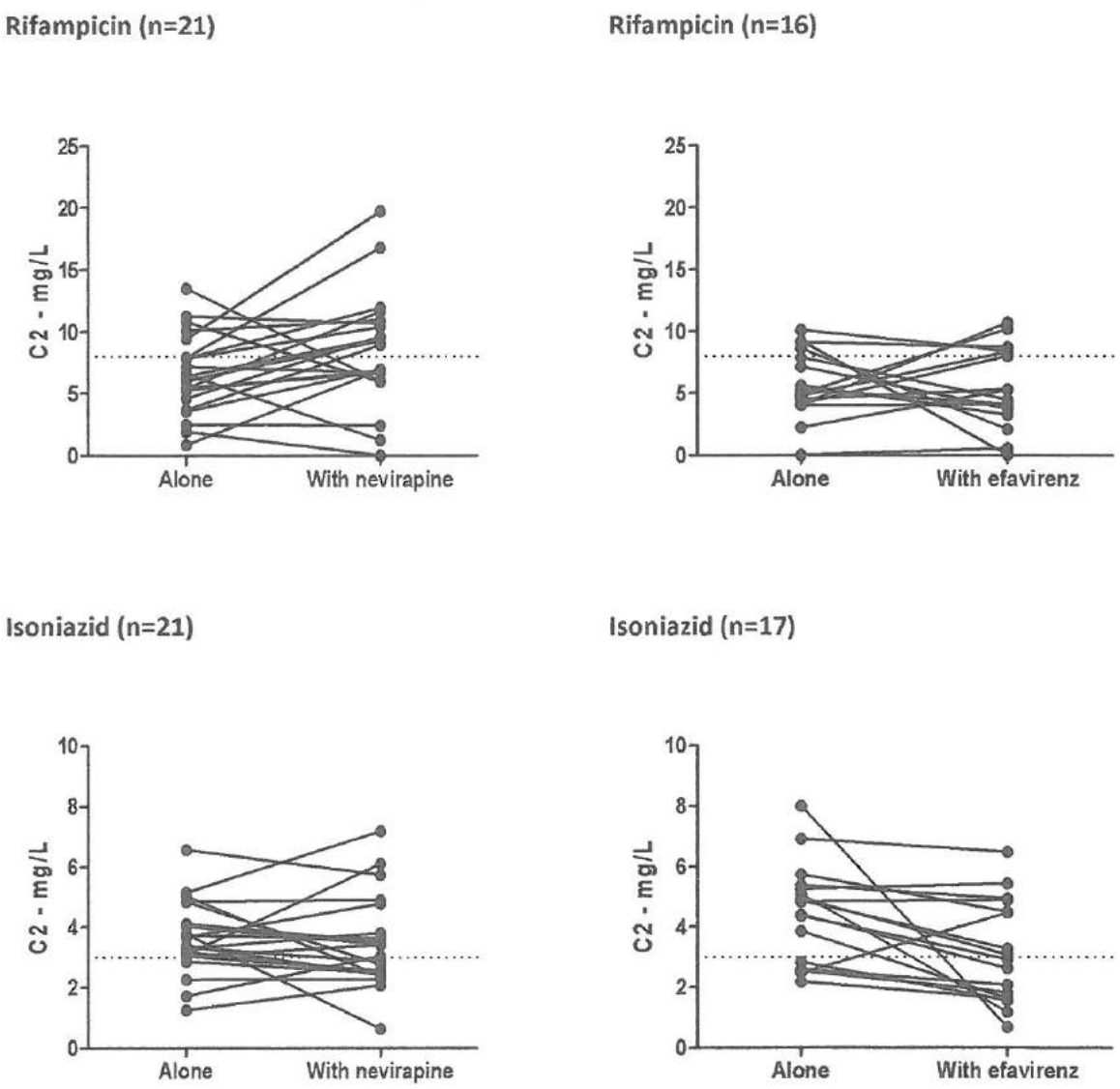


Figure 3: Individual plasma concentrations of rifampicin (upper panels) and isoniazid (lower panels) drawn 2 hours (C2) post-dosing before and after starting antiretroviral treatment with nevirapine (left) or efavirenz (right).

Tables

Table 1 Demographic, clinical and laboratory characteristics of enrolled adult patients at baseline[a]

haracteristics	NVP arm (n=21)	EFV arm (n=17)	Total (n=38)
ge (years)	34 (24-48)	33 (21-50)	33 (21-50)
lale	12 (57.1)	10 (58.8)	22 (57.9)
Veight (kg)	52.6 (40.5-68.5)	51.3 (39.1-72.3)	51.9 (39.1-72.3)
MI (kg/m^2)	19.1 (14.4-24.3)	18.7 (16.7-20.0)	18.9 (14.4-30.1)
laemoglobin (g/dl)	8.9 (7.0-10.5)	9.3 (7.5-11.7)	9.1 (7.0-11.7)
LT (UI/L)	25.3 (7.7-124.2)	32.3 (13.3-123.6)	29.9 (7.7-124.2)
otal bilirubin (mg/dl)	0.4 (0.2-2.2)	0.51 (0.1-1.3)	0.4 (0.1-2.2)
D4 count ($cells/mm^3$)	108 (2-206)	93 (18-214)	104 (2-214)
lasma HIV-1 RNA (log_{10} copies/ml)	5.7 (4.2-7.0)	5.4 (4.3-6.6)	5.5 (4.2-7.0)
ctive hepatitis B infection	1/17 (5.9)	4/17 (23.5)	5/34 (14.7)
ICV infection	0 (0.0)	1 (5.9)	1 (2.6)
astro-Intestinal symptoms	1 (4.8)	1 (5.9)	2 (5.3)
ime between start of tuberculosis therapy nd start of antiretroviral therapy, days	33 (28-40)	33 (28-41)	33 (28-41)
ulmonary tuberculosis	18 (85.7)	10 (58.8)	28 (73.7)
ulmonary tuberculosis smear results			
Positive	7 (33.3)	8 (47.0)	15 (39.5)
Negative or missing results	14 (66.7)	9 (53.0)	23 (60.5)
avitary disease	1/18 (5.6)	0/16 (0)	1/34 (2.9)

[a] Data are n (%), median (range), or n/N (%). ALT = alanine aminotransferase.

HBV= hepatitis B virus. HCV= hepatitis C virus.

Table 2 Pharmacokinetic parameters of rifampicin administered alone and with addition of nevirapine or efavirenz[a]

Drug and time	$C_{through}$ (mg/L)	C_{max} (mg/L)	T_{max} (h)	AUC (mg.h/L)
Nevirapine arm				
N	21	21	21	16
Alone	<0.1	6.59	2	30.10
	(<0.1-0.52)	(3.49-14.07)	(0.7-4.0)	(12.48-109.75)
With nevirapine	<0.1	8.94	2	34.49
	(<0.1-0.70)	(1.49-19.74)	(1.0-6.0)	(13.96-74.95)
GMR[b]	-	1.18	-	1.14
(90% CI)		(0.95-1.47)		(0.89-1.47)
Efavirenz arm				
N	16	16	16	12
Alone	<0.1	6,69	2	29.77
		(2.85-12.29)	(1.0-4.0)	(14.06-62.95)
With efavirenz	<0.1	6.27	2	24.17
		(2.07-12.43)	(1.0-8.0)	(13.73-55.85)
GMR	-	0.94	-	0.94
(90% CI)		(0.71-1.24)		(0.76-1.16)

[a] Data are expressed as median (range)

[b] GMR = is the geometric mean ratio for the parameters with NNRTI to those without NNRTI

$C_{through}$ = trough concentration; C_{max} = maximum concentration; T_{max} = time to achieve maximum concentration; AUC = area under the plasma concentration vs. time curve during a dosing interval.

Table 3 Pharmacokinetic parameters of isoniazid administered alone and with addition of nevirapine or efavirenz)[a]

Drug and time	$C_{through}$ (mg/L)	C_{max} (mg/L)	T_{max} (h)	AUC (mg.h/L)
Nevirapine arm				
N	20	21	21	19
Alone	0.42	4.83	1.0	19.70
	(<0.1-1.12)	(1.26-7.75)	(0.5-4.0)	(8.32-39.53)
With Nevirapine	0.26	5.96	0.5	17.41
	(<0.1-3.01)	(1.88-9.80)	(0.4-2.2)	(12.56-43.90)
GMR[b]	-	1.21	-	1.03
(90% CI)		(1.01-1.45)		(0.95-1.11)
Efavirenz arm				
N	17	17	17	17
Alone	0.36	5.20	1.5	23.56
	(<0.1-1.26)	(2.19-11.51)	(1.0-4.0)	(7.73-56.95)
With Efavirenz	0.28	4.91	0.5	14.06
	(<0.1-0.54)	(1.05-13.47)	(0.5-2.0)	(4.94-34.51)
GMR		0.89	-	0.71
(90% CI)		(0.68-1.15)		(0.55-0.92)

[a] Data are expressed as median (min-max)

[b] GMR = is the geometric mean ratio for the parameters with NNRTI to those without NNRTI.

$C_{through}$ = trough concentration; C_{max} = maximum concentration; T_{max} = time to achieve maximum concentration; AUC = area under the plasma concentration vs. time curve during a dosing interval.

3.2 Artigo 2 - Nevirapine 400mg without lead-in dose or efavirenz 600mg for treatment of tuberculosis and HIV co-infected patients: relationship between exposure and virological failure in the ANRS12146/12214 – CARINEMO study

Nevirapine or efavirenz for tuberculosis and HIV co-infected patients: exposure and virological failure relationship

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Short running title: Nevirapine and efavirenz with TB drugs

Key words: TB/HIV coinfection, nevirapine, efavirenz, drug-drug interaction

Synopsis

Objectives: We described nevirapine and efavirenz exposure on- and off-tuberculosis treatment and consequences on virological efficacy and tolerance in patients included in ANRS12146–CARINEMO trial.

Methods: Participants were randomly selected to receive either nevirapine 200 mg twice daily (n=256) or efavirenz at 600 mg daily (n=270) both combined with two nucleosides analogues. Blood samples were drawn 12-hours post nevirapine or efavirenz administration, while on tuberculosis treatment and after tuberculosis treatment discontinuation. In 62 participants, 12-h samples were drawn weekly for the first month of antiretroviral therapy. Sixteen participants participated to an extensive pharmacokinetic study of nevirapine. Concentrations were compared to therapeutic ranges, 3000-8000 ng/mL for nevirapine and 1000-4000 ng/mL for efavirenz.

Results: Nevirapine concentrations at the end of the first week of treatment (on-antituberculosis drugs) did not differ from concentrations off-tuberculosis treatment, but declined thereafter. Concentrations at steady state were 4111 ng/mL (week 12) *vs* 6095 ng/mL (week 48) ($p<0.0001$). Nevirapine concentrations below 3000 ng/mL were found to be a risk factor for virological failure. Efavirenz concentrations were higher on- than off-tuberculosis treatment (2700 ng/mL *vs* 2450 ng/mL, $p<0.0001$).

Conclusions: The omission of the two-week lead-in dose of nevirapine prevented low concentrations at treatment initiation but did not prevent the risk of virological failure. Results support the WHO recommendation to use efavirenz 600 mg daily dose in patients on rifampicin-based antituberculosis therapy.

Introduction

Tuberculosis is a leading opportunistic infection and a major cause of mortality among individuals infected with Human Immunodeficiency Virus (HIV). Substantial reduction of tuberculosis related morbidity and mortality among individuals with HIV can be achieved with early initiation of antiretroviral treatment (ART).[1-3] Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) drug recommended by the World Health Organization (WHO) as a first-line ART for individuals coinfected with HIV-tuberculosis.[4] Nevirapine has been widely used in resource-limited countries with a high burden of HIV due to convenience and affordability of generic fixed-dose combinations (FDC).

Nevirapine could be an alternative NNRTI for HIV-tuberculosis infected individuals and may be preferential over efavirenz in some cases where efavirenz presents central nervous system toxicity that requires discontinuation of early treatment.[5] However, nevirapine has other risks, and studies have found that clearance of nevirapine is more sensitive than efavirenz, to rifampicin potent enzyme induction; as a result nevirapine-based regimens carry greater risk of sub-therapeutic NNRTI concentrations.[6-8] This is related to differences in their biotransformation pathways, as nevirapine is metabolized by several cytochromes-P450 (CYP), CYP2B6, CYP3A and CYP2C and efavirenz is metabolized mainly by CYP2B6.[9, 10] Reduction in nevirapine levels when combined with rifampicin-based antituberculosis therapy is more pronounced during the first two weeks of ART, when nevirapine is typically prescribed at half dose (200mg) (lead-in dose) as a way of preventing hypersensitivity.[11] There is debate over the optimal dose of efavirenz when combined with rifampicin-based antituberculosis therapy.[12] Therefore, the best dose regimen of both NNRTIs remains a subject of discussion.

68 The ANRS12146-CARINEMO was a multicentre, open-label, randomised, non-inferiority
69 clinical trial conducted at three health centres in Maputo. It is the first trial conducted in
70 Mozambique Africa, comparing the efficacy and safety of nevirapine and efavirenz ARTs in
71 HIV-tuberculosis coinfected patients. In patients who were on antituberculosis therapy,
72 nevirapine was initiated at the full dose 200 mg twice daily and efavirenz, at 600 mg daily.
73 Although the non-inferiority of the nevirapine-regimen was not shown, results led investigators
74 to conclude that nevirapine at full dose could be a safe alternative for patients unable to tolerate
75 efavirenz.[13] The present analysis uses data from the ANRS 12146-CARINEMO to describe
76 nevirapine and efavirenz plasma concentrations during and post rifampicin-based tuberculosis
77 treatment from early treatment initiation up to the end of the first year of ART and to analyse
78 whether these concentrations could be related to virological failure or to occurrence of side-
79 effects.
80
81 **Methods**
82 **Study design and participants**
83 Study design, eligibility criteria and study procedures for the ANRS 12146-CARINEMO clinical
84 trial are described elsewhere.[13] The research was conducted in accordance with the Declaration
85 of Helsinki and national and institutional standards. The research protocol was approved by two
86 ethics committee: the Comité Nacional de Bioética para a Saúde, Mozambique
87 (228/CNBS/2007) and the Medecins Sans Frontieres Ethics Review Board, Zurich, Switzerland
88 (approval letter dated 2, May 2007). All participants provided signed informed consent form. In
89 brief, 573 HIV-tuberculosis coinfected patients were enrolled about four to six weeks after
90 initiation of tuberculosis treatment to receive either nevirapine (200 mg bid) without a lead-in
91 dose or efavirenz (600mg qd). All participants received stavudine (30mg bid) and lamivudine

92 (150mg bid), and tuberculosis treatment consisting of an initial two month long intensive phase
93 of FDC treatment containing rifampicin 150 mg, isoniazid 75 mg, ethambutol 275 mg, and
94 pyrazinamide 400 mg, followed by a four month long maintenance phase of FDC treatment
95 containing rifampicin and isoniazid. Dosage was adjusted based on participants' body weight:
96 rifampicin (10 mg/kg), isoniazid (5 mg/kg), ethambutol (15mg/kg) and pyrazinamide (25
97 mg/kg).
98
99 Clinical examination and laboratory analysis were performed at enrolment, on a weekly basis
100 during the first eight weeks, and every four weeks thereafter until study completion at 48 weeks.
101 CD4+T cell counts were obtained at screening and at weeks 24 and 48. HIV-1 RNA were
102 determined in plasma (limit of quantification=50 copies/mL) at enrolment and at weeks 12, 24,
103 36 and 48. Adverse events were coded according to MedDRA and graded according to the ANRS
104 scale (www.anrs.fr) as previously described.[13]
105
106 **Pharmacokinetic studies**
107 Pre-dose concentrations of nevirapine and morning concentrations after evening intake of
108 efavirenz (C_{12}) were measured at week 12 (while participants were on-tuberculosis drugs) and at
109 weeks 36 and 48 (when participants were off-tuberculosis drugs). C_{12} were measured at the end
110 of week two of antiretroviral treatment to monitor drug exposure in the first 100 participants
111 enrolled in the nevirapine treatment arm. A sub-group of participants were selected to be in the
112 early sample group for additional blood samples drawn on days 7 (week one), 14 (week two), 21
113 (week three) and 28 (week four) of ART to assess nevirapine and efavirenz concentrations.
114 An additional sub-group of participants being treated with nevirapine were selected to be in the
115 the pharmacokinetic (PK) group. They participated in an extensive nevirapine PK study and had

116 blood samples collected during a dosing interval at steady-state four weeks after initiation of
117 ART (while on-antituberculosis drugs) and four weeks after completion of antituberculosis
118 treatment (while off-antituberculosis drugs). Blood was drawn before drug intake (time 0) and
119 after drug intake (0.5 hour, 1 hour, 1.5 hours, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours and 12
120 hours). Plasma concentrations of nevirapine and efavirenz were assayed by validated high
121 performance liquid chromatography methods with lower limit of quantifications (LOQ) of 25
122 ng/mL and 50 ng/mL, respectively.[14, 15] Plasma concentrations were compared with previously
123 described therapeutic ranges, which are, for nevirapine, between 3000-8000 ng/mL [16] and, for
124 efavirenz, between 1000-4000 ng/mL.[17]
125
126 Non-compartmental method was used to estimate nevirapine pharmacokinetic parameters
127 (WinNonlin software, Pharsight Corporation, Mountain View, California, USA). Plasma
128 maximum concentration (C_{max}), time to plasma peak concentration (T_{max}) and plasma minimum
129 concentration (C_{min}) were observed values. The areas under the concentration-versus-time curves
130 during the 12 hour-dosing intervals at steady state (AUC_{12}) were estimated using linear up-log
131 down trapezoidal method. Two-sided 90% confidence intervals (CIs) were constructed for the
132 ratios of the geometric mean values (with tuberculosis treatment *versus* alone) of AUC_{12}, C_{min}
133 and C_{max}.
134
135 **Statistical analysis**
136 Unless otherwise indicated, descriptive data were reported using median and interquartile range
137 (IQR). Nevirapine or efavirenz plasma concentrations were excluded from analysis in the
138 following cases: treatment switch in patients with adverse event or pregnancy; patients still on-
139 antituberculosis drugs at weeks 36 and 48; and blood samples collected outside time period range

of 11.5h-15.5h after the last drug intake. Concentrations below the LOQ were included as LOQ/2 for analysis. Plasma concentrations of NNRTIs on- and off-antituberculosis therapy were compared by Wilcoxon signed-rank test. Mixed models were used to analyse the change in log-transformed concentrations, with time being a fixed effect in the model. Logistic regression was used to identify predictors of virological failure (HIV1-RNA≥50copies/mL) at week 48 and safety binary outcomes of interest (occurrence of central neurological adverse event and hepatitis grade 2 or more). Factors with an association of p-value<0.2 were used for multivariate analyses. A sensitivity analysis was performed on predictors of virological failure after excluding NNRTI plasma concentration below the LOQ used as a surrogate marker of poor treatment adherence. All statistical analysis was conducted with StataSE™ software (StataCorp. 2005. Stata Statistical Software: Release 12.1. College Station, TX: StataCorp, USA). The level of statistical significance was set at 0.05.

Results

Participant characteristics

From 573 patients enrolled in the ANRS 12146-CARINEMO trial, 526 participants had at least one 12-h (13.5±2h) post dose concentration measure available for nevirapine or efavirenz at weeks 12, 36 and 48 of study follow up. One hundred and fourteen participants on nevirapine had an available concentration measure at week two. Sixty-two patients were enrolled in the nevirapine and efavirenz early sample group. Sixteen patients (8 males) participated in the two periods of the extensive nevirapine pharmacokinetic studies. Baseline characteristics of these patients are shown in Table 1. Participant age and weight at enrollment were 33 years and 52.1 kg, and 57% were male. CD4+T cell count was 92/mm^3 and HIV-1 RNA was 5.6 $\log_{10}$

copies/mL. The baseline characteristics of participants in each subgroup were similar to the characteristics of all participants.

Nevirapine and efavirenz exposure at treatment initiation (Early sample group). Thirty-two patients were on nevirapine and 30 patients were on efavirenz. During co-administration, nevirapine concentrations decreased over time from week 1 (5721 ng/mL) to week 12 (4003 ng/mL) ($p=0.001$) and increased after completion of antituberculosis therapy (6271 ng/mL) ($p<0.001$) (Figure 1). Nevirapine concentrations remained steady from week three (3844 ng/mL) to week 12 when combined with antituberculous therapy. Conversely, there was a non-significant increase in efavirenz concentration from week one (2509 ng/mL) to week three (3555 ng/mL) and week 12 (2994 ng/mL), then a significant decrease after antituberculosis therapy discontinuation at week 48 (2329 ng/mL) when compared to week 12 ($p<0.001$). Importantly, when nevirapine was initiated at full dose, plasma concentrations one week after starting nevirapine during co-administration with antituberculosis therapy were not significantly different from those after completion of antituberculosis therapy at week 48. Similar findings were obtained with efavirenz.

Nevirapine and efavirenz exposure on- and off-antituberculosis therapy. Concentrations on- and off-antituberculosis therapy in the whole population are presented in Table 2. Nevirapine and efavirenz concentrations were not different when measured at weeks 36 and 48 after tuberculosis therapy discontinuation, therefore concentrations at week 48 were considered for further comparisons. Nevirapine plasma concentrations at week two and 12 were significantly lower than at week 48 ($p=0.0003$ and $p<0.0001$, respectively). Importantly, 22% and 25% of patients had concentrations <3000 ng/mL at weeks two and 12 *versus* 10% at week 48. In the 16 patients

187 who participated in the extensive nevirapine pharmacokinetic study, nevirapine concentrations
188 were lower while on-antituberculosis drugs than after discontinuation of antituberculosis drugs
189 (Figure 2). Nevirapine AUC_{0-12} and C_{min} were reduced by 13% and 17% respectively, but the
190 90% confidence interval of GMR parameters failed to lie within the 0.80-1.25 bioequivalence
191 range as indicated in Table 3. Efavirenz concentrations decreased slightly but significantly after
192 tuberculosis drugs discontinuation between weeks 12 to week 48 ($p<0.0001$). Efavirenz
193 concentrations were <1000 ng/mL in 9% of participants at weeks 12 and 48. Noteworthy, about
194 25% of the participants had concentrations of efavirenz and nevirapine above the therapeutic
195 range when off-antituberculosis drugs, and was as high as 37% when efavirenz was combined
196 with antituberculosis drugs.
197
198 **Concentrations-efficacy relationship**
199 Plasma HIV-1 RNA was <50 copies/mL at weeks 12, 24 and 48 in 77.2% (156/202), 78.3%
200 (155/198) and 77.3% (157/203) of the participants on nevirapine and 85.0% (170/200), 85.5%
201 (171/200) and 88.2% (164/186) of the participants on efavirenz, respectively. Analysis within
202 each treatment arm demonstrated that in the nevirapine arm, having reactive HBsAg, being a
203 male and having C12<3000ng/mL at week 12 were independently associated with the risk of
204 virological failure as shown in Table 4. Interestingly, such association was unchanged when
205 concentrations below the LOQ were removed. The only predictor of virological failure in the
206 efavirenz arm was having concentrations <1000 ng/mL at week 12 (Table 4). The association
207 was no longer significant after excluding concentrations below LOQ.
208
209 **Safety issues related to concentrations**

Fourteen participants on nevirapine and 2 participants on efavirenz switched treatment because of adverse events. There were 11 hepatitis (for 7 participants concentrations ranged from 3417 up to 30321 ng/mL) and 3 rashes (concentrations not available) in the nevirapine arm and 2 acute psychiatric disorders in the efavirenz arm (786 ng/mL and 5863 ng/mL). There was no significant association between the occurrence of grade 2 or more CNS adverse events reported within the first 12 weeks of ART in 6 participants on efavirenz and efavirenz concentrations >4000 ng/mL at week 12 (p=0.293). Among the factors analysed for association with the occurence of hepatitis (increase in alanine aminotransferase) of grade 2 or more which occurred in 32 patients, only efavirenz concentrations during the same time periods [OR 5.25 (95% CI: 2.1-13.2, p=0.0002)] was significant.

Discussion

To our knowledge, this is the first study describing nevirapine and efavirenz exposure from initiation of ART in HIV-tuberculosis coinfected patients' on-antituberculosis therapy until six months after completion of antituberculosis treatment in a large cohort of participants. We have demonstrated marked differences in two NNRTIs exposure when combined with rifampicin-isoniazid based antituberculosis therapy in an African population. Nevirapine metabolism, but not efavirenz, is induced by this concomitant treatment. However, both nevirapine (omitting the lead-in dose) and efavirenz plasma concentrations at the end of the first week of ART were in the same range as they were after discontinuation of antituberculosis drugs, which is of importance for optimal antiretroviral efficacy at ART initiation. Nevirapine concentrations declined thereafter reaching a steady state from week three up to the end of antituberculosis therapy. Our extensive pharmacokinetic study supports these pre-dose concentration findings. Despite such moderate decrease, 25% of the participants on nevirapine-based ART had concentrations below

234 3000 ng/mL, on-antituberculosis therapy (week 12) versus 11% after antituberculosis therapy
235 discontinuation. Such decrease was a predictor of virological failure.
236 Nevirapine concentrations reported here differ from previous studies, whose all participants
237 received the standard lead-in dose and where as many as 59% to 79% of HIV-tuberculosis
238 coinfected patients had low pre-dose concentrations during the first two weeks of rifampicin-
239 based antituberculosis therapy.[18, 19] Our results support our study design with full dose of
240 nevirapine at initiation of treatment to avoid the first two-week period of treatment with low
241 nevirapine concentrations. Concentrations measured at the end of the first two weeks of
242 nevirapine treatment at 200 mg twice daily were slightly lower (4759 ng/mL) than the one
243 reported by Avihingsanon *et al.*, (5300 ng/mL) in which 16 Thai patients included in the 600 mg
244 nevirapine daily dose group received a lead-in dose of 200 mg twice daily[18] but higher than those
245 reported by Lamorde *et al.* in 9 Ugandan adults (2920 ng/mL).[20] Those two studies are limited by
246 the small number of included patients and the absence of relationship with virological response.
247 To point out, the study conducted in Thai patients was prematurely discontinued as the lead-in
248 strategy with nevirapine 200 mg twice daily was associated with a high rate of nevirapine
249 hypersensitivity.[18] In our study, treatment tolerance was good and did not differ between the
250 nevirapine and efavirenz groups.[13] Nevirapine concentrations that we observed at steady state of
251 induction were similar to those reported in one study conducted in Africa[21] but lower than others
252 conducted in different countries.[19, 22] Differences in population weight and pharmacogenetics
253 could explain the differences nevirapine exposure between African and South-East Asian
254 populations. This decrease in nevirapine concentrations is surprising as it is generally accepted
255 that the enzyme induction process is maximal after 10 to 15 days of drug inducer administration.
256 Nevirapine metabolism involved different CYP enzymes which may be induced differently by
257 rifampicin and nevirapine. Interestingly, the autoinduction process of efavirenz was

258 demonstrated to continue up to 16 weeks of therapy; whether such mechanism could occur for
259 nevirapine combined to rifampicin remains to be investigated.[23]
260 Recent studies, mainly conducted in sub-Saharan Africa, showed that at least in some sub-groups
261 of patients, efavirenz concentrations were higher on- than off-antituberculosis therapy.[24, 25]
262 Bertand *et al.* recently demonstrated that the efavirenz-antituberculosis drugs interaction depends
263 on *CYP2B6* and *NAT2* genetic polymorphism suggesting that isoniazid which has inhibiting
264 properties on some non CYP2B6 biotransformation pathways, could play a role,
265 counterbalancing the inducing properties of rifampicin.[26] Eighty-seven of our patients having
266 efavirenz concentrations above 4000 ng/mL on-antituberculosis therapy which decreased by 44%
267 after antituberculosis discontinuation are likely to carry one of the *CYP2B6* loss of function
268 variant.
269 Importantly, the association between low nevirapine concentrations at week 12 and virological
270 failure at 48 weeks was not affected by the exclusion of concentrations below the LOQ (used as
271 surrogate marker of poor adherence) from the analysis, which was not the case for efavirenz.
272 This finding supports the main results of the CARINEMO trial, which failed to show the non-
273 inferiority of nevirapine compared to efavirenz and points out the different drug-drug interaction
274 mechanism that explains at least in part the difference in virological response between the two
275 antiretroviral regimens.[13]
276 Drug liver injury has been previously reported in African patients with high efavirenz
277 concentrations when efavirenz was combined to antituberculosis therapy.[27, 28] In contrast to some
278 studies,[17, 29] others and ours failed to relate the few recorded CNS adverse effects to high
279 efavirenz concentrations.[25]
280 Our study had some limitations. First C_{12} and not pre-dose trough concentrations were collected
281 as a surrogate of efavirenz exposure. However, such approximation is acceptable as efavirenz has

282 an elimination half-life longer than the 24h-dosing interval, which would minimize fluctuations
283 between peak and trough concentrations.[30] Second, it is now well demonstrated that nevirapine
284 and efavirenz concentrations are highly dependent on *CYP2B6* genetic polymorphism.[6, 31-35]
285 Indeed the frequency of the *CYP2B6* loss of function variants was reported to be higher in
286 African than in European descents.[6, 26, 32, 33] The frequency of *CYP2B6516T* loss of function
287 allele in the Mozambican population is as high as 40%[36] and explains, at least in part, the high
288 concentrations observed in our study. The exact mechanism of the nevirapine-antituberculosis
289 drugs interaction warrants further study, and pharmacogenetics could be a useful tool. Third,
290 none of the metabolites of nevirapine or efavirenz were quantified. Several nevirapine
291 metabolites involving different CYP pathways were identified. The 8-hydroxy efavirenz in the
292 main CYP2B6 mediated metabolite of efavirenz. Metabolite concentrations in plasma were
293 found to be below those of the parent drug and therefore their contribution to nevirapine or
294 efavirenz efficacy is unlikely.[23, 37]
295 In conclusion, this pharmacokinetic study conducted in 526 HIV-tuberculosis coinfected patients,
296 adds new evidence on the nevirapine or efavirenz exposure and drug-drug interaction when
297 combined to rifampicin and isoniazid based antituberculosis treatment. Our efavirenz data are in
298 agreement with most recent studies and support the WHO recommendation. Omitting the 200 mg
299 once daily for the 2-first weeks of nevirapine treatment allows concentrations to be within the
300 therapeutic range at initiation of treatment when combined with antituberculosis drugs and this
301 drug regimen was well tolerated. However, such strategy does not avoid decrease in nevirapine
302 concentrations after the first two weeks of treatment and supports the results of the main trial
303 which recommends using efavirenz whenever possible.
304
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316

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334

335 **Transparency declarations**

336 None of the authors have conflicts of interest to declare related to this study.

337 MB and AMT conceived and designed the study. NBB and MB implemented and led the study.
338 EB coordinated the data management of the study and performed the statistical analysis. EB, CS,
339 BM, VF, ABT and BG critically revised the study design, contributed to the interpretation of
340 results. CS, BM, VF and ABT coordinated the laboratory analysis and supervised efavirenz and
341 nevirapine assays in accordance to good laboratory practices. CS supported the implementation
342 and running of the study. NBB and AMT wrote and prepared the manuscript. All authors
343 reviewed and approved the final version of the manuscript.

344

345 **References**

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Table 1 Demographic, clinical and laboratory characteristics of patients at baseline [a]

Characteristics	All studied population [b]		Early samples group		Extensive PK
	NVP group (n=256)	EFV group (n=270)	NVP group (n=32)	EFV group (n=30)	NVP group (n=16)
Age, years	33 (29-41)	33 (28-40)	36 (31-42)	34 (29-38)	36 (31-47)
Sex, male	142 (55.5)	160 (59.3)	20 (62.5)	17 (56.7)	8/16 (50.0)
Weight, Kg	52.0 (46.6-57.5)	52.3 (47.2-58.7)	51.9 (46.0-58.9)	50.9 (47.2-55.1)	52.6 (46.6-55.6)
Body-mass index, Kg/m^2	18.7 (17.2-20.3)	18.9 (17.6-20.3)	19.1 (16.9-20.4)	18.5 (16.9-20.1)	18.3(17.0-19.5)
Haemoglobin, g/dl	9.4 (8.5-10.3)	9.4 (8.3-10.4)	9.0 (8.1-9.8)	9.4 (8.3-10.4)	9.4 (7.9-10.3)
ALT, UI/L	22.6 (14.7-36.8)	23.0 (15.6-37.7)	26.4 (12.8-44.4)	28.7 (22.1-38.3)	13.0 (9.0-20.0)
Total bilirubin, mg/dl	0.4 (0.3-0.6)	0.5 (0.3-0.6)	0.4 (0.3-0.5)	0.5 (0.3-0.7)	0.8 (0.7-0.9)
CD4+T cell count, $cells/mm^3$	94 (44-152)	86 (44-144)	106 (47-153)	91 (69-127)	86 (57-169)
HIV-1 RNA, log10 copies/ml	5.7 (5.1-6.0)	5.5 (5.2-6.1)	5.7 (5.3-5.9)	5.4 (5.2-6.0)	5.5 (5.2-5.7)
HBsAg, reactive	53/255 (20.8)	57/266 (21.4)	1/32 (3.1)	4/30 (13.3)	1(6.3)
HCV antibody, reactive	4 (1.6)	5 (1.9)	1 (3.1)	1 (3.3)	1 (6.3)
Pulmonary tuberculosis	202 (78.9)	203 (75.2)	29 (90.6)	20 (66.7)	11 (69.0)
Smear positive pulmonary tuberculosis	93 (46.3)	111 (56.7)	7 (24.1)	16 (80.0)	9 (81.8)

[a] Data are n (%), median (IQR), or n/N (%). ALT = alanine aminotransferase. HBsAg = hepatitis B surface antigen. HCV= hepatitis C virus. PK = pharmacokinetic. [b]At least one 12 hours post-dosing concentrations available for nevirapine or efavirenz at weeks 12, 36 and 48 of study follow up.

Table 2 Concentration of nevirapine and efavirenz at weeks 2 and 12 (on-tuberculosis drugs) and at weeks 36 and 48 (off-tuberculosis drugs) measured 12-h post dose (trough concentrations for nevirapine and mid-dose concentrations for efavirenz).

Time (weeks)	2	12	36	48
Nevirapine, n	114	225	218	205
Median (IQR), ng/mL	4759 (3201-7327)	4111(2970-5534)	5970 (4261-7898)	6095 (4521-8504)*,**
n (%)				
< 25, ng/mL	3 (2.6)	6 (2.7)	6 (2.8)	3 (1.5)
≥ 25 and <3 000, ng/mL	22 (19.3)	51 (22.6)	15 (6.8)	20 (9.7)
≥ 3 000 and < 8 000, ng/mL	68 (59.6)	148 (65.8)	144 (66.1)	125 (61.0)
≥ 8 000, ng/mL	21 (18.5)	20 (8.9)	53 (24.3)	57 (27.8)
Efavirenz, n	-	235	199	189
Median (IQR), ng/mL	-	2700 (1701-6965)	2604 (1742-4412)	2450 (1742-4086)**
n (%)				
< 50, ng/mL	-	12 (5.1)	6 (3.0)	5 (2.6)
≥ 50 and < 1 000, ng/mL	-	9 (3.9)	9 (4.6)	12 (6.3)
≥ 1 000 and <4 000, ng/mL	-	127 (54.0)	131 (65.8)	122 (64.6)
≥ 4 000, ng/mL	-	87 (37.0)	53 (26.6)	50 (26.5)

*week 2 *vs* week 48 (p=0.0003) and **week 12 *vs* week 48 (p<0.0001)

Table 3 Nevirapine pharmacokinetic parameters (16 patients in the nevirapine treatment group) [a]

Time	C_{min} (ng/mL)	C_{max} (ng/mL)	T_{max} (h)	AUC (ng.h/mL)
With antituberculosis drugs	4 513 (2 527-8 797)	6 561 (4 744-10 311)	2.0 (1.5-4.2)	66 743 (46 817-114 072)
Alone	5 025 (3 557-10 662)	7 283 (5 246-13 637)	1.5 (1.0-4.0)	71 332 (53 440-146 908)
GMR[b] (90% CI)	0.83 (0.71-0.97)	0.89 (0.79-1.00)		0.87 (0.77-0.99)

[a] Data are expressed as median (ranges)

[b] GMR = is the ratio of the geometric means for the parameters with rifampicin to those without rifampicin

C_{min} = minimum concentration; C_{max} = maximum concentration; T_{max} = time to achieve maximum concentration; AUC = area under the plasma concentration vs. time curve during a 12-h dosing interval.

Table 4 Factors associated with virological failure after 48 weeks of antiretroviral therapy among participants in the nevirapine and efavirenz treatment groups

Possible risk factors	Treatment outcome at week 48, proportion of patients (%)		Univariate analysis		Multivariate analysis	
	Success	Failure	OR (95% CI)	p	OR (95% CI)	p
Nevirapine						
C_{12}< 3000 ng/mL at week12	30/156 (19.2)	20/46 (43.5)	3.23 (1.59-6.54)	0.001	3.44 (1.65-7.17)	0.001
Male	87/175 (49.7)	37/53 (69.8)	2.34 (1.21-4.51)	0.011	2.18 (1.03-4.61)	0.036
Weight ≤50 Kg	70/175 (40.0)	20/53 (37.7)	0.92 (0.95-3.29)	0.767	-	-
HBsAg reactive	15/175 (18.9)	15/53 (28.3)	1.69 (0.83-3.42)	0.148	2.51 (1.15-5.51)	0.035
Baseline CD4+ cell counts, <50 cells/mm^3	48/175 (27.4)	13/53 (24.5)	0.86 (0.42-1.75)	0.674	-	-
HIV-1 RNA viral load at baseline, ≥5.5 log	77/175 (44.0)	17/53 (32.1)	1.66 (0.87-3.19)	0.118	-	NS
Efavirenz						
C_{12}< 1000 ng/mL at week12	7/170 (4.1)	5/30 (16.7)	4.70 (1.37-15.81)	0.020	4.70 (1.37-15.81)	0.020

Male	109/198 (55.1)	18/33 (54.6)	0.98 (0.47-2.05)	0.957	-	-	
Weight≤50 Kg	77/198 (38.9)	21/33 (63.6)	0.90 (0.42-1.93)	0.782	-	-	
HBsAg reactive	46/198 (23.2)	4/33 (12.1)	0.44 (0.15-1.33)	0.116	-	NS	
Baseline CD4+ cell counts, <50 cells/mm^3	51/198 (25.8)	12/33 (36.4)	1.65 (0.76-3.58)	0.216	-	-	
HIV-1 RNA viral load at baseline, ≥5.5 log	101/198 (51.0)	11/33 (33.3)	2.08 (0.96-4.52)	0.058	-	NS	

C_{12} = 12 hours post-dosing concentrations. HBsAg=Hepatitis B surface antigen. NS = Not significant.

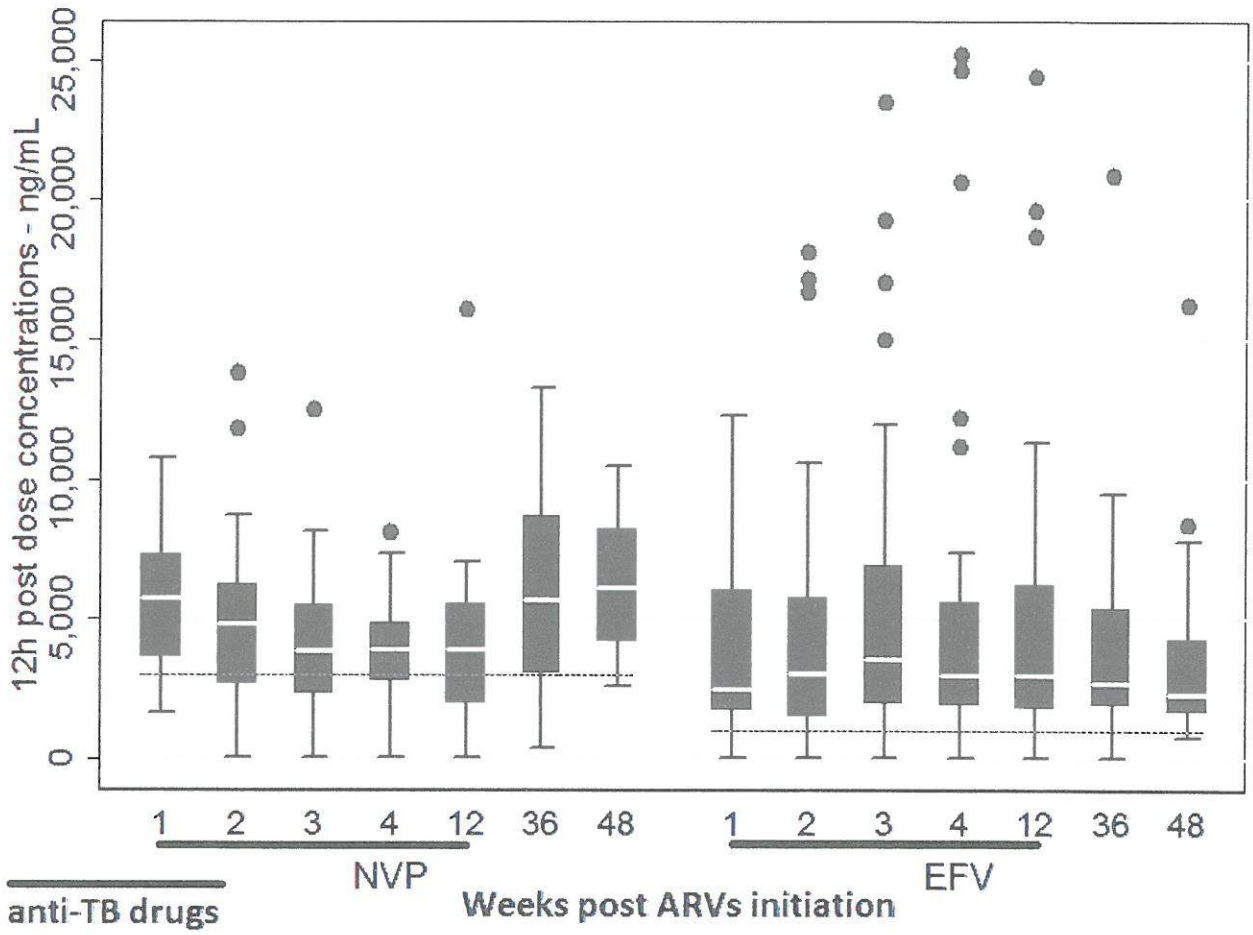

Figure 1 Plasma trough concentrations 12 hours post dosing (C_{12}) of nevirapine in 32 patients and efavirenz in 30 patients during 48 weeks of antiretroviral therapy. Medians are bold line, interquartile ranges are boxes and adjacent lines are minimum and maximum without outliers; dots are outliers. Dotted lines are the lower targets of the therapeutic ranges (3000 ng/mL and 1000 ng/mL for NVP and EFV, respectively). EFV = efavirenz. NVP = nevirapine. ARV = antiretroviral. TB = Tuberculosis.

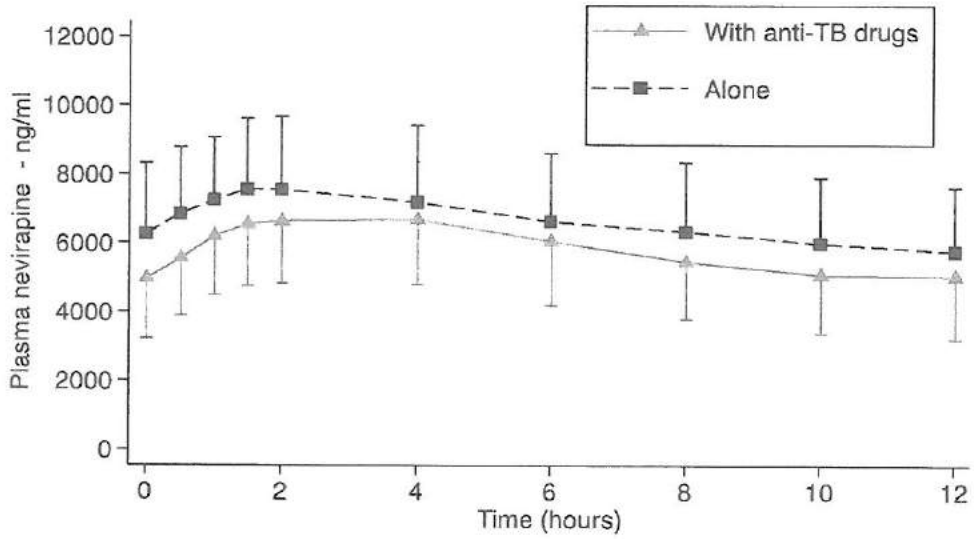

Figure 2 Mean±SD nevirapine plasma concentrations measured during anti-tuberculosis drugs 4 weeks after ART initiation (closed triangles and solid lines) and 4 four weeks after completion of tuberculosis treatment (closed squares and dotted lines). anti-TB = antituberculosis.

4. CONSIDERAÇÕES FINAIS

Nestes dois estudos caracterizamos as concentrações plasmáticas de ITRNN (nevirapine e efavirenz) e de medicamentos antituberculose, em particular da rifampicina e da isoniazida, usados em pacientes moçambicanos com coinfecção por HIV e tuberculose. Estes foram subestudos do ensaio clínico CARINEMO-ANRS 12146, no qual os pacientes em tratamento com medicamentos antituberculose eram aleatoriamente alocados para iniciar a TARV com regime contendo nevirapina ou efavirenz, ambos combinados com a lamivudina e a estavudina. Há que se realçar que a dose escalonada da nevirapina não foi usada neste ensaio clínico para evitar concentrações subterapêuticas no início da TARV. Os resultados do ensaio clínico CARINEMO-ANRS 12146 foram recentemente publicados (Bonnet M et al. *Lancet Infect Dis* 2013; 13:303-12).

O nosso artigo 1 foi o primeiro de gênero a comparar os parâmetros farmacocinéticos da rifampicina e da isoniazida quando coadministradas com e sem nevirapina (200mg, duas vezes ao dia, sem a dose escalonada), em pacientes co-infectados pelo HIV/TB, e o nosso estudo também contribuiu para a fornecer dados, que são limitados na literatura especializada, sobre a farmacocinética de medicamentos antituberculose em pacientes infectados pelo HIV tratados com efavirenz (600mg por dia). Os achados deste estudo indicaram que a exposição à rifampicina não foi alterada de forma clinicamente significativa, quando combinada com nevirapina ou efavirenz. No entanto, verificamos uma redução significativa de 30% na exposição isoniazida (AUC) quando co-administrada com efavirenz. Esta redução, provavelmente, não tem qualquer impacto no resultado de tratamento da tuberculose uma vez que o parâmetro de eficácia é determinado pela relação AUC/MIC e todas as AUC estão bem acima da concentração efetiva máxima de 50 (EC50), que foi estimada em 1 mg.h/L.

O artigo 2 é o primeiro estudo que descreve as concentrações plasmáticas de nevirapina, sem a dose escalonada, e de efavirenz no início da terapia antiretroviral em pacientes em tratamento antituberculose. Note-se que as concentrações plasmáticas da nevirapina e efavirenz no final da primeira semana de TARV encontram-se nos mesmos níveis observados após a descontinuação do tratamento antituberculose. Os pacientes incluídos neste estudo apresentavam uma imunodepressão severa com carga viral HIV-1 elevada na inclusão, e a demonstração de que as concentrações plasmáticas dos ITRNN não são inferiores aos limites terapêuticos é de importancial crucial para sustentar a eficácia da TARV. No entanto, as concentrações plasmáticas da nevirapina diminuem, atingido o *steady state* três semanas após o início da TARV, mantiveram-se estáveis até o fim do tratamento antituberculose, e aumentaram significativamente após a descontinuação do tratamento da tuberculose. Por outro lado, as concentrações plasmáticas de efavirenz reduziram-se após a interrupção dos medicamentos antituberculose, mas a quando da coadministração, as mesmas foram mais elevadas. Na semana 48 após o início da TARV, 76.8% (175/228) dos pacientes do grupo da nevirapina e 85.7% (198/231) do grupo do efavirenz alcançaram níveis de HIV-1 RNA<50 cópias/mL, com as respectivas medianas de células T CD4+ de 249 e 252 células/mm^3 ($p>0.05$). A análise multivariada revelou que os pacientes que receberam nevirapina com valores baixos de C12 na semana 12, do sexo masculino, e com HBsAg positivo apresentaram, respectivamente, 3.4, 2.2 e 2.5 vezes mais possibilidade de desenvolver falência virológica ($p<0.05$).

5. CONCLUSÕES

1.- Nos indivíduos com imunossupressão severa associada ao HIV, as concentrações plasmáticas de medicamentos antituberculose, em particular da rifampicina, demonstraram ser reduzidas em relação aos pacientes não infectados pelo HIV. No entanto, os parâmetros farmacocinéticos da rifampicina e da isoniazida e sua exposição não estiveram alterados de forma clinicamente significativa, quando coadministrados com a nevirapina e o efavirenz, apesar de se notar um aumento discreto da C_{max} da rifampicina após a introdução da nevirapina, e uma redução da C_{max} da isoniazida após a introdução do efavirenz. O resultado clínico do tratamento nos pacientes foi favorável devido a uma possível consequência da terapia múltipla da tuberculose e aumento de células CD4+ com a TARV.

2.- A análise farmacocinética e das concentrações pré-dose demonstraram uma redução das concentrações plasmáticas da nevirapina quando esta é administrada simultaneamente à medicação antituberculose. A suspensão do tratamento antituberculose aumenta de forma siginificativa a C_{min} da nevirapina (em 17%) e as C_{max} e AUC da nevirapina (em 11% e 13%, respectivamente).

3.- A não utilização da dose escalonada da nevirapina no início da TARV permite obter concentrações plasmáticas da droga dentro do intervalo terapêutico no final da primeira semana de TARV. A partir daí, é notável a redução progressiva da concentração plasmática da nevirapina, que atinge o *steady state* a partir da semana 3 até o fim de tratamento da tuberculose. Porém, após a suspensão do tratamento antituberculose, as concentrações plasmáticas da nevirapina aumentam. As concentrações plasmáticas do efavirenz, ao contrário, são reduzidas com a suspensão do tratamento antituberculose.

4.- Na semana 12 após o início da TARV, as concentrações plasmáticas da nevirapina foram reduzidas nos indivíduos com falência virológica quando comparadas às dos indivíduos sem falência virológica. Factores independentemente associados a falência virológica, nos indivíduos com coinfecção HIV/TB, em TARV com regime contendo nevirapina, são a baixa concentração de nevirapina na semana 12 após o início do TARV, o sexo masculino e o HBsAg reactivo. No entanto, para o regime TARV contendo efavirenz, a baixa concentração desta droga na semana 12 após o início do TARV foi único factor independente associado à falência virológica.

6. RECOMENDAÇÕES

1.- Os medicamentos antituberculose comummente usados para o tratamento da tuberculose activa podem ser usados em regimes de dosagem padrão nos pacientes com coinfecção HIV/TB em TARV com nevirapina (sem a dose escalonada) ou com efavirenz (dose padrão de 600 mg/dia), sem que haja um comprometimento do resultado do tratamento da TB.

2.- Considerar o uso, em pacientes com coinfecção HIV/TB, da nevirapina sem a dose escalonada (200 mg, duas vezes ao dia), sempre que o regime TARV com base em efavirenz for contraindicado, ou que regimes alternativos do TARV sejam limitados. Não há necessidade de aumentar a dose do efavirenz para os pacientes em tratamento antituberculose contendo a rifampicina.

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8. ANEXO

8.1 Aprovação do estudo pelo Comité de Ética em Pesquisa

REPÚBLICA DE MOÇAMBIQUE

MINISTÉRIO DA SAÚDE

COMITÉ NACIONAL DE BIOÉTICA PARA A SAÚDE

Exmo Senhor
Dr. Nilesh Bhatt
Imunologia

Ref.318/CNBS Data 21 de Setembro de 2009

Assunto: Parecer sobre o estudo "*Nevirapine (or efavirenz) and rifampin / co-administration: impact of genetic polymorphism of drug metabolizing enzymes and transporters in interindividual variability of concentrations and tolerance ", versão 1.0 de 10 de Agosto de 2009.*

O Comité Nacional de Bioética para a Saúde (CNBS) analisou as correcções efectuadas no protocolo intitulado: **"Nevirapine (or efavirenz) and rifampin / co-administration: impact of genetic polymorphism of drug metabolizing enzymes and transporters in interindividual variability of concentrations and tolerance" versão 1.0 de 10 de Agosto de 2009.**", sobre o mesmo o CNBS chegou a seguinte conclusão:

Não havendo nenhum inconveniente de ordem ética que impeça a realização do estudo, o CNBS dá a sua devida aprovação.

Recomenda aos investigadores que o mantenham informado do decurso do estudo.

E faz notar que a aprovação ética não substitui a autorização administrativa.

Sem mais de momento as nossas cordiais saudações.

O Presidente

Dr. João Manuel de Carvalho Fumane

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