COMMENTARY

Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms Based on Biopharmaceutics Classification System (BCS) Literature Data: Chloroquine Phosphate, Chloroquine Sulfate, and Chloroquine Hydrochloride

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ABSTRACT: Literature data on the properties of chloroquine phosphate, chloroquine sulfate, and chloroquine hydrochloride related to the Biopharmaceutics Classification System (BCS) are reviewed. The available information indicates that these chloroquine salts can be classified as highly soluble and highly permeable, i.e., BCS class I. The qualitative composition of immediate release (IR) tablets containing these Active Pharmaceutical Ingredients (APIs) with a Marketing Authorization (MA) in Belgium (BE), Germany (DE), Finland (FI), and The Netherlands (NL) is provided. In view of these MA's and the critical therapeutic indication of chloroquine, it is assumed that the registration authorities had evidence that these formulations are bioequivalent to the innovator. It is concluded that IR tablets formulated with these excipients are candidates for a biowaiver. © 2005 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 94:1389–1395, 2005

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INTRODUCTION

A monograph based on literature data is presented on the three salt forms of chloroquine in therapeutic use, concerning their properties related to the Biopharmaceutics Classification System (BCS). Purpose and scope of these monographs were discussed previously.[1] The working procedure was identical as described earlier.[1] The objectives of these monographs are to evaluate all data from various literature sources and to come to a conclusion whether or not to recommend a biowaiver for immediate release (IR) solid oral dosage forms containing one of the water-soluble chloroquine salts.

GENERAL CHARACTERISTICS

Chloroquine, chemical name: 7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline;possesses an asymmetric carbon atom and therefore exists as two enantiomers, S(+)-chloroquine and R(−)-chloroquine.[2] Its structure is shown in Figure 1. Only preparations containing the racemic mixture are commercially available. In this monograph, chloroquine is understood to be the racemic form, unless otherwise indicated. Only salts are normally used: the diphosphate (usually called chloroquine phosphate), the sulfate, and hydrochloride.[3] For the phosphate, the sulfate and the hydrochloride, 100 mg chloroquine base is equivalent to 161, 136, and 123 mg of these salts, respectively.[3] The wording chloroquine is used to describe attributes that all three substances have in common, otherwise the specific salt is mentioned.

Therapeutic Indication

Chloroquine is used in the treatment and prophylaxis of malaria and has also been used in the treatment of hepatic amoebiasis, lupus erythematosus, and light-sensitive skin eruptions.[3] Chloroquine possesses anti-inflammatory properties and rheumatoid arthritis is a further indication for this drug.[3,4] Frequent Adverse Drug Reactions (ADRs) of chloroquine include headache, various skin eruptions, pruritus, and gastrointestinal (GI) disturbances such as nausea, vomiting, and diarrhea.[3] More rarely, mental changes including psychotic episodes, agitation, and personality changes may occur.[3] Retinopathy is a severe ADR of chloroquine and can result in visual impairment.[3] Acute overdose with chloroquine is extremely dangerous and death can occur within a few hours.[3]
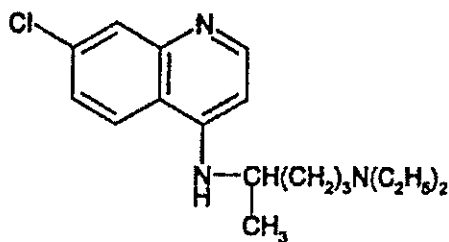

Figure 1.   Structure of chloroquine.

CHEMICAL PROPERTIES

Solubility

Choroquine Phosphate

Chloroquine phosphate is freely soluble in water.[3,5,6] An aqueous solubility of 1 in 4 was reported.[7] It is not clear if this means 1 part dissolved in 4 part solution, i.e., 250 mg/ml, or 1 part dissolvable in 4 parts of water, i.e., 200 mg/ml. Other workers reported a solubility of 100 mg/ml in water.[8]

Chloroquine Sulfate

Chloroquine sulfate is freely soluble in water.[3,5] Other workers reported an aqueous solubility of 1 in 3, i.e., 250 or 333 mg/ml, see above.[7]

Chloroquine Hydrochloride

No literature data were found.

Polymorphism

Chloroquine phosphate exists in two polymorphic forms which have melting points at approximately 195 and 218°C.[6]

Partition Coefficient

For the uncharged chloroquine base, a log P (n-octanol/water) of 3.73 was reported, this value being calculated using a fragmentation method based on atomic contributions to lipophilicity.[8] ClogP, calculated by using the ClogP program (version 3.0, Biobyte Corp., Clalement, CA, http://www.biobyte.com.) was 5.06.[8] Augustijns measured partitioning of chloroquine in octanol/transport medium pH 7.2 at different temperatures.[9] The logarithm of the distribution coefficient, log D, at pH 7.2 and 37°C was 0.83.

pKa

Chloroquine possesses two basic ionization sites. pKa values of 8.1 and 10.4 at 37°C and 8.4 and 10.8 at 20°C, respectively, were reported.[2,10] Other workers reported values of 8.10 and 9.94 without referring to temperature.[6]

Available Dose/Tablet

The usual tablet strength is the equivalent of 100 mg chloroquine base.[3] The dose recommend by the WHO for tablets is 150 mg base (as phosphate or sulfate).[11]

PHARMACOKINETIC PROPERTIES

Permeability

Augustijns studied the transport of chloroquine phosphate in Caco-2 cell monolayers and concluded that chloroquine crosses the intestinal epithelium by passive diffusion.[9] Apparent permeability coefficients (P_{app}) reported are 2.3×10^{-6} and 20.0×10^{-6} cm/s at pH 6.0 and 7.2, respectively.[9] However, these data were obtained without establishing an in-house correlation between P_{app} and the fraction dose absorbed (fa) for a set of model drugs.[12,13] Also, no internal standards as proposed by the FDA Biowaiver guideline were used.[14]

Absorption

Chloroquine is rapidly and almost completely absorbed from the gastro-intestinal (GI) tract when given orally.[15] The average oral bioavailability (BA) is approximately 89%.[16] However, the intersubject variability in chloroquine absorption is high; oral BA values between 67% and 114% have been reported.[15,17] The oral BA of chloroquine was significantly reduced, by more than 60% i.e., to 40%, in malnourished children.[18]

The absorption of chloroquine is increased when taken with food.[3] For example, following administration of an oral dose with a standard breakfast, the plasma peak concentration (C_{max}) and area under the curve (AUC) of chloroquine were 152% and 142% respectively, compared to administration on an empty stomach.[19]

In contrast, a significant reduction in BA was observed when chloroquine phosphate was administered with three common Sudanese beverages.[20] Co-administration of chloroquine phosphate with any one of these three beverages reduced C_{max} and AUC both by approximately 70%. All three beverages were fairly acidic (pH 2.6–2.8) and the authors postulated that the intake of these beverages increased the ionization of chloroquine in the GI tract and hence reduced the absorption of chloroquine base. Moreover, acidification of the urine by these beverages may have reduced the tubular reabsorption of chloroquine and consequently increased its renal clearance. The authors suggested that both mechanisms may have contributed to the observed significant reduction in the BA.

Distribution

The pharmacokinetics of chloroquine have been reviewed.[15,17] Chloroquine is bound to an extent of 60% to plasma proteins.[21,22] Chloroquine is much more extensively bound to various body tissues, including the cellular components of blood.[23,24] The combination of moderate plasma protein binding and extensive binding to tissues explains its extremely large distribution volume of 200–800 L/kg.[15,17] The total body clearance of chloroquine is approximately 0.10 L/h/kg based on whole blood concentrations, and 0.7–1.0 L/h/kg based on plasma concentrations.[15,17] The long terminal plasma half-life of chloroquine, ranging from 20 to 60 days, is due to its large distribution volume. The pharmacokinetics of chloroquine were shown to be linear following administration of single oral doses between 2 and 15 mg/kg chloroquine base.[25]

Metabolism and Excretion

Metabolism and renal excretion contribute equally to the elimination of chloroquine: approximately 40%–50% of the administered dose has been reported to be excreted unchanged in the urine in individuals with normal renal function.[24] Chloroquine is rapidly dealkylated to the pharmacologically active N-desethylchloroquine, bisdesethylchloroquine, and 7-chloro-4-aminoquinoline.[15,17,26] Additional metabolites, such as chloroquine N-oxide and chloroquine di-N-oxide have been detected in plasma and/or urine. In vitro studies on human liver microsomes have identified CYP2C8, CYP3A4, and CYP2D6 as the main cytochrome P450 isoforms catalyzing the formation of N-desethylchloroquin.[27] Augustijns et al.[28] studied the pharmacokinetics of both chloroquine enantiomers and showed moderate, but statistically significant differences in their terminal elimination half-lives and body clearances. The clinical consequences of the stereoselective pharmacokinetics of R- and S-chloroquine on efficacy and toxicity are not known.

DOSAGE FORM PERFORMANCE

Excipients

The excipients present in IR tablets having a marketing authorization (MA) in Belgium (BE), Germany (DE), Finland (FI), and The Netherlands (NL) are shown in Table 1. In previous

Table 1. Excipients Present in Chloroquine IR Tablets with a MA in Belgium (BE), Germany (DE), Finland (FI), and The Netherlands (NL)

Excipient	Country
Chloroquine sulfate	
Gelatine	BE[a], NL[b]
Magnesium stearate	BE[a], NL[b]
Silica, hydrated	BE[a], NL[b]
Sucrose	BE[a], NL[b]
Wheat starch	BE[a], NL[b]
Chloroquine phosphate	
Cellulose (microcrystalline)	DE[c], FI[d]
Gelatin	DE[e], FI[d]
Hypromellose	DE[37,38], FI[d]
Lactose anhydrous/monohydrate	DE[c], FI[d]
Macrogol	DE[e,f], FI[d]
Magnesium stearate	DE[c,e,f], FI[d]
Maize starch	DE[e,f], FI[d]
Pregelatinised starch	FI[d]
Povidon	DE[c], FI[d]
Sodium starch glycollate	DE[c], FI[d]
Silica, hydrated	FI[d]
Talc	DE[e,f]
Titandioxide	DE[e,f], FI[d]

[a]Nivaquine®, SmPC in Belgium.

[b]Nivaquine®, SmPC in The Netherlands, http://www.cbg-meb.nl/IB-teksten/00303.PD.

[c]Weimer®quin/-forte Tabletten. ROTE LISTE® 2004 Arzneimittelverzeichnis für Deutschland, ed., Aulendorf Germany: ECV Editio Cantor Verlag, http://www.rote-liste.de.

[d]Heliopar®, SmPC in Finland, www.nam.fi/laakeinformaatio/index.html.

[e]Chlorochin 250 mg Berlin-Chemie. ROTE LISTE® 2004 Arzneimittelverzeichnis für Deutschland, ed., Aulendorf Germany: ECV Editio Cantor Verlag, http://www.rote-liste.de.

[f]Resochin® junior/Resochin® Tabletten. ROTE LISTE® 2004 Arzneimittelverzeichnis für Deutschland, ed., Aulendorf Germany: ECV Editio Cantor Verlag, http://www.rote-liste.de.

monographs, such an MA was taken as an indication that this formulation had passed an *in vivo* bioequivalence test.[1] However, it appears that this assumption is not so straightforward. In most countries, regulatory authorities have accepted and do accept small changes in a formulation without an *in vivo* bioequivalence study. Therefore, it is not certain that all the formulations shown in Table 1 have passed an *in vivo* bioequivalence study. However, in view of the critical therapeutic indication of chloroquine, we suppose that the registration authorities of BE, DE, FI, and NL were in receipt of sufficient evidence to conclude to bioequivalence, even for formulations that were not actually tested in an *in vivo* bioequivalence study.

Dissolution

Chloroquine Phosphate Tablets USP 27 are required to dissolve in the paddle apparatus at 100 rpm not less than 75%(Q) of the labeled amount in 45 min in 900 mL water.[29] Risha et al.[30] evaluated the quality of the innovator and generic chloroquine phosphate tablets on the Tanzanian market as described in USP 27. In all cases not less than 90% dissolution was observed at 45 min.

DISCUSSION

Solubility

It seems safe to suppose that the solubility of the hydrochloride salt is comparable to that of the phosphate and the sulfate and hence these three chloroquine salts are freely soluble in water. However, solubility data over the full pH range are lacking. For a biowaiver, the FDA and EMEA guidances require the API to be "highly soluble" over the pH range 1.0–7.5 (FDA) or within the range of pH 1–8, preferably at or about pH 1.0, 4.6, and 6.8 (EMEA).[14,31] Moreover, when a biowaiver is granted, comparative dissolution testing of the test formulation and the reference formulation is to be carried out at three different pH values between 1.0 and 6.8.[14,31] The test formulation has to be "rapidly dissolving" in each of the three media, which is an additional indication that the solubility of the API is sufficiently high over the pH-range 1.0–6.8.

Absorption and Permeability

The permeability data for chloroquine based on Caco-2 experiments are inconclusive because the method was not validated by using reference compounds as proposed by the FDA guidance.[9,14] Caco-2 permeability determinations are known to display tremendous inter-laboratory variability and the use of reference compounds is therefore essential. However, the permeability can also be estimated from BA data. The FDA Guidance defines "highly permeable" when the fraction of dose absorbed (fa) is not less than 90%.[14] The reported BA of chloroquine, which is on average 89%, is slightly below that critical limit. It was suggested that a limit of 85% might be appropriate in defining high permeability.[32] Also, during a recent workshop consensus was reached that the minimum value of fa can be lowered to 85%.[33] The permeability can also be estimated

from the partition coefficient, as was shown by Kasim et al.[8] In their report, the permeability of 123 substances on the WHO Essential drugs list was estimated based on correlations of experimentally determined human intestinal permeabilities of select compounds with log P, ClogP, or log D values.[8] Substances with a log P, ClogP, or log D greater than the corresponding values of the reference substance metoprolol, i.e., 1.72, 1.35, and −1.48, respectively, were classified as highly permeable. Chloroquine phosphate with values for log P, ClogP, and Log D of 3.73, 5.06, and 0.83,[9] respectively, was therefore classified to be "highly permeable".

All evidence taken together, it is concluded that chloroquine is highly permeable.

Risks of Bioinequivalence Caused by Excipient and/or Manufacturing Conditions

In the tablets which have an MA in several European countries, a wide range of excipients is used. Although, as discussed above, there is no solid proof that all the formulations shown in Table 1 have actually passed an *in vivo* bioequivalence study, it can be assumed that the registration authorities had evidence they would be bioequivalent, if tested *in vivo*. This suggests that the risk of an excipient effect on the BA of chloroquine for the excipients listed in Table 1 is small for the amounts normally present in IR tablets.

Food interaction itself has no influence on the bioequivalence as long as the BA of the test product and the reference product are influenced to the same degree, but food interactions can indicate a potential risk for an excipient interaction. For instance, the reported reduction of the BA caused by acidic beverages could indicate that there is a risk that a test product, containing acidic excipients, may be bioinequivalent when the reference product does not contain such excipients. The same holds for the reported increase of the BA with food, indicating a potential risk for bioequivalence caused by very lipophilic excipients. However, the excipients shown in Table 1 are neither highly acidic nor strongly lipophilic. All taken together, it is concluded that for excipients listed in Table 1, used in amounts normally present in IR tablets, the risk of bioinequivalence is small.

Patient's Risks Associated with Bioinequivalence

When considering a biowaiver for a drug substance, its therapeutic index and indication also need to be taken into account.[14,31,32] Chloroquine is indicated for serious diseases and very serious ADRs have been reported. The latter, however, have been documented in cases of overdose, and not as a result of relatively minor fluctuations in plasma concentrations such as those which could be seen in case of bioinequivalence.

In malaria therapy, resistant parasites are most likely to be selected if the parasite population is exposed to subtherapeutic drug concentrations.[34] Consequently, assuring the BA of chloroquine tablets is of utmost importance.

Considerations of the therapeutic index and the pharmacokinetics of chloroquine led in 1998 the German regulatory authorities to catogorize chloroquine as an API for which biowaivers could not be granted.[35]

Dissolution

The *in vitro* dissolution test for chloroquine phosphate tablets described in the USP 27 uses water. This unbuffered medium is very sensitive to pH changes. In view of the insolubility of chloroquine and its salts at alkaline pH-values, a buffered medium, with a pH corresponding to the limits of the solubility of chloroquine in water seems to be more discriminating. However, once bioequivalence has been established, *in vivo* or *in vitro*, the test USP 27, when applied for batch-to-batch testing, will provide sufficient assurance of batch-to-batch bioequivalence.

CONCLUSION

Although the data do not provide full proof, there can be little doubt that neither the solubility, nor the permeability of these salts of chloroquine are limiting factors in the GI absorption. Consequently, they are classified as BCS Class I. Other workers also classified chloroquine phosphate as BCS class I.[8,36] This suggests that these chloroquine salts are candidates for a biowaiver.

When considering a biowaiver, difficulties associated with carrying out *in vivo* bioequivalence studies with chloroquine is also to be considered. Because of its exceptionally long plasma half-life, long washout periods are needed. This supports biowaiving.

The potential consequences of an incorrect biowaiver decision, leading to a bioinequivalent product, should also be considered. These consequences are serious. However, the risk of bioinequivalence that cannot be detected with comparative dissolution testing in pH 1.0. 4.5,

and 6.8, is estimated to be very low. This risk is even lower when formulations contain only the excipients shown in Table 1.

We conclude that for chloroquine hydrochloride, chloroquine phosphate, and chloroquine sulfate IR tablets granting a biowaiver is justified for formulations containing the excipients shown in Table 1, comply with the requirements for "rapidly dissolving" at pH 1.0, pH 4.5, and pH 6.8[14,31] and also comply with the similarity requirements for comparative dissolution testing versus the reference product at pH 1.0, pH 4.5, and pH 6.8.[14,31]

REFERENCES

1. Vogelpoel H, Welink J, Amidon GL, Junginger HE, Midha KK, Moller H, Olling M, Shah VP, Barends DM. 2004. 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 93:1945–1956.

2. Tariq M, Al-Badr AA. 1984. Chloroquine. In: Florey K, editor. Analytical profiles of drug substances. New York: Academic Press. pp 95–125.

3. Sweetman S, editor. 2004. Martindale: The complete drug reference. Electronic version. London UK: Pharmaceutical Press, Thomson MICRO-MEDEX Healthcare Series Vol. 123, expires 3/2005, Greenwood Village, Colorado.

4. Furst DE. 1996. Pharmacokinetics of hydroxychloroquine and chloroquine during treatment of rheumatic diseases. Lupus 5:S11–S15.

5. European Pharmacopoeia, 4th edn. Strasbourg, France: Council of Europe, European Directorate for the Quality of Medicines.

6. Hong DD. 1976. Chloroquine phosphate. In: Florey K, editor. Analytical profiles of drug substances. New York: Academic Press. pp 61–85.

7. Moffat AC, Jackson JV, Moss MS, Widdop B, editors. 1986. Clarke's isolation and identification of drugs, 2nd edn. London UK: The Pharmaceutical Press.

8. Kasim NA, Whitehouse M, Ramachandran C, Bermejo M, Lennernäs H, Hussain AS, Junginger HE, Stavchansky SA, Midha KK, Shah VP, Amidon GL. 2004. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. Mol Pharm 1:85–96.

9. Augustijns PF. 1996. Uptake and transport characteristics of chloroquine in an in vitro cell culture system of the intestinal mucosa, Caco-2. J Pharm Pharmacol 48:277–280.

10. Ferrari V, Cutler DJ. 1987. Temperature dependence of the acid dissociation constants of chloroquine. J Pharm Sci 76:554–556.

11. WHO Model List of Essential Medicines 13th edn. Available form URL: www.who.int/medicines/organization/par/edl/expcom13/eml13_en.doc.

12. Artursson P, Palm K, Luthman K. 2001. Caco-2 monolayers in experimental and theoretical predictions of drug transport. Adv Drug Deliv Rev 46:27–43.

13. Ungell AL, Karlsson J. 2003. Cell cultures in drug discovery: An industrial perspective. In: Waterbeemd Hvd, Lennernäs H, Artursson P, editors. Drug bioavailability: Estimation of solubility, permeability, absorption and bioavailability. Weinheim, Germany: Wiley-VCH. pp 90–131.

14. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2000. Guidance for industry: Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. Available form URL: http://www.fda.gov/cder/guidance/3618fnl.pdf.

15. Krishna S, White NJ. 1996. Pharmacokinetics of quinine, chloroquine and amodiaquine. Clinical implications. Clin Pharmacokinet 30:263–299.

16. Benet LZ, Øie S, Schwartz JB. 1996. Design and optimization of dosage regimens; pharmacokinetic data. Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th edn. New York: McGraw-Hill. pp 1707–1792.

17. Ducharme J, Farinotti R. 1996. Clinical pharmacokinetics and metabolism of chloroquine. Focus on recent advancements. Clin Pharmacokinet 31:257–274.

18. Walker O, Dawodu AH, Salako LA, Alvan G, Johnson AO. 1987. Single dose disposition of chloroquine in kwashiorkor and normal children—Evidence for decreased absorption in kwashiorkor. Br J Clin Pharmacol 23:467–472.

19. Tulpule A, Krishnaswamy K. 1982. Effect of food on bioavailability of chloroquine. Eur J Clin Pharmacol 23:271–273.

20. Mahmoud BM, Ali HM, Homeida MM, Bennett JL. 1994. Significant reduction in chloroquine bioavailability following coadministration with the Sudanese beverages Aradaib, Karkadi and Lemon. J Antimicrob Chemother 33:1005–1009.

21. Walker O, Birkett DJ, Alvan G, Gustafsson LL, Sjoqvist F. 1983. Characterization of chloroquine plasma protein binding in man. Br J Clin Pharmacol 15:375–377.

22. Ofori-Adjei D, Ericsson O, Lindstrom B, Sjoqvist F. 1986. Protein binding of chloroquine enantiomers and desethylchloroquine. Br J Clin Pharmacol 22:356–358.

23. Ferrari V, Cutler DJ. 1990. Uptake of chloroquine by human erythrocytes. Biochem Pharmacol 39:753–762.

24. Gustafsson LL, Walker O, Alvan G, Beermann B, Estevez F, Gleisner L, Lindstrom B, Sjoqvist F.