JÚLIO CÉSAR SACHET

**Cinética plasmática de emulsão lipídica semelhante à
lipoproteína de baixa densidade (LDL) no lúpus
eritematoso sistêmico com e sem difosfato de
cloroquina**

Tese apresentada à Faculdade de Medicina da
Universidade de São Paulo para obtenção do
título de Doutor em Ciências

Área de Concentração: Reumatologia

Orientador: Prof. Dr. Eduardo Ferreira Borba Neto

São Paulo
2006

Para Marina,

Um lindo raio de sol em minha vida

AGRADECIMENTOS

A meus pais...

Pelo exemplo de integridade e perseverança, possibilitando que eu pudesse
estudar e me formar como médico.

A minha esposa Cristiane...

Por estar sempre ao meu lado, me apoiando e incentivando a prosseguir
com esta tese.

Ao Dr. Eduardo Ferreira Borba...

Mais do que um orientador, um grande amigo e exemplo de profissional.

A Carmem G. C. Vinagre...

Pela amizade e orientação.

Aos pacientes e controles voluntários...

Por disporem de um dia de suas vidas em benefício deste estudo.

Aos funcionários e assistentes do Ambulatório de Reumatologia...
Pela disponibilidade em resolver dúvidas e no auxílio precioso na seleção de
pacientes.

Aos funcionários do Laboratório de Lípidos, em especial Vanessa e Lisa...
Pela grande ajuda na realização do ensaio.

Aos professores doutores Raul C. Maranhão e Eloísa Bonfá...
Pela imensa ajuda na elaboração do artigo e revisão do trabalho.

Esta tese está de acordo com as seguintes normas, em vigor no momento de sua publicação:

Referências: adaptado de *International Committee of Medical Journals Editors* (Vancouver)

Universidade de São Paulo. Faculdade de Medicina. Serviço de Biblioteca e Documentação. Guia de apresentação de dissertações, teses e monografias. Elaborado por Anneliese Carneiro da Cunha, Maria Júlia de A. L. Freddi, Maria F. Crestana, Marinalva de Souza Aragão, Suely Campos Cardoso, Valéria Vilhena. 2ª ed. São Paulo: Serviço de Biblioteca e Documentação; 2005

Abreviaturas dos títulos dos periódicos de acordo com *List of Journals Indexed in Index Medicus*.

SUMÁRIO

Resumo

Summary

1. INTRODUÇÃO.....	1
2. OBJETIVOS.....	4
3. PACIENTES E MÉTODOS.....	5
3.1. População de estudo.....	5
3.2. Preparação da LDE.....	6
3.3. Cinética plasmática da LDE.....	6
3.4. Cálculo da TFR da emulsão de lípidos radioativos.....	7
3.5. Consentimento informado e segurança radiológica.....	8
3.6. Perfil lipídico.....	8
3.7. Análise estatística.....	9
4. RESULTADOS.....	10
4.1. Cinética plasmática da LDE.....	12
5. DISCUSSÃO.....	15
6. CONCLUSÕES.....	19
7. REFERÊNCIAS	20

Apêndice

RESUMO

Sachet JC. Cinética plasmática de emulsão lipídica semelhante à lipoproteína de baixa densidade (LDL) no lúpus eritematoso sistêmico com e sem difosfato de cloroquina [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2006. 68p.

OBJETIVO: A via metabólica da lipoproteína de baixa densidade (LDL) em pacientes com lúpus eritematoso sistêmico (LES) em uso de difosfato de cloroquina (DFC) foi avaliada através do comportamento cinético de uma nanoemulsão radioativa rica em colesterol (LDE) que se assemelha à estrutura lipídica da LDL. **MÉTODOS:** LDE foi marcada com ^{14}C -colesterol éster (^{14}C -CE), sendo a seguir injetada endovenosamente em pacientes do sexo feminino com LES inativo: 10 tomando DFC (grupo DFC), 10 sem tratamento (grupo SEM TRATAMENTO); e 10 mulheres normais (grupo CONTROLE). Os grupos foram pareados pela idade e seguiram rigorosos critérios de seleção de condições que pudessem interferir no perfil lipídico. Amostras de sangue foram coletadas em intervalos pré-estabelecidos após a infusão para mensuração da radioatividade. Níveis séricos de jejum de lipoproteínas foram determinados no início dos estudos cinéticos. **RESULTADOS:** Idade e índice de massa corpórea (IMC) foram similares nos grupos estudados. A taxa fracional de remoção (TFR) de ^{14}C -CE foi significativamente maior no grupo DFC comparada ao grupo SEM

TRATAMENTO ($0,076 \pm 0,037$ vs. $0,046 \pm 0,021 \text{ h}^{-1}$; $p < 0,05$) e CONTROLE ($0,0516 \pm 0,0125 \text{ h}^{-1}$; $p < 0,05$). Em concordância, níveis significativamente menores de colesterol total e LDL foram observados no grupo DFC (156 ± 16 e $88 \pm 16 \text{ mg/dl}$) comparando-se com SEM TRATAMENTO (174 ± 15 e $108 \pm 17 \text{ mg/dl}$; $p < 0,05$) e CONTROLE (200 ± 24 e $118 \pm 23 \text{ mg/dl}$; $p < 0,05$). Além disso, o incremento em 50% na TFR de ^{14}C -CE no grupo DFC foi acompanhado por uma redução em 20% no LDL-C comparando-se a SEM TRATAMENTO. CONCLUSÃO: Esta é a primeira demonstração *in vivo* que a remoção de LDE do plasma encontra-se aumentada em pacientes com LES em uso de DFC. Estes dados suportam o benefício desta droga no tratamento do LES e identificam o receptor de LDL como um mecanismo promissor de DFC na redução de lípidos em pacientes tomando corticosteróides.

Descritores: 1.Lúpus eritematoso sistêmico/metabolismo 2.Cloroquina 3.Emulsões 4.Arteriosclerose 5.Lipoproteínas 6.Lipoproteínas do colesterol LDL 7.Hiperlipidemia

SUMMARY

Sachet JC. Plasma kinetics of a lipid emulsion resembling low-density lipoprotein (LDL) in systemic lupus erythematosus with or without chloroquine diphosphate [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2006. 68p.

OBJECTIVE: Low-density lipoprotein (LDL) pathway in systemic lupus erythematosus (SLE) patients taking chloroquine diphosphate (CDP) was evaluated through the kinetic behavior of a radioactive cholesterol-rich nanoemulsion (LDE) that resembles the LDL lipidic structure. METHODS: LDE was labeled with ^{14}C -cholesteryl ester (^{14}C -CE), then IV injected in inactive female SLE patients: 10 taking CDP (CDP), 10 without therapy (NO THERAPY); and 10 normal subjects (CONTROL). Groups were age-matched and followed rigorous selection criteria of conditions that interfere in the lipid profile. Blood samples were collected in pre-established intervals after infusion for radioactivity measurement. Fasting lipoproteins were determined in the beginning of kinetic studies. RESULTS: Age and body mass index (BMI) were similar in the studied groups. Fractional clearance rate (FCR) of ^{14}C -CE was significantly greater in CDP compared to NO THERAPY (0.076 ± 0.037 vs. $0.046 \pm 0.021 \text{ h}^{-1}$; $p < 0.05$) and CONTROL ($0.0516 \pm 0.0125 \text{ h}^{-1}$; $p < 0.05$). Accordingly, a significant lower total and LDL cholesterol were observed in CDP (156 ± 16 and $88 \pm 16 \text{ mg/dl}$) compared to NO THERAPY

(174 ± 15 and 108 ± 17 mg/dl; $p < 0.05$) and CONTROL (200 ± 24 and 118 ± 23 mg/dl; $p < 0.05$). Moreover, the 50% increase in ^{14}C -CE FCR in CDP was paralleled by 20% decrease in LDL-c compared to NO THERAPY. CONCLUSION: This is the first *in vivo* demonstration that removal of LDE from plasma was increased in SLE patients taking CDP. These data support its beneficial use in SLE and identify the LDL receptor as a promising CDP mechanism for lowering lipids in patients taking corticosteroids.

Descriptors: 1.Systemic lupus erythematosus/metabolism 2.Chloroquine 3.Emulsions 4.Arteriosclerosis 5.Lipoproteins 6.LDL cholesterol lipoproteins 7.Hyperlipidemia

1. INTRODUÇÃO

Drogas antimaláricas contendo o radical 4-aminoquinolina têm um efeito modulador no processo inflamatório das doenças reumáticas¹⁻³. Em relação ao lúpus eritematoso sistêmico (LES), a hidroxicloroquina (HCQ) e o difosfato de cloroquina (DFC) são amplamente usados⁴⁻⁷ para prevenir novos surtos inflamatórios^{7,8}.

Adicionalmente, os antimaláricos têm um efeito redutor dos níveis plasmáticos de lípides em pacientes com LES⁹⁻¹¹, o qual é interessante do ponto de vista terapêutico devido ao elevado risco de desenvolver aterosclerose prematura a que os pacientes com LES são expostos, manifestada mais freqüentemente pelo infarto agudo do miocárdio¹². As dislipidemias são muito freqüentes no LES¹²⁻¹⁴ e certamente têm um papel central no risco cinquenta vezes maior para o desenvolvimento de doença arterial coronariana (DAC)¹⁵, uma importante causa de mortalidade no LES^{12,16,17}. A dislipoproteinemia do LES^{18,19} é agravada pela atividade de doença¹⁹ e é caracterizada por distúrbios no catabolismo de triglicérides relacionados à baixa atividade de lipoproteína lipase²⁰, a enzima que catalisa a hidrólise intravascular dos triglicérides das lipoproteínas de muito baixa densidade (VLDL) e quilomícrons. Tal redução de atividade pode estar associada à presença de autoanticorpos contra esta enzima²¹. O colesterol HDL encontra-se reduzido, provavelmente em conseqüência da hipertrigliceridemia¹⁹.

Por outro lado, é bem conhecido que a terapia com corticosteróides aumenta a síntese de lipoproteínas levando à elevação preferencial de triglicérides e colesterol total²²⁻²⁴, particularmente LDL, que é reconhecido como um dos grandes fatores de risco para a aterosclerose²⁵. De fato, tem sido demonstrado um aumento progressivo nos níveis de colesterol total com maiores doses de prednisona em pacientes com LES¹⁰.

Em contraste, os antimaláricos parecem reduzir o LDL colesterol em muitos estudos com pacientes com LES recebendo esteróides^{9,10,26-31}. Estudos *in vitro* sugerem que a cloroquina diminui a síntese de colesterol através da inibição da ciclização de oxiescaleno a lanosterol em células de mamíferos^{32,33}.

A fim de melhor esclarecer os possíveis mecanismos pelos quais os antimaláricos afetam o metabolismo plasmático de colesterol no LES, nós avaliamos a via do LDL colesterol em pacientes com lúpus tratados ou não com DFC e em controles normais através da determinação da cinética plasmática de uma nanoemulsão artificial rica em colesterol marcada radioativamente (LDE), a qual se assemelha à estrutura lipídica da LDL³⁴⁻³⁶. Ela é elaborada sem proteína, mas quando injetada na corrente sanguínea adquire várias apolipoproteínas, principalmente a Apo E³⁴. Esta apolipoproteína permite que as partículas de LDE se liguem aos receptores de LDL, uma vez que estes reconhecem não só a Apo B presente na LDL nativa, mas também a Apo E, ligando-se a esta com maior afinidade³⁴. Desta maneira, a emulsão é removida do plasma mais rapidamente que a lipoproteína natural. Isto representa uma vantagem metodológica, uma vez

que encurta o período de tempo necessário para a realização do estudo cinético³⁴.

O compartilhamento pela LDE do mesmo receptor utilizado pela LDL nativa é demonstrado em estudos de hipercolesterolemia familiar, doença autossômica dominante que se caracteriza por defeitos na expressão e função destes receptores, reduzindo a remoção plasmática de LDL³⁷. Nesta patologia, o “clearance” plasmático da emulsão encontra-se muito lentificado na comparação com os controles normais³⁷. Em contrapartida, patologias como a leucemia mielóide aguda não-tratada, na qual há uma hiperexpressão de receptores de LDL pelas células neoplásicas, ocorre importante aumento da remoção plasmática da emulsão, observando-se a equiparação aos valores dos controles normais após a obtenção de remissão da neoplasia com o tratamento específico³⁸.

Deste modo, a LDE tem sido usada como um substituto da LDL nativa para se investigar o processo de remoção de LDL do plasma em estudos clínicos, ressaltando-se sua praticidade e custo-efetividade^{37,39,40}.

2. OBJETIVOS

- 1- Avaliar a cinética plasmática da emulsão lipídica semelhante à LDL em pacientes com LES sem atividade de doença, mas em uso contínuo de difosfato de cloroquina, comparando-se com a das pacientes com LES sem uso desta droga e as controles, a fim de verificar a influência desta droga no metabolismo da LDL;
- 2- Comparar a cinética plasmática desta emulsão lipídica nas pacientes com LES sem atividade de doença e sem nenhum tratamento com as controles, no sentido de avaliar alterações da doença no metabolismo da LDL colesterol.

3. PACIENTES E MÉTODOS

3.1. População de estudo

Vinte pacientes consecutivas com LES foram selecionadas entre os 900 pacientes em acompanhamento no Ambulatório de LES da Disciplina de Reumatologia da Universidade de São Paulo. Todas as pacientes tinham menos de 50 anos de idade e preenchiam quatro ou mais dos critérios revisados do Colégio Americano de Reumatologia para a classificação de LES⁴¹. As pacientes com LES foram divididas em dois grupos pareados pela idade de acordo com o tratamento à época da avaliação: DFC – uso exclusivo de 250 mg de difosfato de cloroquina diariamente por pelo menos 1 ano; e SEM TRATAMENTO – doença inativa sem qualquer terapia que nunca usaram DFC ou descontinuaram esta droga há no mínimo 1 ano. As pacientes com LES não fizeram uso de corticosteróides ou drogas imunossupressoras durante o último ano, e nenhuma delas ingeriu quaisquer medicações que pudessem alterar os níveis de lípidos, tais como: anticonvulsivantes, antihipertensivos (beta-bloqueadores e diuréticos), estrógenos e/ou progestágenos, ou agentes hipolipemiantes. Critérios de exclusão foram presença de evidência clínica e/ou bioquímica de Diabetes Mellitus, DAC, doença hepática ou tireoidiana, e história de uso de álcool. Nenhuma paciente estava grávida ou em menopausa, todas apresentando ciclos menstruais regulares. Todas as pacientes tinham níveis séricos de creatinina abaixo de 1,5 mg/dl e proteinúria menor que 0,3 g/L/dia. Atividade

clínica foi mensurada de acordo com o Índice de Atividade de Doença do LES (SLEDAI)⁴² e todas as pacientes apresentavam doença inativa com escore zero no SLEDAI. Dez pessoas do sexo feminino, pareadas pela idade (grupo CONTROLE), foram selecionadas para comparação, sendo estudadas em paralelo às pacientes com LES. Nenhuma das pacientes com LES e controles normais tinha índice de massa corpórea (IMC) maior do que 30 kg/m².

3.2. Preparação da LDE

LDE foi preparada de uma mistura lipídica composta de 40 mg de oleato de colesterol, 20 mg de fosfatidilcolina de ovo, 1 mg de trioleína, e 0,5 mg de colesterol adquiridos de Nu-Check Prep (Elysian). [¹⁴C] colesterol éster adquirido de Amersham International (Amersham, Reino Unido) foi adicionado à mistura. A emulsificação de lípides por irradiação ultrassônica prolongada em meio aquoso e o procedimento de ultracentrifugação em dois passos da emulsão com ajuste de densidade pela adição de KBr a fim de obter a microemulsão de LDE foi realizada pelo método de Ginsburg⁴³ modificado por Maranhão *et al*³⁴. LDE foi dialisada contra solução salina e passada através de um filtro de 0,22 µm antes de ser injetada nos pacientes, assegurando uma preparação estéril e apirogênica.

3.3. Cinética plasmática da LDE

Os participantes encontravam-se em jejum por, pelo menos, 12 horas no início do teste aproximadamente às 8:00h da manhã, mas permitiram-se

duas refeições-padrão (total de 1800 kcal) durante o estudo por volta de 12:30h e 19:00h. LDE contendo 37 kBq de [^{14}C] colesterol éster em uma massa lipídica total de 5-6 mg (em um volume de 200 μl) foi injetada endovenosamente em bolus. Amostras de plasma foram coletadas durante 24 horas, em intervalos de 5 min, 1, 2, 4, 6, 8 e 24 horas após a injeção. Alíquotas (1 ml) de plasma foram transferidas para tubos de contagem contendo 7,0 ml de solução de cintilação (ULTIMA GOLD XR, Packard Bioscience, Meriden, USA). Radioatividade foi contada usando-se espectrômetro Packard 1660 TR (Meriden, USA).

3.4. Cálculo da TFR da emulsão de lípidos radioativos

A TFR do [^{14}C] colesterol éster foi calculada de acordo com o método descrito por Matthews⁴⁴ como TFR, onde a_1 , a_2 , b_1 e b_2 foram estimados a partir de curvas biexponenciais obtidas da radiação remanescente encontrada no plasma após a injeção, traçadas pelo procedimento dos mínimos quadrados:

$$y = (a_1 \cdot e^{-b_1 t}) + (a_2 \cdot e^{-b_2 t})$$

onde y representa o decaimento da radioatividade plasmática em função do tempo (t); a indica o coeficiente linear e b , o coeficiente angular, que representa a TFR em h^{-1} . As TFR foram estimadas a partir dos parâmetros a_1 , b_1 , e b_2 pela seguinte equação: $\text{TFR} = (a_1 / b_1 + a_2 / b_2)^{-1}$. Os cálculos foram realizados através de análise compartimental usando-se o software ANACOMP⁴⁵.

3.5. Consentimento informado e segurança radiológica

O protocolo do experimento foi aprovado pelo Comitê de Ética local e um consentimento informado foi dado por todas as participantes. Um teste de gravidez foi realizado na véspera do estudo cinético e todos os exames resultaram negativos. A segurança da dose radioativa injetada endovenosamente nas pacientes foi assegurada de acordo com as normas da Comissão Internacional de Segurança Radiológica, conforme descrito em estudo prévio⁴⁶. A dose equivalente absorvida em cada experimento foi 0,03 mSV, a qual fica abaixo do limite anual de 50 mSV para exposição a radionuclídeos.

3.6. Perfil lipídico

Todas as pacientes com LES e controles estavam em suas dietas normais e se encontravam em jejum por, pelo menos, 12 horas no início do estudo. Os níveis de lipoproteínas em jejum foram determinados no mesmo dia dos estudos cinéticos. Colesterol plasmático total (CT) e triglicérides (TG) foram medidos enzimaticamente (Boehringer Mannheim, Argentina e Merck, Alemanha, respectivamente) em um analisador RA 1000 (Technicon Instruments Corp)^{47,48}. O colesterol da lipoproteína de alta densidade (HDL-c) foi obtido após precipitação do colesterol da lipoproteína de muito baixa densidade (VLDL-c) e do colesterol da lipoproteína de baixa densidade (LDL-c) pelo ácido fosfotúngstico e cloreto de magnésio⁴⁹. O colesterol de muito baixa densidade (VLDL-c) e o colesterol de baixa densidade (LDL-c)

foram estimados, uma vez que todos os participantes tinham níveis de triglicérides menores que 400 mg/dl⁵⁰. Os níveis de VLDL-c foram calculados a partir da divisão dos triglicérides séricos por cinco (TG/5)⁵⁰. Os níveis de LDL-c foram estimados a partir da seguinte equação⁵⁰:

$$CT = HDL-c + TG/5 + LDL-c$$

3.7. Análise estatística

Os resultados são apresentados como média $\pm$ desvio padrão (SD). Os dados foram comparados por análise de variância (ANOVA) e, se um resultado significativo fosse obtido ($p < 0,05$), o teste de Tukey era realizado a fim de avaliar as diferenças entre os grupos. Correlações entre as frações de lípidos plasmáticos e TFR foram calculadas pelo teste de correlação de Spearmann. A significância estatística foi considerada com valores de $p < 0,05$.

4. RESULTADOS

A distribuição de idade, peso, altura e índice de massa corpórea (IMC) dos três grupos é ilustrada na Tabela 1. Como esperado, a idade foi similar nos grupos DFC, SEM TRATAMENTO e CONTROLE ($p=0,93$). Além disso, nenhuma diferença significativa foi encontrada entre os grupos em relação ao peso ($p=0,39$) e altura ($p=0,96$). Dessa maneira, o IMC não diferiu significativamente entre os grupos ($p=0,31$). Os pacientes com LES do grupo DFC estavam em uso desta droga por $7,1 \pm 2,9$ anos e tinham duração de doença similar ao grupo SEM TRATAMENTO ($9,5 \pm 4,6$ e $8,2 \pm 6,6$ anos respectivamente, $p=0,10$). Ressalta-se que os pacientes com LES de ambos os grupos demonstraram escore zero no SLEDAI, o que reflete a ausência de sinais clínicos e laboratoriais de atividade de doença.

TABELA 1 - Achados demográficos, duração de doença e SLEDAI nos grupos LES (DFC e SEM TRATAMENTO) e CONTROLE

GRUPO	DFC (n=10)	SEM TRATAMENTO (n=10)	CONTROLE (n=10)	P
Idade (anos)	35,4 ± 7,5	36,5 ± 6,9	35,6 ± 8,9	0,93
Peso (kg)	60,2 ± 6,8	63,0 ± 8,8	57,5 ± 10,4	0,39
Altura (m)	1,61 ± 0,04	1,60 ± 0,06	1,60 ± 0,10	0,96
I.M.C. (kg/m ²)	23,0 ± 2,5	24,3 ± 2,4	22,2 ± 3,7	0,31
Tempo de doença (anos)	9,5 ± 4,6	8,2 ± 6,6	-	0,10
SLEDAI	0	0	-	-

Valores expressos em média ± DP; I.M.C. = índice de massa corpórea; SLEDAI = *Systemic Erythematosus Lupus Disease Activity Index*

O perfil de lipoproteínas dos grupos DFC, SEM TRATAMENTO e CONTROLE é demonstrado na Tabela 2. DFC apresentou níveis de LDL-c mais baixos, comparando-se com o grupo SEM TRATAMENTO (88 ± 16 vs. 108 ± 17 mg/dl; $p < 0,05$) e CONTROLE (88 ± 16 vs. 118 ± 23 mg/dl; $p < 0,05$). Similarmente, os níveis de colesterol total (CT) no grupo DFC (156 ± 16 mg/dl) foram mais baixos se comparados aos de SEM TRATAMENTO (174 ± 15 mg/dl; $p < 0,05$) e CONTROLE (200 ± 24 mg/dl; $p < 0,05$). Em contraste, maiores níveis de HDL-c foram encontrados no grupo DFC se comparados a SEM TRATAMENTO (54 ± 8 vs. 46 ± 9 mg/dl; $p < 0,05$), embora fossem similares aos dos controles. Além disso, o grupo SEM TRATAMENTO também evidenciou menor HDL-c comparado aos controles saudáveis, assim como menores níveis de CT ($p < 0,05$). TG e VLDL-c tenderam a ser menores no grupo DFC comparando-se aos outros dois grupos, embora esta

diferença não tenha atingido significância estatística ($p=0,06$).

TABELA 2 - Perfil lipídico plasmático dos grupos LES (DFC e SEM TRATAMENTO) e CONTROLE

PARÂMETRO	CONTROLE (N=10)	SEM TRATAMENTO (N=10)	DFC (N=10)	<i>P</i>
Colesterol				
Total	200 ± 24	174 ± 15 *	156 ± 16 *†	<0,001
HDL-c	64 ± 7	46 ± 9 *	54 ± 8 †	<0,001
LDL-c	118 ± 23	108 ± 17	88 ± 16 *†	<0,001
VLDL-c	17 ± 7	20 ± 7	13 ± 4	0,06
Triglicérides	86 ± 33	103 ± 38	68 ± 19	0,06

Valores expressos com média ± SD, em mg/dl

* $p < 0,05$ vs. CONTROLE

† $p < 0,05$ vs. SEM TRATAMENTO

4.1. Cinética plasmática da LDE

A Figura 1 mostra as curvas de decaimento plasmático do colesterol éster da LDE radioativamente marcado durante as 24 horas do estudo. Deve-se enfatizar que a curva de desaparecimento de [^{14}C] colesterol éster

do grupo SEM TRATAMENTO foi quase igual àquela dos controles ($p>0,05$). Em contraste, a curva obtida no grupo DFC foi pronunciadamente mais rápida do que as outras duas curvas (Figura 1). De fato, conforme ilustrado na Tabela 3, a TFR de $[^{14}\text{C}]$ colesterol éster não diferiu entre SEM TRATAMENTO e CONTROLE, sendo maior no grupo DFC comparando-se com os outros dois grupos ($p=0,03$).

FIGURA 1

FIGURA 1 LEGENDA: “Clearance” de LDE em pacientes do grupo DFC (linha contínua), pacientes SEM TRATAMENTO (linha tracejada) e grupo CONTROLE (linha pontilhada) durante 24 horas após a infusão endovenosa. A curva de desaparecimento da emulsão $[^{14}\text{C}]$ colesterol éster de DFC foi mais rápida do que as dos grupos SEM TRATAMENTO e CONTROLE ($p=0,03$), indicando um aumento significativo do “clearance” em pacientes com LES tratados com DFC.

TABELA 3 - Taxa fracional de remoção (TFR) de [^{14}C] colesterol éster nos grupos LES (DFC e SEM TRATAMENTO) e CONTROLE

TFR	CONTROLE (N=10)	SEM TRATAMENTO (N=10)	DFC (N=10)	P
[^{14}C] colesterol éster	0,051 $\pm$ 0,012	0,046 $\pm$ 0,021	0,076 $\pm$ 0,037 *	0,03

Valores em h^{-1} , expressos como média $\pm$ DP

* $p < 0,05$ vs. CONTROLE e vs. SEM TRATAMENTO

É interessante frisar a correlação negativa em pacientes com lúpus (DFC e SEM TRATAMENTO) entre a TFR de [^{14}C] colesterol éster e os níveis plasmáticos de CT ($r = -0,581$, $p = 0,007$) e LDL-c ($r = -0,495$, $p = 0,026$). Não se pôde relacionar com HDL-c ($r = 0,132$, $p = 0,57$), VLDL-c ($r = -0,356$, $p = 0,12$) ou TG ($r = -0,352$, $p = 0,12$). Além disso, a TFR não mostrou correlação com a idade ($r = -0,050$, $p = 0,83$) ou IMC ($r = -0,016$, $p = 0,94$).

5. DISCUSSÃO

Esta é a primeira demonstração *in vivo* que, em pacientes com LES tratados com DFC, a cinética plasmática de LDE encontra-se acelerada em paralelo à redução dos níveis de LDL colesterol.

Para chegar a estes achados, rigorosos critérios de seleção foram estabelecidos a fim de eliminar as principais doenças sistêmicas que promovessem alterações específicas no metabolismo lipídico⁵¹⁻⁵⁵. Adicionalmente, o aumento progressivo nos níveis de colesterol total e LDL-c com o envelhecimento⁵⁶ e menopausa⁵⁷ foi evitado pelos critérios de exclusão. Todos os pacientes incluídos no estudo tinham doença inativa, uma vez que se sabe que a atividade pode agravar a dislipoproteinemia do lúpus¹⁹, não havendo também uso de drogas que pudessem interferir no metabolismo de lipoproteínas⁵⁸. Ressalta-se, também, que o período de administração de DFC foi longo o suficiente para assegurar que as alterações no metabolismo lipídico pelo DFC fossem sustentadas, o que ocorre após 1 ano de tratamento^{31,59}.

A grande vantagem do sistema de LDE usado neste estudo é a avaliação específica da função *in vivo* dos receptores de LDL, que removem a lipoproteína do plasma até as células. A LDE é produzida sem proteína, mas quando injetada na circulação e em contato com o plasma ela adquire várias pequenas apolipoproteínas de baixo peso molecular (apos), incluindo apo E³⁴. Apo E capacita a ligação das partículas aos receptores de LDL,

uma vez que os mesmos reconhecem não só a apo B presente na LDL, mas também a apo E, não encontrada na fração LDL^{36,60}. Apo E liga-se ao receptor com uma maior afinidade do que apo B100 sendo, desta forma, removida mais rapidamente do que a LDL nativa^{36,60}.

Além disso, as conhecidas diferenças nas subclasses de LDL entre as pessoas⁶¹⁻⁶³ que poderiam influenciar na remoção da lipoproteína do plasma são excluídas pelo presente estudo, uma vez que LDE é uma preparação padrão, permitindo uma definição mais precisa da função do receptor^{37,40}.

Ressalta-se a revelação, por este procedimento, que a função do receptor de LDL no LES não está alterada pela doença, o que é suportado pelos níveis normais de LDL observados em pacientes com lúpus inativo não-tratados¹⁹. Em contraste, a remoção acelerada de LDE tem sido descrita na leucemia mielocítica aguda^{38,64} e a função defeituosa do receptor foi detectada na hipercolesterolemia familiar³⁷.

O achado mais interessante em nosso estudo foi a descoberta de que o DFC realiza uma “upregulation” nos receptores de LDL, aumentando a cinética plasmática *in vivo* desta emulsão rica em colesterol no LES. Este efeito foi forte o suficiente para determinar uma queda pronunciada nos níveis de LDL-c. A remoção plasmática duas vezes maior no grupo DFC foi acompanhada por uma redução em 20% na concentração de LDL-c, comparando-se com pacientes não-tratados. De fato, tem sido sugerido previamente que o uso de hidroxycloquina leva a menores níveis de LDL-c em pacientes com LES tomando corticosteróides⁹⁻¹⁰. Uma diminuição nos níveis de LDL-c foi também identificada em estudos randomizados do uso de

hidroxicloroquina em pacientes diabéticos^{65,66}.

De acordo com este conceito, uma correlação negativa entre a TFR de [¹⁴C] colesterol éster e LDL-c foi identificada em pacientes com LES, confirmando-se que a droga influencia poderosamente a função do receptor de LDL, grande determinante da concentração plasmática desta lipoproteína⁶⁷.

Inversamente, outros grupos de pesquisadores, bem como o nosso, têm demonstrado uma redução de VLDL-c e TG, levando a aumento nos níveis de HDL-c pelo uso de antimaláricos em pacientes com LES tratados com prednisona^{26,31}. Neste estudo, a comparação com pacientes com LES não-tratados também revelou tendência à redução de TG no grupo DFC com aumento concomitante no HDL-c, possivelmente relacionado à redução da síntese hepática de lipoproteínas, conforme previamente relatado³¹.

É bem reconhecido que níveis baixos de HDL-c e elevação de LDL-c são fatores de risco clássicos para o desenvolvimento de aterosclerose na população geral²⁵ e também no LES^{13,68-70}. Além disso, exposição aumentada a corticosteróides, especialmente em doses mais altas, é a principal causa de hipercolesterolemia nesta doença^{10,13,68}. Nesse sentido, as estatinas são atualmente o tratamento de eleição para esta condição. Evidências crescentes sugerem que essas drogas também têm efeitos anti-inflamatórios e/ou imunomodulatórios nas doenças reumáticas, reduzindo o LDL-c⁷¹. Além da inibição da enzima limitante no processo de síntese de colesterol, as estatinas também aumentam a remoção plasmática de tanto a LDL nativa quanto da LDE^{37,40}.

De acordo com os achados acima, este novo mecanismo de redução do LDL-c por parte da cloroquina através do aumento de sua remoção plasmática documentado neste estudo, enfatiza a utilidade desta droga em pacientes com lúpus com níveis elevados de LDL, em particular aqueles tomando corticosteróides. Um possível sinergismo entre DFC e estatinas levando a um efeito hipolipemiante mais intenso e melhor proteção antiaterogênica deve também ser considerado. À luz desses achados, esses efeitos podem ser de valor e devem ser explorados em estudos futuros.

6. CONCLUSÕES

- 1- Existe um aumento da remoção plasmática da emulsão lipídica semelhante à LDL em pacientes com LES em uso contínuo de difosfato de cloroquina comparada às pacientes com LES sem droga e às controles, o que determina uma redução dos níveis de LDL colesterol nas pacientes em uso desta droga;
- 2- A cinética plasmática desta emulsão lipídica é semelhante nas pacientes com LES sem atividade de doença e sem nenhuma droga comparando-se às controles, mostrando que a doença não promove alteração no metabolismo da LDL colesterol.

7. REFERÊNCIAS

1. Avina-Zubieta JA, Galindo-Rodriguez G, Newman S, Suarez-Almazor ME, Russell AS. Long-term effectiveness of antimalarial drugs in rheumatic diseases. *Ann Rheum Dis*. 1998;57:582-7.
2. Rynes RI. Antimalarial drugs in the treatment of rheumatological diseases. *Br J Rheumatol*. 1997;36:799-805.
3. Wallace DJ. Antimalarials – the ‘real’ advance in lupus. *Lupus*. 2001;10:385-7.
4. Rothfield N. Efficacy of antimalarials in systemic lupus erythematosus. *Am J Med*. 1988;85:53-6.
5. Rudnicki RD, Gresham GE, Rothfield NF. The efficacy of antimalarials in systemic lupus erythematosus. *J Rheumatol*. 1975;2:323-30.
6. Meinao IM, Sato EI, Andrade LE, Ferraz MB, Atrá E. Controlled trial with chloroquine diphosphate in systemic lupus erythematosus. *Lupus*. 1996;5:237-41.
7. The Canadian Hydroxychloroquine Study Group: A randomized study of the effect of withdrawing hydroxychloroquine sulfate in systemic lupus erythematosus. *N Engl J Med*. 1991;324:150-4.
8. Tsakonas E, Joseph L, Esdaile JM, Choquette D, Senecal JL, Cividino A, Danoff D, Osterland CK, Yeadon C, Smith CD. A long-term study of hydroxychloroquine withdrawal on exacerbations in systemic lupus erythematosus. The Canadian Hydroxychloroquine Study Group. *Lupus*.

1998;7:80-5.

9. Wallace DJ, Metzger AL, Stecher VJ, Tumbull BA, Kern PA. Cholesterol-lowering effect of hydroxychloroquine in patients with rheumatic disease: reversal of deleterious effects of steroids on lipids. *Am J Med.* 1990;89:322-6.
10. Petri M, Lakatta C, Magder L, Goldman D. Effect of prednisone and hydroxychloroquine on coronary artery disease risk factors in systemic lupus erythematosus: a longitudinal data analysis. *Am J Med.* 1994;96:254-9.
11. Petri M. Hydroxychloroquine use in the Baltimore Lupus Cohort: effects on lipids, glucose and thrombosis. *Lupus.* 1996;5(Suppl 1):S16-22.
12. Bruce IN. 'Not only...but also': factors that contribute to accelerated atherosclerosis and premature coronary heart disease in systemic lupus erythematosus. *Rheumatology.* 2005; 44:1492-502.
13. Petri M, Perez-Gutthann S, Spence D, Hochberg MC. Risk factors for coronary artery disease in patients with systemic lupus erythematosus. *Am J Med.* 1992;93:513-9.
14. Bruce IN, Gladman DD, Urowitz MB. Premature atherosclerosis in systemic lupus erythematosus. *Rheum Dis Clin North Am.* 2000;26:257-78.
15. Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA Jr, Jansen-McWilliams L, D'Agostino RB, Kuller LH. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J*

Epidemiol. 1997;145:408-15.

16. Ward MM. Premature morbidity from cardiovascular and cerebrovascular diseases in women with systemic lupus erythematosus. *Arthritis Rheum.* 1999;42:338-46.
17. Ward MM. Outcomes of hospitalizations for myocardial infarctions and cerebrovascular accidents in patients with systemic lupus erythematosus. *Arthritis Rheum.* 2004;50:3170-6.
18. Ilowite NT, Samuel P, Ginzler E, Jacobson MS. Dyslipoproteinemia in pediatric systemic lupus erythematosus. *Arthritis Rheum.* 1988;31:859-63.
19. Borba EF, Bonfa E. Dyslipoproteinemias in systemic lupus erythematosus: influence of disease, activity, and anticardiolipin antibodies. *Lupus.* 1997;6:533-9.
20. Borba EF, Bonfá E, Vinagre CG, Ramires JA, Maranhao RC. Chylomicron metabolism is markedly altered in systemic lupus erythematosus. *Arthritis Rheum.* 2000;43:1033-40.
21. de Carvalho JF, Borba EF, Viana VS, Bueno C, Leon EP, Bonfa E. Anti-lipoprotein lipase antibodies: a new player in the complex atherosclerotic process in systemic lupus erythematosus? *Arthritis Rheum.* 2004;50:3610-5.
22. Ettinger WH, Goldberg AP, Applebaum-Bowden D, Hazzard WR. Dyslipoproteinemia in systemic lupus erythematosus: Effect of corticosteroids. *Am J Med.* 1987;83:503-8.
23. Ettinger WH Jr, Hazzard WR. Elevated apolipoprotein-B levels in

- corticosteroid-treated patients with systemic lupus erythematosus. *J Clin Endocrinol Metab.* 1988;67:425-8.
24. MacGregor AJ, Dhillon VB, Binder A, Forte CA, Knight BC, Betteridge DJ, Isenberg DA. Fasting lipids and anticardiolipin antibodies as risk factors for vascular disease in systemic lupus erythematosus. *Ann Rheum Dis.* 1992;51:152-5.
 25. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA.* 2001;285:2486-97.
 26. Hodis HN, Quismorio FP Jr, Wickham E, Blankenhorn DH. The lipid, lipoprotein, and apolipoprotein effects of hydroxychloroquine in patients with systemic lupus erythematosus. *J Rheumatol.* 1993;20:661-5.
 27. Kavanaugh A, Adams-Huet B, Jain R, Denke M, McFarlin J. Hydroxychloroquine effects on lipoprotein profiles (the HELP trial): A double-blind, randomized, placebo-controlled, pilot study in patients with systemic lupus erythematosus. *J Clin Rheumatol.* 1997;3:3-8.
 28. Rahman P, Gladman DD, Urowitz MB, Yuen K, Hallett D, Bruce IN. The cholesterol lowering effect of antimalarial drugs is enhanced in patients with lupus taking corticosteroid drugs. *J Rheumatol.* 1999;26:325-30.
 29. Tam LS, Li EK, Lam CW, Tomlinson B. Hydroxychloroquine has no significant effect on lipids and apolipoproteins in Chinese systemic lupus erythematosus patients with mild or inactive disease. *Lupus.*

2000;9:413-6.

30. Tam LS, Gladman DD, Hallett D, Rahman P, Urowitz MB. Effect of antimalarial agents on the fasting lipid profile in systemic lupus erythematosus. *J Rheumatol.* 2000;27:2142-5.
31. Borba EF, Bonfa E. Longterm beneficial effect of chloroquine diphosphate on lipoprotein profile in lupus patients with and without steroid therapy. *J Rheumatol.* 2001;28:780-5.
32. Goldstein JL, Brunschede GY, Brown MS. Inhibition of the proteolytic degradation of low density lipoprotein in human fibroblasts by chloroquine, concanavalin A, and Triton WR 1339. *J Biol Chem.* 1975;250:7854-62.
33. Chen HW, Leonard DA. Chloroquine inhibits cyclization of squalene oxide to lanosterol in mammalian cells. *J Biol Chem.* 1984;259:8156-62.
34. Maranhao RC, Cesar TB, Pedroso-Mariani SR, Hirata MH, Mesquita CH. Metabolic behavior in rats of a nonprotein microemulsion resembling low-density lipoprotein. *Lipids.* 1993;28:691-6.
35. Maranhao RC, Roland IA, Toffoletto O, Ramires JA, Gonçalves RP, Mesquita CH, Pileggi F. Plasma kinetic behavior in hyperlipidemic subjects of a lipidic microemulsion that binds to low density lipoprotein receptors. *Lipids.* 1997;32:627-33.
36. Hirata RD, Hirata MH, Mesquita CH, Cesar TB, Maranhao RC. Effects of apolipoprotein B-100 on the metabolism of a lipid microemulsion model in rats. *Biochim Biophys Acta.* 1999;1437:53-62.
37. Santos RD, Chacra AP, Morikawa A, Vinagre CC, Maranhao RC. Plasma

- kinetics of free and esterified cholesterol in familial hypercholesterolemia: effects of simvastatin. *Lipids*. 2005;40:737-43.
38. Maranhao RC, Garicochea B, Silva EL, Llacer PD, Pileggi FJ, Chamone DA. Increased plasma removal of microemulsions resembling the lipid phase of low-density lipoproteins (LDL) in patients with acute myeloid leukemia: a possible new strategy for the treatment of the disease. *Braz J Med Biol Res*. 1992;25:1003-7.
 39. Pinto LB, Wajngarten M, Silva EL, Vinagre CC, Maranhao RC. Plasma kinetics of a cholesterol-rich emulsion in young, middle-aged and elderly subjects. *Lipids*. 2001;36:1307-11.
 40. Santos RD, Hueb W, Oliveira AA, Ramires JA, Maranhao RC. Plasma kinetics of a cholesterol-rich emulsion in subjects with or without coronary artery disease. *J Lipid Res*. 2003;44:464-9.
 41. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1982;25:1271-7.
 42. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum*. 1992;35:630-40.
 43. Ginsburg GS, Small DM, Atkinson D. Microemulsion of phospholipids and cholesterol esters. Protein-free models of low density lipoprotein. *J Biol Chem*. 1982;257:8216-27.

44. Matthews CME. The theory of tracer experiments with ^{131}I -labeled plasma proteins. *Phys Med Biol* 1957;2:36-41.
45. Marchese SRM, Mesquita CH, Cunha IIL. Anacomp program application to calculate ^{137}C transfer rates in marine organisms and dose in man. *J Radioan Nucl Chem*.1998;232:233-6.
46. Maranhão RC, Feres MC, Martins MT, Mesquita CH, Toffoletto O, Vinagre CG, Gianinni SD, Pileggi F. Plasma kinetics of a chylomicron-like emulsion in patients with coronary artery disease. *Atherosclerosis*. 1996;126:15-25.
47. Siedel J, Hägele EO, Ziegenhorn J, Wahlefeld AW. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin Chem*. 1983;29:1075-80.
48. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem*. 1982;28:2077-80.
49. Warnick GR, Cheung MC, Albers JJ. Comparison of current methods for high-density lipoprotein cholesterol quantitation. *Clin Chem*. 1979;25:596-604.
50. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502.
51. Brunzell JD, Albers JJ, Haas LB, Goldberg AP, Agadoa L, Sherrard DJ. Prevalence of serum lipid abnormalities in chronic hemodialysis. *Metabolism*. 1977;26:903-10.

52. Attman PO, Alaupovic P. Lipid and apolipoprotein profiles of uremic dyslipoproteinemia - relation to renal function and dialysis. *Nephron*. 1991;57:401-10.
53. Appel GB, Blum CB, Chien S, Kunis CL, Appel AS. The hyperlipidemia of the nephrotic syndrome. Relation to plasma albumin concentration, oncotic pressure and viscosity. *N Engl J Med*. 1985;312:1544-8.
54. Thompson GR, Soutar AK, Spengel FA, Jadhav A, Gavigan SJ, Myant NB. Defects of receptor-mediated low density lipoprotein catabolism in homozygous familial hypercholesterolemia and hypothyroidism *in vivo*. *Proc Natl Acad Sci (USA)*. 1981;78:2591-5.
55. Brown WV. Lipoprotein disorders in diabetes mellitus. *Med Clin North Am*. 1994;78:143-61.
56. Heiss G, Tamir I, Davis CE, Tyroler HA, Rifkind BM, Schonfeld G, Jacobs D, Frantz ID Jr. Lipoprotein-cholesterol distributions in selected North American populations: the lipid research clinics program prevalence study. *Circulation*. 1980;61:302-15.
57. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. *Ann Intern Med*. 1976;85:447-52.
58. Henkin Y, Como JA, Oberman A. Secondary dyslipidemia. Inadvertent effects of drugs in clinical practice. *JAMA*. 1992;267:961-8.
59. Munro R, Morrison E, McDonald AG, Hunter JA, Madhok R, Capell HA. Effect of disease modifying agents on the lipid profiles of patients with rheumatoid arthritis. *Ann Rheum Dis*. 1997;56:374-7.

60. Innerarity TL, Mahley RW. Enhanced binding by cultured human fibroblasts of apo-E-containing lipoproteins as compared with low density lipoproteins. *Biochemistry*. 1978;17:1440-7.
61. Capell WH, Zambon A, Austin MA, Brunzell JD, Hokanson JE. Compositional differences of LDL particles in normal subjects with LDL subclass phenotype A and LDL subclass phenotype B. *Arterioscler Thromb Vasc Biol*. 1996;16:1040-6.
62. Ohmura H, Mokuno H, Sawano M, Hatsumi C, Mitsugi Y, Watanabe Y, Daida H, Yamaguchi H. Lipid compositional differences of small, dense low-density lipoprotein particle influence its oxidative susceptibility: possible implication of increased risk of coronary artery disease in subjects with phenotype B. *Metabolism*. 2002;51:1081-7.
63. Chapman MJ, Guerin M, Bruckert E. Atherogenic, dense low-density lipoproteins. Pathophysiology and new therapeutic approaches. *Eur Heart J*. 1998;19(Suppl A):A24-30.
64. Maranhao RC, Garicochea B, Silva EL, Dorlhiac-Llacer P, Cadena SM, Coelho IJ, Meneghetti JC, Pileggi FJ, Chamone DA. Plasma kinetics and biodistribution of a lipid emulsion resembling low-density lipoprotein in patients with acute leukemia. *Cancer Res*. 1994;54:4660-6.
65. Powrie JK, Shojaee-Moradie F, Watts GF, Smith GD, Sönksen PH, Jones RH. Effects of chloroquine on the dyslipidemia of non-insulin-dependent diabetes mellitus. *Metabolism*. 1993;42:415-9.
66. Gerstein HC, Thorpe KE, Taylor DW, Haynes RB. The effectiveness of hydroxychloroquine in patients with type 2 diabetes mellitus who are

- refractory to sulfonylureas – a randomized trial. *Diabetes Res Clin Pract.* 2002;55:209-19.
67. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science.* 1986;232:34-47.
 68. Petri M, Spence D, Bone LR, Hochberg MC. Coronary artery disease risk factors in the Johns Hopkins Lupus Cohort: prevalence, recognition by patients, and preventive practices. *Medicine (Baltimore).* 1992;71:291-302.
 69. Bruce IN, Urowitz MB, Gladman DD, Hallett DC. Natural history of hypercholesterolemia in systemic lupus erythematosus. *J Rheumatol.* 1999;26:2137-43.
 70. Svenungsson E, Jensen-Urstad K, Heimbürger M, Silveira A, Hamsten A, de Faire U, Witztum JL, Frostegård J. Risk factors for cardiovascular disease in systemic lupus erythematosus. *Circulation.* 2001;104:1887-93.
 - 71 Abeles AM, Pillinger MH. Statins as antiinflammatory and immunomodulatory agents: a future in rheumatologic therapy? *Arthritis Rheum.* 2006;54:393-407.

APÊNDICE

TRABALHO ENCAMINHADO PARA PUBLICAÇÃO NA REVISTA

ARTHRITIS & RHEUMATISM

CHLOROQUINE INCREASES LOW-DENSITY LIPOPROTEIN (LDL) REMOVAL FROM PLASMA IN SYSTEMIC LUPUS PATIENTS

Julio C. Sachet, Eduardo F. Borba, Eloísa Bonfá, Carmen G.C. Vinagre, Vanessa M. Silva, Raul C. Maranhão.

Rheumatology Division and Lipid Metabolism Laboratory of the Heart Institute (InCor) of the Medical School Hospital and Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil.

J.C. Sachet, MD: Research Fellow; E. F. Borba, MD, PhD: Assistant Professor; E. Bonfá, MD, PhD: Professor of Medicine; C.G.C. Vinagre, PhD: Associate Investigator; V.M. Silva: Research Fellow; R.C. Maranhão, MD, PhD: Professor of Clinical Biochemistry.

Dr. Bonfá and Dr. Maranhão are recipients of Research Awards from Conselho Nacional de Desenvolvimento Científico e Tecnológico (grants 304756/2003-2 and 300771/2003-7, respectively). This research was supported by Fundação do Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant 04/08048-3.

Address reprint requests to:
Dr. Eduardo F. Borba
Faculdade de Medicina USP, Reumatologia
Av. Dr. Arnaldo, 455 - 3º andar – Sala 3133.
São Paulo - SP - Brazil. CEP:01246-903
Email: reumato@edu.usp.br

ABSTRACT

Objective: LDL pathway in SLE patients taking chloroquine diphosphate (CDP) was evaluated through the kinetic behavior of a radioactive cholesterol-rich nanoemulsion (LDE) that resembles the LDL lipidic structure.

Patients and Methods: LDE was labeled with ^{14}C -cholesteryl ester (^{14}C -CE), then IV injected in inactive female SLE patients: 10 taking CDP (CDP), 10 without therapy (NO THERAPY); and 10 normal subjects (CONTROL). Groups were age-matched and followed rigorous selection criteria of conditions that interfere in the lipid profile. Blood samples were collected in pre-established intervals after infusion for radioactivity measurement. Fasting lipoproteins were determined in the beginning of kinetic studies.

Results: Age and BMI were similar in the studied groups. Fractional clearance rate (FCR) of ^{14}C -CE was significantly greater in CDP compared to NO THERAPY (0.076 ± 0.037 vs. $0.046 \pm 0.021 \text{ h}^{-1}$; $p < 0.05$) and CONTROL ($0.0516 \pm 0.0125 \text{ h}^{-1}$; $p < 0.05$). Accordingly, a significant lower total and LDL cholesterol were observed in CDP (156 ± 16 and $88 \pm 16 \text{ mg/dl}$) compared to NO THERAPY (174 ± 15 and $108 \pm 17 \text{ mg/dl}$; $p < 0.05$) and CONTROL (200 ± 24 and $118 \pm 23 \text{ mg/dl}$; $p < 0.05$). Moreover, the 50% increase in ^{14}C -CE FCR in CDP was paralleled by 20% decrease in LDL-c compared to NO THERAPY.

Conclusion: This is the first *in vivo* demonstration that removal of LDE from plasma was increased in SLE patients taking CDP. These data support its beneficial use in SLE and identify the LDL receptor as a promising CDP

mechanism for lowering lipids in patients taking corticosteroids.

Key Indexing Terms: Chloroquine, Dyslipoproteinemia, Systemic Lupus Erythematosus, Atherosclerosis, Lipoproteins, Emulsions, Low-Density Lipoprotein.

INTRODUCTION

Antimalarial drugs containing the four amino-quinilone radicals have a modulating effect on the inflammatory process in rheumatic diseases (1-3). Regarding systemic lupus erythematosus (SLE), hydroxychloroquine (HCQ) and chloroquine diphosphate (CDP) are widely used (4-7) in order to prevent new inflammatory flares (7,8).

Furthermore, antimalarials show a plasma lipid lowering effect in SLE patients (9-11) that is therapeutically interesting due to increased risk of developing premature atherosclerosis that SLE patients are exposed (12). Dyslipidemias are very frequent in SLE (12-14) and certainly play a pivotal role in the fifty times greater risk of developing coronary artery disease (CAD) (15), an important cause of mortality in SLE (12,16,17). SLE dyslipoproteinemia (18,19) is aggravated by disease activity (19) and is characterized by disturbances of triglyceride catabolism related with decreased lipoprotein lipase activity (20), the enzyme that catalyzes the intravascular breaking-down of triglycerides of very-low density lipoproteins (VLDL) and chylomicrons. This may be associated to the presence of auto-antibodies against this enzyme (21). HDL cholesterol is reduced, probably as a consequence of hypertriglyceridemia (19).

On the other hand, it is well-known that corticosteroid therapy enhances lipoprotein synthesis leading to a preferential increase of triglycerides and total cholesterol (22-24), particularly LDL which is recognized as one of the main risk factor for atherosclerosis (25). In fact, it

has been demonstrated a continuous increase in total cholesterol levels with higher prednisone doses in lupus patients (10).

In contrast, antimalarials seems to reduce LDL cholesterol in most studies with SLE patients taking steroids (9,10,26-31). *In vitro* studies suggest that chloroquine diminishes the cholesterol synthesis by inhibiting the cyclization of squalene oxide to lanosterol in mammalian cells (32,33).

To further clarify possible mechanisms whereby antimalarials affect the plasma cholesterol metabolism in SLE, we evaluated the LDL pathway in lupus patients with or without CDP and in controls through the determination of plasma kinetics of a radioactively labeled artificial cholesterol-rich nanoemulsion (LDE) that resembles LDL lipidic structure (34-36). LDE has been used as a surrogate for native LDL to investigate in clinical studies the process of LDL removal from the plasma (37-39).

PATIENTS AND METHODS

Study population. Twenty consecutive female SLE patients were selected among 900 patients attending the Outpatient Lupus Clinics at the Rheumatology Division of the University of São Paulo. All patients were under 50 years old and fulfilled 4 or more of the revised American College of Rheumatology criteria for the classification of SLE (40). SLE patients were divided in two age-matched groups according to current therapy at the time of evaluation: CDP - exclusive use of 250 mg of chloroquine diphosphate daily for at least 1 year; and NO THERAPY – inactive disease without any therapy that never used CDP or have stopped this drug for at least 1 year. SLE patients were not under corticosteroids nor immunosuppressive drugs in the last year, and none of them were in use of any lipid-raising drugs, such as anticonvulsants, antihypertensives (beta blockers and diuretics), estrogen and/or progesterone containing agents, or lipid-lowering agents. Exclusion criteria were presence of clinical and/or biochemical evidence of Diabetes Mellitus, CAD, liver and thyroid disease, and history of alcohol use. No patient was pregnant or had menopause. All patients had serum creatinine levels below 1.5 mg/dl and proteinuria less than 0.3 g/L/day. Clinical activity was measured according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (41) and all SLE patients have inactive disease with SLEDAI scores of 0. Ten healthy and age-matched female subjects (CONTROL) were selected for comparison and were studied in parallel with lupus patients. None of the SLE patients and controls had body mass index

(BMI) greater than 30 kg/m².

LDE preparation. LDE was prepared from a lipid mixture composed of 40mg cholesteryl oleate, 20mg egg phosphatidylcholine, 1mg triolein, and 0.5mg cholesterol purchased from Nu-Check Prep (Elysian). [¹⁴C] cholesteryl ester purchased from Amersham International (Amersham, UK) was added to the mixture. The emulsification of lipids by prolonged ultrasonic irradiation in aqueous media and the two-step ultracentrifugation procedure of the crude emulsion with density adjustment by addition of KBr to obtain LDE microemulsion was carried out by the method of Ginsburg (42) modified by Maranhão et al (34). LDE was dialysed against saline solution and passed through 0.22µm filter for injection into the patients.

LDE plasma kinetics. The participants were fasting for 12h at the beginning of the test at approximately 8:30 a.m., but they were allowed two standard meals (total of 1,800 kcal) during the study at ~12:30 PM and 7 PM. LDE containing 37 kBq [¹⁴C]cholesteryl ester in a total lipid mass of 5-6mg (in a 200 µl volume) was intravenously injected in a bolus. Plasma samples were collected during 24h, in intervals of 5 min, 1, 2, 4, 6, 8 and 24h after the injection. Aliquots (1 ml) of blood plasma were transferred to counting vials containing 7.0ml scintillation solution (ULTIMA GOLD XR, Packard Bioscience, Meriden, USA). Radioactivity was counted using a Packard 1660 TR (Meriden, USA) Liquid Scintillation Analyzer.

Calculation of FCR of the emulsion radioactive lipids. FCR of the LDE [¹⁴C]cholesteryl ester, was calculated according to the method described by Matthews as FCR, where a_1 , a_2 , b_1 and b_2 were estimated from biexponential curves obtained from the remaining radioactivity found in plasma after injection, fitted by least squares procedure:

$$y = (a_1 \cdot e^{-b_1 t}) + (a_2 \cdot e^{-b_2 t})$$

where y represents the radioactivity plasma decay in function of time (t); a indicates the linear coefficient and b , the angular coefficient, which represents the FCR in h^{-1} . The FCR were estimated from parameters a_1 , b_1 , and b_2 by the following equation: $FCR = (a_1 / b_1 + a_2 / b_2)^{-1}$. Calculations were performed by compartmental analysis using the ANACOMP software (43).

Informed consent and radiological safety. The experimental protocol was approved by the local Ethical Committee and a written informed consent was given by all participants. A pregnancy test was performed one day before the kinetic study and all tests were negative. The safety of the radioactive dose intravenously injected into the patients was assured according to the regulations of the International Commission on Radiological Safety as described in our previous study (44). The equivalent absorbed dose on each experiment was 0.03 mSV which is below the 50 mSV annual limit for intake of radionuclides.

Lipid profile. All SLE patients and controls were in their normal diet and were fasting for at least 12-h at the beginning of the study. Fasting

lipoproteins were determined on the same day of the kinetic studies. Plasma total cholesterol (TC) and triglycerides (TG) were measured enzymatically (Boehringer Mannheim, Argentina and Merck, Germany, respectively) on a RA 1000 analyser (Technicon Instruments Corp) (45,46). High density lipoprotein cholesterol (HDL-c) was obtained after precipitation of very low density lipoprotein cholesterol (VLDL-c) and low density lipoprotein cholesterol (LDL-c) by phosphotungstic acid and magnesium chloride (47). Very low-density lipoprotein cholesterol (VLDL-c) and low-density lipoprotein cholesterol (LDL-c) were estimated since all subjects had triglycerides less than 400 mg/dl (48). VLDL-c levels were calculated from the division of serum triglyceride by 5 (TG/5) (48). LDL-c levels were estimated using the equation (48):

$$TC = HDL-c + TG/5 + LDL-c$$

Statistical analysis. Results are presented as the mean $\pm$ standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) and if a significant result was obtained ($p < 0.05$), Tukey's test was performed to evaluate differences between groups. Correlations among plasma lipid fractions and FCRs were calculated using Spearman's rank correlation test. Statistical significance was set as $p < 0.05$.

RESULTS

The distribution of age, weight, height, and body mass index (BMI) of the three groups is shown in Table 1. As expected, age was similar in CDP, NO THERAPY, and CONTROL groups ($p=0.93$). In addition, no significant difference was found between groups concerning weight ($p=0.39$) and height ($p=0.96$). Likewise, BMI did not significantly differ between groups ($p=0.31$). SLE patients of CDP group were in use of this drug for 7.1 ± 2.9 years and had a similar disease duration compared to NO THERAPY (9.5 ± 4.6 and 8.2 ± 6.6 years respectively, $p=0.10$). Importantly, SLE patients of both groups demonstrated a SLEDAI score of zero which reflects no clinical or laboratorial signs of disease activity.

The lipoprotein profile of CDP, NO THERAPY, and CONTROL are shown in Table 2. CDP had lower LDL-c levels compared to NO THERAPY (88 ± 16 vs. 108 ± 17 mg/dl; $p<0.05$) and CONTROL (88 ± 16 vs. 118 ± 23 mg/dl; $p<0.05$). Similarly, total cholesterol (TC) levels in CDP (156 ± 16 mg/dl) were lower compared to NO THERAPY (174 ± 15 mg/dl; $p<0.05$) and healthy controls (200 ± 24 mg/dl; $p<0.05$). In contrast, higher HDL-c levels were found in CDP compared to NO THERAPY (54 ± 8 vs. 46 ± 9 mg/dl; $p<0.05$) although they were similar to those of controls. In addition, NO THERAPY group also had lower HDL-c compared to healthy controls, as well as lower TC levels ($p<0.05$). TG and VLDL-c tended to be lower in CDP compared to the other two groups, although this difference did not reach statistical significance ($p=0.06$).

LDE PLASMA KINETICS

Figure 1 shows the plasma decaying curves of the radioactively labeled LDE cholesteryl ester during the 24 hours of the study. It should be noticed that the [^{14}C]cholesteryl ester disappearance curve of the NO THERAPY group was nearly equal to that of the controls ($p>0.05$). In contrast, the curve obtained in CDP was pronouncedly faster than the other two curves (Figure 1). Indeed, as shown in Table 3, the [^{14}C]cholesteryl ester FCR did not differ between NO THERAPY and controls and was greater in the CDP group compared with the two other groups ($p=0.03$).

Interestingly, a negative correlation in lupus patients (CDP + NO THERAPY) was found between [^{14}C]cholesteryl ester FCR and the plasma levels of TC ($r= - 0.581$, $p=0.007$) and LDL-c ($r= - 0.495$, $p=0.026$). FCR did not correlate with HDL-c ($r= 0.132$, $p=0.57$), VLDL-c ($r= - 0.356$, $p=0.12$) or TG ($r= - 0.352$, $p=0.12$). In addition, FCR also did not correlate with age ($r= - 0.050$, $p=0.83$) or BMI ($r= - 0.016$, $p=0.94$).

DISCUSSION

This is the first *in vivo* demonstration that in SLE patients treated with CDP the LDE plasma kinetics is accelerated whereas the LDL cholesterol simultaneously decreases.

To achieve these findings, rigorous selection criteria were established to eliminate the main systemic diseases that promote specific alterations on lipid metabolism (49-53). Additionally, the progressive increase in total and LDL cholesterol with aging (54) and menopause (55) was avoided by the exclusion criteria. Moreover, all patients included in the study had inactive disease since it is known that activity can aggravate lupus dyslipoproteinemia (19) and were not in use of drugs that could interfere in lipoprotein metabolism (56). Also important, the CDP administration period was long enough to assure that the changes in lipid metabolism by CDP were sustained, which occurs after one year of treatment (31,57).

The great advantage of the LDE system used herein is to specifically evaluate the *in vivo* function of LDL receptors that remove the lipoprotein from plasma into cells. LDE is made without protein but when injected into the circulation and in contact with plasma it acquires several small molecular weight apolipoproteins (apo), including apo E (34). Apo E endows LDE particles to bind to the LDL receptors, since those receptors recognize not only the apo B present in LDL-c, but also apo E that is not found in the LDL fraction (36,58). Apo E binds to the LDL receptor with a greater affinity than apo B100 and is thus removed faster than the native LDL (36,58).

Moreover, the known differences in LDL subclasses among subjects (59-61) which may influence the removal of the lipoprotein from the plasma are excluded by the present approach since LDE is a standard preparation allowing a more accurate definition of receptor function (38,39).

Importantly, this procedure revealed that LDL receptor function in SLE is not altered by the disease, which is supported by normal LDL levels observed in inactive untreated lupus patients (19). In contrast, accelerated LDE removal has been described for acute myelocytic leukemia (62,63) and a defective function was detected in familial hypercholesterolemia (38).

The most interesting finding of our study was the disclosure that CDP up-regulates LDL receptors increasing the *in vivo* plasma kinetics of cholesterol rich emulsion in SLE. This effect was strong enough to determine a pronounced fall in the LDL-c levels. The twofold greater LDE removal from the plasma in CDP was paralleled by a 20% reduction in the LDL-c concentration compared to the untreated patients. Indeed, it has previously been suggested that hydroxychloroquine use elicits lower LDL cholesterol levels in SLE patients taking corticosteroids (9-10). Interestingly, a decrease in LDL-c levels were also identified in randomized trials after the use of hydroxychloroquine in diabetic patients (64,65).

According to this concept a positive correlation between [¹⁴C]cholesteryl ester FCR and LDL cholesterol was identified in SLE patients confirming that the drug powerfully influences the status of LDL receptor function, that is a major determinant of LDL concentration (66).

Conversely, others and we have demonstrated a reduction in VLDL-c

and TG leading to an increase of HDL-c levels with the use of antimalarials in SLE patients taking prednisone (26,31). In this study, the comparison with untreated SLE patients also revealed a trend towards a TG reduction in the CPD group with a concomitant increase in HDL cholesterol, possibly related with reduction of lipoprotein hepatic synthesis, as previously reported (31).

It is well recognized that low HDL and high LDL levels are classic risk factors on the development of atherosclerosis in the general population (25) and also in SLE (13,67-69). In addition, increased exposure to corticosteroids especially in higher doses is the main cause of hypercholesterolemia in this disease (10,13,67). In this regard, statins (or HMG-CoA) are currently the preferential treatment for this condition but a growing body of evidence suggests that these drugs also have antiinflammatory and/or immunomodulatory effects in rheumatic diseases besides lowering LDL cholesterol (70). Besides inhibiting the rate-limiting step of cholesterol synthesis, these drugs also increase the removal from the plasma of both native LDL and LDE (38,39).

Consistent with these perceptions, this novel CDP mechanism of lowering LDL cholesterol by increasing its removal from the plasma documented herein, emphasizes the useful of this drug in lupus patients with high LDL levels particularly those taking steroids. A possible synergism between CDP and statins leading to a more intense antilipidemic effect and better antiatherogenic protection should also be considered. In the light of these findings, those effects can be valuable and should be explored in future studies.

REFERENCES

1. Avina-Zubieta JA, Galindo-Rodriguez G, Newman S, Suarez-Almazor ME, Russell AS. Long-term effectiveness of antimalarial drugs in rheumatic diseases. *Ann Rheum Dis*. 1998;57:582-7.
2. Rynes RI. Antimalarial drugs in the treatment of rheumatological diseases. *Br J Rheumatol*. 1997;36:799-805.
3. Wallace DJ. Antimalarials – the ‘real’ advance in lupus. *Lupus*. 2001;10:385-7.
4. Rothfield N. Efficacy of antimalarials in systemic lupus erythematosus. *Am J Med*. 1988;85:53-6.
5. Rudnicki RD, Gresham GE, Rothfield NF. The efficacy of antimalarials in systemic lupus erythematosus. *J Rheumatol*. 1975;2:323-30.
6. Meinao IM, Sato EI, Andrade LE, Ferraz MB, Atra E. Controlled trial with chloroquine diphosphate in systemic lupus erythematosus. *Lupus*. 1996;5:237-41.
7. The Canadian Hydroxychloroquine Study Group: A randomized study of the effect of withdrawing hydroxychloroquine sulfate in systemic lupus erythematosus. *N Engl J Med*. 1991;324:150-4.
8. Tsakonas E, Joseph L, Esdaile JM, Choquette D, Senecal JL, Cividino A, Danoff D, Osterland CK, Yeadon C, Smith CD. A long-term study of hydroxychloroquine withdrawal on exacerbations in systemic lupus erythematosus. The Canadian Hydroxychloroquine Study Group. *Lupus*. 1998;7:80-5.

9. Wallace DJ, Metzger AL, Stecher VJ, Tumbull BA, Kern PA. Cholesterol-lowering effect of hydroxychloroquine in patients with rheumatic disease: reversal of deleterious effects of steroids on lipids. *Am J Med.* 1990;89:322-6.
10. Petri M, Lakatta C, Magder L, Goldman D. Effect of prednisone and hydroxychloroquine on coronary artery disease risk factors in systemic lupus erythematosus: a longitudinal data analysis. *Am J Med.* 1994;96:254-9.
11. Petri M. Hydroxychloroquine use in the Baltimore Lupus Cohort: effects on lipids, glucose and thrombosis. *Lupus.* 1996;5(Suppl 1):S16-22.
12. Bruce IN. 'Not only...but also': factors that contribute to accelerated atherosclerosis and premature coronary heart disease in systemic lupus erythematosus. *Rheumatology.* 2005; 44:1492-502.
13. Petri M, Perez-Gutthann S, Spence D, Hochberg MC. Risk factors for coronary artery disease in patients with systemic lupus erythematosus. *Am J Med.* 1992;93:513-9.
14. Bruce IN, Gladman DD, Urowitz MB. Premature atherosclerosis in systemic lupus erythematosus. *Rheum Dis Clin North Am.* 2000;26:257-78.
15. Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA Jr, Jansen-McWilliams L, D'Agostino RB, Kuller LH. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol.* 1997;145:408-15.

16. Ward MM. Premature morbidity from cardiovascular and cerebrovascular diseases in women with systemic lupus erythematosus. *Arthritis Rheum.* 1999;42:338-46.
17. Ward MM. Outcomes of hospitalizations for myocardial infarctions and cerebrovascular accidents in patients with systemic lupus erythematosus. *Arthritis Rheum.* 2004;50:3170-6.
18. Ilowite NT, Samuel P, Ginzler E, Jacobson MS. Dyslipoproteinemia in pediatric systemic lupus erythematosus. *Arthritis Rheum.* 1988;31:859-63.
19. Borba EF, Bonfa E. Dyslipoproteinemias in systemic lupus erythematosus: influence of disease, activity, and anticardiolipin antibodies. *Lupus.* 1997;6:533-9.
20. Borba EF, Bonfá E, Vinagre CG, Ramires JA, Maranhao RC. Chylomicron metabolism is markedly altered in systemic lupus erythematosus. *Arthritis Rheum.* 2000;43:1033-40.
21. de Carvalho JF, Borba EF, Viana VS, Bueno C, Leon EP, Bonfa E. Anti-lipoprotein lipase antibodies: a new player in the complex atherosclerotic process in systemic lupus erythematosus? *Arthritis Rheum.* 2004;50:3610-5.
22. Ettinger WH, Goldberg AP, Applebaum-Bowden D, Hazzard WR. Dyslipoproteinemia in systemic lupus erythematosus: Effect of corticosteroids. *Am J Med.* 1987;83:503-8.
23. Ettinger WH Jr, Hazzard WR. Elevated apolipoprotein-B levels in corticosteroid-treated patients with systemic lupus erythematosus. *J*

Clin Endocrinol Metab. 1988;67:425-8.

24. MacGregor AJ, Dhillon VB, Binder A, Forte CA, Knight BC, Betteridge DJ, Isenberg DA. Fasting lipids and anticardiolipin antibodies as risk factors for vascular disease in systemic lupus erythematosus. *Ann Rheum Dis.* 1992;51:152-5.
25. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA.* 2001;285:2486-97.
26. Hodis HN, Quismorio FP Jr, Wickham E, Blankenhorn DH. The lipid, lipoprotein, and apolipoprotein effects of hydroxychloroquine in patients with systemic lupus erythematosus. *J Rheumatol.* 1993;20:661-5.
27. Kavanaugh A, Adams-Huet B, Jain R, Denke M, McFarlin J. Hydroxychloroquine effects on lipoprotein profiles (the HELP trial): A double-blind, randomized, placebo-controlled, pilot study in patients with systemic lupus erythematosus. *J Clin Rheumatol.* 1997;3:3-8.
28. Rahman P, Gladman DD, Urowitz MB, Yuen K, Hallett D, Bruce IN. The cholesterol lowering effect of antimalarial drugs is enhanced in patients with lupus taking corticosteroid drugs. *J Rheumatol.* 1999;26:325-30.
29. Tam LS, Li EK, Lam CW, Tomlinson B. Hydroxychloroquine has no significant effect on lipids and apolipoproteins in Chinese systemic lupus erythematosus patients with mild or inactive disease. *Lupus.* 2000;9:413-6.

30. Tam LS, Gladman DD, Hallett D, Rahman P, Urowitz MB. Effect of antimalarial agents on the fasting lipid profile in systemic lupus erythematosus. *J Rheumatol.* 2000;27:2142-5.
31. Borba EF, Bonfa E. Longterm beneficial effect of chloroquine diphosphate on lipoprotein profile in lupus patients with and without steroid therapy. *J Rheumatol.* 2001;28:780-5.
32. Goldstein JL, Brunschede GY, Brown MS. Inhibition of the proteolytic degradation of low density lipoprotein in human fibroblasts by chloroquine, concanavalin A, and Triton WR 1339. *J Biol Chem.* 1975;250:7854-62.
33. Chen HW, Leonard DA. Chloroquine inhibits cyclization of squalene oxide to lanosterol in mammalian cells. *J Biol Chem.* 1984;259:8156-62.
34. Maranhão RC, Cesar TB, Pedroso-Mariani SR, Hirata MH, Mesquita CH. Metabolic behavior in rats of a nonprotein microemulsion resembling low-density lipoprotein. *Lipids.* 1993;28:691-6.
35. Maranhao RC, Roland IA, Toffoletto O, Ramires JA, Gonçalves RP, Mesquita CH, Pileggi F. Plasma kinetic behavior in hyperlipidemic subjects of a lipidic microemulsion that binds to low density lipoprotein receptors. *Lipids.* 1997;32:627-33.
36. Hirata RD, Hirata MH, Mesquita CH, Cesar TB, Maranhao RC. Effects of apolipoprotein B-100 on the metabolism of a lipid microemulsion model in rats. *Biochim Biophys Acta.* 1999;1437:53-62.
37. Pinto LB, Wajngarten M, Silva EL, Vinagre CC, Maranhao RC. Plasma kinetics of a cholesterol-rich emulsion in young, middle-aged and elderly

subjects. *Lipids*. 2001;36:1307-11.

38. Santos RD, Chacra AP, Morikawa A, Vinagre CC, Maranhao RC. Plasma kinetics of free and esterified cholesterol in familial hypercholesterolemia: effects of simvastatin. *Lipids*. 2005;40:737-43.
39. Santos RD, Hueb W, Oliveira AA, Ramires JA, Maranhao RC. Plasma kinetics of a cholesterol-rich emulsion in subjects with or without coronary artery disease. *J Lipid Res*. 2003;44:464-9.
40. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1982;25:1271-7.
41. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum*. 1992;35:630-40.
42. Ginsburg GS, Small DM, Atkinson D. Microemulsion of phospholipids and cholesterol esters. Protein-free models of low density lipoprotein. *J Biol Chem*. 1982;257:8216-27.
43. Marchese SRM, Mesquita CH, Cunha IIL. Anacomp program application to calculate ^{137}C transfer rates in marine organisms and dose in man. *J Radioan Nucl Chem*. 1998;232:233-6.
44. Maranhão RC, Feres MC, Martins MT, Mesquita CH, Toffoletto O, Vinagre CG, Gianinni SD, Pileggi F. Plasma kinetics of a chylomicron-like emulsion in patients with coronary artery disease. *Atherosclerosis*.

1996;126:15-25.

45. Siedel J, Hägele EO, Ziegenhorn J, Wahlefeld AW. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin Chem*. 1983;29:1075-80.
46. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem*. 1982;28:2077-80.
47. Warnick GR, Cheung MC, Albers JJ. Comparison of current methods for high-density lipoprotein cholesterol quantitation. *Clin Chem*. 1979;25:596-604.
48. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502.
49. Brunzell JD, Albers JJ, Haas LB, Goldberg AP, Agadoa L, Sherrard DJ. Prevalence of serum lipid abnormalities in chronic hemodialysis. *Metabolism*. 1977;26:903-10.
50. Attman PO, Alaupovic P. Lipid and apolipoprotein profiles of uremic dyslipoproteinemia - relation to renal function and dialysis. *Nephron*. 1991;57:401-10.
51. Appel GB, Blum CB, Chien S, Kunis CL, Appel AS. The hyperlipidemia of the nephrotic syndrome. Relation to plasma albumin concentration, oncotic pressure and viscosity. *N Engl J Med*. 1985;312:1544-8.
52. Thompson GR, Soutar AK, Spengel FA, Jadhav A, Gavigan SJ, Myant NB. Defects of receptor-mediated low density lipoprotein catabolism in

homozygous familial hypercholesterolemia and hypothyroidism *in vivo*.

Proc Natl Acad Sci (USA). 1981;78:2591-5.

53. Brown WV. Lipoprotein disorders in diabetes mellitus. *Med Clin North Am*. 1994;78:143-61.
54. Heiss G, Tamir I, Davis CE, Tyroler HA, Rifkind BM, Schonfeld G, Jacobs D, Frantz ID Jr. Lipoprotein-cholesterol distributions in selected North American populations: the lipid research clinics program prevalence study. *Circulation*. 1980;61:302-15.
55. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. *Ann Intern Med*. 1976;85:447-52.
56. Henkin Y, Como JA, Oberman A. Secondary dyslipidemia. Inadvertent effects of drugs in clinical practice. *JAMA*. 1992;267:961-8.
57. Munro R, Morrison E, McDonald AG, Hunter JA, Madhok R, Capell HA. Effect of disease modifying agents on the lipid profiles of patients with rheumatoid arthritis. *Ann Rheum Dis*. 1997;56:374-7.
58. Innerarity TL, Mahley RW. Enhanced binding by cultured human fibroblasts of apo-E-containing lipoproteins as compared with low density lipoproteins. *Biochemistry*. 1978;17:1440-7.
59. Capell WH, Zambon A, Austin MA, Brunzell JD, Hokanson JE. Compositional differences of LDL particles in normal subjects with LDL subclass phenotype A and LDL subclass phenotype B. *Arterioscler Thromb Vasc Biol*. 1996;16:1040-6.

60. Ohmura H, Mokuno H, Sawano M, Hatsumi C, Mitsugi Y, Watanabe Y, Daida H, Yamaguchi H. Lipid compositional differences of small, dense low-density lipoprotein particle influence its oxidative susceptibility: possible implication of increased risk of coronary artery disease in subjects with phenotype B. *Metabolism*. 2002;51:1081-7.
61. Chapman MJ, Guerin M, Bruckert E. Atherogenic, dense low-density lipoproteins. Pathophysiology and new therapeutic approaches. *Eur Heart J*. 1998;19(Suppl A):A24-30.
62. Maranhao RC, Garicochea B, Silva EL, Llacer PD, Pileggi FJ, Chamone DA. Increased plasma removal of microemulsions resembling the lipid phase of low-density lipoproteins (LDL) in patients with acute myeloid leukemia: a possible new strategy for the treatment of the disease. *Braz J Med Biol Res*. 1992;25:1003-7.
63. Maranhao RC, Garicochea B, Silva EL, Dorlhiac-Llacer P, Cadena SM, Coelho IJ, Meneghetti JC, Pileggi FJ, Chamone DA. Plasma kinetics and biodistribution of a lipid emulsion resembling low-density lipoprotein in patients with acute leukemia. *Cancer Res*. 1994;54:4660-6.
64. Powrie JK, Shojaee-Moradie F, Watts GF, Smith GD, Sönksen PH, Jones RH. Effects of chloroquine on the dyslipidemia of non-insulin-dependent diabetes mellitus. *Metabolism*. 1993;42:415-9.
65. Gerstein HC, Thorpe KE, Taylor DW, Haynes RB. The effectiveness of hydroxychloroquine in patients with type 2 diabetes mellitus who are refractory to sulfonylureas – a randomized trial. *Diabetes Res Clin Pract*. 2002;55:209-19.

66. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science*. 1986;232:34-47.
67. Petri M, Spence D, Bone LR, Hochberg MC. Coronary artery disease risk factors in the Johns Hopkins Lupus Cohort: prevalence, recognition by patients, and preventive practices. *Medicine (Baltimore)*. 1992;71:291-302.
68. Bruce IN, Urowitz MB, Gladman DD, Hallett DC. Natural history of hypercholesterolemia in systemic lupus erythematosus. *J Rheumatol*. 1999;26:2137-43.
69. Svenungsson E, Jensen-Urstad K, Heimbürger M, Silveira A, Hamsten A, de Faire U, Witztum JL, Frostegård J. Risk factors for cardiovascular disease in systemic lupus erythematosus. *Circulation*. 2001;104:1887-93.
70. Abeles AM, Pillinger MH. Statins as antiinflammatory and immunomodulatory agents: a future in rheumatologic therapy? *Arthritis Rheum*. 2006;54:393-407.

TABLE 1: Demographic features, disease duration, and SLEDAI in SLE groups (CDP and NO THERAPY) and CONTROL.

GROUP	CDP (n=10)	NO THERAPY (n=10)	CONTROL (n=10)	<i>P</i>
AGE (years)	35.4 ± 7.5	36.5 ± 6.9	35.6 ± 8.9	0.93
WEIGHT (kg)	60.2 ± 6.8	63.0 ± 8.8	57.5 ± 10.4	0.39
HEIGHT (m)	1.61 ± 0.04	1.60 ± 0.06	1.60 ± 0.10	0.96
B.M.I. (kg/m ²)	23.0 ± 2.5	24.3 ± 2.4	22.2 ± 3.7	0.31
Disease duration (years)	9.5 ± 4.6	8.2 ± 6.6	-	0.10
SLEDAI	0	0	-	-

Values were expressed in mean ± SD; B.M.I. = body mass index ; SLEDAI = Systemic lupus erythematosus disease activity index

TABLE 2: Plasma lipid profile of SLE groups (CDP and NO THERAPY) and CONTROL

PARAMETER	CONTROL (N=10)	NO THERAPY (N=10)	CDP (N=10)	<i>P</i>
Cholesterol				
Total	200 ± 24	174 ± 15 *	156 ± 16 *†	<0.001
HDL-c	64 ± 7	46 ± 9 *	54 ± 8 †	<0.001
LDL-c	118 ± 23	108 ± 17	88 ± 16 *†	<0.001
VLDL-c	17 ± 7	20 ± 7	13 ± 4	0.06
Triglycerides	86 ± 33	103 ± 38	68 ± 19	0.06

Values expressed in mean ± SD and range (in parenthesis), in mg/dl

* *p* < 0.05 vs. CONTROL

† *p* < 0.05 vs. NO THERAPY

TABLE 3: Fractional clearance rate (FCR) of [¹⁴C]cholesteryl ester in SLE groups (CDP and NO THERAPY) and CONTROL

FCR	CONTROL	NO THERAPY	CDP	<i>P</i>
[¹⁴C]cholesteryl ester	0.051 ± 0.012	0.046 ± 0.021	0.076 ± 0.037 *	0.03

Values in h⁻¹, expressed in mean ± SD
 * *p* < 0.05 vs. CONTROL and vs. NO THERAPY

FIGURE 1

FIGURE 1 LEGEND: LDE clearance in CDP patients (continuous line), NO THERAPY patients (dotted line) and CONTROL subjects (dashed line) during 24 hours after intravenous infusion. Disappearance curves of the emulsion [^{14}C]cholesteryl ester of CDP were faster than those of NO THERAPY and CONTROL ($p=0.03$), indicating a significant increase of clearance in SLE patients taking CDP.