UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

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Biowaiver Monograph for Immediate-Release Solid Oral Dosage Forms: Oseltamivir Phosphate – a Draft Document

Márcia Nunes da Silva

Porto Alegre, novembro de 2016.

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Aos meus pais, pelo incentivo e por sempre me fazerem acreditar na minha capacidade. Obrigada pelo carinho, apoio e compreensão.

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SUMÁRIO

1. CONTEXTUALIZAÇÃO

Produtos genéricos devem demonstrar, em primeira instância, equivalência farmacêutica em relação ao medicamento referência para serem aprovados pelos órgãos reguladores. A avaliação da bioequivalência geralmente é realizada através de estudos farmacocinéticos em humanos, entretanto, entre outras formas, é possível utilizar os testes de dissolução *in vitro* para demonstrar a equivalência entre um produto teste e seu comparador.

Testes de dissolução são ferramentas úteis no processo de desenvolvimento de medicamentos. Eles auxiliam na identificação de problemas relacionados aos excipientes utilizados na formulação e ao processo produtivo. Inicialmente, os testes de dissolução *in vitro* foram desenvolvidos para serem utilizados no controle de qualidade de medicamentos, de forma a garantir a consistência lote a lote. Há uma diferença evidente entre os testes de dissolução utilizados no controle de qualidade e aqueles que evidenciam a equivalência *in vitro.* O primeiro envolve a análise de um único ponto de coleta do meio de dissolução, enquanto o segundo, envolve a comparação de perfis de dissolução de um medicamento referência e um teste, na faixa de pH fisiológico relevante para a absorção no trato gastrintestinal.

Bioisenção consiste na dispensa do teste de bioequivalência *in vivo* solicitado pelas autoridades sanitárias para novas cápsulas, comprimidos ou novas formulações de formas farmacêuticas orais de liberação imediata já existentes e aplicável a determinadas situações. O produto é considerado bioequivalente através da realização de um estudo comparativo de dissolução *in vitro* (VOGELPOEL, 2004).

No contexto da crescente popularização do uso de medicamentos genéricos, a isenção de estudos de bioequivalência/biodisponibilidade é uma forma de facilitar o registro de produtos, facilitando o seu acesso para a população e ao mesmo tempo evitando problemas éticos e de custo que envolvem os estudos em humanos.

A bioisenção pode ser aplicável em alguns casos, dependendo da legislação de cada país:

- quando um mesmo medicamento possui diferentes dosagens, as quais apresentam perfis de dissolução semelhantes, é possível a realização de apenas um estudo *in vivo* que demonstre bioequivalência, utilizando uma única dosagem;

- em alterações no processo produtivo e pós-registro o teste de dissolução *in vitro* pode ser utilizado para demonstrar que o produto permanece semelhante à sua versão anterior e ao produto referência.

- para novos produtos, utilizando como critério de avaliação o Sistema de Classificação Biofarmacêutica.

O Sistema de Classificação Biofarmacêutica (SCB) foi proposto por Amidon e colaboradores, em 1995, e classifica os fármacos em quatro classes diferentes com base nas características de solubilidade e de permeabilidade através da membrana intestinal (AMIDON, 1995), conforme demonstrado na Tabela 1. Essa sistematização permite estimar a absorção de fármacos administrados via oral e tem sido empregada pelas agências reguladoras, em conjunto com dados de estudos de dissolução, para a avaliação dos candidatos à isenção de estudos de biodisponibilidade (BD) e/ou bioequivalência (BE) (DAVIT, 2016).

Tabela 1. Sistema de Classificação Biofarmacêutica, adaptado de Amidon e colaboradores (1995).

Classe	Solubilidade	Permeabilidade	Correlação IVIV Esperada
I	Alta	Alta	Correlação se a taxa de dissolução for mais lenta do que a taxa de esvaziamento gástrico, caso contrário, limitada ou sem correlação.
II	Baixa	Alta	Correlação esperada se a taxa de dissolução *in vitro* for semelhante à taxa de dissolução *in vivo*, a menos que a dose seja muito alta
III	Alta	Baixa	A absorção (permeabilidade) é determinante e correlação limitada ou sem correlação com a taxa de dissolução.
IV	Baixa	Baixa	Limitada ou não esperada

Em 2000, a *Food and Drug Administration* (FDA) foi a primeira agência reguladora a publicar um guia com orientações para a isenção de testes de bioequivalência baseado no SCB para formas farmacêuticas sólidas orais de liberação imediata contendo substâncias pertencentes à Classe I. Alguns anos mais tarde, a Organização Mundial da Saúde (OMS) (2006) e a Agência Europeia de Medicamentos (EMA) (2010) também permitiram a bioisenção para substâncias da Classe III. Em 2011, uma diretriz brasileira sobre o assunto foi publicada pela Agência Nacional de Vigilância Sanitária (ANVISA). Ela permite a isenção de estudos de BD e/ou BE exclusivamente para os medicamentos descritos na lista que faz parte do documento (RDC 37, 2011). Inicialmente, a lista continha 9 substâncias e, atualmente, compreende 21 fármacos: ácido acetilsalicílico; cafeína; capecitabina; cloridrato de doxiciclina; cloridrato de memantina; cloridrato de propanolol; cloridrato de venlafaxina; dicloridrato de pramipexol; dipirona; estavudina; fluconazol; fumarato de bisoprolol; hemitartrato de rivastigmina; isoniazida; levofloxacino; metoprolol; metronidazol; paracetamol; pregabalina; sotalol e temozolomida (IN 10, 2016).

Somente em 2015, a FDA começou a considerar os medicamentos de Classe III como candidatos à bioisenção (FDA, 2015).

Monografias de bioisenção de fármacos são revisões da literatura científica que reúnem dados e avaliam se os mesmos permitem ou não que uma determinada forma farmacêutica oral, contendo uma determinada substância ativa, pode ser isenta dos estudos de biodisponibilidade e/ou bioequivalência *in vivo* com base na classe à qual pertence a substância no SCB. As informações pertinentes a serem compiladas para a elaboração dessas monografias incluem dados sobre solubilidade, permeabilidade, dissolução, farmacocinética, indicação terapêutica, índice terapêutico, excipientes empregados nos diversos produtos registrados mundialmente e problemas com biodisponibilidade e/ou bioequivalência.

Uma iniciativa da Federação Internacional de Farmacêuticos (FIP) em conjunto com a Organização Mundial da Saúde vem publicando essas monografias de medicamentos presentes na lista de Medicamentos Essenciais da OMS, desde 2004, no *Journal of Pharmaceutical Sciences***.** O projeto está sob a liderança da Professora Jennifer Dressman da Universidade de Goethe, Frankfurt am Main, na Alemanha e permite que pesquisadores de diversos países participem da elaboração das publicações. O projeto é uma ação que visa prover acesso universal a medicamentos de qualidade e, para isso, é necessário promover uma redução dos custos de desenvolvimento e registro de produtos. A bioisenção é uma conduta que visa facilitar a inserção de medicamentos genéricos no mercado e ainda reduzir os custos com pesquisas, mas com a preocupação de garantir que os produtos apresentem bom desempenho. A intenção é publicar monografias para todos os medicamentos presentes na Lista de Medicamentos Essenciais da OMS, assim como para alguns outros que não estão presentes nela, mas são considerados de grande importância. Atualmente, 45 monografias estão disponíveis para acesso na página da FIP.

O fármaco escolhido para realizar a monografia de bioisenção apresentada foi o fosfato de oseltamivir, um fármaco utilizado na prevenção e tratamento da influenza tipo A e B, utilizado em mais de 100 países e presente na Lista de Medicamentos Essenciais da OMS. Essa infecção é altamente contagiosa e está associada a altas taxas de hospitalização e mortalidade devido às complicações que ela acarreta. Embora a vacinação venha sendo utilizada como uma forma de prevenção da doença,

problemas relacionados ao seu acesso e à sua eficácia justificam o uso dos medicamentos antivirais para o tratamento da influenza. O medicamento referência Tamiflu® foi lançado em 2000, pela fabricante Roche. Em alguns países, como Brasil e Canadá e na União Europeia, versões genéricas foram aprovadas, o que pode significar um benefício para a saúde pública em termos de redução de custos para o tratamento da doença (Smith, 2011). Nesse contexto, a bioisenção baseada no SCB pode ser útil para incentivar o registro de novos produtos contendo este ativo.

Este artigo foi elaborado de acordo com as normas do *Journal of Pharmaceutical Sciences* apresentadas em anexo.

2. PROPOSTA DE ARTIGO

Biowaiver Monographs for Immediate-Release Solid Oral Dosage Forms: Oseltamivir Phosphate – a Draft Document

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ABSTRACT: Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate release (IR) solid oral dosage forms containing oseltamivir are reviewed. Available data suggest that oseltamivir is highly soluble and exhibits low permeability, hence, it would likely be assigned to BCS Class III. No BE studies comparing oseltamivir capsules formulations were identified in the literature. It is concluded that a biowaiver for oseltamivir IR solid dosage forms could be justified provided that (a) more replicates of the solubility data demonstrating high solubility are generated; (b) the test and reference product show very rapid dissolution profiles; (c) the test product formulation is qualitatively the same and quantitatively very similar to the reference.

Keywords: oseltamivir; dissolution; absorption; Biopharmaceutics Classification System (BCS); permeability; regulatory science; solubility.

INTRODUCTION

A monograph based on the literature data is presented for oseltamivir with respect to its biopharmaceutical properties. The purpose and scope of these monographs have been discussed previously. [1] To illustrate the various aspects that are considered to determine whether the biowaiver procedure can be recommended or not, biowaiver monographs for active pharmaceutical ingredients (APIs) belonging to all biopharmaceutics classification system (BCS) classes have been published (www.fip.org/bcs). The monographs have no formal regulatory status but they provide

a summary of relevant information found in the scientific literature and based on these data the authors discuss whether IR dosage forms containing a given active substance could be eligible for a biowaiver. This recommendation is dependent on the drug BCS class, molecule characteristics, formulations available in the international market which contain the drug and the risks to the patient associated with an incorrect biowaiver decision. Oseltamivir is a drug used in the treatment and prevention of influenza types A and B and is present in the World Health Organization (WHO) Model List of Essential Medicines. [2] This work aims to present biopharmaceutical characteristics of oseltamivir phosphate based on the literature data and discuss them in the context of pharmaceutical biowaiver, evaluating the possibility of exemption from bioequivalence testing for new immediate release capsules containing oseltamivir as the single API.

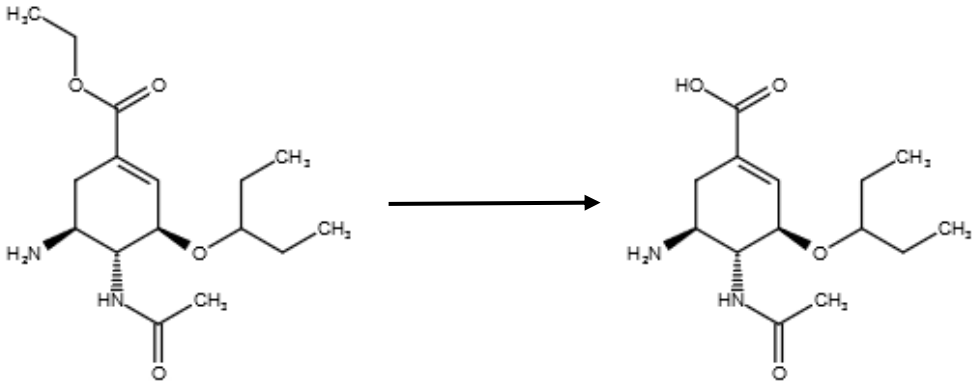
EXPERIMENTAL

A literature search was performed in the Pubmed, Web of Science and International Pharmaceutical Abstracts databases. Documents of international regulatory agencies were also consulted.

The keyword "oseltamivir" was used alone and/or in combination with one or more of the following terms: "bioequivalence", "bioavailability", "pharmacokinetics", "solubility", "permeability", "dissolution", "polymorphism", "log P", "intestinal absorption", "toxicity", "pKa" and "lipophilicity". There was no restriction to the publication period.

GENERAL CHARACTERISTICS

Oseltamivir phosphate (OP) chemical name is ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate, phosphate (1:1), [3,4] its molecular formula is $C_{16}H_{28}N_2O_4.H_3PO_4$, its molar weight is 410,4 g/mol [3,4,5] and its melting range is 192-195°C, with degradation.[6] The active drug oseltamivir carboxylate (OC) has its carboxylic group changed to ethyl ester to generate the prodrug OP which results in certain lipophilicity. The structure of oseltamivir is shown in Figure 1.



Oseltamivir Oseltamivir carboxylate

Figure 1. Chemical structure of oseltamivir and oseltamivir carboxylate.

Therapeutic Indications, Therapeutic Index and Toxicity

OP is an antiviral agent, it is supplied as a prodrug which, through its active form, OC, acts as an inhibitor of neuraminidase, a surface glycoprotein responsible for the release of progeny virions from infected host cells. Oseltamivir is indicated for the treatment and prevention of infections caused by influenza A and B viruses.[7,8]

Commonly observed adverse effects are mild to moderate in intensity and involve especially the gastrointestinal tract and include nausea and vomiting. [9,10,11] In clinical studies, oral doses up to 1000 mg daily of oseltamivir were well tolerated, resulting in no serious adverse effects. [12,13]

PHYSICOCHEMICAL PROPERTIES

Salts, Isomers and Polymorphs

Oseltamivir phosphate is a salt, each 1.3 mg is equivalent to about 1 mg of oseltamivir. The enantiomer (3R, 4R, 5S) is employed as the active substance. It is reported that oseltamivir phosphate may exhibit polymorphism, [5] nevertheless no more information in the literature was obtained as to whether the polymorphic form affects bioavailability. The production method is validated to ensure that the substance is the (3R,4R,5S)-enantiomer and that less than 100 ppm of the impurity ethyl-(1R,2R,3S,4R,5S)-4-acetamido-5-amino-2-azido-3-(1-ethylpropoxy)

cyclohexanecarboxylate is present, when determined by a suitable method such as liquid chromatography combined with mass spectrometry. [5]

Solubility

Oseltamivir phosphate is freely soluble in water (a part is soluble in 1 to 10 parts of solvent) [3,5] and its aqueous solubility is >250 mg/mL [14] and 588 mg/mL at 25°C. [15] No data on its solubility over the physiological pH range was found in the literature.

Experiments were conducted by our research group to evaluate oseltamivir API saturation point in aqueous media over distinct pH values, the results are shown in Table 1. Details of the procedure are presented in Appendix I.

Table 1. Solubility of oseltamivir phosphate over different pH values.

pH	Aqueous solvents	Solubility (mg/mL)	Dose/Solubility Ratio Calculated (mL) [a]
1.2	0.1 M HCl	618	0.121
4.5	0.05 M acetate buffer	581	0.129
6.8	0.025 M phosphate buffer	559	0.134

[a] Calculated for WHO highest recommended dose (75 mg)

Partition Coefficient

The partition coefficient (*P*) between octanol and water is commonly used as measure of lipophilicity. The distribution coefficient (log D) is appropriate to represent the partition of an ionized compound between octanol and phosphate buffer saline (pH 7.4). The active form of oseltamivir (OC) is highly polar, hence, the prodrug is administered to improve intestinal permeability and oral bioavailability. [16]

The following log *P* values have been reported to the prodrug in the literature 0.36 [17], 0.54 [18] and 1.1 [15]. Other studies reported a log *D* value of 0.36 at pH 7.4. [14,16]

p*K*a

An acid dissociation constant (p*K*a) value of 7.7 has been reported in the literature for oseltamivir. [4,14] The prodrug prevents the ionization of the carboxylic acid group present in the active form, the only ionized group in the intestinal pH is a protonated amine.

Dose and Dosage Forms Strenghts

In Brazil, oseltamivir phosphate is commercially available in the form of 30, 45 and 75 mg capsules. [19] In the US, capsules are available at the same doses, and a powder for oral suspension resulting in 6 mg/ml is also marketed. [20]

PHARMACOKINETIC PROPERTIES

Absorption and BA

After oral administration, oseltamivir is absorbed from the gastrintestinal tract and extensively converted to its active form, OC, by carboxylesterase 1 present in the liver. The drug exhibits bioavailability of 80%. [13] Another study showed approximately 93% of the orally administered dose is converted to OC in healthy Thai volunteers. [21] The pharmacokinetic profile of the active metabolite is linear and dose-proportional. Maximal concentrations of OC occur at approximately 3-4 hours after oral dosing. The plasma half-life of OC is 6-10 hours. [13]

Permeability

Oseltamivir phosphate is converted to its active form predominantly after permeation through the intestinal membrane, therefore the permeability of the prodrug should be evaluated.

It is reported that OP has moderately low coefficient of permeability in Caco-2 cells ($1.2 \pm 2.2 \times 10^{-5}$ cm/s). [17] In a physiologically based pharmacokinetic modelling study the absorption of oseltamivir in monkeys cells was predicted to be 1.5×10^{-4} cm/s which is considered a relatively high permeability, nevertheless, the same study

reports these results were calculated based on a permeability in LLC-PK1 cells of 40 nm/s (0.4 x 10^{-5} cm/s) which is considered intermediate. [14]

Distribution

OC exhibits low protein binding of 3% and 42% for the prodrug. The volume of distribution of the active form after intravenous administration is 23 L to 26 L. [13]

Metabolism and Excretion

The prodrug undergoes extensive metabolism to its active form through ester hydrolysis, the metabolite is then eliminated through glomerular filtration and renal tubular secretion. Elimination occurs mainly through renal excretion, approximately 60 to 70% of the oral dose is found in the urine as OC and less than 5% as OP. Fecal excretion of less than 20% of the oral dose also occurs (50% as OP and 50% as OC). Hence, up to 80% of an oral dose of OP can be excreted as OC. The half-life of the active form is 6 to 10 hours and 1 to 3 hours for the prodrug. No observed oxidative metabolites of OC have been reported in humans. [13] Studies have shown oseltamivir is suitable for use in diverse patient populations such as critically ill patients, [22,23,24] obese subjects. [25,26,27] Its use has also been studies in Asian populations, such as the Japanese. [28]

A study that investigated the effect of hepatic impairment on the pharmacokinetics of oseltamivir and its active form showed differences in C_{max} and AUC(0,∞) but they were considered within safety limits for the drug. Oseltamivir was considered adequately metabolized and no dose adjustment was indicated for patients with mild and moderate hepatic impairment. [29] Studies suggest dose reduction in patients with moderate levels of renal impairment [30] and severe renal failure. [13,30]

Elderly individuals are exposed to approximately 25% higher levels of OC at steady-state when compared with young individuals; however, no dosage adjustment is necessary. [12,13]

Food Effect and Interactions

Oseltamivir phosphate may be administered with or without food. The administration under altered gastric pH conditions do not change the rate or extent of absorption. [13] A study showed oral absorption of oseltamivir was not impaired in the presence of antacids containing magnesium, aluminium or calcium. [31] Another study showed coadministration with milk reduces the rate of absorption but has little effect on the overall extent of absorption of the drug. [18] There is no evidence of clinically relevant drug interactions based on *in vitro* and *in vivo* studies. [13]

DOSAGE FORM PERFORMANCE

Excipients

Oseltamivir phosphate is available as Tamiflu® capsules, manufactured by Roche in many countries. In our search, we found some similar and generic products which are also approved for use in Brazil [19], Canada [32] and in the European Union. [33] All dosage forms found until the moment are constituted by the same capsule core excipients: pregelatinized starch, povidone, croscarmellose sodium, sodium stearyl fumarate and talc.

In Vivo BE Studies

No publications were identified in the literature reporting results from bioequivalence studies between different oseltamivir capsules formulations. Nevertheless, it is reported by regulatory agencies that approved generic versions of Tamiflu® have demonstrated to be bioequivalent to the reference medicine. [32, 33]

Dissolution

The US Pharmacopoeia specifies in the monograph of oseltamivir capsules that not less than 75% of the dose should be dissolved in 900 ml of 0.1N HCl, in 20 minutes, using the apparatus 2 at 50 rpm. [3] Comparative dissolution studies between reference and test products have not been identified.

A document regarding Tamifu® 45 mg capsules equivalence to 75 mg capsules was published on EMA's website. It states that the lower dosage complies with the requirements for a fast dissolving immediate release dosage form (more than 85% of drug substance is dissolved in 15 minutes). 45 mg capsules are also considered equivalent to the 75 mg regarding their *in vitro* performance, which means they are both very rapidly dissolving. [34]

DISCUSSION

Solubility

Data show oseltamivir has a high solubility in water. Solubility tests over the physiological pH range indicate oseltamivir is highly soluble and were performed in our laboratory in small scale due to drug amount available.

The FDA [35] considers an API is highly soluble if its largest dose is soluble in 250 ml or less of aqueous media over a pH range of 1 to 6.8, at 37 °C. European Medicines Agency (EMA), [36] WHO [37] and Brazil National Health Surveillance Agency (ANVISA) [38] use the same definition, except that the pH range is 1.2 to 6.8. EMA also requires investigation at the pKa, if it is within the specified pH range. Three replicate determinations at each pH condition are indicated.

Dose/solubility ratio over the pH range of interest is around 0.1 mL, calculated based on 75 mg of oseltamivir as the highest dose strength. Thus, oseltamivir can be considered highly soluble, however tests should be carried out in triplicate in order to fulfill conditions described in current guidelines.

Permeability

Oseltamivir phosphate is a prodrug and its conversion to the active form is shown to occur predominantly after intestinal membrane permeation, in the liver, thus the permeability of the prodrug should be measured.

The FDA, EMA and OMS consider a highly permeable substance when its extent of absorption in humans is 85 percent or more of an administered dose based on human studies. Supportive data may include results from *in vitro* studies.

Data suggest oseltamivir exhibits low-intermediate permeability based on *in vitro* studies. The bioavailability of 80% also fails to meet FDA criteria of a highly permeable substance. Hence, oseltamivir cannot be typically classified as a BCS class I drug.

BCS Classification

It is not possible to establish with absolute certainty the BCS class of oseltamivir phosphate according to current guidelines. However, data suggest it belongs to BCS class III (high solubility, low permeability).

Surrogate techniques for *in vivo* BE testing

Compendial dissolution tests have been designed for quality control purposes and are not suitable to demonstrate bioequivalence between products. It is desirable that class III drugs display rapid dissolution profile to maximize the time of contact between the drug and the absorption membrane.

The FDA, EMA and WHO require that a candidate to a biowaiver which API belongs to BCS class III demonstrate that test and comparator products display very rapid *in vitro* dissolution characteristics, which means at least 85% of the labelled amount is released from the product within 15 minutes. Comparative *in vitro* dissolution should ensure similar dissolution profiles over the pH range considered relevant for absorption in the gastrointestinal tract. Test parameters to establish a very rapid dissolving profile vary for each guideline. FDA is the most conservative agency regarding this aspect and requires a lower volume of dissolution media, 500 mL, in contrast with up to 900 mL allowed by EMA, OMS and ANVISA.

Risk of Bioinequivalence Caused by Excipients and/or Manufacturing

Excipients in a pharmaceutical formulation may affect drug dissolution and, consequently, its absorption rate and bioavailability.

The FDA, EMA and WHO determine that IR solid oral dosage forms containing Class III drugs may be candidates for a biowaiver if the excipients used in the formulation do not significantly affect the absorption of the active ingredient. There is a concern that the excipients may have a greater impact on the absorption of low permeability drugs. Therefore, the product composition must be very similar quantitatively and qualitatively equal to the reference product. The generic products approved contain the exact same excipients as the reference medicine, hence, provided that they are present at the approximate same amounts, composition should not affect absorption.

Patient Risks Associated with Bioinequivalence

Considerations regarding potential risks to patients must be taken into account in case of an inappropriate decision with respect to bioequivalence.

A treatment using a bioinequivalent drug could expose the patient to subtherapeutic or overtherapeutic doses, which are related to therapeutic failure and adverse events, respectively.

Oseltamivir phosphate is not considered a narrow therapeutic index drug, its use has been shown to be safe and the adverse effects observed are mostly mild to moderate. Therefore, the risk to patients appear to be greater in the case of exposure to subtherapeutic drug levels which may result in low antiviral efficacy.

CONCLUSIONS

Solubility data were generated for oseltamivir over distinct pH values of interest. The results showed that OP fulfills the criteria of is a highly soluble drug. Proper permeability data are lacking, but the API show no evidence of high permeability through the intestinal membrane. Thus, it is suggested that oseltamivir belongs to BCS Class III. In summary, solid oral dosage forms of oseltamivir phosphate could be granted a biowaiver of BE if dissolution profile assays comparing the reference and test drug products demonstrate very rapid dissolution and if the composition of both products are qualitatively the same and quantitatively similar.

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REFERENCES

1. Vogelpoel HWJ, Amidon GL, Junginger HE, Midha KK, Moller H, Olling M, Shah VP, Barends DM. Biowaiver monographs for immediate release solid oral dosage forms based on biopharmaceutics classification system (BCS) literature data: Verapamil hydrochloride, propranolol hydrochloride, and atenolol. J Pharm Sci 2004; 93:1945–1956.
2. World Health Organization (WHO). WHO model list of essential medicines, 19th ed. Available at: http://www.who.int/medicines/publications/essentialmedicines/EML_2015_FINAL_amended_NOV2015.pdf?ua=1. Accessed Nov 20, 2016.
3. United States Pharmacopoeia-National Formulary (USP39/NF34) Rockville: Maryland, The United States Pharmacopoeial Convention, Inc.; 2016:5175-5178.
4. The Merck Index Online. Available at: https://www.rsc.org/Merck-Index/. Accessed Nov 20, 2016.
5. The International Pharmacopoeia (Ph.Int.). 5th ed. Available at: http://apps.who.int/phint/en/p/about/. Accessed Nov 10, 2016.
6. Tamiflu Product Monograph. Available at: http://www.rochecanada.com/en/products/pharmaceuticals/consumer_information/tamiflu.html. Acessed Nov 10, 2016
7. Hayden FG1, Treanor JJ, Fritz RS, Lobo M, Betts RF, Miller M, Kinnersley N, Mills RG, Ward P, Straus SE. Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: randomized controlled trials for prevention and treatment. JAMA 1999;282(13):1240-6.

8. Smith JR, Rayner CR, Donner B, Wollenhaupt M, Klumpp K, Dutkowski R. Oseltamivir in seasonal, pandemic, and avian influenza: a comprehensive review of 10-years clinical experience. Adv Ther. 2011;28(11):927-59.
9. Brewster M, Smith JR, Dutkowski R, Robson R. Active metabolite from Tamiflu solution is bioequivalent to that from capsule delivery in healthy volunteers: a cross-over, randomised, open-label study. Vaccine 2006;24(44-46):6660-3.
10. Treanor JJ1, Hayden FG, Vrooman PS, Barbarash R, Bettis R, Riff D, Singh S, Kinnersley N, Ward P, Mills RG. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. JAMA 2000;283(8):1016–24.
11. Nicholson KG, Aoki FY, Osterhaus AD, Trottier S, Carewicz O, Mercier CH, Rode A, Kinnersley N, Ward P. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomized controlled trial. Lancet 2000;355:1845–50.
12. Massarella JW, He GZ, Dorr A, Nieforth K, Ward P, Brown A. The pharmacokinetics and tolerability of the oral neuraminidase inhibitor oseltamivir (Ro 64-0796/GS4104) in healthy adult and elderly volunteers. J Clin Pharmacol 2000;40(8):836-43.
13. He G, Massarella J, Ward P. Clinical pharmacokinetics of the prodrug oseltamivir and its active metabolite Ro 64-0802. Clin Pharmacokinet 1999;37(6):471-84.
14. Parrott N, Davies B, Hoffmann G, Koerner A, Lave T, Prinssen E, Theogaraj E, Singer T. Development of a physiologically based model for oseltamivir and simulation of pharmacokinetics in neonates and infants. Clin Pharmacokinet 2011;50(9):613-623.

15. Singer AC, Nunn MA, Gould EA, Johnson AC. Potential risks associated with the proposed widespread use of Tamiflu. Environ Health Perspect. 2007;115(1):102-6.
16. Liu KC, Lee PS, Wang SY, Cheng YS, Fang JM, Wong CH. Intramolecular ion-pair prodrugs of zanamivir and guanidino-oseltamivir. Bioorg Med Chem 2011;19(16):4796-802.
17. Oo C, Snell P, Barrett J, Dorr A, Liu B, Wilding I.Pharmacokinetics and delivery of the anti-influenza prodrug oseltamivir to the small intestine and colon using site-specific delivery capsules. Int J Pharm 2003; 257(1-2):297-299.
18. Morimoto K, Kishimura K, Nagami T, Kodama N, Ogama Y, Yokoyama M, Toda S, Chiyoda T, Shimada R, Inano A, Kano T, Tamai I, Ogihara T. Effect of milk on the pharmacokinetics of oseltamivir in healthy volunteers. J Pharm Sci 2011;100(9):3854-61.
19. ANVISA. Bulário Eletrônico. Available at: http://www.anvisa.gov.br/datavisa/fila_bula/index.asp. Accessed Aug 22, 2016.
20. FDA. Orange Book. Available at: http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm?resetfields=1. Accessed Nov 23, 2016.
21. Wattanagoon Y, Stepniewska K, Lindegårdh N, Pukrittayakamee S, Silachamroon U, Piyaphanee W, Singtoroj T, Hanpithakpong W, Davies G, Tarning J, Pongtavornpinyo W, Fukuda C, Singhasivanon P, Day NP, White NJ. Pharmacokinetics of high-dose oseltamivir in healthy volunteers. Antimicrob Agents Chemother 2009;53(3):945-52.

22. Ariano RE, Sitar DS, Zelenitsky SA, Zarychanski R, Pisipati A, Ahern S, Kanji S, Rello J, Kumar A. Enteric absorption and pharmacokinetics of oseltamivir in critically ill patients with pandemic (H1N1) influenza. CMAJ 2010;182(4):357-63.
23. Taylor WR, Thinh BN, Anh GT, Horby P, Wertheim H, Lindegardh N, de Jong MD, Stepniewska K, Hanh TT, Hien ND, Bien NM, Chau NQ, Fox A, Ngoc NM, Crusat M, Farrar JJ, White NJ, Ha NH, Lien TT, Trung NV, Day N, Binh NG. Oseltamivir is adequately absorbed following nasogastric administration to adult patients with severe H5N1 influenza. PLoS One 2008;3(10):e3410.
24. Giraud C, Manceau S, Oualha M, Chappuy H, Mogenet A, Duchêne P, Ducrocq S, Hubert P, Treluyer JM. High levels and safety of oseltamivir carboxylate plasma concentrations after nasogastric administration in critically ill children in a pediatric intensive care unit. Antimicrob Agents Chemother 2011;55(1):433-5.
25. Pai MP, Lodise TP Jr. Oseltamivir and oseltamivir carboxylate pharmacokinetics in obese adults: dose modification for weight is not necessary. Antimicrob Agents Chemother 2011;55(12):5640-5.
26. Jittamala P, Pukrittayakamee S, Tarning J, Lindegardh N, Hanpithakpong W, Taylor WR, Lawpoolsri S, Charunwattana P, Panapipat S, White NJ, Day NP. Pharmacokinetics of orally administered oseltamivir in healthy obese and nonobese Thai subjects. Antimicrob Agents Chemother 2014;58(3):1615-21.
27. Chairat K, Jittamala P,Hanpithakpong W, Day NP, White NJ, Pukrittayakamee S, Tarning J. Population pharmacokinetics of oseltamivir and oseltamivir carboxylate in obese and non-obese volunteers. Br J Clin Pharmacol 2016;81(6):1103-12.

28. Schentag JJ, Hill G, Chu T, Rayner CR. Similarity in pharmacokinetics of oseltamivir and oseltamivir carboxylate in Japanese and Caucasian subjects. J Clin Pharmacol 2007;47(6):689-96.
29. Snell P, Dave N, Wilson K, Rowell L, Weil A, Galitz L, Robson R. Lack of effect of moderate hepatic impairment on the pharmacokinetics of oral oseltamivir and its metabolite oseltamivir carboxylate. Br J Clin Pharmacol 2005;59(5):598-601.
30. Gibiansky L, Giraudon M, Rayner CR, Brennan BJ, Subramoney V, Robson R, Kamal MA. Population pharmacokinetic analysis of oseltamivir and oseltamivir carboxylate following intravenous and oral administration to patients with and without renal impairment. J Pharmacokinet Pharmacodyn 2015;42(3):225-36.
31. Snell P, Oo C, Dorr A, Barrett J. Lack of pharmacokinetic interaction between the oral anti-influenza neuraminidase inhibitor prodrug oseltamivir and antacids. Br J Clin Pharmaco 2002;54(4):372-7.
32. Health Canada. Drug Product Database. Available at: https://health-products.canada.ca/dpd-bdpp/index-eng.jsp Accessed November 23, 2016.
33. European Medicines Agency. Product information. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003717/human_med_001770.jsp&mid=WC0b01ac058001d124. Accessed November 23, 2016.
34. EMA. Oseltamivir product-specific bioequivalence guidance. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000625.jsp&mid=WC0b01ac0580848f74. Accessed November 23, 2016.

35. US Food and Drug Administration. Draft guidance for industry, dissolution testing and specification criteria for immediate release solid oral dosage forms containing BCS class 1 and class 3 drugs. Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformaon/Guidances/ UCM456594.pdf. Accessed 20 Nov 2016.
36. European Medicines Agency (EMA), Committee for Medicinal Products for Human Use (CHMP). 2010. Guideline on the Investigation of Bioequivalence. Accessed March 25, 2010, at: http://www.ema.europa.eu/pdfs/human/qwp/140198enrev1fin.pdf.
37. World Health Organization (WHO). 2006. Proposal to waive in vivo bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms. Technical Report Series, No. 937, 40th Report, Annex 8 of WHO Expert Committee on Specifications for Pharmaceutical Preparations. Available at: http:// whqlibdoc.who.int/trs/WHO TRS 937 eng.pdf. Accessed Nov 20, 2016
38. ANVISA. Resolução da Diretoria Colegiada - RDC nº 37 de 03/08/2011. Available at: http://portal.anvisa.gov.br/legislacao#. Accessed Nov 23, 2016.

Appendix I – Solubility test procedure

Oseltamivir phosphate (OP) saturation point was evaluated in usual aqueous dissolution media over different pH values. Three distinct aqueous media usually applied to drug dissolution assessment: 0.1 M HCl, 0.05 M acetate buffer pH 4.5 and 0.025 M phosphate buffer pH 6.8 were prepared according Brazilian Pharmacopoeia 5th edition to determine OP solubility.

OP chemical standard 100.3%, from Roche®, was kindly donated by Farmanguinhos Laboratory (Rio de Janeiro, Brazil), was used to obtain the UV spectrum of the drug and applied to construct three standard curves for analytical quantitation. OP standard stock solution was prepared in water to achieve 1.0 mg/mL and dilution in each aqueous media was performed to reach concentrations from 50 to 150 µg/mL of the drug. Solutions were analyzed on UV spectrophotometer (Shimadzu) and the absorbance was initially measured at 240 nm, once USP 39 preconizes this wavelength in dissolution tests of OP capsules, even though in this region a maximum of energy absorption was not observed. In all media, the slope of the standard curves obtained was around 0.0061 $UA.\mu g^{-1}.mL$ and $R^2 > 0.9999$.

For the solubility procedure, sequential additions of weighted amounts of OP bulk material (Farmanguinhos, Rio de Janeiro, Brazil) was added to 1 mL of each media in duplicate, until saturation was visually observed. An increase of solution viscosity was noticed at each addition, as a considerable amount of solute (over 0.5 g) was incorporated to only one mL of each medium.

After visual observation of OP precipitation, systems were kept under agitation for 24 hours at 37°C . Next, samples were centrifuged for 10 min and the supernatant filtered with 0.45 µm syringe filters. Aliquots were diluted (5000 fold) with respective medium and analyzed by UV spectrophotometer. Concentration of samples solutions were assayed through the standard equation. Taking into consideration the dilution factor of the samples, the exact amount of solubilized OP in 1 mL of each medium studied was calculated.

3. ANEXO 1 - Regras do *Journal of Pharmaceutical Sciences*

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For edited books: Rall TW, Schleifer LS. Drugs effective in the therapy of the epilepsies. In: Gilman AG, Goodman LS, Rall TW, Murad F, eds. *The Pharmacological Basis of Therapeutics, 7th ed.*, New York: Macmillan Publishing Co.; 1985:446-472.

For web references: Health Care Financing Administration. 1996 statistics at a

glance. Available at: http://www. hcfa.gov/stats/stathili.htm. Accessed December 2, 1996.

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