HILTON OLIVEIRA DOS SANTOS FILHO

ESTUDO CLÍNICO FASE I DE CARBONATO DE LODENAFILA, UM NOVO TIPO DE INIBIDOR DE FOSFODIESTERASE 5 (PDE5), EM VOLUNTÁRIOS SAUDÁVEIS DO SEXO MASCULINO.

A PHASE I CLINICAL TRIAL OF LODENAFIL CARBONATE, A NEW PHOSPHODIESTERASE TYPE 5 (PDE5) INHIBITOR, IN HEALTHY MALE VOLUNTEERS.

Campinas
2013

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Ciências Médicas

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Orientador (a) / Supervisor: Prof. Dr. Gilberto De Nucci

A PHASE I CLINICAL TRIAL OF LODENAFIL CARBONATE, A NEW PHOSPHODIESTERASE TYPE 5 (PDE5) INHIBITOR, IN HEALTHY MALE VOLUNTEERS

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Clínica Médica da Faculdade de Ciências Médicas da Universidade de Campinas para obtenção de título de Doutor em Clínica Médica, área de concentração Clínica Médica.

Doctoral Thesis presented to the Internal Medicine Posgraduate Program of the Faculty of Medical Sciences, State University of Campinas, for obtainment of the Ph.D. degree in Internal Medicine/Medical Sciences, specialization in Internal Medicine.

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO HILTON OLIVEIRA DOS SANTOS FILHO E ORIENTADO PELO PROF. DR. GILBERTO DE NUCCI.

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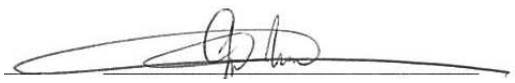

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FICHA CATALOGRÁFICA ELABORADA POR
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S237e

Santos Filho, Hilton Oliveira dos, 1956-
Estudo clínico fase I de carbonato de lodenafila, um novo tipo de inibidor de fosfodiesterase 5 (PDE5), em voluntários saudáveis do sexo masculino / Hilton Oliveira dos Santos Filho. -- Campinas, SP : [s.n.], 2013.

Orientador : Gilberto De Nucci.
Tese (Doutorado) - Universidade Estadual de Campinas, Faculdade de Ciências Médicas.

1. Disfunção erétil. 2. Inibidores da fosfodiesterase tipo 5. 3. Farmacocinética. 4. Segurança. I. De Nucci, Gilberto, 1958-. II. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. III. Título.

Informações para Biblioteca Digital

Título em inglês: A phase I clinical trial of lodenafil carbonate, a new phosphodiesterase type 5 (PDE5) inhibitor, in healthy male volunteers.
Palavras-chave em inglês:
Erectile dysfunction
Phosphodiesterase type 5 (PDE5) inhibitors
Pharmacokinetics
Safety
Área de concentração: Clínica Médica
Titulação: Doutor em Clínica Médica
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Data da defesa: 13-05-2013
Programa de Pós-Graduação: Clínica Médica

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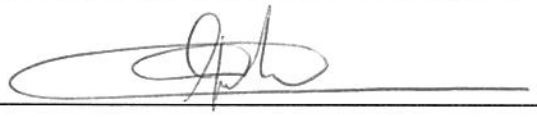
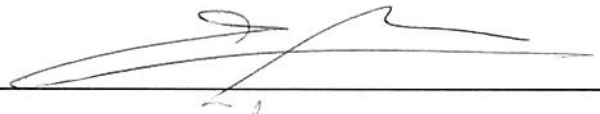
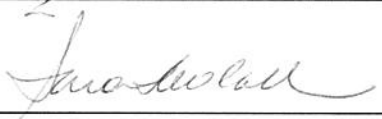
1. **PROF. DR. GILBERTO DE NUCCI** 
2. **PROF. DR. UBIRAJARA FERREIRA** 
3. **PROFA. DRA. SARA TERESINHA OLALLA SAAD** 
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Data: 13 de maio de 2013

DEDICATÓRIA

Aos meus pais, Hilton e Dalva:

De quem Deus me deu a honra de ser filho, responsáveis por minha formação ética e profissional, meus grandes exemplos de vida. Meu amor por vocês é infinito e eterno.

A minha esposa Fátima, meus filhos Carla e Bruno:

Razões de minha vida, e a quem devo muito do que consegui até o momento.

Meu amor incondicional e gratidão.

AGRADECIMENTOS

Agradeço a minha esposa, Fátima, pela enorme paciência, amor incondicional e companheirismo.

Aos meus pais, Hilton e Dalva, pelo apoio em todos os meus projetos, por mais ousados que tenham sido. Sempre me estimularam a ir mais adiante e a acreditaram em meu potencial.

Em especial ao orientador Prof. Dr. Gilberto De Nucci pela confiança; amizade, oportunidade de aprendizado que tive a honra de receber. Sua experiência e seu conhecimento serão reconhecidos eternamente por toda a comunidade acadêmica nacional e internacional.

É uma honra imensa poder ter este grande cientista e pessoa excepcional como meu orientador.

Em especial ao Dr. Márcio Falci, pela grande amizade, confiança,

aprendizado profissional e de vida durante vários anos.

Agradeço a todos que direta ou indiretamente contribuíram para a realização desse trabalho.

Especialmente, aos que participam ou participaram dos grupos de pesquisa do

Prof. Dr. Gilberto De Nucci.

A TODOS, O MEU MAIS SINCERO MUITO OBRIGADO!

Carbonato de Lodenafila é um novo tipo de inibidor de fosfodiesterase 5 (PDE5) utilizado no tratamento da disfunção erétil. O presente estudo foi realizado para avaliar a segurança, tolerabilidade e farmacocinética do carbonato de lodenafila após a administração de doses orais únicas ascendentes (de 1 a 100 mg) a voluntários saudáveis do sexo masculino (n = 33). O estudo foi um estudo clínico fase I, aberto, de escalonamento de dose, utilizando a administração de doses orais únicas de carbonato de lodenafila. Carbonato de Lodenafila foi administrado sequencialmente escalonado em doses únicas de 1 mg a 100 mg com um período sem uso do medicamento (*washout)* de pelo menos 1 semana, entre cada dose. A progressão para a próxima dose foi permitida se após a avaliação dos exames clínicos, laboratoriais e Monitorização Ambulatorial da Pressão Arterial (MAPA), não demonstrassem alterações e eventos adversos sem relevância clínica. As amostras de sangue foram coletadas na pré-dose e em intervalos determinados e 24 horas após a administração. As amostras de plasma para a mensuração de carbonato lodenafila e lodenafila foram analisadas por cromatografia líquida acoplada à espectrometria de massa. Não foram observados eventos adversos graves, e nenhum dos voluntários abandonou o estudo devido à intolerância. As medições do MAPA, exames clínicos e laboratoriais e ECG não revelaram alterações significativas mesmo em doses mais elevadas. Carbonato Lodenafila não foi detectado em qualquer amostra, indicando que ele atua como um pró-droga. Os parâmetros farmacocinéticos médios de lodenafila para tmax e t1 / 2 foram 1,6 (± 0,4) horas e 3,3 (± 1,1) horas, respectivamente. Este estudo demonstrou que o carbonato de lodenafila é bem tolerado e apresentou um bom perfil de segurança em voluntários saudáveis do sexo masculino.

Palavras-chave: inibidores da fosfodiesterase tipo 5 (PDE5), farmacocinética, segurança.

Lodenafil carbonate is a new phosphodiesterase type 5 (PDE5) inhibitor used in treatment of erectile dysfunction. The present study was conducted to evaluate the safety, tolerability, and pharmacokinetics of lodenafil carbonate after administering ascending (1 to 100 mg) single oral doses to healthy male volunteers (n=33). The study was an open-label, dose-escalation, phase I clinical trial involving the administration of single oral doses of lodenafil carbonate. Lodenafil carbonate was administered sequentially, escalating in single doses of 1 mg to 100 mg with a washout period of at least 1 week between each dose. The progression to the next dose was allowed after clinical and laboratory exams, Ambulatory Monitoring of Arterial Pressure (AMAP) without relevant clinical modifications and adverse events without clinical relevancy. Blood samples were collected at pre-dose, determined intervals and 24h post-dosing. Plasma samples for measurement of lodenafil carbonate and lodenafil were analyzed by liquid chromatography coupled to tandem mass spectrometry. No serious adverse events were observed, and none of the subjects discontinued the study due to intolerance. The AMAP measurements, clinical and laboratory exams and ECG revealed no significant changes even at higher doses**.** Lodenafil carbonate was not detected in any samples, indicating that it acts as a pro-drug. The mean lodenafil pharmacokinetic parameters for tmax and $t_{1/2}$ were 1.6 (±0.4) hr and 3.3 (±1.1) hr, respectively. This study demonstrated that lodenafil carbonate was well tolerated and showed a good safety profile in healthy male volunteers.

Key words: phosphodiesterase type 5 (PDE5) inhibitor, pharmacokinetics, safety

Lista de abreviaturas

AMPc: Monofosfato cíclico de adenosina

ANVISA: Agência Nacional de Vigilância Sanitária

AVC: Acidente vascular cerebral

BNDES: Banco Nacional do Desenvolvimento Econômico e Social

CEMIB: Centro Multidisciplinar para Investigação Biológica na Área da Ciência em Animais de Laboratório

CTC: *Common toxicity criteria* - critério comum de toxicidade

DE: Disfunção erétil

$DL_{50:}$ dose letal 50%

DLT: *dose-limiting toxicity* - dose limite de toxicidade

EC50: Efeito máximo 50% - concentração de droga necessária para produzir 50% do efeito máximo

FINEP: Financiadora de Estudos e Projetos

FOB: Functional observation battery - bateria de observação funcional

GMPc: Monofosfato cíclico de guanosina

HED: *Human equivalent dose* - dose equivalente humana

IIEF: International Index for Erectile Function- Índice internacional de Função Erétil

IIEF5: Índice Internacional de Função Erétil-5

MAPA: Monitorização ambulatorial da pressão arterial

MRSD: *Maximum recommended starting dose* - dose máxima inicial

MTD: *Maximum tolerable dosage* - dose máxima tolerada

NOAEL: *No observed adverse effect level* – nível de efeito adverso não observado

P&D: Pesquisa e Desenvolvimento

PDE5: inibidores da fosfodiesterase tipo 5

pEC50: antilog da concentração de droga necessária para produzir 50% do efeito máximo

TRPN: Tumescência e rigidez peniana noturna

UNICAMP: Universidade Estadual de Campinas

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SUMÁRIO

1. Introdução Geral

A disfunção erétil (DE) é uma condição clínica definida como a incapacidade persistente de obter ou manter uma ereção suficiente para permitir um desempenho sexual satisfatório (1, 2).

É considerada por muitos como uma doença, tendo em vista seu amplo espectro de dano ao indivíduo. Apesar de benigna, acomete o indivíduo e seus familiares tendo em vista que tanto a saúde física como a psicossocial se encontram afetada (3).

Em relação à sua epidemiologia, a literatura demonstra dados de incidência e prevalência com variações amplas. Este fato deve-se provavelmente a diversos fatores, como diferentes metodologias utilizadas, idade e condição socioeconômica das populações estudadas.

No entanto, uma revisão dos estudos epidemiológicos, realizada em 2003, sugere que a DE de intensidade moderada a severa, afeta 5% a 20% dos homens (4).

Estudo realizado na população brasileira demonstrou que 25 milhões de homens com mais de 18 anos apresentam algum grau de DE e aproximadamente 11 milhões tenham disfunção erétil moderada ou severa. A incidência de DE na população brasileira foi de 65,6 casos por 1.000 pessoas /ano. Com a projeção de um milhão de novos casos anualmente demonstra que a DE deve ser considerada um problema de saúde pública (5,6).

Como será abordado em maiores detalhes a seguir, o desenvolvimento de um novo fármaco é extremamente complexo e com taxa de sucesso baixa, mesmo em países onde este procedimento já é muito bem conhecido e executado.

O medicamento abordado neste trabalho foi desenvolvido, patenteado e registrado na Agência Nacional de Vigilância Sanitária (ANVISA) para comercialização, integralmente no Brasil, processo pioneiro em nosso país.

A denominada inovação colaborativa foi utilizada com muita propriedade durante todo o processo de desenvolvimento. A integração entre a universidade, indústria e órgãos governamentais, foi determinante para que o projeto fosse executado e o resultado esperado, alcançado. Um medicamento inovador com desenvolvimento inteiramente brasileiro.

Podemos afirmar, sem receio de cometermos excessos, que a universidade, indústria, governo e principalmente a população brasileira podem se orgulhar deste projeto, exemplo a ser seguido por todos os setores envolvidos na pesquisa e desenvolvimento (P&D) brasileiro.

1.1 Fisiologia e fisiopatologia da Disfunção Erétil

A ereção é resultado de uma complexa integração de funções neurais, hormonais e vasculares.

Resumidamente, uma ereção ocorre quando o fluxo de sangue para o pênis excede o fluxo para fora do pênis, conforme detalhado a seguir.

As artérias cavernosas fornecem sangue para o corpo cavernoso do pênis (através da artéria pudenda), enquanto as veias emissárias drenam o corpo cavernoso (para a veia pudenda interna). Durante a ereção, as fibras da musculatura lisa do corpo cavernoso se encontram relaxadas, diminuindo a resistência ao fluxo arterial para o corpo cavernoso e consequente expansão dos sinusoides nele contidos. Esta distensão causa compressão mecânica sobre as veias emissárias , o que impede a sua capacidade para drenar o sangue resultando em rigidez peniana. Durante a ereção, o pênis atua como um capacitor, acumulando sangue sob pressão devido ao relaxamento dos corpos cavernosos (7).

O fluxo sanguíneo do pênis é controlado pelo centro da ereção autonômica, através dos nervos parassimpáticos (S2-S4), simpáticos (T12-L2) e plexo pélvico incluindo o nervo cavernoso, que inerva as artérias e musculatura lisa trabecular do corpo cavernoso.

Apesar de existir uma grande sinergia entre eles, dois mecanismos podem desencadear as alterações vasculares envolvidas na ereção peniana: psicogênicas e reflexogênicas.

As psicogênicas agem através de estímulos como, som, odor, visão e tato. As reflexogênicas através da estimulação peniana direta.

Nos dois mecanismos neurotransmissores são envolvidos na ereção e detumescência, entre eles a dopamina e noradrenalina.

Os hormônios androgênicos atuam modulando a libido e comportamento sexual. A testosterona aumenta o interesse e a frequência dos atos sexuais, no entanto não afeta as ereções reflexogênicas e psicogênicos.

O neurotransmissor óxido nítrico, um dos mais importantes sinalizadores intra e extracelular é, em última análise, o responsável pelo mecanismo de ereção e relaxamento peniano.

Este sinalizador se difunde através da membrana do músculo liso e ativa a guanilato ciclase para produzir o monofosfato cíclico de guanosina (GMPc) que desencadeia uma cascata de reações bioquímicas levando a uma alteração da permeabilidade dos canais iônicos de cálcio e potássio. Esta alteração diminui o cálcio intracelular promovendo o relaxamento do músculo liso, aumento do fluxo sanguíneo e consequentemente ereção peniana.

As enzimas fosfodiesterases, principalmente a isoforma 5 (PDE5), regulam o mecanismo descrito acima catalisando a reação inversa das guanilato ciclases e inativando o GMPc através da sua transformação em GMP (8).

Pelo menos 11 famílias de PDEs foram descritas em mamíferos, mas como alguns tipos estão associados a mais de um gene e podem diferir quanto à afinidade e especificidade pelos possíveis substratos (GMPc ou AMPc), o resultado é pelo menos 50 isotipos de PDEs. A PDE5 é a fosfodiesterase predominante no corpo cavernoso do homem (9,10).

1.2 Diagnóstico da Disfunção Erétil

Conforme já descrito, a ereção é um fenômeno neurovascular sob controle hormonal. A DE compartilha fatores de risco com as doença cardiovascular como sedentarismo, obesidade, tabagismo, hipercolesterolemia e síndrome metabólica. Este fato é importante pois o conhecimento destes fatores de risco tem impacto importante no tratamento da DE (11, 12).

A etiologia da DE é dividada em: vasculogênica, neurogênica, hormonal, anatômica, medicamentosa, ou psicogênica. É comum mais de uma etiologia estar presente em um mesmo paciente. Por este motivo, a classificação que inclui o termo mista, se aproxima muito da realidade clínica da doença.

As vasculogênicas estão relacionadas às doenças cardiovasculares, hipertensão, diabetes mellitus, hiperlipidemia, tabagismo, cirurgias de grande porte ou radioterapia (pélvis ou retroperitônio).

As neurogênicas são subdivididas em: **causas centrais:** como por exemplo, a esclerose múltipla; atrofia múltipla; doença de Parkinson; tumores; acidente vascular cerebral (AVC), doenças discais; **causas periféricas:** como por exemplo: diabetes mellitus; alcoolismo; uremia, olineuropatia e cirúrgicas (pélvica, retroperitônio, prostatectomia radical); **causas anatômicas ou estruturais:** doença de Peyronie; fratura peniana; curvatura congênita do pênis; micropênis; hipospádias, epispádias.

Hormonal: hipogonadismo; hiperprolactinemia, hiper e hipotireoidismo e doença de Cushing.

Medicamentosa ou induzida por drogas: anti-hipertensivos (diuréticos e betabloqueadores), antidepressivos, antipsicóticos, antiandrogênicos, anti-histamínicos e drogas recreacionais (heroína, cocaína, metadona).

Psicogênica: tipo generalizado: falta de excitabilidade e disfunções na intimidade sexual, tipo situacional: relacionamento com parceira, problemas relacionados ao desempenho sexual.

O diagnóstico é eminentemente clínico, realizado após anamnese detalhada e exame clínico. Exames complementares podem auxiliar no diagnóstico, tais como, medida sérica da testosterona, glicemia de jejum e perfil lipídico, Ultrassonografia Doppler colorida com

ereção fármaco induzida prévia, tumescência e rigidez peniana noturna (TRPN) utilizando Rigiscan (3, 13). Atualmente utiliza-se como ferramenta, tanto no diagnóstico como para avaliação em estudos clínicos o International Index for Erectile Function (IIEF), validada para o português como Índice Internacional de Função Erétil-5 (IIFE-5) (14).

1.3 Tratamento da disfunção erétil

O tratamento, como na maioria das doenças, deve seguir o principio de ser individualizado e escalonado do menos invasivo e mais tolerado ao mais invasivo e menos tolerado.

A orientação do homem e do casal sobre sua sexualidade é um ponto fundamental no tratamento da DE independente da etiologia.

A psicoterapia e tratamento com psicofármacos têm apresentado bons resultados, tendo em vista que, principalmente em pacientes jovens, a etiologia mais frequente é a psicogênica (15).

Doenças concomitantes e principalmente as cardiovasculares que compartilham dos mesmos fatores de risco, devem ser devidamente tratadas.

Estudo demonstrou que se os fatores de risco para doenças cardiovasculares forem manejados adequadamente, além do risco cardíaco diminuir a DE pode ser controlada (12).

1.3.1 Tratamento de primeira linha

Os inibidores da Fosfodiesterase 5 (PDE5) são considerados o tratamento de primeira linha para quase todas as etiologias.

A escolha do inibidor da PDE5 deve ser adequada à expectativa do paciente. Estes devem ser adequadamente informados quanto às vantagens e possíveis desvantagens em seu uso.

Como existem inibidores da PDE5 com meia vida variada comercializados no Brasil, é importante adaptar o tratamento aos hábitos sexuais do paciente, frequência de relações sexuais e a previsibilidade destas.

O primeiro inibidor da PDE5, sildenafila (*Viagra ®)*, introduzido no mercado em 1998, era utilizado de acordo com a necessidade (sob demanda) por suas características farmacológicas, principalmente a meia vida curta. Em 2008 a tadalafila (*Cialis®)* foi aprovada para uso diário continuo, facilitando pacientes que tenha atividade sexual mais frequente ou não previsível (3).

São comercializados no Brasil, além dos já descritos, Vardenafila (Levitra®, Vivanza®) e Lodenafila (Helleva®).

Outros inibidores da PDE5 se encontram registrados ou em fase de registro em outros países, como: Udenafila (Coreia e Rússia, pendente FDA), Avanafila (em avaliação FDA), Mirodenafila (Coreia), SLX-2101(fase II finalizado e em avaliação FDA) (16, 17).

Os inibidores da PDE5 registrados no Brasil são medicamentos com perfil de segurança adequados com os eventos adversos e contra indicações, formais e relativas, bem estabelecidas.

Por serem vasodilatadores os eventos adversos mais comuns se relacionam a esta ação, como cefaleia, vermelhidão, tontura e congestão nasal. As menos frequentes são anormalidades visuais, dor nas costas /mialgias, taquicardia, hipotensão, entre outros.

Infartos do miocárdio não aumentaram após o uso de PDE5 em diversos estudos (18). Hipotensão ortostática é descrita com o uso concomitante a alfa-bloqueadores e finalmente, a

contra indicação formal do uso com nitratos, por seus efeitos hipotensores imprevisíveis, podendo levar a baixa perfusão coronariana ao miocárdio.

Doses menores de inibidores de PDE5 podem ser necessárias quando os pacientes estiverem recebendo cetoconazol, itraconazol, eritromicina, claritromicina e inibidores da protease do HIV (ritonavir, saquinavir). Doses maiores de inibidores de PDE5 podem vir a ser necessárias em pacientes que estão recebendo rifampicina, fenobarbital, fenitoína ou carbamazepina. A presença de insuficiência renal ou hepática pode levar à necessidade de ajuste da dose dos inibidores de PDE.

Antes de se iniciar um tratamento de segunda ou terceira linha recomenda-se a reposição de testosterona, o que restabelece a eficácia em pacientes hipogonádicos não respondedores aos inibidores de PDE5, observadas todas as contra indicações formais e relativas desta reposição.

1.3.2 Tratamentos de segunda linha

Pacientes que não respondem adequadamente ao tratamento por via oral podem se beneficiar com o uso de medicamentos injetáveis no corpo cavernoso, como o alprostadil e a associação de medicamentos alprostadil + papaverina + fentolamina, menos indicada por seus maiores efeitos adversos, relacionados à papaverina, principalmente a fibrose.

Ainda pode-se utilizar a prostaglandina E1 administrada por via intrauretral.

1.3.3 Tratamento de terceira linha (prótese peniana)

O implante cirúrgico de uma prótese peniana pode ser considerado para pacientes que não respondem a farmacoterapia ou que desejam uma solução permanente. As principais complicações do método são falhas mecânicas e infecção (3, 13, 19, 20).

1.4 O desenvolvimento de um novo medicamento

O desenvolvimento de um novo medicamento é um grande desafio. Desde a concepção da ideia até o registro de uma nova molécula o tempo despendido é extremamente longo e a taxa de sucesso é muito baixa (21, 22).

No Brasil, este desafio se torna ainda maior, graças a processos regulatórios morosos e cultura de pesquisa e desenvolvimento ainda embrionários. No entanto, este cenário vem se modificando rapidamente com a maior e efetiva participação das universidades e órgãos de fomentos governamentais importantes, como FINEP - Agência Brasileira da Inovação e BNDES – Banco Nacional do Desenvolvimento Econômico e Social (23).

Uma invenção nem sempre se tornará uma inovação, este conceito é muito importante para que oriente grupos de pesquisa em nosso país.

Se uma invenção não é apropriada comercialmente para gerar valor de uso não se tornará uma inovação.

Para exemplificar, se a descoberta de nova entidade química (invenção) não seguir os preceitos acima não se tornará um medicamento inovador (24).

O desenvolvimento sem o foco na apropriação comercial entra no processo de esquecimento e não melhora a qualidade de vida da população. No entanto uma invenção, mesmo não se transformando em uma inovação, pode gerar conhecimento para que outras possam ser verdadeiras inovações no futuro (25).

As inovações são classicamente descritas como radical e incremental. As radicais costumam mudar paradigmas, enquanto que as incrementais estão relacionadas à melhoria da qualidade e aperfeiçoamento de produtos, processos e serviços. Apesar de sua complexidade menos aparente, as inovações incrementais são necessárias, tanto para melhorar a qualidade de vida da população, como para gerar ideias que poderão levar a uma inovação radical (23).

Nas ciências da saúde, especialmente na medicina, estes conceitos são bastante úteis. Atualmente, toda inovação incremental é avaliada em relação ao seu custo-efetividade, comparada aos tratamentos tradicionais. A farmacoeconomia hoje é uma ciência e auxilia na decisão de utilização de um novo medicamento, tanto pelo médico, como, e principalmente, em termos de saúde pública (26, 27).

Em relação ao tempo, custo e riscos inerentes processo inovador de um novo medicamente, sabe-se que;

Se construirmos um gráfico onde em X plotamos o tempo de desenvolvimento (fases) e no Y o risco, observaremos que no início do processo o tempo e custo são menores, no entanto o risco é maior. Em contrapartida, em fases mais tardias do desenvolvimento – após a prova de conceito e estudos pré-clínicos – esta relação se inverte, o tempo e principalmente os custos aumentam exponencialmente.

Quando se fala em medicamentos, sabe-se que de cada 10.000 moléculas prospectadas, 10 a 20 terminam satisfatoriamente os estudos pré-clínicos, de 1 a 5 iniciam a fase de estudos clínicos e apenas uma será efetivamente aprovada pelos órgãos regulatórios (24).

Ainda, acontecimentos recentes demonstram que, na fase IV o monitoramento da farmacovigilância e estudos de farmacoepidemiolgia podem retirar um medicamento do mercado por questões de risco sanitário, não detectados nas fases de estudos clínicos I, II e II.

Atualmente, tornou-se mais fácil demonstrar a eficácia de um medicamento do que garantir a sua segurança.

1.4.1 Estudos clínicos: fase I; II, III e IV.

Um novo medicamento, antes de iniciar a fase de estudos clínicos passa por estudos pré-cínicos, *in vitro* e em animais.

O objetivo destes estudos é determinar o possível mecanismo de ação de um novo medicamento, a eficácia e principalmente a segurança para que o mesmo possa ser testado em seres humanos. Nesta fase se determina a dose segura que se iniciará os estudos clínicos fase I.

"Um ensaio clínico é um estudo sistemático de medicamentos e/ou especialidades medicinais em voluntários humanos que seguem estritamente as diretrizes do método científico. Seu objetivo é descobrir ou confirmar os efeitos e/ou identificar as reações adversas ao produto investigado e/ou estudar a farmacocinética dos ingredientes ativos, de forma a determinar sua eficácia e segurança" (28).

Os ensaios clínicos são realizados, na maioria das vezes, para o desenvolvimento de novos medicamentos.

São classicamente divididos em:

Fase I – onde se avalia a tolerabilidade, segurança e farmacocinética de um medicamento, geralmente são conduzidos em voluntários saudáveis.

Fase II – conduzidos em pacientes portadores da patologia a qual o medicamento se destinará, geralmente o número de voluntários não é muito elevado e o objetivos destes estudos é avaliar a eficácia e principalmente a segurança do novo medicamento.

Fase III - são estudos terapêuticos com um número maior de doentes, para determinação do risco-benefício do tratamento.

Fase IV – estudos também conhecidos como pós-marketing onde o objetivo é acompanhar o medicamento no mercado, visando principalmente o perfil de segurança do novo medicamento (29).

Estudo Clínico Fase I

Os estudos de Fase I, como já sumarizado acima, têm como objetivo testar a tolerabilidade, segurança e perfil farmacocinético de um novo medicamento.

Como é o primeiro estudo onde seres humanos serão expostos, o delineamento deste deve levar sempre em consideração os dados de estudos pré-clínicos, principalmente relacionados às doses que apresentaram toxicidade nos modelos animais estudados.

Existem várias maneiras de se calcular a dose inicial que será utilizada no estudo clínico fase I.

O preconizado em vários *guidelines* e na literatura, inicialmente, calcula-se a dose máxima inicial (MRSD - maximum recommended starting dose -) – que é calculada após a determinação da dose equivalente human (HED - human equivalent dose), que por sua vez é calculada utilizando-se a NOAEL- no observed adverse effect levels – da espécie animal mais adequada, dentre as testadas durante a fase pré-clinica. Em seguida se aplica um fator de segurança, uma fração da HED, dependendo da classe do novo medicamento e do perfil de segurança esperado em seres humanos (30, 31, 32).

Em seguida, o delineamento de um estudo fase I deve prever um escalonamento para atingir, se possível, a dose máxima tolerada, MTD - maximum tolerable dosage. Existem

alguns modelos estatísticos para se aplicar ao escalonamento da dose, ou seja, as doses subsequentes à inicial, calculada conforme descrido acima.

Habitualmente, se utiliza para estabelecer a próxima dose, em um estudo de escalonamento de dose, a sequência de Fibonacci. Esta foi formulada por Leonardo Pisano em 1202 em um de seus problemas (problema dos coelhos) descritos no clássico Liber Abbaci, considerado um dos melhores tratados de aritmética e álgebra.

Esta sequência, definida como, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, 144,... é obtida, a partir do terceiro número, pela soma dos dois antecessores. Simplificadamente pode ser obtida multiplicando-se o termo anterior por 1,618 (33, 34).

Ainda, é importante estabelecer uma regra de escalonamento da dose que garanta segurança ao sujeito de pesquisa. Em estudos com medicamentos oncológicos esta regra é bastante criteriosa, detalhada e padronizada, pois estes estudos clínicos envolvem medicamentos com alto grau de toxicidade.

Esta regra leva em consideração a dose limite de toxicidade (DLT- dose-limiting toxicity), definida como a dose que apresenta acima de grau 3 de toxicidade, exceto neutropenia de grau 3, acompanhados ou não por febre ou infecção.

A escala em questão é a critério comum de toxicidade (CTC - common toxicity criteria) padronizada pelo Cancer Therapy Evaluation Program. Este critério estabelece variações de gravidade do evento adverso observado entre 0 e 4 e os categoriza por sistemas/órgãos. É utilizada na avaliação de eventos adversos durante o tratamento oncológico e em estudos clínicos envolvendo medicamentos desta classe, como já salientado (35).

Resumidamente, a regra segue o seguinte esquema:

- Inicialmente, organiza-se uma coorte de 3-6 voluntários por dose. Hipoteticamente utilizaremos uma coorte de 3 voluntários para exemplificar.

Três voluntários são tratados com a dose inicial. Se, pelo menos, dois pacientes apresentam sinais de DLT, a dose prévia é definida como a dose máxima tolerada (MTD). Se nenhum dos 3 pacientes apresentam sinais e sintomas de DLT, a dose é aumentada para etapa seguinte em uma nova coorte de 3 voluntários, onde os critérios anteriores serão utilizados. Se nenhum dos três pacientes tratados apresentam sinais e sintomas de DLT, 3 pacientes adicionais são tratados com a dose atual. Se nenhum destes 3 voluntários adicionais apresentam sinais e sintomas de DLT, a dose é aumentada para o próximo grupo de 3 pacientes e o processo continua. A MTD é considerada como atingida quando pelo menos 2 pacientes de um total de 6 pacientes são tratados (30).

Apesar do perfil de segurança de outras classes de medicamentos, em geral , ser mais favorável, métodos de escalonamento de doses seguros devem sempre ser adotados, baseados nos resultados de estudos anteriosres não clínicos e perfil de segurança da classe farmacológica a que pertence o novo medicamento, quando possível (31, 36, 37).

1.5 Estudos preliminares com o Carbonato de lodenafila

Com o objetivo de pesquisar a possibilidade de um análogo da sildenafila com maior potência, seletividade e melhor perfil de segurança, foram sintetizados pelo Dipartimento di Chimica Farmacêutica da Università di Napoli "Federico II", vinte moléculas (6 a-v), caracterizados pela presença de um grupo sulfonil ou de um substituinte n'n'etilendiamina na posição do grupo metil da piperazina. Estes análogos foram testados no departamento de farmacologia da Faculdade de Ciências Médicas – UNICAMP.

Em plaquetas e músculo liso há elevada concentração de PDE5, é por este motivo que ensaios funcionais e bioquímicos em plaquetas e músculo liso têm sido bastante usados para o desenvolvimento de novos medicamentos visando seletividade na degradação do GMPc. Sendo assim, a metodologia adotada para testar estes análogos e selecionar os mais viáveis

para o desenvolvimento de um novo medicamento inibidor da PDE5 foi utilizar, os seguintes ensaios: a atividade inibitória da PDE5 de plaquetas humanas, o efeito relaxante dos análogos do sildenafila em corpo cavernoso de coelho e o efeito relaxante dos análogos do sildenafila em anéis de aorta de coelho. Nos estudos com plaquetas para avaliar a atividade inibitória da PDE5 foi utilizado sangue de voluntários sadios. Nos estudos funcionais foram utilizados coelhos New Zealand machos (2-3 kg) procedentes do CEMIB-UNICAMP (38).

Os compostos 6m, 6n e 6q demonstraram valores elevados de concentração efetiva 50% (IC50) para inibir a PDE5 plaquetária. Os 6a, 6b, 6d, 6g e 6p produziram inibição inferior a 50%. A IC50 dos compostos 6c, 6e, 6f, 6h, 6l e 6o foram similares ao da sildenafila.

Nos estudos funcionais, todos análogos da sildenafila, exceto o 6m, relaxaram preparações de corpo cavernoso de coelho de maneira dependente da concentração.

O análogo 6f demonstrou o melhor perfil farmacológico no relaxamento, com potência similar ao sildenafila.

Vários dos análogos testados demonstraram ser mais lipofílicos do que o sildenafila. Estes achados podem contribuir para o desenvolvimento de medicamentos para patologias que apresentem disfunção endotelial em sua etiopatogenia.

Dando continuidade à definição da melhor molécula para atingir os objetivos propostos, avaliaram-se os efeitos dos análogos hidroxisetilildenafil, e os dímeros, carbonato, ureia e uretano. Estudou-se também o efeito máximo 50% (EC50), antilog da concentração de droga necessária para produzir 50% do efeito máximo (pEC50) em corpo cavernoso de rato e cães da raça beagle, a estabilidade e metabolização dos dímeros no plasma de rato, humano e cão. Avaliou-se também a farmacocinética dos dímeros em cão e o efeito da administração endovenosa sobre a pressão arterial e frequência cardíaca em cães da raça beagle.

Com base nos resultados obtidos foi decidido que o dímero hidroetilsildenafil (em sua forma carbonato) foi o mais indicado para a próxima etapa da avaliação da toxicidade pré-clínica (38, 39, 40).

A patente do composto denominado CRIS035 foi depositada pelo laboratório Cristália Produtos Químicos Farmacêuticos Ltda. durante a fase experimental.

1.5.1 Estudos pré-clínicos de toxicidade

Foram realizados os ensaios de atividade mutagênica *in vitro* (Teste de Ames), estudos de toxicidade aguda em roedor (ratos) e estudos em um roedor (rato) e um não roedor (cão) com duração de 14 dias.

No teste de Ames concluiu-se que:

- O composto CRIS031 não demonstrou efeitos mutagênicos sobre as linhagens TA 98, TA 100, TA 1535 e TA 1537 de *Salmonella typhimurium* testadas na presença ou na ausência da fração microssomal metabolicamente ativa S9 de fígado de rato.

Na toxidade aguda em ratos concluiu-se que:

- Em condições experimentais a dose oral DL_{50} do teste é maior do que 2000mg/Kg. Não foram observados sinais de toxicidade nestas doses.

Estudo de toxicidade de doses repetidas duas semanas em ratos com doses orais (Gavagem):

- Não foram observados sinais de toxicidade em doses de tolerabilidade máxima (MTD) de 1000 mg/Kg.

Estudo de toxidade em doses repetidas em 2 semanas por via oral (Gavagem) em cães (Beagle):

- Não foram observados sinais de toxicidade em doses de 1000 mg/Kg

Avaliação sobre a atividade do sistema nervosa central após dose oral única (gavagem) em roedores (ratos):

- Nenhuma mortalidade ou morbidade foi observada após a administração de doses únicas (100, 550,775 e 1000 mg/Kg).

- Nenhum distúrbio do comportamento foi observado, utilizando-se o functional observation battery (FOB).

- Nenhuma alteração macroscópica foi observada - post-mortem (38, 39, 40).

1.6 Objetivo

O estudo foi conduzido para avaliar a segurança, tolerabilidade e farmacocinética do carbonato de lodenafil após administração em doses crescentes (1 a 100 mg), em dose única, em voluntários saudáveis.

2. Capítulo I

A phase I clinical trial of lodenafil carbonate, a new phosphodiesterase type 5 (PDE5) inhibitor, in healthy male volunteers.

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Running title: Lodenafil carbonate phase I study.

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Abstract

Lodenafil carbonate is a new phosphodiesterase type 5 (PDE5) inhibitor used in treatment of erectile dysfunction. *Objective:* The present study was conducted to evaluate the safety, tolerability, and pharmacokinetics of lodenafil carbonate after administering ascending (1 to 100 mg) single oral doses to healthy male volunteers (n=33). *Methods*: The study was an open-label, dose-escalation, phase I clinical trial involving the administration of single oral doses of lodenafil carbonate. Lodenafil carbonate was administered sequentially, escalating in single doses of 1 mg to 100 mg with a washout period of at least 1 week between each dose. The progression to the next dose was allowed after clinical and laboratory exams, Ambulatory Monitoring of Arterial Pressure (AMAP) without relevant clinical modifications and adverse events without clinical relevancy. Blood samples were collected at pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12, 14, 16, 20 and 24h post-dosing. Plasma samples for measurement of lodenafil carbonate and lodenafil were analyzed by liquid chromatography coupled to tandem mass spectrometry. *Results:* No serious adverse events were observed, and none of the subjects discontinued the study due to intolerance. The AMAP measurements, clinical and laboratory exams and ECG revealed no significant changes even at higher doses. Lodenafil carbonate was not detected in any samples, indicating that it acts as a pro-drug. The mean lodenafil pharmacokinetic parameters for tmax and $t_{1/2}$ were 1.6 (±0.4) hr and 3.3 (±1.1) hr, respectively. This study demonstrated that lodenafil carbonate was well tolerated and showed a good safety profile in healthy male volunteers.

Key words: phosphodiesterase type 5 (PDE5) inhibitor, pharmacokinetics, safety

1. Introduction

Lodenafil carbonate is a new phosphodiesterase type 5 (PDE5) inhibitor for treatment of erectile dysfunction [1]-[4], a common sexual problem for males over 40-50 years of age. Lodenafil relaxes isolated human and animal cavernous tissues through inhibition of cyclic guanosine monophosphate hydrolysis [1]. Lodenafil carbonate was approved by Brazilian Sanitary Surveillance Agency (ANVISA) in 2007 [5].

The pharmacokinetic parameters for lodenafil carbonate and lodenafil following oral administration (10 mg) of lodenafil carbonate in male beagle dogs were (mean±S.D): lodenafil carbonate: 11±14 ng/mL, 23±21 ng*h/mL, 2.1±3.0 h, 1.67±1.53 h; lodenafil: 1357±961 ng/mL, 9,091±5,526 ng*h/mL, 4.3±0.5 h, 4.3±0.5 h for Cmax, AUC, $t_{1/2}$, tmax, respectively [1]. The above results indicate that lodenafil carbonate acts as a prodrug, delivering lodenafil *in vivo* as the active moiety, since very small amounts of lodefanil carbonate reached the systemic circulation.

The tmax for PDE5 inhibitors is between 0.33 – 1.8 hours (sildenafil 1 h, tadalafil 1.8 h, vardenafil 0.75 h, udenafil 1 h and avanafil 0.33 h) and the half-life time for PDE5 inhibitors varies from 2.6 to 21.1 hours (sildenafil 2.76 h, vardenafil 2.63 h, avanafil 5.36 h, udenafil 11 h and tadalafil 21.1 h) [6]-[10]. After single oral administrations of lodenafil carbonate (160 mg) in healthy male volunteers, lodenafil is rapidly absorbed with peak of concentration (157 ng/mL) occurring at ≅ 1.2 hours, half-life time of 2.4 hours and area under the curve (AUC) of 530 ng*h/mL [4].

The present study was conducted to evaluate the safety, tolerability, and pharmacokinetics of lodenafil carbonate after administering ascending (1 to 100 mg) single oral doses in normal healthy male volunteers.

2. Materials and methods

2.1. Clinical protocol

The following inclusion criteria were used: Thirty-three healthy male volunteers, aged between 18 and 55 years old and within ≥ 19 and ≤ 28.75 of the ideal body weight were selected for the study [11]. The volunteers were selected for the study after having their health status previously assessed by a clinical evaluation and laboratory tests (biochemical and hematological parameters, and urinalysis). The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal and hematological diseases, as assessed by clinical examination, ECG and the following laboratory tests: blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, γGT, total bilirrubin and fractions, uric acid, total cholesterol, triglycerides, albumin and total protein, hemoglobin, hematocrit, total and differential white cell counts and routine urinalysis. All subjects were negative for HIV, HBV (except for serological scar) and HCV. The subjects were able to comprehend the full nature and purpose of the study, including possible risks and side effects and were willing to cooperate with the Investigator and comply with the requirements of the entire study. All subjects provided signed consent forms.

The following exclusion criteria were used: laboratory values outside the accepted normal ranges, unless considered not clinically significant by the investigator; participation in an experimental study or had ingested an experimental drug three months prior to the initiation of the study; any significant clinical illness or surgery within eight weeks

immediately prior to the initiation of the study or maintenance therapy with any drug; hospitalizing for any reason within eight weeks prior to the initiation of the study; history of drug, medication or alcohol abuse, or had ingested alcohol within 48 hours prior to the beginning of the confinement portion of the study; history of hepatic, renal, epileptic or hemopoietic disease; hypo or hypertension of any etiology (outside the range of 100-140 mmHg for systolic and 60-90 mmHg for diastolic blood pressure), or heart rate not between 50-90 bpm; myocardial infarction, angina pectoris and/or congestive heart failure; the subject had donated or lost blood within three months prior to the study or more than 1500mL within 12 months preceding the study; any other condition as per investigator judgment; high caffeine intake (more than 5 cups of tea or coffee/day); subject who smoked; history or presence of gastrointestinal, liver or kidney disease or any other condition known to interfere with the absorption, distribution, metabolism or excretion of drugs.

Concomitant medication was to be avoided where possible during the study. Any medication, including over-the-counter medication, was to be recorded.

The male volunteers had the following clinical characteristics (mean ± SD [range]): age 31.1± 5.7 yr. [23 – 40]; height 174 ± 6.1 cm [160.0 – 184.0]; body weight 71.2 ± 9.7 kg [54.5 – 90.5]. All subjects provided written informed consent, and the Ethics Committee of the State University of Campinas approved the protocol. Any major changes required approval from the Committee. The study was conducted in accordance with Good Clinical Practices and provisions of the Declaration of Helsinki (1964) and all revisions.

After screening and a washout period (of at least 2 weeks), the individuals who qualified were confined for 3 periods of approximately 40 hours. Each confinement was intervaled for 1 week. The study was conducted in an open, randomized, dose-escalation, three-period open-label experimental design, involving the administration of increasing single

oral doses (1 mg, 2 mg, 3 mg, 5 mg, 8 mg, 10 mg, 20 mg, 30 mg, 50 mg, 80 mg, 100 mg) of lodenafil carbonate (Table 1). A Fibonacci-like scheme was used for dose escalation (from 1-10, and from 10-100 mg). The progression to the next dose was allowed by clinical and laboratory exams, Ambulatory Monitoring of Arterial Pressure (AMAP) without relevant clinical modifications, and adverse events without clinical relevancy. The adverse event evaluation was performed during confinement period and in a clinical visit 24h before the next confinement. The medical investigator classified the event intensity as mild, moderate, severe, and serious, and the causal relationship to the formulation as unrelated, unlikely, possible and probable.

During each period, the volunteers were hospitalized between 4:00 and 7:00 p.m., having already eaten a normal evening meal. After overnight fasting, they received at 7:00 a.m., a single dose of appropriate lodenafil carbonate (tablet) along with 240 ml of tap water. All volunteers were required to remain fasting until 2 hours after dose administration, when a xanthine-free standard breakfast was available. A xanthine-free standard lunch was provided five (lunch), eight (afternoon snack), twelve (lunch) and fifteen (supper) hours after dose. A standard meal (lunch) of rice, beans, vegetables, and fried chicken, plus a fruit as dessert was consumed. The breakfast, afternoon snack and supper included crackers, bread, jelly, cake and apple. No other food was permitted during the "in-house" period and liquid consumption was allowed *ad libitum* after lunch (with the exception of xanthine-containing drinks, including tea, coffee, and cola). No comedication was permitted during the study.

Blood samples (5 mL) from a suitable antecubital vein were collected by an indwelling catheter into heparin containing tubes at pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12, 14, 16, 20 and 24h post-dosing.

The Ambulatory Monitoring of Arterial Pressure (AMAP) was measured for 24 hours after drug administration, with 15-minute intervals in the first two hours and then every hour until the end of the internment (24 hours after the administration of the lodenafil carbonate). Two ECGs were obtained one hour and twelve hours after the administration of the lodenafil carbonate.

2.2 Formulations

The following formulations were employed: lodenafil carbonate test formulation tablet in 1 mg (lot 318/04), 2 mg (lot 319/04), 3 mg (lot 320/04), 5 mg (lot 321/04), 8 mg (lot 378/04), 10 mg (lot 379/04), 20 mg (lot 392/04), 30 mg (lot 391/04), 50 mg (lot 394/04), 80 mg (lot 396/04) and 100 mg (lot 397/04) from Cristália Produtos Químicos e Farmacêuticos Ltda, Itapira, Brazil.

2.3 Chemicals and reagents

Lodenafil carbonate (lot number: SL24-024), lodenafil (lot number: not available), sildenafil (lot number: 20227/99) were provided by Cristália Produtos Químicos e Farmacêuticos Ltda, Itapira, Brazil. Acetonitrile, formic acid and methanol were purchased from J. T. Baker, USA. Diethyl ether and hexane were purchased from Mallinckrodt, USA. Blank human blood was collected from healthy, drug-free volunteers. Plasma was obtained by centrifugation of blood treated with sodium heparin. Pooled plasma was prepared and stored at approximately -20 ºC until needed.

2.4 Calibration standards and quality controls

Stock solutions of lodenafil carbonate, lodenafil and the internal standard (sildenafil) were prepared in acetonitrile/water (50/50, v/v) at concentrations of 1 mg/mL. Calibration curves of lodenafil carbonate and lodenafil were prepared by spiking blank plasma at concentrations of 0.5, 1.0, 5.0, 20.0, 50.0, 200, 1,000 and 2,000 ng/mL and each concentration was analyzed in duplicate. The quality control samples were prepared in blank plasma at concentrations of 3, 30, 180 and 1,800 ng/mL (QCA, QCB, QCC and QCD, respectively). The spiked plasma samples (standards and quality controls) were extracted in each analytical batch along with the unknown samples.

2.5 Drug analysis

The blood samples (5 mL) were centrifuged at 2500 g for 10 min at -4^{o}C and the plasma decanted and stored at –20^{0} C until assayed for lodenafil carbonate and lodenafil contents. Lodenafil carbonate, lodenafil, and the IS were extracted from plasma samples and quantified by liquid chromatography tandem mass spectrometry (LC-MS-MS) with positive electrospray ionization using multiple reaction monitoring (MRM).

Briefly, 0.2 mL of each plasma sample was pipetted into a glass tube followed by 0.05 mL of the internal standard solution (1,000 ng/mL of sildenafil (Cristália Produtos Químicos e Farmacêuticos Ltda, Itapira, Brazil.) and 4 mL of hexane/diethyl-ether (20/80; v/v) (HPLC grade, J. T. Baker, Phillipsburg, NJ, USA). The tubes were briefly vortex-mixed (40 s). The upper organic layers were removed and transferred into another sterile tube, and evaporated to dryness under N_2 (at 40 ºC). The dry residues were dissolved with 0.2 mL of acetonitrile/water (50/50; v/v) and the solutions were transferred to glass microvials using

automatic pipettes with disposable plastic tips. The vials were caped and placed into the autosampler racks.

High performance liquid chromatography (HPLC) was performed on C_{18}, 4 mm (100 x 2.1 mm i.d. (Jones Chromatography, Genesis, USA) at a flow rate of 0.45 mL/min and pressure of the system was approximately 120 bar. The mobile phase was acetonitrile/water (90/100; v/v) + 0.1% formic acid (Analysis Grade, J. T. Baker, USA). The column operated at room temperature. The temperature of the auto sampler operated at 10 ± 2 ^{o}C and the injection volume was 10 μL.

Typical standard retention times were 1.1 ± 0.2 min for lodenafil carbonate, 0.8 ± 0.1 min for lodenafil and 0.8 ± 0.1 min for the IS, and the total run-time was 2.4 min. The mass spectrometer (model API 4000, Sciex/Applied Biosystems, Foster City, CA, USA) equipped with a turbospray source using a crossflow counter electrode, was run in positive mode (ES+) with multiple reaction monitoring (MRM). The mass spectrometer was set as follows: 1035.2 > 487.1, 505.2 > 487.0 and 475.1 >283.2, for lodenafil carbonate, lodenafil and sildenafil, as the precursor ions and the respective product ions *m/z*. The limit of quantification in plasma was 0.5 ng/mL for both lodenafil carbonate and lodenafil.

2.6 Assay performance

A linear regression with a weighting index of 1/x^2 was performed on the peak area ratios of lodenafil carbonate and the internal standard versus the lodenafil carbonate concentrations of eight human plasma standards (in duplicate) to generate a calibration curve. A linear regression with a weighting index of 1/x^2 was performed on the peak area ratios of lodenafil and the internal standard versus the lodenafil concentrations of the eight human plasma standards (in duplicate) to generate a calibration curve.

Precision and accuracy: Within- and between-run precision was determined as the relative standard deviation, RSD (%) = 100(SD/M), and the accuracy as the percentage relative error, RE (%) = (E-T)(100/T), where M is the mean, SD is the standard deviation of M, E is the experimentally determined concentration and T is the theoretical concentration.

2.7 Pharmacokinetics

Individual plasma level-time curves were constructed and pharmacokinetic parameters were obtained according to a non-compartmental approach. The first-order terminal elimination rate constant (ke) was estimated by linear regression from the points describing the elimination phase in a log-linear plot, and the half-life ($t_{1/2}$) was derived from this rate constant ($t_{1/2}$ = ln (2)/ke). The maximum plasma concentration (C_{max}) and the time taken to achieve this concentration (tmax) were obtained directly from the curves. The areas under the lodenafil plasma concentration vs. time curves from time zero to the last detectable concentration (AUC_{last}) were calculated by applying the linear trapezoid rule. Extrapolation of these areas to infinity (AUC_{inf}) was done by adding the value C_{last}/ke to the calculated AUC_{last} (where C_{last} = the last detectable concentration). Clearance (CL) was calculated by the formula dose/AUC0-inf. Volume of distribution (Vd) was calculated by the formula Dose/Ke(AUC_{inf}). The software used included WinNonLin Professional Network Edition (Scientific Consulting, v. 3.0), Microsoft Excel (v. 7.0) and GraphPad Prism (v. 3.02).

3. Results

All biochemical parameters monitored presented no clinically relevant alterations during the phase I study. No significant changes were observed in the laboratory exams and in the ECGs intra-study. Tolerance of both formulations (1 to 100 mg) was good and no

significant adverse event were observed or reported. One volunteer had an adverse event (dysuria not related to therapy and classified as not serious) after the discharge of the third confinement period during the phase I study. Two volunteers related spontaneous erections after administration of lodenafil carbonate (30 and 50 mg): one during the clinical evaluation for discharge of the study and one during the final clinical evaluation in the first period of confinement.

Laboratory tests after administration of lodenafil carbonate are shown in Table 4. Blood pressure and heart rate were assessed for 24 hours after treatment with lodenafil carbonate (Figure 2; Table 5).

The mean lodenafil plasma concentrations vs. time profiles after testing single oral doses of lodenafil carbonate in males are shown in Figure 1. Calculated mean pharmacokinetic parameters of lodenafil are given in Table 3.

The method was linear for lodenafil carbonate and lodenafil concentrations from 0.5 to 2000 ng/mL. No significant matrix effect was observed. The limit of quantification (LOQ), defined as the lowest concentration at which both the precision and accuracy were <20%, was 0.5 ng/mL for lodenafil carbonate and lodenafil. The within- and between-run precision and accuracy for the quality controls are summarized in Table 2.

4. Discussion

High-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (LC-MS-MS) in animal and human plasma has been used for lodenafil and/or lodenafil carbonate quantification [1][4]. However, no full validation method has been previously published for lodenafil and lodenafil carbonate quantification**.** Our method employed sildenafil as internal standard instead of theophylline, having a shorter retention

time of 1.1 min for lodenafil carbonate and 0.8 min for lodenafil. The limit of quantification (0.5 ng/ml) was adequate for pharmacokinetic evaluation of lodenafil carbonate and lodenafil.

The results obtained following oral administration of lodenafil carbonate in healthy male volunteers show that lodenafil carbonate acts as a prodrug when given orally, since no quantifiable lodefanil carbonate concentration has been detected in the systemic circulation. Similar results were observed in male beagles following oral administration of lodenafil carbonate [1].

The adverse event of dysuria was evaluated as not serious since the volunteer spontaneously recovered and only reported in the medical final evaluation.

After the oral administration of the lodenafil carbonate 80 mg tablets to the volunteers, the tmax (1.6h) and t1/2 (3.3h) were similar to the reported in the literature (tmax: 1.2 -1.5h; t1/2: 2.4 – 3h) [2]. The Cmax (17.62 ng/mL) and AUC (42.80 hr*ng/mL) values were different to those reported in the literature (Cmax 158 ng/mL; AUC 528 hr*ng/mL) for a lodenafil carbonate tablet (dose 160 mg; two tablets of 80 mg) formulation [2].

As shown in Table 3, the pk parameters calculated for 1 mg are not reliable due to low plasma lodenafil after a dose of 1 mg. The major differences for t1/2, Cl and Vd over the dose range can be explained by the low number (n=3 to 1 mg; n=6 to 2mg and n=9 between 3 mg and 100 mg) of volunteer per dose and by the variability inter-subject.

The mean lodenafil pharmacokinetic parameters for tmax (1.6±0.4 hr) was similar to the PDE5 (sildenafil 1 h, tadalafil 1.8 h, vardenafil 0.75 h, udenafil 1 h, and avanafil 0.33 h). The mean lodenafil pharmacokinetic parameters for t1/2 (3.3h) were similar to the sildenafil and vardenafil (sildenafil 2.76 h, vardenafil 2.63 h, avanafil 5.36 h, udenafil 11 h and tadalafil 21.1 h) [6]-[10].

No serious adverse events were observed, and none of the subjects discontinued the study because of lack of tolerance. As shown in Tables 4 and 5, the AMAP measurements and clinical tests revealed no significant alteration even at a higher dose. The lodenafil carbonate presented good tolerance and safety for healthy male volunteers in phase I study. The recommended dose of 80 mg was used in following trials and it is the commercial formulation dose marketed in Brazil.

Acknowledgements: The Phase I trial was sponsored by Cristália Produtos Químicos e Farmacêuticos Ltda, Itapira, Brazil. AcademicEnglishSolutions.com revised the English.

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Table 1: Treatment scheme with lodenafil in 1 mg, 2 mg, 3 mg, 5 mg, 8 mg, 10 mg, 20 mg, 30 mg, 50 mg, 80 mg and 100 mg in healthy male volunteers.

Volunteer	1 mg	2 mg	3 mg	5 mg	8 mg	10 mg	20 mg	30 mg	50 mg	80 mg	100 mg
I	x	x	x								
II	x	x	x								
III	x	x	x								
IV		x	x	x							
V		x	x	x							
VI		x	x	x							
VII			x	x	x						
VIII			x	x	x						
IX			x	x	x						
X				x	x	x					
XI				x	x	x					
XII				x	x	x					
XIII					x	x	x				
XIV					x	x	x				
XV					x	x	x				
XVI						x	x	x			
XVII						x	x	x			
XVIII						x	x	x			
XIX							x	x	x		
XX							x	x	x		
XXI							x	x	x		
XXII								x	x	x	
XXIII								x	x	x	
XXIV								x	x	x	
XXV									x	x	x
XXVI									x	x	x
XXVII									x	x	x
XXVIII										x	x
XXIX										x	x
XXX										x	x
XXXI											x
XXXII											x
XXXIII											x

Table 2: Accuracy and precision data for lodenafil carbonate and lodenafil from the pre-study validation in human plasma.

Lodenafil carbonate						
Nominal concentration (in ng/mL)	Intra-batch mean (in ng/mL)	Intra-batch precision (CV %)	Intra-batch accuracy (%)	Inter-batch mean (in ng/mL)	Inter-batch precision (CV%)	Inter-batch accuracy (%)
0.5	0.55	12.55%	109.29%	0.52	15.38%	103.42%
0.5	0.47	14.16%	93.70%			
0.5	0.53	17.01%	105.89%			
3	3.1	3.61%	102.42%	3.2	8.70%	105.17%
3	3.1	4.01%	104.50%			
3	3.3	14.98%	109.72%			
30	32.8	4.25%	109.25%	33.3	9.32%	110.89%
30	33.9	10.23%	113.08%			
30	33.1	12.38%	110.33%			
180	187.3	2.33%	104.03%	189.5	6.16%	105.30%
180	191.4	3.11%	106.32%			
180	190.0	10.30%	105.56%			
1800	1923	6.06%	106.81%	1894	5.91%	105.23%
1800	1935	5.39%	107.50%			
1800	1825	5.03%	101.39%			
Lodenafil						
Nominal concentration (in ng/mL)	Intra-batch mean (in ng/mL)	Intra-batch precision (CV %)	Intra-batch accuracy (%)	Inter-batch mean (in ng/mL)	Inter-batch precision (CV%)	Inter-batch accuracy (%)
0.5	0.54	19.67%	107.13%	0.51	16.39%	101.02%
0.5	0.48	19.02%	96.37%			
0.5	0.50	7.95%	100.33%			
3	2.8	11.33%	92.29%	2.7	9.66%	90.26%
3	2.7	7.36%	89.75%			
3	2.7	10.79%	88.52%			
30	28.7	5.25%	95.63%	27.6	8.42%	91.97%
30	27.8	10.50%	92.75%			
30	25.8	5.18%	86.06%			
180	163.5	5.48%	90.83%	157.7	8.69%	87.59%
180	157.9	6.61%	87.71%			
180	151.6	12.27%	84.24%			
1800	1604	3.60%	89.10%	1623	5.32%	90.14%
1800	1685	4.41%	93.61%			
1800	1579	5.88%	87.71%			

Legends: CV = coefficient of variation;

Table 3: Mean pharmacokinetic parameters obtained from volunteers after administration of lodenafil carbonate formulation (1 mg, 2 mg, 3 mg, 5 mg, 8 mg, 10 mg, 20 mg, 30 mg, 50 mg, 80 mg and 100 mg) in 33 healthy volunteers.

Dose (mg)	**1.00**		**2.00**		**3.00**		**5.00**	
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
AUC Extrap (%)	65.65	36.92	32.61	23.63	31.39	18.59	30.15	23.04
AUCinf (hr*ng/mL)	15.99	13.19	7.02	3.65	12.82	5.26	12.13	6.02
AUClast (hr*ng/mL)	2.07	1.98	3.67	2.37	8.11	3.84	6.45	5.89
Cmax (ng/mL)	1.76	1.61	2.87	1.67	2.77	0.42	3.71	2.36
tmax (hr)	0.75	0.00	1.17	0.49	2.06	2.46	2.22	2.06
Ke (1/hr)	0.17*	0.20	0.47	0.25	0.30	0.29	0.33	0.20
$t_{1/2}$ (hr)	14.62*	17.54	2.37	2.38	4.02	2.25	3.33	2.61
Vd (L)	1009.00*	750.00	925.00	463.00	1266.00	472.00	1951.00	1299.00
Cl (L/hr)	95.00*	78.00	355.00	189.00	283.00	147.00	497.00	228.00
Dose (mg)	**8.00**		**10.00**		**20.00**		**30.00**	
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
AUC Extrap (%)	31.08	23.41	24.27	24.18	6.75	4.08	14.82	18.71
AUCinf (hr*ng/mL)	22.62	15.73	29.69	24.85	55.53	39.71	86.69	85.97
AUClast (hr*ng/mL)	17.54	16.34	24.99	24.45	46.18	39.87	82.40	86.41
Cmax (ng/mL)	8.08	6.92	10.92	12.20	19.21	13.74	24.39	19.37
tmax (hr)	2.11	1.29	1.38	0.50	1.86	1.99	1.44	0.92
Ke (1/hr)	0.35	0.20	0.41	0.35	0.42	0.30	0.25	0.13
$t_{1/2}$ (hr)	2.97	2.23	2.66	1.92	2.59	1.75	3.78	2.61
Vd (L)	2348.00	1908.00	2435.00	2435.00	1477.00	789.00	6037.00	7740.00
Cl (L/hr)	613.00	553.00	796.00	821.00	490.00	257.00	1007.00	1175.00
Dose (mg)	**50.00**		**80.00**		**100.00**		**Mean**	
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
AUC Extrap (%)	5.90	4.83	21.07	29.71	5.23	3.53	-	-
AUCinf (hr*ng/mL)	65.91	51.90	49.16	39.69	89.72	73.32	-	-
AUClast (hr*ng/mL)	63.37	51.36	42.80	41.95	86.66	73.25	-	-
Cmax (ng/mL)	24.09	15.39	17.62	15.40	37.27	29.02	-	-
tmax (hr)	1.67	1.04	1.86	1.08	1.42	1.02	1.6	0.4
Ke (1/hr)	0.32	0.12	0.25	0.16	0.33	0.16	0.3	0.1
$t_{1/2}$ (hr)	2.59	1.25	5.93	9.12	2.56	1.24	3.3	1.1
Vd (L)	4137.00	2902.00	22971.00	28532.00	5581.00	3189.00	4913	6590.5
Cl (L/hr)	1361.00	1189.00	3274.00	2955.00	1784.00	1120.00	1046	914.7

*Legends: Ke = elimination rate constant; $t_{1/2}$ = half-life; Cmax = maximum plasma concentration; tmax = time to the maximum plasma concentration, AUClast - area under curve from time zero to the last detectable concentration AUCinf = extrapolation of area to infinity, AUC Extrap = % extrapolation of area to infinity; CL = clearance; VD = volume of distribution; Clast = last detectable concentration, tlast = time of last detectable concentration, * = excluded of descriptive statistic.*

Table 4: Laboratory tests in healthy male volunteers after administration of lodenafil carbonate.

Type		Unit	Normal range	Pre-study				Pos-study			
				Mean	SD	Min	Max	Mean	SD	Min	Max
Hematological	Hemoglobin	g%	13.5 - 17.5	15.4	0.8	14.1	17.2	14.6	0.8	12.9	16.8
	Hematocrit	%	39 - 50	44.7	1.9	42.2	49.4	42.9	2.1	40.1	47.7
	Red Blood Count	106/mm3	4.3 - 5.7	5.2	0.6	4.7	8.0	4.8	0.2	4.5	5.2
	VCM	Micra3	81 - 95	84.4	17.1	5.2	93.6	89.0	2.4	85.3	93.3
	HbCM	pg	26 - 34	29.6	3.4	14.9	33.4	30.3	1.3	27.4	32.9
	White Blood Count	103/mm3	3500 - 10500	6.4	1.3	3.7	8.4	6.9	1.7	4.2	10.6
	Band Neutrophils	%	≤ 5	1.7	1.0	1.0	5.0	1.7	0.7	1.0	3.0
	Segmented	%	48 - 76	55.0	8.6	36.1	66.7	54.6	8.7	32.1	65.8
	Lymphocytes	%	25 - 30	31.9	8.6	18.4	50.3	31.8	7.9	21.9	51.5
	Monocytes	%	1 - 9	7.7	1.7	4.7	10.3	7.8	2.2	4.8	12.0
	Basophils	%	0 - 1	0.6	0.4	0.0	1.8	0.5	0.3	0.0	1.0
	Eosinophils	%	1 - 5	3.8	2.8	0.6	10.7	4.0	2.5	1.0	9.9
	Platelet Count	-	150 – 400	238.5	50.0	153.0	363.0	247.3	44.4	162.0	328.0
Biochemical	Total Cholesterol	mg/dL	≤ 239	167.3	30.9	112.0	239.0	165.5	42.0	22.0	248.0
	Triglycerids	mg/dL	≤ 150	102.4	44.5	45.0	191.0	99.6	54.1	24.0	303.0
	Total Proteins	g/dL	6 - 8	7.8	0.3	7.3	8.4	7.7	0.3	7.2	8.3
	Albumin	g/dL	3.5 - 5.5	4.7	0.2	4.4	5.0	4.6	0.2	4.2	5.1
	Uric Acid	mg/dL	2.5 – 7.0	5.5	1.5	3.1	9.0	5.4	1.3	3.0	8.8
	Total Bilirubins	mg/dL	0 - 1.1	0.8	0.5	0.4	2.6	0.6	0.4	0.0	1.4
	Alkaline Phosphatase	U/L	27 - 100	76.2	18.9	49.0	115.0	67.6	24.5	0.8	116.0
	gT	U/L		24.1	11.9	8.0	63.0	23.8	11.2	5.2	47.0
	SGOT (AST)	U/L	≤ 43	21.9	5.5	15.0	42.0	20.2	4.1	12.0	34.0
	SGPT (ALT)	U/L	≤ 43	23.2	11.0	13.0	64.0	23.1	9.7	10.0	53.0
	Urea	mg/dL	10 - 50	26.6	5.4	14.4	36.7	29.1	5.5	17.2	38.0
	Creatinine	mg/dL	0.4 - 1.3	1.1	0.8	0.8	4.7	1.1	0.9	0.7	4.5
	Fasting Blood Glucose	mg/dL	60 - 100	87.2	8.1	74.0	108.0	87.9	6.9	74.0	101.0
Urine	Specific Gravity	-	1.015 - 1.028	1019.3	5.8	1008.0	1029.0	1019.6	6.1	1006.0	1032.0
	pH	-	5.5 - 6.5	6.2	0.6	5.5	7.0	6.2	0.4	5.5	6.5
	WBC	/mL	≤ 10000	7124.2	16901.9	300.0	97000.0	3277.0	1924.3	1000.0	9400.0
	RBC	/mL	≤ 5000	4150.0	4784.6	300.0	19430.0	2900.0	3924.6	100.0	22900.0

Table 5: Mean sitting systolic and diastolic blood pressures and mean heart rates after lodenafil carbonate administration.

Time	Mean diastolic blood pressures				Mean systolic blood pressures				Mean heart rate			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
0	71	7	56	89	113	8	91	132	63	7	41	86
1	70	7	54	93	113	8	86	134	59	7	42	77
2	72	8	52	96	114	8	88	134	61	8	43	85
3	69	9	45	99	115	8	88	145	65	10	45	100
4	68	9	41	93	115	10	95	138	66	10	43	107
5	71	10	44	97	118	9	89	143	72	11	48	107
6	65	9	40	89	113	10	77	135	67	8	49	85
7	66	10	40	96	112	9	85	133	68	10	46	99
8	67	9	40	93	112	10	90	135	66	10	46	92
9	65	11	43	86	112	16	90	140	65	12	46	97
10	66	9	43	88	112	11	84	143	68	10	48	92
11	68	11	44	90	114	10	86	138	65	10	48	92
12	74	10	50	98	117	11	94	149	70	14	47	111
13	70	11	44	95	117	10	93	146	69	11	47	104
14	69	10	44	89	116	10	82	143	70	10	49	102
15	64	10	46	94	112	11	91	142	66	10	46	99
16	65	8	46	84	113	10	83	144	67	11	45	115
17	62	9	44	80	110	10	84	135	64	10	44	89
18	63	10	41	89	110	11	86	136	62	11	42	98
19	63	8	43	81	108	8	87	127	62	13	42	138
20	65	8	45	88	109	10	88	133	62	10	42	87
21	62	10	43	91	105	11	83	142	60	10	46	105
22	62	10	40	84	105	12	79	132	59	9	41	82
23	63	11	42	91	106	11	74	127	61	11	39	98
24	73	8	54	91	116	7	98	132	70	9	49	91

Figure 1: The mean lodenafil plasma concentrations vs. time profiles after a single oral dose (1 mg, 2 mg, 3 mg, 5 mg, 8 mg, 10 mg, 20 mg, 30 mg, 50 mg, 80 mg, 100 mg) tablet formulation of lodenafil carbonate in male volunteers.

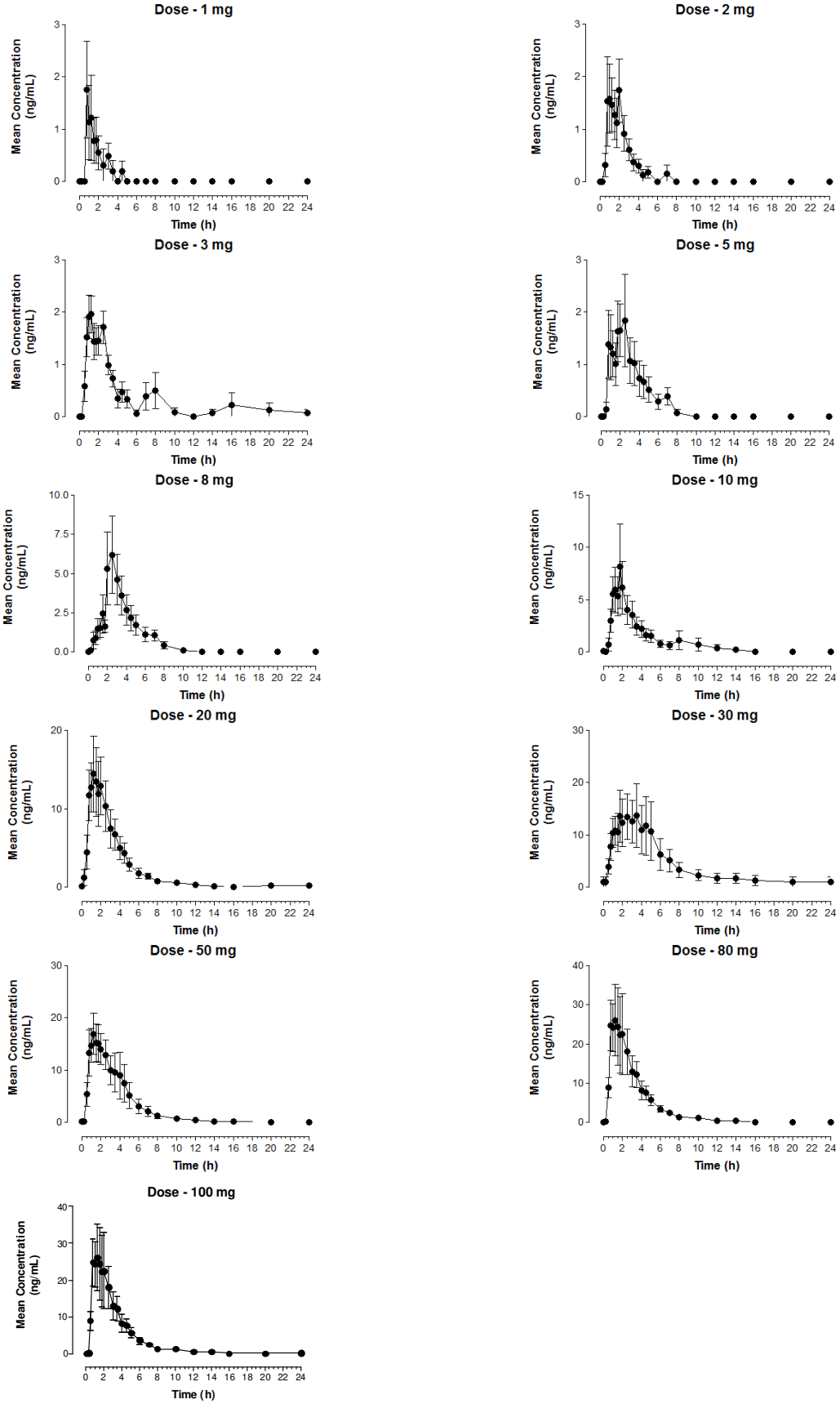
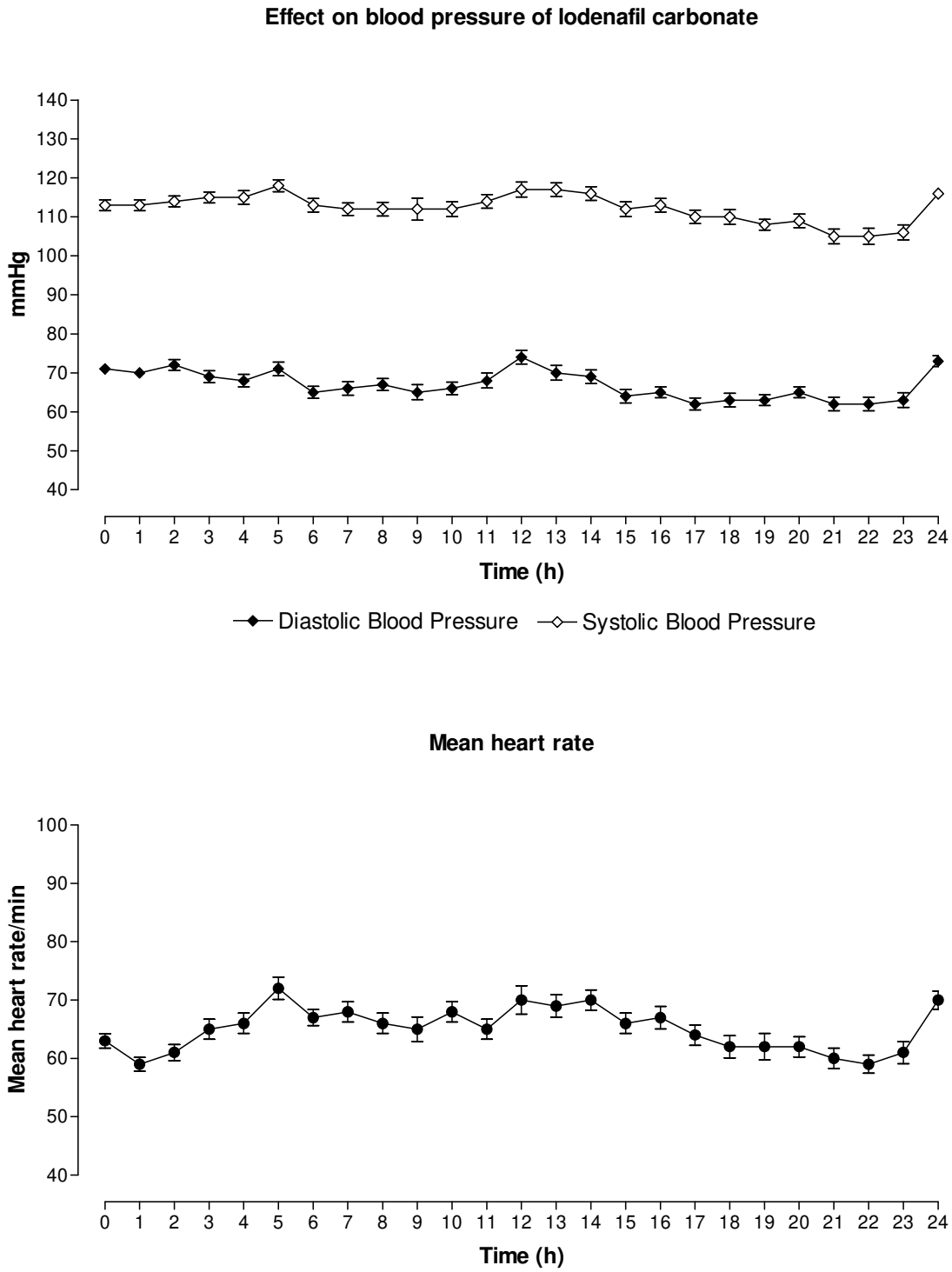


Figure 2: Mean sitting systolic and diastolic blood pressures and sitting heart rates after lodenafil carbonate administration.



3. Discussão

A cromatografia líquida de alto desempenho (HPLC), acoplado com espectrometria de massa (LC-MS-MS) tem sido utilizada para quantificação de carbonato de lodenafila e ou lodenafila no plasma humano e de animais. No entanto, nenhum método de validação completo foi publicado anteriormente para quantificação de lodenafila e carbonato de lodenafila. O nosso método emprega sildenafila como padrão interno em vez de teofilina, tendo um tempo de retenção mais curto de 1,1 min. para o carbonato de lodenafila e 0,8 min. para lodenafila. O limite de quantificação (0,5 ng / ml) foi adequado para avaliação de farmacocinética de carbonato lodenafila e lodenafila.

Os resultados obtidos após a administração oral de carbonato de lodenafila em voluntários saudáveis do sexo masculino mostraram que o carbonato de lodenafila atua como um pró-droga quando administrado por via oral, uma vez que nenhuma concentração quantificável de carbonato de lodefanila foi detectada na circulação sistêmica. Resultados semelhantes foram observados em beagles machos após a administração oral de carbonato lodenafila.

O evento adverso disúria não foi considerado como relacionado à terapia uma vez que ocorreu 74 horas após a administração da droga (20 mg) e a média do tempo de meia-vida para lodenafila é de 3,3 horas. Foi avaliado como não grave, pois ocorreu recuperação espontânea e o voluntário apenas o relatou na avaliação médica final.

Após a administração oral de comprimidos de carbonato de lodenafila 80 mg, para os voluntários, o tmax (1.6h) e t1/2 (3.3h) foram similares ao descrito na literatura (tmax: 1.2 - 1.5h; t1/2: 2.4 – 3h). Os valores de Cmax (17.62 ng/mL) e a AUC (42.80 hr*ng/mL) foram diferentes daqueles relatados na literatura (Cmax 158 ng/mL; AUC 528 hr*ng/mL) para a

formulação de comprimidos de carbonato de lodenafila (dose 160 mg; dois comprimidos de 80 mg).

Os parâmetros farmacocinéticos calculados para 1 mg não são confiáveis devido à baixa concentração de lodenafila no plasma após uma dose de 1 mg. A maior diferença para t1/2, Cl e Vd ,ao longo do intervalo de dose, pode ser explicada pelo baixo número de voluntários por dose (n=3 para 1 mg; n=6 para 2mg e n=9 entre 3 mg and 100 mg) e pela variabilidade inter-sujeitos.

As médias dos parâmetros farmacocinéticos de lodenafila para Tmax (1,6 ± 0,4 hr) foi semelhante aos PDE5 (sildenafila 1 h, tadalafila1, 8 h, vardenafila 0,75 h, udenafila 1 h, e avanafila 0,33 h). A média dos parâmetros farmacocinéticos de lodenafila de t1 / 2 (3.3 h) foi similar à sildenafila e vardenafila (sildenafila 2,76 h, vardenafila 2,63 h, avanafila 5,36 h, udenafila 11 h e tadalafila 21,1 h).

4. Conclusão

Não foi observado evento adverso grave e nenhum dos voluntários descontinuou o estudo por falta tolerabilidade.

O Carbonato de Lodenafila apresenta boa tolerabilidade e segurança no estudo fase I com voluntários saudáveis.

A dose recomendada de 80 mg foi usada em estudos clínicos posteriores a este e é a formulação comercializada no Brasil.

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ANEXO I

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que contam da minha Tese de Doutorado, intitulada "Estudo clínico fase I de carbonato de lodenafila, um novo tipo de inibidor de fosfodiesterase 5 (PDE5), em voluntários saudáveis do sexo masculino.", não infringem os dispositivos da Lei nº 9,610/98 nem o direito autoral de qualquer editora.

Campinas, 13 de maio de 2013.

Autor: Hilton Oliveira dos Santos Filho
RG nº.: 6.198.671

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ANEXO II

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Enviada em: segunda-feira, 10 de junho de 2013 03:20
Para: 'Hilton Oliviera Santos'
Cc: 'Gilberto De Nucci'
Assunto: AW: A phase I clinical trial of lodenafil carbonate, a new phosphodiesterase type 5 (PDE5) inhibitor, in healthy male volunteers. - (Manuscript-ID 201624 - 3)

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ANEXO III

Estudo publicado:

Int J Clin Pharmacol Ther. 2012 Dec;50(12):896-906.

A Phase I clinical trial of lodenafil carbonate, a new phosphodiesterase Type 5 (PDE5) inhibitor, in healthy male volunteers.

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Abstract

Lodenafil carbonate is a new phosphodiesterase Type 5 (PDE5) inhibitor used in treatment of erectile dysfunction. Objective: The present study was conducted to evaluate the safety, tolerability, and pharmacokinetics of lodenafil carbonate after administering ascending (1 - 100 mg) single oral doses to healthy male volunteers (n = 33). Methods: The study was an open label, dose-escalation, Phase I clinical trial involving the administration of single oral doses of lodenafil carbonate. Lodenafil carbonate was administered sequentially, escalating in single doses of 1 mg - 100 mg with a washout period of at least 1 week between each dose. The progression to the next dose was allowed after clinical and laboratory exams, Ambulatory Monitoring of Arterial Pressure (AMAP) without relevant clinical modifications and adverse events without clinical relevancy. Blood samples were collected at pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12, 14, 16, 20 and 24 h post-dosing. Plasma samples for measurement of lodenafil carbonate and lodenafil were analyzed by liquid chromatography coupled to tandem mass spectrometry. Results: No serious adverse events were observed, and none of the subjects discontinued the study due to intolerance. The AMAP measurements, clinical and laboratory exams and ECG revealed no significant changes even at higher doses. Lodenafil carbonate was not detected in any samples, indicating that it acts as a prodrug. The mean lodenafil pharmacokinetic parameters for tmax and t1/2 were 1.6 (± 0.4) h and 3.3 (± 1.1) h, respectively. This study demonstrated that lodenafil carbonate was well tolerated and showed a good safety profile in healthy male volunteers.

PMID:23073140[PubMed - in process]

ISSN 0946-1965

DOI 10.5414/CP201624
e-pub: October 17, 2012

A Phase I clinical trial of lodenafil carbonate, a new phosphodiesterase Type 5 (PDE5) inhibitor, in healthy male volunteers

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Key words
phosphodiesterase Type 5 (PDE5) inhibitor- pharmacokinetics – erectile disfunction – iodenofil carbonate

Abstract. Lodenafil carbonate is a new phosphodiesterase Type 5 (PDE5) inhibitor used in treatment of erectile dysfunction. Objective: The present study was conducted to evaluate the safety, tolerability, and pharmacokinetics of lodenafil carbonate after administering ascending (1 – 100 mg) single oral doses to healthy male volunteers (n = 33). Methods: The study was an open-label, dose-escalation, Phase I clinical trial involving the administration of single oral doses of lodenafil carbonate. Lodenafil carbonate was administered sequentially, escalating in single doses of 1 mg – 100 mg with a washout period of at least 1 week between each dose. The progression to the next dose was allowed after clinical and laboratory exams, Ambulatory Monitoring of Arterial Pressure (AMAP) without relevant clinical modifications and adverse events without clinical relevancy. Blood samples were collected at pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12, 14, 16, 20 and 24 h post-dosing. Plasma samples for measurement of lodenafil carbonate and lodenafil were analyzed by liquid chromatography coupled to tandem mass spectrometry. Results: No serious adverse events were observed, and none of the subjects discontinued the study due to intolerance. The AMAP measurements, clinical and laboratory exams and ECG revealed no significant changes even at higher doses. Lodenafil carbonate was not detected in any samples, indicating that it acts as a prodrug. The mean lodenafil pharmacokinetic parameters for t_{max} and $t_{1/2}$ were 1.6 (± 0.4) h and 3.3 (± 1.1) h, respectively. This study demonstrated that lodenafil carbonate was well tolerated and showed a good safety profile in healthy male volunteers.

Received
July 18, 2011;
accepted
May 28, 2012

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Introduction

Lodenafil carbonate is a new phosphodiesterase Type 5 (PDE5) inhibitor for treatment of erectile dysfunction [1, 2, 3, 4], a common sexual problem for males over 40 – 50 years of age. Lodenafil relaxes isolated human and animal cavernous tissues through inhibition of cyclic guanosine monophosphate hydrolysis [1]. Lodenafil carbonate was approved by Brazilian Sanitary Surveillance Agency (ANVISA) in 2007 [5].

The pharmacokinetic parameters for lodenafil carbonate and lodenafil following oral administration (10 mg) of lodenafil carbonate in male beagle dogs were (mean ± S.D): lodenafil carbonate: 11 ± 14 ng/ml, 23 ± 21 ng×h/ml, 2.1 ± 3.0 h, 1.67 ± 1.53 h; lodenafil: 1,357 ± 961 ng/ml, 9,091 ± 5,526 ng×h/ml, 4.3 ± 0.5 h, 4.3 ± 0.5 h for C_{max}, AUC, $t_{1/2}$, t_{max}, respectively [1]. The above results indicate that lodenafil carbonate acts as a prodrug, delivering lodenafil *in vivo* as the active moiety, since very small amounts of lodefanil carbonate reached the systemic circulation.

The t_{max} for PDE5 inhibitors is between 0.33 – 1.8 h (sildenafil 1 h, tadalafil 1.8 h, vardenafil 0.75 h, udenafil 1 h and avanafil 0.33 h) and the half-life time for PDE5 inhibitors varies from 2.6 to 21.1 h (sildenafil 2.76 h, vardenafil 2.63 h, avanafil 5.36 h, udenafil 11 h and tadalafil 21.1 h) [6, 7, 8, 9, 10]. After single oral administrations of lodenafil carbonate (160 mg) in healthy male volunteers, lodenafil is rapidly absorbed with

Table 1. Treatment scheme with lodenafil in 1 mg, 2 mg, 3 mg, 5 mg, 8 mg, 10 mg, 20 mg, 30 mg, 50 mg, 80 mg and 100 mg in healthy male volunteers.

Volunteer	1 mg	2 mg	3 mg	5 mg	8 mg	10 mg	20 mg	30 mg	50 mg	80 mg	100 mg
I	x	x	x								
II	x	x	x								
III	x	x	x								
IV		x	x	x							
V		x	x	x							
VI		x	x	x							
VII			x	x	x						
VIII			x	x	x						
IX			x	x	x						
X				x	x	x					
XI				x	x	x					
XII				x	x	x					
XIII					x	x	x				
XIV					x	x	x				
XV					x	x	x				
XVI						x	x	x			
XVII						x	x	x			
XVIII						x	x	x			
XIX							x	x	x		
XX							x	x	x		
XXI							x	x	x		
XXII								x	x	x	
XXIII								x	x	x	
XXIV								x	x	x	
XXV									x	x	x
XXVI									x	x	x
XXVII									x	x	x
XXVIII										x	x
XXIX										x	x
XXX										x	x
XXXI											x
XXXII											x
XXXIII											x

peak of concentration (157 ng/ml) occurring at 1.2 h, half-life time of 2.4 h and area under the curve (AUC) of 530 ng×h/ml [4].

The present study was conducted to evaluate the safety, tolerability, and pharmacokinetics of lodenafil carbonate after administering ascending (1 – 100 mg) single oral doses in normal healthy male volunteers.

Materials and methods

Clinical protocol

The following inclusion criteria were used: 33 healthy male volunteers, aged between 18 and 55 years old and within ≥ 19 and ≤ 28.75 of the ideal body weight were selected for the study [11]. The volunteers were selected for the study after having their health status previously assessed by a clinical evaluation and laboratory tests (biochemical and hematological parameters, and urinalysis). The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal and hematological diseases, as assessed by clinical examination, ECG and the following laboratory tests: blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, γGT, total bilirrubin and fractions, uric acid, total cholesterol, triglycerides, albumin and total protein, hemoglobin, hematocrit, total and differential white cell counts and routine urinalysis. All subjects were negative for HIV, HBV (except for serological scar) and HCV. The subjects were able to comprehend the full nature and purpose of the study, including possible risks

Table 2. Accuracy and precision data for lodenafil carbonate and lodenafil from the pre-study validation in human plasma.

Lodenafil carbonate						
Nominal concentration (in ng/ml)	Intra-batch mean (in ng/ml)	Intra-batch precision (CV %)	Intra-batch accuracy (%)	Inter-batch mean (in ng/ml)	Inter-batch precision (CV%)	Inter-batch accuracy (%)
0.5	0.55	12.55%	109.29%	0.52	15.38%	103.42%
0.5	0.47	14.16%	93.70%			
0.5	0.53	17.01%	105.89%			
3	3.1	3.61%	102.42%	3.2	8.70%	105.17%
3	3.1	4.01%	104.50%			
3	3.3	14.98%	109.72%			
30	32.8	4.25%	109.25%	33.3	9.32%	110.89%
30	33.9	10.23%	113.08%			
30	33.1	12.38%	110.33%			
180	187.3	2.33%	104.03%	189.5	6.16%	105.30%
180	191.4	3.11%	106.32%			
180	190.0	10.30%	105.56%			
1,800	1,923	6.06%	106.81%	1894	5.91%	105.23%
1,800	1,935	5.39%	107.50%			
1,800	1,825	5.03%	101.39%			

Lodenafil						
Nominal concentration (in ng/ml)	Intra-batch mean (in ng/ml)	Intra-batch precision (CV %)	Intra-batch accuracy (%)	Inter-batch mean (in ng/ml)	Inter-batch precision (CV%)	Inter-batch accuracy (%)
0.5	0.54	19.67%	107.13%	0.51	16.39%	101.02%
0.5	0.48	19.02%	96.37%			
0.5	0.50	7.95%	100.33%			
3	2.8	11.33%	92.29%	2.7	9.66%	90.26%
3	2.7	7.36%	89.75%			
3	2.7	10.79%	88.52%			
30	28.7	5.25%	95.63%	27.6	8.42%	91.97%
30	27.8	10.50%	92.75%			
30	25.8	5.18%	86.06%			
180	163.5	5.48%	90.83%	157.7	8.69%	87.59%
180	157.9	6.61%	87.71%			
180	151.6	12.27%	84.24%			
1,800	1,604	3.60%	89.10%	1623	5.32%	90.14%
1,800	1,685	4.41%	93.61%			
1,800	1,579	5.88%	87.71%			

CV = coefficient of variation.

and side effects and were willing to cooperate with the investigator and comply with the requirements of the entire study. All subjects provided signed consent forms.

The following exclusion criteria were used: laboratory values outside the accepted normal ranges, unless considered not clinically significant by the investigator; participation in an experimental study or ingestion of an experimental drug 3 months prior to the initiation of the study; any significant clinical illness or surgery within 8 weeks immediately prior to the initiation of the study or maintenance therapy with any drug; hospitalization for any reason within 8 weeks prior to the initiation of the study; history of drug, medication or alcohol abuse, or had ingested alcohol within 48 hours prior to the beginning of the confinement portion of the study; history of hepatic, renal, epileptic or hemopoietic disease; hypo- or hypertension of any etiology (outside the range of 100 – 140 mmHg for systolic and 60 – 90 mmHg for diastolic blood pressure), or heart rate not between 50 and 90 bpm; myocardial infarction, angina pectoris and/or congestive heart

Table 3. Mean pharmacokinetic parameters obtained from volunteers after administration of lodenafil carbonate formulation (1 mg, 2 mg, 3 mg, 5 mg, 8 mg, 10 mg, 20 mg, 30 mg, 50 mg, 80 mg and 100 mg) in 33 healthy volunteers.

Dose (mg)	1.00		2.00		3.00		5.00	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC Extrap (%)	65.65	36.92	32.61	23.63	31.39	18.59	30.15	23.04
AUC_{∞} (h×ng/ml)	15.99	13.19	7.02	3.65	12.82	5.26	12.13	6.02
AUC_{last} (h×ng/ml)	2.07	1.98	3.67	2.37	8.11	3.84	6.45	5.89
C_{max} (ng/ml)	1.76	1.61	2.87	1.67	2.77	0.42	3.71	2.36
t_{max} (h)	0.75	0.00	1.17	0.49	2.06	2.46	2.22	2.06
Ke (1/h)	0.17*	0.20	0.47	0.25	0.30	0.29	0.33	0.20
$t_{1/2}$ (h)	14.62*	17.54	2.37	2.38	4.02	2.25	3.33	2.61
Vd (l)	1,009.00*	750.00	925.00	463.00	1,266.00	472.00	1,951.00	1,299.00
Cl (l/h)	95.00*	78.00	355.00	189.00	283.00	147.00	497.00	228.00

Dose (mg)	8.00		10.00		20.00		30.00	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC Extrap (%)	31.08	23.41	24.27	24.18	6.75	4.08	14.82	18.71
AUC_{∞} (h×ng/ml)	22.62	15.73	29.69	24.85	55.53	39.71	86.69	85.97
AUC_{last} (h×ng/ml)	17.54	16.34	24.99	24.45	46.18	39.87	82.40	86.41
C_{max} (ng/ml)	8.08	6.92	10.92	12.20	19.21	13.74	24.39	19.37
t_{max} (h)	2.11	1.29	1.38	0.50	1.86	1.99	1.44	0.92
Ke (1/h)	0.35	0.20	0.41	0.35	0.42	0.30	0.25	0.13
$t_{1/2}$ (h)	2.97	2.23	2.66	1.92	2.59	1.75	3.78	2.61
Vd (l)	2,348.00	1,908.00	2,435.00	2,435.00	1,477.00	789.00	6,037.00	7,740.00
Cl (l/h)	613.00	553.00	796.00	821.00	490.00	257.00	1,007.00	1,175.00

Dose (mg)	50.00		80.00		100.00		Mean	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC Extrap (%)	5.90	4.83	21.07	29.71	5.23	3.53	–	–
AUC_{∞} (h×ng/ml)	65.91	51.90	49.16	39.69	89.72	73.32	–	–
AUC_{last} (h×ng/ml)	63.37	51.36	42.80	41.95	86.66	73.25	–	–
C_{max} (ng/ml)	24.09	15.39	17.62	15.40	37.27	29.02	–	–
t_{max} (h)	1.67	1.04	1.86	1.08	1.42	1.02	1.6	0.4
Ke (1/h)	0.32	0.12	0.25	0.16	0.33	0.16	0.3	0.1
$t_{1/2}$ (h)	2.59	1.25	5.93	9.12	2.56	1.24	3.3	1.1
Vd (l)	4,137.00	2,902.00	22,971.00	28,532.00	5,581.00	3,189.00	4,913	6,590.5
Cl (l/h)	1,361.00	1,189.00	3,274.00	2,955.00	1,784.00	1,120.00	1,046	914.7

Ke = elimination rate constant; $t_{1/2}$ = half-life; C_{max} = maximum plasma concentration; tmax = time to the maximum plasma concentration, AUC_{last} = area under curve from time zero to the last detectable concentration AUC_{∞} = extrapolation of area to infinity, AUC Extrap = % extrapolation of area to infinity; CL = clearance; VD = volume of distribution; Clast = last detectable concentration, t_{last} = time of last detectable concentration, *excluded of descriptive statistic.

failure; the subject had donated or lost blood within 3 months prior to the study or more than 1,500 ml within 12 months preceding the study; any other condition as per investigator judgment; high caffeine intake (more than 5 cups of tea or coffee/day); subject who smoked; history or presence of gastrointestinal, liver or kidney disease or any other condition known to interfere with the absorption, distribution, metabolism or excretion of drugs.

Concomitant medication was to be avoided where possible during the study. Any medication, including over-the-counter medication, was to be recorded.

The male volunteers had the following clinical characteristics (mean ± SD (range)): age 31.1 ± 5.7 y (23 – 40); height 174 ± 6.1 cm (160.0 – 184.0); body weight 71.2 ± 9.7 kg (54.5 – 90.5). All subjects provided written informed consent, and the Ethics Committee of the State University of Campinas approved the protocol. Any major changes required approval from the Committee. The study was conducted in accordance with Good Clinical Practices and provisions of the Declaration of Helsinki (1964) and all revisions.

After screening and a washout period (of at least 2 weeks), the individuals who qualified were confined for 3 periods of ~ 40

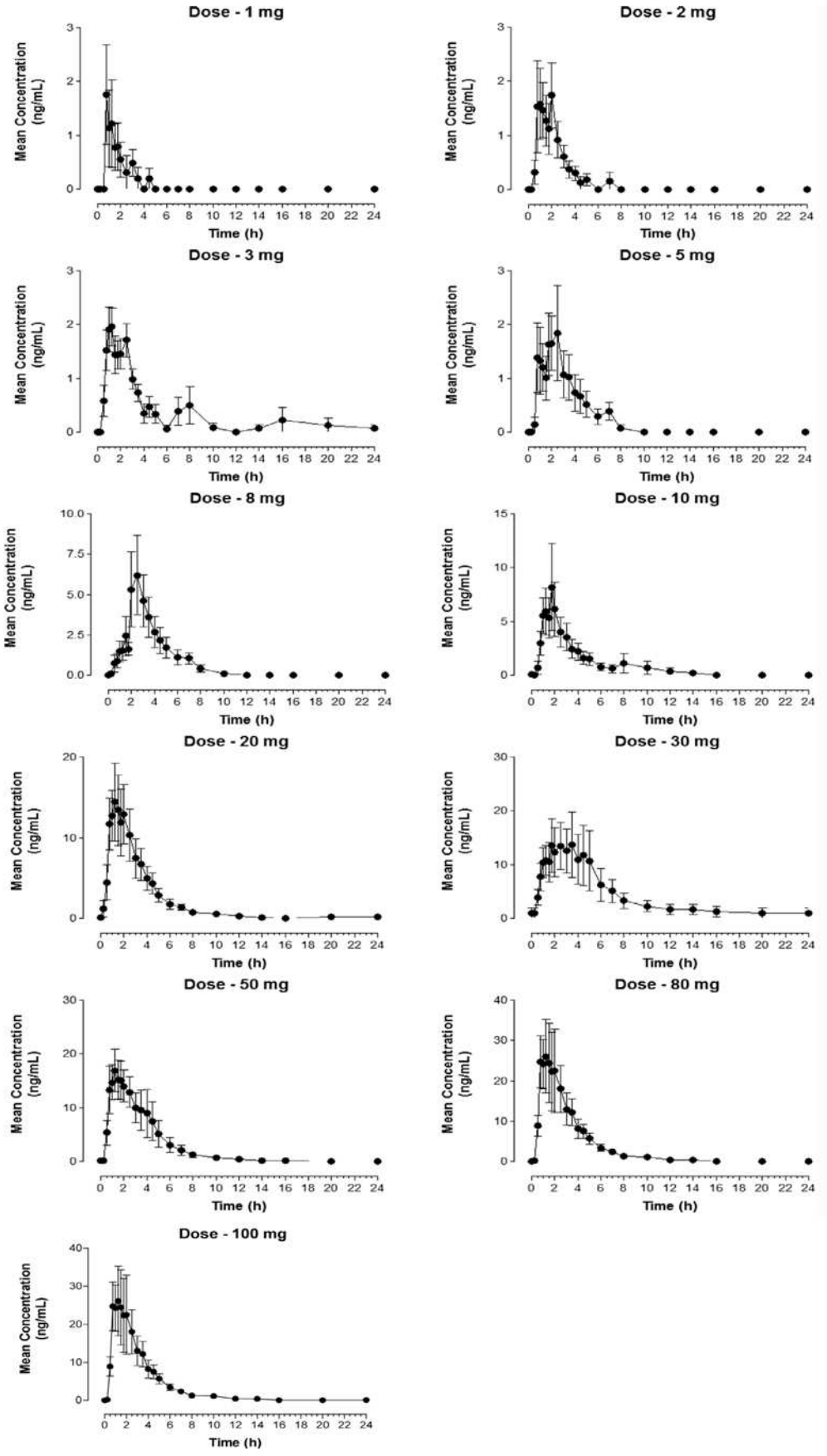

Figure 1. The mean lodenafil plasma concentrations vs. time profiles after a single oral dose (1 mg, 2 mg, 3 mg, 5 mg, 8 mg, 10 mg, 20 mg, 30 mg, 50 mg, 80 mg, 100 mg) tablet formulation of lodenafil carbonate in male volunteers.

Table 4. Laboratory tests in healthy male volunteers before and after administration of lodenafil carbonate.

Type		Unit	Normal range	Pre-study				Post-study			
				Mean	SD	Min	Max	Mean	SD	Min	Max
Hematological	Hemoglobin	g%	13.5 – 17.5	15.4	0.8	14.1	17.2	14.6	0.8	12.9	16.8
	Hematocrit	%	39 – 50	44.7	1.9	42.2	49.4	42.9	2.1	40.1	47.7
	Red blood count	106/mm^3	4.3 – 5.7	5.2	0.6	4.7	8.0	4.8	0.2	4.5	5.2
	VCM	Micra3	81 – 95	84.4	17.1	5.2	93.6	89.0	2.4	85.3	93.3
	HbCM	Pg	26 – 34	29.6	3.4	14.9	33.4	30.3	1.3	27.4	32.9
	White blood count	103/mm^3	3,500 – 10,500	6.4	1.3	3.7	8.4	6.9	1.7	4.2	10.6
	Band neutrophils	%	≤ 5	1.7	1.0	1.0	5.0	1.7	0.7	1.0	3.0
	Segmented	%	48 – 76	55.0	8.6	36.1	66.7	54.6	8.7	32.1	65.8
	Lymphocytes	%	25 – 30	31.9	8.6	18.4	50.3	31.8	7.9	21.9	51.5
	Monocytes	%	1 – 9	7.7	1.7	4.7	10.3	7.8	2.2	4.8	12.0
	Basophils	%	0 – 1	0.6	0.4	0.0	1.8	0.5	0.3	0.0	1.0
	Eosinophils	%	1 – 5	3.8	2.8	0.6	10.7	4.0	2.5	1.0	9.9
	Platelet count	–	150 – 400	238.5	50.0	153.0	363.0	247.3	44.4	162.0	328.0
Biochemical	Total cholesterol	mg/dl	≤ 239	167.3	30.9	112.0	239.0	165.5	42.0	22.0	248.0
	Triglycerids	mg/dl	≤ 150	102.4	44.5	45.0	191.0	99.6	54.1	24.0	303.0
	Total proteins	g/dl	6 – 8	7.8	0.3	7.3	8.4	7.7	0.3	7.2	8.3
	Albumin	g/dl	3.5 – 5.5	4.7	0.2	4.4	5.0	4.6	0.2	4.2	5.1
	Uric acid	mg/dl	2.5 – 7.0	5.5	1.5	3.1	9.0	5.4	1.3	3.0	8.8
	Total bilirubins	mg/dl	0 – 1.1	0.8	0.5	0.4	2.6	0.6	0.4	0.0	1.4
	Alkaline phosphatase	U/L	27 – 100	76.2	18.9	49.0	115.0	67.6	24.5	0.8	116.0
	gT	U/L		24.1	11.9	8.0	63.0	23.8	11.2	5.2	47.0
	SGOT (AST)	U/L	≤ 43	21.9	5.5	15.0	42.0	20.2	4.1	12.0	34.0
	SGPT (ALT)	U/L	≤ 43	23.2	11.0	13.0	64.0	23.1	9.7	10.0	53.0
	Urea	mg/dl	10 – 50	26.6	5.4	14.4	36.7	29.1	5.5	17.2	38.0
	Creatinine	mg/dl	0.4 – 1.3	1.1	0.8	0.8	4.7	1.1	0.9	0.7	4.5
	Fasting blood glucose	mg/dl	60 – 100	87.2	8.1	74.0	108.0	87.9	6.9	74.0	101.0
Urine	Specific bravity	–	1.015 – 1.028	1019.3	5.8	1,008.0	1,029.0	1,019.6	6.1	1,006.0	1,032.0
	pH	–	5.5 – 6.5	6.2	0.6	5.5	7.0	6.2	0.4	5.5	6.5
	WBC	/ml	≤ 10,000	7,124.2	16,901.9	300.0	97,000.0	3,277.0	1,924.3	1,000.0	9,400.0
	RBC	/ml	≤ 5,000	4,150.0	4,784.6	300.0	19,430.0	2,900.0	3,924.6	100.0	22,900.0

hours. Each confinement was intervaled for 1 week. The study was conducted in an open, randomized, dose-escalation, three-period open-label experimental design, involving the administration of increasing single oral doses (1 mg, 2 mg, 3 mg, 5 mg, 8 mg, 10 mg, 20 mg, 30 mg, 50 mg, 80 mg, 100 mg) of lodenafil carbonate (Table 1). A Fibonacci-like scheme was used for dose escalation (from 1 to 10, and from 10 to 100 mg). The progression to the next dose was allowed by clinical and laboratory exams, Ambulatory Monitoring of Arterial Pressure (AMAP) without relevant clinical modifications, and adverse events without clinical relevancy. The adverse event evaluation was performed during confinement period and in a clinical visit 24 hours before the next confinement. The medical investigator classified the event intensity as mild, moderate, severe, and serious, and the causal relationship to the formulation as unrelated, unlikely, possible and probable.

During each period, the volunteers were hospitalized between 4:00 p.m. and 7:00 p.m., having already eaten a normal evening meal. After overnight fasting, they received at 7:00 a.m., a single dose of appropriate lodenafil carbonate (tablet) along with 240 ml of tap water. All volunteers were required to remain fasting until 2 hours after dose administration, when a xanthine-free standard breakfast was available. A xanthine-free standard lunch was provided 5 (lunch), 8 (afternoon snack), 12 (lunch) and 15 (supper) hours after dose. A standard meal (lunch) of rice, beans, vegetables, and fried chicken, plus a fruit as dessert was consumed. The breakfast, afternoon snack and supper included crackers, bread, jelly, cake and apple. No other food was permitted during the "in-house" period and liquid consumption

was allowed *ad libitum* after lunch (with the exception of xanthine-containing drinks, including tea, coffee, and cola). No comedication was permitted during the study.

Blood samples (5 ml) from a suitable antecubital vein were collected by an indwelling catheter into heparin containing tubes at pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12, 14, 16, 20 and 24 h post-dosing.

The Ambulatory Monitoring of Arterial Pressure (AMAP) was measured for 24 hours after drug administration, with 15-min intervals in the first 2 hours and then every hour until the end of the internment (24 hours after the administration of the lodenafil carbonate). Two ECGs were obtained 1 hour and 12 hour after the administration of the lodenafil carbonate.

Formulations

The following formulations were employed: lodenafil carbonate test formulation tablet in 1 mg (lot 318/04), 2 mg (lot 319/04), 3 mg (lot 320/04), 5 mg (lot 321/04), 8 mg (lot 378/04), 10 mg (lot 379/04), 20 mg (lot 392/04), 30 mg (lot 391/04), 50 mg (lot 394/04), 80 mg (lot 396/04) and 100 mg (lot 397/04) from Cristália Produtos Químicos e Farmacêuticos Ltda, Itapira, Brazil.

Chemicals and reagents

Lodenafil carbonate (lot number: SL24-024), lodenafil (lot number: not available), sildenafil (lot number: 20227/99) were provided by Cristália Produtos Químicos e Farmacêuticos Ltda, Itapira, Brazil. Acetonitrile, formic acid and methanol were purchased from J. T. Baker, USA. Diethyl ether and hexane were purchased from Mallinckrodt, USA. Blank human blood was collected from healthy, drug-free volunteers. Plasma was obtained by centrifugation of blood treated with sodium heparin. Pooled plasma was prepared and stored at approximately –20 °C until needed.

Calibration standards and quality controls

Stock solutions of lodenafil carbonate, lodenafil and the internal standard (sildenafil) were prepared in acetonitrile/water (50/50, v/v) at concentrations of 1 mg/ml. Calibration curves of lodenafil carbonate and lodenafil were prepared by spiking blank plasma at concentrations of 0.5, 1.0, 5.0, 20.0, 50.0, 200, 1,000 and 2,000 ng/ml and each concentration was analyzed in duplicate. The quality control samples were prepared in blank plasma at concentrations of 3, 30, 180 and 1,800 ng/ml (QCA, QCB, QCC and QCD, respectively). The spiked plasma samples (standards and quality controls) were extracted in each analytical batch along with the unknown samples.

Drug analysis

The blood samples (5 ml) were centrifuged at 2,500 g for 10 min at 4 °C and the plasma decanted and stored at –20 °C until assayed for lodenafil carbonate and lodenafil contents. Lodenafil carbonate, lodenafil, and the IS were extracted from plasma samples and quantified by liquid chromatography tandem mass spectrometry (LC-MS-MS) with positive electrospray ionization using multiple reaction monitoring (MRM).

Briefly, 0.2 ml of each plasma sample was pipetted into a glass tube followed by 0.05 ml of the internal standard solution (1,000 ng/ml of sildenafil (Cristália Produtos Químicos e Farmacêuticos Ltda, Itapira, Brazil.) and 4 ml of hexane/diethyl-ether (20/80; v/v) (HPLC grade, J. T. Baker, Phillipsburg, NJ, USA). The tubes were briefly vortex-mixed (40 s). The upper organic layers were removed and transferred into another sterile tube, and evaporated to dryness under N_2 (at 40 °C). The dry residues were dissolved with 0.2 ml of acetonitrile/water (50/50; v/v) and the solutions were transferred to glass microvials using automatic pipettes with disposable plastic tips. The vials were caped and placed into the autosampler (10 °C) racks.

High performance liquid chromatography (HPLC) was performed on C_{18}, 4 µm (100 × 2.1 mm i.d. (Jones Chromatography, Genesis, USA) at a flow rate of 0.45 ml/min

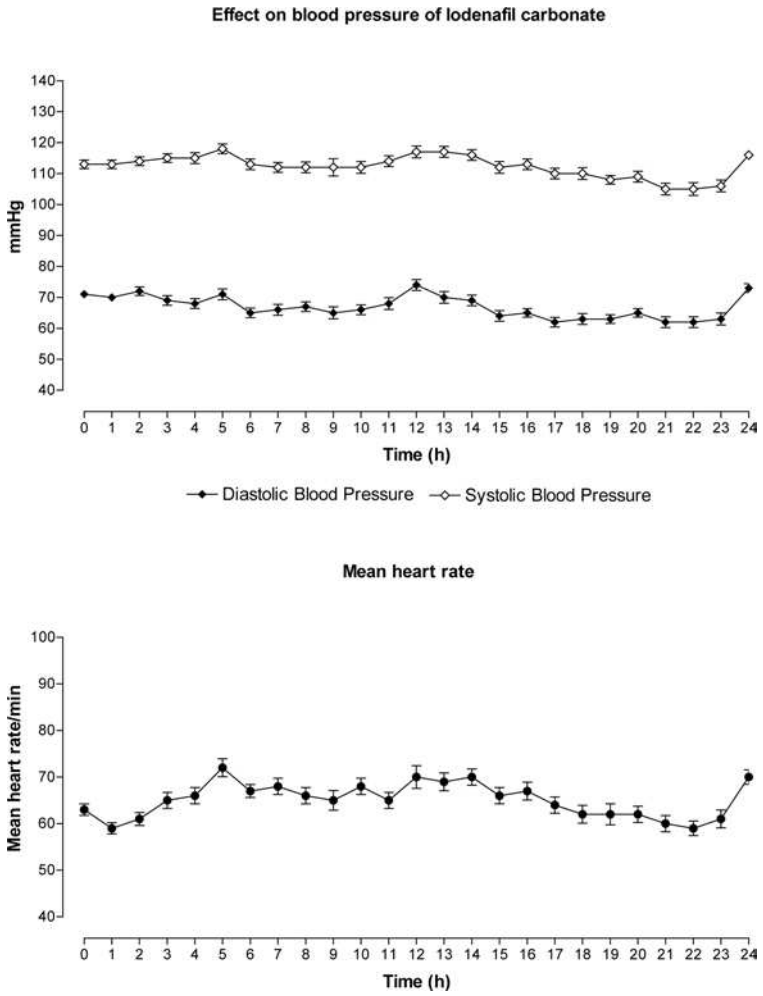

Figure 2. Mean sitting systolic and diastolic blood pressures and sitting heart rates after lodenafil carbonate administration.

and pressure of the system was ~ 120 bar. The mobile phase was acetonitrile/water (90/100; v/v) + 0.1% formic acid (Analysis Grade, J. T. Baker, USA). The column operated at room temperature. The temperature of the auto sampler operated at 10° ± 2 °C and the injection volume was 10 µl.

Typical standard retention times were 1.1 ± 0.2 min for lodenafil carbonate, 0.8 ± 0.1 min for lodenafil and 0.8 ± 0.1 min for the IS, and the total run-time was 2.4 min. The mass spectrometer (model API 4000, Sciex/Applied Biosystems, Foster City, CA, USA) equipped with a turbospray source using a crossflow counter electrode, was run in positive mode (ES+) with multiple reaction monitoring (MRM). The mass spectrometer was set as follows: 1035.2 > 487.1, 505.2 > 487.0 and 475.1 > 283.2, for lodenafil carbonate, lodenafil and sildenafil, as the precursor ions and the respective product ions *m/z*. The limit of quantification in plasma was 0.5 ng/ml for both lodenafil carbonate and lodenafil.

Assay performance

A linear regression with a weighting index of 1/x^2 was performed on the peak area ratios of lodenafil carbonate and the internal standard versus the lodenafil carbonate concentrations of eight human plasma standards (in duplicate) to generate a calibration curve. A linear regression with a weighting index of 1/x^2 was performed on the peak area ratios of lodenafil and the internal standard versus the lodenafil concentrations of the eight human plasma standards (in duplicate) to generate a calibration curve.

Precision and accuracy: Within- and between-run precision was determined as the relative standard deviation, RSD (%) = 100 (SD/M), and the accuracy as the percentage relative error, RE (%) = (E-T)(100/T), where M is the mean, SD is the standard deviation of M, E is the experimentally determined concentration and T is the theoretical concentration.

Pharmacokinetics

Individual plasma level-time curves were constructed and pharmacokinetic parameters were obtained according to a non-compartmental approach. The first-order terminal elimination rate constant (ke) was estimated by linear regression from the points describing the elimination phase in a log-linear plot, and the half-life ($t_{1/2}$) was derived from this rate constant ($t_{1/2}$ = ln (2)/ke). The maximum plasma concentration (C_{max}) and the time taken to achieve this concentration (t_{max}) were obtained directly from the curves. The areas under the lodenafil plasma concentration vs. time curves from time zero to the last detectable concentration (AUC_{last}) were calculated by applying the linear trapezoid rule. Extrapolation of these areas to infinity (AUC_{∞}) was done by adding the value C_{last}/ke to the calculated AUC_{last} (where C_{last} = the last detectable concentration). Clearance (CL) was calculated by the formula dose/$AUC_{0-\infty}$. Volume of distribution (Vd) was cal-

Table 5. Mean sitting systolic and diastolic blood pressures and mean heart rates after lodenafil carbonate administration.

Time	Mean diastolic blood pressures				Mean systolic blood pressures				Mean heart rate			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
0	71	7	56	89	113	8	91	132	63	7	41	86
1	70	7	54	93	113	8	86	134	59	7	42	77
2	72	8	52	96	114	8	88	134	61	8	43	85
3	69	9	45	99	115	8	88	145	65	10	45	100
4	68	9	41	93	115	10	95	138	66	10	43	107
5	71	10	44	97	118	9	89	143	72	11	48	107
6	65	9	40	89	113	10	77	135	67	8	49	85
7	66	10	40	96	112	9	85	133	68	10	46	99
8	67	9	40	93	112	10	90	135	66	10	46	92
9	65	11	43	86	112	16	90	140	65	12	46	97
10	66	9	43	88	112	11	84	143	68	10	48	92
11	68	11	44	90	114	10	86	138	65	10	48	92
12	74	10	50	98	117	11	94	149	70	14	47	111
13	70	11	44	95	117	10	93	146	69	11	47	104
14	69	10	44	89	116	10	82	143	70	10	49	102
15	64	10	46	94	112	11	91	142	66	10	46	99
16	65	8	46	84	113	10	83	144	67	11	45	115
17	62	9	44	80	110	10	84	135	64	10	44	89
18	63	10	41	89	110	11	86	136	62	11	42	98
19	63	8	43	81	108	8	87	127	62	13	42	138
20	65	8	45	88	109	10	88	133	62	10	42	87
21	62	10	43	91	105	11	83	142	60	10	46	105
22	62	10	40	84	105	12	79	132	59	9	41	82
23	63	11	42	91	106	11	74	127	61	11	39	98
24	73	8	54	91	116	7	98	132	70	9	49	91

culated by the formula Dose/Ke(AUC_{∞}). The software used included WinNonLin Professional Network Edition (Scientific Consulting, v. 3.0), Microsoft Excel (v. 7.0) and GraphPad Prism (v. 3.02).

Results

All biochemical parameters monitored presented no clinically relevant alterations during the Phase I study. No significant changes were observed in the laboratory exams and in the ECGs intra-study. Tolerance of both formulations (1 – 100 mg) was good and no significant adverse events were observed or reported. One volunteer had an adverse event (dysuria not related to therapy and classified as not serious) after the discharge of the third confinement period during the Phase I study. Two volunteers related spontaneous erections after administration of lodenafil carbonate (30 and 50 mg): one during the clinical evaluation for discharge of the study and one during the final clinical evaluation in the first period of confinement.

Laboratory tests after administration of lodenafil carbonate are shown in Table 4. Blood pressure and heart rate were assessed for 24 h after treatment with lodenafil carbonate (Figure 2) (Table 5).

The mean lodenafil plasma concentrations vs. time profiles after testing single oral doses of lodenafil carbonate in males are shown in Figure 1. Calculated mean pharmacokinetic parameters of lodenafil are given in Table 3.

The method was linear for lodenafil carbonate and lodenafil concentrations from 0.5 to 2,000 ng/ml. No significant matrix effect was observed. The limit of quantification (LOQ), defined as the lowest concentration at which both the precision and accuracy were < 20%, was 0.5 ng/ml for lodenafil carbonate and lodenafil. The within- and between-run precision and accuracy for the quality controls are summarized in Table 2.

Discussion

High-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (LC-MS-MS) in animal and human plasma has been used for lodenafil and/or lodenafil carbonate quantification [1, 4]. However, no full validation method has been previously published for lodenafil and lodenafil carbonate quantification. Our method employed sildenafil as internal standard instead of theophylline, having a shorter retention time of 1.1 min for lodenafil carbonate and 0.8 min for lodenafil. The limit of quantification (0.5 ng/ml) was adequate for pharmacokinetic evaluation of lodenafil carbonate and lodenafil.

The results obtained following oral administration of lodenafil carbonate in healthy male volunteers show that lodenafil carbonate acts as a prodrug when given orally, since no quantifiable lodefanil carbonate concentration has been detected in the systemic circulation. Similar results were observed in male beagles following oral administration of lodenafil carbonate [1].

The adverse event of dysuria was evaluated as not serious since the volunteer spontaneously recovered and only reported in the medical final evaluation.

After the oral administration of the lodenafil carbonate 80 mg tablets to the volunteers, the t_{max} (1.6 h) and $t_{1/2}$ (3.3 h) were similar to the reported in the literature (t_{max}: 1.2 – 1.5 h; $t_{1/2}$: 2.4 – 3 h) [2]. The C_{max} (17.62 ng/ml) and AUC (42.80 h×ng/ml) values were different to those reported in the literature (C_{max} 158 ng/ml; AUC 528 h×ng/ml) for a lodenafil carbonate tablet (dose 160 mg; two tablets of 80 mg) formulation [2].

As shown in Table 3, the pk parameters calculated for 1 mg are not reliable due to low plasma lodenafil after a dose of 1 mg. The major differences for t1/2, Cl and Vd over the dose range can be explained by the low number (n = 3 to 1 mg; n = 6 to 2 mg and n = 9 between 3 mg and 100 mg) of volunteer per dose and by the variability inter-subject.

The mean lodenafil pharmacokinetic parameters for tmax (1.6 ± 0.4 h) was similar to the PDE5 (sildenafil 1 h, tadalafil 1.8 h, vardenafil 0.75 h, udenafil 1 h, and avanafil 0.33 h). The mean lodenafil pharmacokinetic parameters for $t_{1/2}$ (3.3 h) were similar to the sildenafil and vardenafil (sildenafil 2.76 h, vardenafil 2.63 h, avanafil 5.36 h, udenafil 11 h and tadalafil 21.1 h) [6, 7, 8, 9, 10].

No serious adverse events were observed, and none of the subjects discontinued the study because of lack of tolerance. As shown in Table 4 and Table 5, the AMAP measurements and clinical tests revealed no significant alteration even at a higher dose. The lodenafil carbonate presented good tolerance and safety for healthy male volunteers in Phase I study. The recommended dose of 80 mg was used in following trials and it is the commercial formulation dose marketed in Brazil.

Acknowledgments

The Phase I trial was sponsored by Cristália Produtos Químicos e Farmacêuticos Ltda, Itapira, Brazil. AcademicEnglishSolutions.com revised the English. Gilberto De Nucci is a visiting Professor at the Department of Clinical Pharmacy, King Saud University, Riyadh, Saudi Arabia.

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ANEXO IV

Outras publicações durante o período de doutorado

Curr Drug Metab. 2005 Dec;6(6):519-29.

Pharmacokinetics of dihydroergocristine and its major metabolite 8'-hydroxy-dihydroergocristine in human plasma.

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Abstract

Dihydroergocristine (DHEC) is a semi-synthetic drug mainly used for age-related cognitive impairment. In this study, its major metabolite 8'-hydroxy-dihydroergocristine (8'-OH-DHEC) was produced in incubates of a bovine liver preparation using dihydroergocristine mesylate (DHECM) as substrate. Purification was achieved by flash silica gel column and reverse phase liquid chromatographies, and identification was based on accurate molecular mass measurements, mass fragmentation spectra and NMR ((1)H/(13)C) chemical shifts. By using the substance produced in vitro, a fast, sensitive, specific and robust LC/MS/MS method for the simultaneous determination of DHEC and its major metabolite in human plasma was developed and validated. Bromocriptine was used as internal standard and limits of quantification for DHEC and 8'-OH-DHEC were 10 pg/ml and 20 pg/ml, respectively. Pharmacokinetic parameters were investigated on 12 male healthy volunteers to whom a single dose of 18 mg DHECM was administered in tablets (Iskevert). The peak of DHEC was 0.28 +/- 0.22 microg/l, the t(max) 0.46 +/- 0.26 h, the AUC(last) 0.39 +/- 0.41 microg/l.h and the terminal elimination half-life 3.50 +/- 2.27 h. The peak of 8'-OH-DHEC was 5.63 +/- 3.34

microg/l, the t(max) 1.04 +/- 0.66 h, the AUC(last) 13.36 +/- 5.82 microg/l.h and the terminal elimination half-life 3.90 +/- 1.07 h. Dosing of 18 mg DHECM was well tolerated, causing no adverse events.

PMID: 16379666

Arzneimittelforschung. 2007;57(9):582-90.

Comparative bioavailability study with two amiodarone tablet formulations administered with and without food in healthy subjects.

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Abstract

OBJECTIVE: The aim was to assess the comparative bioavailability of two formulations (200 mg tablet) of amiodarone (CAS 19774-82-4) in healthy volunteers of both sexes, with and without food.

METHODS: The study was conducted using an open, randomized, two-period crossover design with a 3-week washout interval, in two groups, with and without food. Plasma samples were obtained for up to 240 h post dose. Plasma amiodarone concentrations were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS) with positive ion electrospray ionization using multiple reactions monitoring (MRM). From the amiodarone plasma concentration vs. time curves, the following pharmacokinetic parameters were obtained, with and without food: AUC(last), AUC(inf), AUC(0-240h), AUC(0-72h) and C(max).

RESULTS: The limit of quantification was 1 ng/mL for plasma amiodarone analysis. The geometric mean and 90% confidence interval CI of Test/Reference percent ratios were, without and with food, respectively: 107.61 (92.73-124.89) and 100.6 (94.1-107.5) for C(max), 107.05 (95.88-119.51) and 100.2 (96.0-104.7) for AUC(last), 107.27 (95.78-120.15) and 100.8 (97.0-104.8) for AUC(0-72h), 106.76 (95.84-118.94) and 100.2 (96.0-104.7) for AUC(0-240h) and AUC(inf) 105.15 (94.18-117.41) and 100.7 (96.6-105.0).

CONCLUSION: Since the 90% CI for AUC(0-72) and C(max) ratios were within the 80-125% interval proposed by the US FDA, it was concluded that the amiodarone 200 mg tablet (test formulation) with and without food was bioequivalent to the reference 200 mg tablet for both the rate and extent of absorption.

PMID:17966757