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General Commentary

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Biowaiver monograph for immediate-release dosage Forms: Levamisole hydrochloride

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ABSTRACT

This work describes the potential applicability of the BCS-based Biowaiver to oral solid dosage forms containing Levamisole hydrochloride, an anthelmintic drug on the WHO List of Essential Medicines. Solubility and permeability data of levamisole hydrochloride were searched in the literature and/or measured experimentally. Levamisole hydrochloride is a highly soluble drug, but there is no clear evidence of high permeability in humans, indicating that it should provisionally be assigned to BCS class III. The biowaiver procedure would thus be applicable for solid oral dosage forms containing levamisole hydrochloride as the only active ingredient. Due to the lack of data in the literature regarding excipient effects on the bioequivalence of products containing levamisole, it is currently recommended that the products comply with the ICH and WHO guidelines: the test formulation should have the same qualitative composition as the comparator, contain very similar quantities of those excipients, and be very rapidly dissolving at pH 1.2, 4.5, and 6.8. However, for certain well-studied excipients, there appears to be opportunity for additional regulatory relief in future versions of the ICH BCS Guidance M9, such as not requiring that the quantities of these common excipients in the test and comparator be the same.

Introduction

The present monograph reviews the available data on the biopharmaceutical properties of levamisole hydrochloride with the aim of evaluating the applicability of a BCS-based biowaiver. According to the biowaiver approach, in vitro dissolution studies can serve as a surrogate for establishing in vivo bioequivalence.[1,2] This is considered appropriate only for immediate-release formulations which contain highly soluble drug substances. Approval according to the biowaiver approach has the advantage of avoiding unnecessary and expensive in vivo bioequivalence (BE) studies for formulation changes, manufacturing changes, additional dosage strengths, and generic formulations. With the advent of the internationally recognized ICH

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Guidance (M9) on approvals via the BCS-based biowaiver concept,[1] it is expected that there will be an uptick in applications based on this concept across the globe.

This monograph is part of a series of biowaiver monographs for essential drugs, in particular those which are listed on the WHO model list of essential medicines.[3] The series of biowaiver monographs was initiated by the International Pharmaceutical Federation (FIP) .[4] Current biowaiver monographs can be found at the FIP website (http://www.fip.org/bcs_monographs) or at the Journal of Pharmaceutical Sciences website (https://jpharmsci.org/biowaiver-monographs) free of charge. In each monograph, relevant literature and experimental data are collected and used to evaluate whether a biowaiver can be recommended for use in future applications. In this monograph the requirements for such biowaivers and possible risks of the biowaiver approach are considered for levamisole hydrochloride.

Materials and Methods

Materials

Levamisole hydrochloride was obtained from Shaanxi Hanjiang Pharmaceutical Group Co., Ltd. (Hantai, China).

Methods

Different databases, such as PubMed, Google Scholar and Scopus, were used to obtain data for solubility, permeability, stability, absorption, bioavailability, bioequivalence, etc. of levamisole hydrochloride in the literature. Both the solubility and stability of levamisole hydrochloride were evaluated in the present study.

To establish the “highly soluble” classification of levamisole hydrochloride, solubility of the pure API at 37 °C was studied using the paddle apparatus at 50 rpm. Levamisole hydrochloride (500 mg as levamisole base) housed in a capsule was added to 250 mL of an aqueous solution with a pH of 1.2, 4.5, 6.8, or 7.6. Since the recommended highest single dose of levamisole is 150 mg, the amount applied corresponds to more than twice the concentration required to qualify levamisole as a highly soluble compound. Samples were withdrawn from the vessels at 45 min and the drug concentration was measured using a UV spectrophotometry at 214 nm. The dissolution tests were conducted in duplicate at each pH value.

In the stability testing, aqueous solutions of levamisole hydrochloride at a concentration of 0.056 mg/mL (as levamisole base) in pH 1.2 or 6.8 were prepared. After 1 hour of incubation at 37 °C, the remaining drug concentrations in the solutions were measured using an HPLC-UV method.

General Characteristics

Levamisole is the levo-isomer of tetramisole. It was discovered by Janssen Pharmaceutica in 1966 and belongs to the class of synthetic imidazothiazole derivatives. Its chemical name is (6S)−6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b][1,3]thiazole. The chemical structure is shown in Fig. 1. Levamisole is primarily available as a hydrochloride salt, and in this form it has a molecular weight of 240.8 g/mol. An amount of 50.0 mg levamisole base is equivalent to 58.9 mg levamisole hydrochloride.

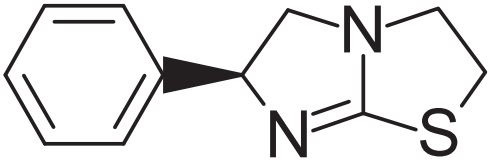

Figure 1. Chemical structure of levamisole.

Therapeutic Indications

Levamisole was originally used as an anthelmintic drug in human and veterinary medicine. It is an effective treatment of intestinal roundworm (ascariasis) and hookworm (ancylostomiasis) infections.[5] It acts through activation of acetylcholine receptors resulting in paralysis and passive elimination of the parasites. Levamisole is listed by the WHO as an essential medicine for treatment of intestinal worm infections in both adults and children.[3,6]

During its development in the 1960s, levamisole was found to exhibit twice as much anthelmintic activity as the racemic form, tetramisole.[7] As the levo- and dextro-isomers showed similar animal toxicity, the more active levo-isomer levamisole was selected for clinical use. While levamisole has been widely used as an anthelmintic drug, it is now more commonly prescribed for its immunomodulating activity. Levamisole was demonstrated to be able to restore impaired host immune responses, mainly through T-cell activation.[8] Thereafter, it was investigated extensively in patients with immunological conditions, such as rheumatoid arthritis,[9] cancer[10] and nephrotic syndrome.[11] However, levamisole is nowadays only rarely prescribed in these therapeutic indications.

In the 1990s, levamisole was approved for the adjuvant treatment of colon cancer in combination with 5-fluoruracil. It was, however, voluntarily withdrawn by the manufacturer after several years, due to the superior efficacy of other drug combinations for adjuvant therapy.[12]

Currently, levamisole is primarily available as a veterinary anthelmintic drug with applications for human use limited to treatment of intestinal worms.

Dose

Doses for levamisole hydrochloride are expressed as the free base. Ascariasis infections are treated with a single oral dosage of 150 mg in adult humans. Children are given a body weight-adjusted dosage of 3 mg/kg. Both adults and children receive a body weight-adjusted dosage of 2.5 mg/kg to treat ancylostomiasis or mixed infections. It may be necessary to administer a second dosage after 1 week in severe ancylostomiasis infections.[5]

Toxicity and Therapeutic Index

Levamisole has cholinergic and immunomodulating activity. The main sites of toxicity are the gastrointestinal system, the central nervous system, and the bone marrow.[13] The adverse drug reactions after single-dose anthelmintic therapy are generally limited to mild gastrointestinal symptoms and headache. Extended treatment is necessary in immunological conditions and in this case toxicity occurs more frequently. Adverse drug reactions may include gastrointestinal upset (abdominal pain, nausea, vomiting, diarrhea, abnormal taste), neurological abnormalities (headache, dizziness, insomnia, convulsions), hematological abnormalities (agranulocytosis, leukopenia, thrombocytopenia), and hypersensitivity reactions (pyrexia, arthralgia, myalgia, rash, cutaneous vasculitis) .[14]

Human fatalities have been reported after 15 mg/kg in a child and 32 mg/kg in an adult.[13] Non-fatal overdoses caused exacerbation of cholinergic and neurological effects.[15–17] Multifocal inflammatory leukoencephalitis has been reported at large overdoses as well as in low incidences at therapeutic dosages of levamisole alone and in combination with 5-fluoruracil.[5,15,17–19]

Although the therapeutic index for levamisole is lower than for other anthelmintics,[20,21] it is not classified as a narrow therapeutic index drug.

Physicochemical Properties

Salt form and polymorphism

Levamisole is used primarily as the hydrochloride salt. Levamisole hydrochloride has a melting point of 227 – 229 °C.[22] No information on polymorphism is available in the open literature.

pKa and Partition Coefficient

Levamisole is a weak base with reported pKa values ranging from 6.75[23] to 6.98.[24] In the fasted stomach, the ionized form of levamisole is the predominant species, while in the small intestine, approximately half of the drug would present as the non-ionized form. The predicted log P value of levamisole is 2.36.[24]

Solubility

Levamisole hydrochloride is freely soluble in water according to the European Pharmacopeial monograph.[25] A 50 mg/mL solution in water is acidic with a pH between 3.0 – 4.5.[25,26] The solubility data (Table 1) generated in the present study (see section 2 for experimental details) demonstrated that the solubility of this drug is 2.0 mg/mL or more in four different pH solutions (pH 1.2, 4.5, 6.8, 7.6).

Stability

In the open literature, levamisole was shown to be 'practically stable' at a low pH of 2 – 3, but the degradation rate rapidly increases at pH 5 – 8 particularly with increasing temperatures.[23,27,28] At a temperature of 37 °C, some degradation products were formed within 4 h at pH 7.0 – 7.5.[28]

The experimental data generated in the present study indicated that the fraction remaining in pH 1.2 and 6.8 solutions of levamisole hydrochloride is 100% and 99% respectively, when the aqueous solutions are stored at 37 °C for 1 hour. Thus, it seems unlikely that there would be more than 10% degradation within the time limits specified in the ICH guidance (one hour for gastric and three hours for intestinal conditions).

Available Dosage Forms

The 22nd WHO model list of essential medicines has listed tablet strengths of 50 and 150 mg for levamisole hydrochloride.[3] The originator product, Ergamisol (50 mg tablets), has been withdrawn from the U.S. and European market. Levamisole tablets have been available through other manufacturers worldwide, but are not currently available in the US and Japanese markets. However, in Eastern Europe, current marketing authorisations (MAs) exist for 50 and 150 mg tablet formulations (see Table 2).

Pharmacokinetic Properties

Absorption and Permeability

Although the absolute BA for levamisole has not been studied in humans, the oral BA is estimated to be 62.5% after a single dosing of 150 mg levamisole[29] or 65% – 68% following 2.5 – 5 mg/kg dosing.[30] The total radioactivity excreted in urine is about 70% and in the feces less than 4% within 72 h after oral dosing of 150 mg of levamisole.[31]

The absorption of levamisole is generally rapid, with a Tmax of about 1 – 2 h.[29,30] The rate and extent of absorption may be influenced by gender differences and gastrointestinal disease.[29,32] An effect of food on oral absorption in humans has not been described in the literature. Comparative BA between tablet and syrup formulations of this drug revealed no significant differences in pharmacokinetic parameters (Cmax and AUC) in humans (n = 3) .[31]

The effective permeability in the intestinal epithelium in humans and the apparent permeability in Caco-2 cell monolayers of levamisole have not been reported in the literature to date.

Table 1
Solubility of levamisole hydrochloride at different pH values.

pH	Solubility (mg/mL)	Dose/Solubility Ratio at a Dose of 150 mg
1.2	≥ 2.0	≤ 75 mL
4.5	≥ 2.0	≤ 75 mL
6.8	≥ 2.0	≤ 75 mL
7.6	≥ 2.0	≤ 75 mL

Table 2
Excipients present in immediate-release formulations containing levamisole hydrochloride with a MA in European countries.

Excipient	Drug product containing excipient by named country *MA in Europe*
Magnesium stearate	HU (1,2), LA (2), LI (2)
Povidone	HU (1,2), LA (2), LI (2)
Talc	HU (1,2), LA (2), LI (2), RO (3,4)
Saccharin sodium	HU (1)
Sucrose	HU (2), LA (2), LI (2)
Lactose monohydrate	HU (2), LA (2), LI (2), RO (3,4)
Maize starch	HU (1,2), LA (2), LI (2)
Microcrystalline cellulose	RO (3,4)
Colloidal silicium dioxide	RO (3,4)

HU: Hungary (http://www.ogyi.hu/drug_database/), LA: Latvia (http://www.zva.gov.lv/zalu-registrs/?lang=en), LI: Lithuania (https://vapris.vvkt.lt/vvkt-web/public/medications?lang=en), RO: Romania (https://www.anm.ro/nomenclator/medicamente) (Accessed April 1st, 2019).
(1) Decaris 50 mg tablets (Gedeon Richter),.
(2) Decaris 150 mg tablets (Gedeon Richter).
(3) Levamisol Arena 50 mg tablets (S.C. Arena Group).
(4) Levamisol Arena 150 mg tablets (S.C. Arena Group).
*Colourants and flavouring agents are not included.
** Sources of data:.

Distribution, Metabolism and Elimination

Levamisole has a large volume of distribution (99 – 266 liters) .[29,30] It is metabolized primarily by the liver. The majority of the dose excreted is in the form of metabolites, which are excreted in the urine, with unchanged levamisole recovered in the urine only accounting for 3.17% of the dose.[29]

The total clearance rate of levamisole is 18 – 34 L/h [29,30], while the elimination half-life has been reported to lie in the range 4.0 to 5.6 h.[29,30] Pharmacokinetics in healthy individuals has been shown to be comparable to those suffering with cancer,[30,32,33] nephrotic syndrome,[34] and malaria.[35] Interindividual variability in pharmacokinetic parameters is high, for example, the coefficient of variation for the total clearance of levamisole in healthy or cancer subjects is 36% – 38% or 36% – 37%, respectively.[30]

Dosage form Performance

Bioequivalence

No bio(in)equivalence reports are available in the open literature.

Excipients

The excipients in tablet formulations containing levamisole hydrochloride with current marketing authorizations (MA) in Europe are given in Table 2. Current MAs were found by searching databases of the national competent authorities (available through the EMA website).

None of the eight excipients in Table 2 are expected to modulate the intestinal permeability, intestinal transit, or gastrointestinal stability of levamisole hydrochloride. Other common excipients are also

not expected to modulate the intestinal permeability, intestinal transit, or gastrointestinal stability of levamisole hydrochloride or highly soluble drugs.[36,37] For example, prior results show that test and reference of Class 3 drugs do not have to be either qualitatively the same nor quantitatively very similar for sodium lauryl sulfate, corn starch, sodium starch glycolate, colloidal silicon dioxide, dibasic calcium phosphate, crospovidone, lactose, povidone, stearic acid, pregelatinized starch, croscarmellose sodium, and magnesium stearate.[38] We are unaware of any expectation, based on experience with other BCS Class III drugs, that talc, saccharin sodium, or sucrose would modulate the intestinal permeability, intestinal transit, or gastrointestinal stability of levamisole hydrochloride. Given the experience to date with several well-known and widely used excipients, there appears to be opportunity for additional regulatory relief in future versions of M9, such as not requiring similar quantities of these common excipients, or even the qualitative sameness of such excipients, in the test and comparator products.

Dissolution

Dissolution criteria for levamisole hydrochloride tablets described in the USP require that 80% (Q) or more of the labeled drug amount is dissolved within 45 min in 900 mL of 0.01 N hydrochloric acid solution using the paddle apparatus with a revolution rate of 50 rpm.[26]

Comparing these dissolution specifications to those for a biowaiver, passing the quality control test would not be sufficient evidence for a product to qualify for a biowaiver-based approval of products containing levamisole: oral solid dosage formulations containing BCS Class I drugs should be at least 'rapidly dissolving', meaning that 85% or more of the labeled amount should be dissolved within 30 min at pH 1.2, 4.5 and 6.8 to consider the formulation "rapidly dissolving". For products containing BCS Class III drugs, 85% or more of the labeled amount should be dissolved within 15 min to consider the formulation "very rapidly dissolving".

Dissolution profiles for immediate-release film-coated tablets of levamisole hydrochloride have been reported in the literature.[39] Four dosage strengths (i.e., 5, 10, 25, and 50 mg) were developed that contained common excipients (lactose, microcrystalline cellulose, sodium starch glycolate, maize starch, hydroxypropylcellulose, sodium stearyl fumarate). Release of levamisole from 5 mg tablet (Fig. 2) and 50 mg tablet (Fig. 3) was "very rapid" at pH 1.2, 4.5 and 6.8 using the paddle method at 50 rpm and 37°C. The dissolution rate found for the 50 mg tablets was slightly slower than that of 5 mg tablets. The highest pH of 6.8 is considered to be the most critical for determining dissolution characteristics, since at this pH the ionized fraction is at its lowest over the pH range of interest i.e. pH 1.2 to 6.8. Further dissolution profiles of levamisole hydrochloride tablets could not be located in the open literature.

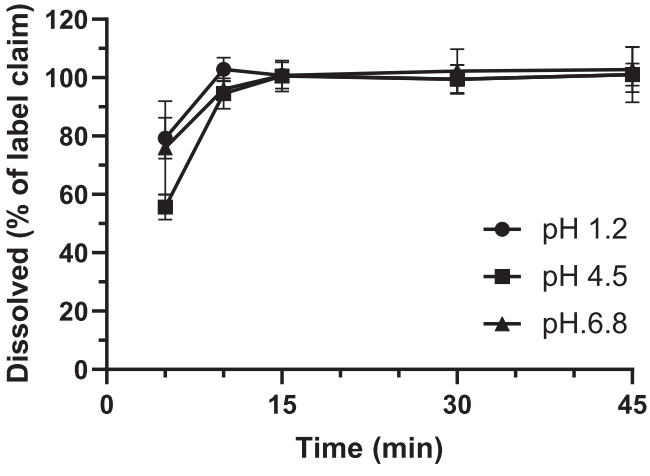

Figure 2. Dissolution profiles of levamisole 5 mg tablet in pH 1.2, pH 4.5, and pH 6.8. This figure is based on the literature.[39]

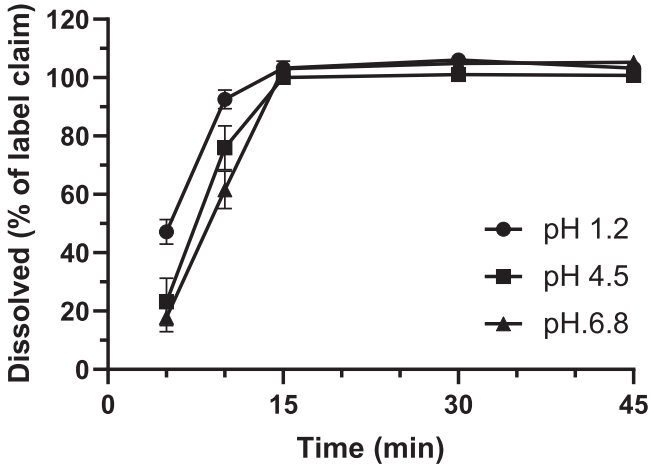

Figure 3. Dissolution profiles of levamisole 50 mg tablet in pH 1.2, pH 4.5, and pH 6.8. This figure is based on the literature.[39]

Discussion

Solubility

Given that the aqueous solubility of levamisole hydrochloride is equal to or more than 2.0 mg/mL in the pH range 1.2 – 7.5 at 37 °C, the highest dose (150 mg) of the drug can be dissolved completely in far less than 250 mL of the aqueous media. It can be concluded that levamisole hydrochloride is a highly soluble drug substance according to the BCS criteria.

Permeability

The oral bioavailability of levamisole hydrochloride is approximately 65% in humans. This means the fraction absorbed (Fa) of the drug must be at least 0.65 (i.e., 0.65 – 1.00). Given the extensive metabolism of levamisole, it is possible that this drug undergoes first pass metabolism, so the Fa value of levamisole may in fact be much closer to 1. Assuming that the hepatic clearance of levamisole is the same as its total clearance (18–34 L/h), the estimated Fa*Fg value of levamisole would be 0.82 – 1.05.

Furthermore, given that levamisole is metabolized extensively, the Biopharmaceutical Drug Disposition Classification System (BDDCS) concept[40] would suggest that this drug may have high permeability. However, it is not possible to definitively state whether this compound meets the criteria for high permeability according to the guidance (i.e., Fa $\geq$ 0.85)[1] based on the current data set.

BCS Dlassification

Levamisole could belong to either BCS class I or III, since it has high solubility but the fraction absorbed has not been precisely determined. At the WHO, it is correspondingly classified as class I/III[2]. However, according to all current Guidances, levamisole hydrochloride would have to be assigned to BCS class III due to the uncertainty of the permeability, which is in line with the EMA's conclusion to this compound.[41] This "worst case" approach is used to mitigate the risk of bioinequivalence if the actual permeability of this compound proves not to be high. Should permeability data for this compound

supporting a high permeability become available in the future, the classification will change to BCS class I.

Levamisole is currently used in the treatment of intestinal worms, which are most likely be paralyzed by the drug in the intestinal tract and eliminated. Although drugs which act locally in the gastrointestinal tract are out of scope for the ICH M9 guideline, in this monograph we have discussed levamisole and the possibility of biowaiver in this framework. This is because the concept of BCS-based biowaiver is thought to be important for not only drugs acting after reaching the systemic blood circulation but also anthelmintics acting in the gastrointestinal tract. However, one could argue that the necessity to include permeability considerations in the biowaiver decision may be moot in such cases.

Risks with Respect to Excipient and/or Manufacturing Variations

A comparative BA study found only minor differences in Cmax and AUC between a liquid and a tablet formulation in humans.[31] This suggests that excipients used in those tablets and the manufacturing process had little influence on the oral absorption of levamisole. Although dissolution performance of the particular tablet used in that clinical study[31] is unknown, levamisole hydrochloride was shown to be released very rapidly from all the tablet formulations investigated in the literature.[39]

The application of BCS Class III specifications for dissolution testing i.e. ≥ 85% dissolution within 15 min in aqueous media with pH of 1.2, 4.5, and 6.8 to levamisole products mitigates any excipient and manufacturing related risks to bioequivalence from a dissolution point of view. Restricting the excipients to those used in the comparator formulation would further decrease the risk of an inappropriate biowaiver decision.

Patients' Risk Associated with Bioinequivalence

Bioinequivalence may result in patient risks either through diminished effectiveness (in case of lower BA) or through higher risk of toxicity (in case of higher BA).

While levamisole has a lower therapeutic index than other anthelmintics, it has not been assigned a narrow therapeutic index. Severe side effects may occur sporadically, even after single doses.[19] However, for levamisole the occurrence of adverse drug reactions seems to be individually determined, and no dose-response studies, which would help clarify whether there is an increase in the side effect incidence with increasing dose, have been performed.

Inconsistent therapeutic effects may be more likely to occur with variations in bioavailability among individuals, given the large variation in volume of distribution. But because therapeutic doses are limited to a range between 2.5−3.0 mg/kg for all age groups and anthelmintic indications, the risk of adverse effects due to a higher amount being absorbed than expected can be considered low.

Bioinequivalence issues are likely to be of even less relevance with the usual single-dose anthelmintic treatment than with the rarely prescribed long-term immunomodulatory treatment.

Conclusion

Levamisole hydrochloride is a highly soluble drug, but pending clear evidence of high permeability in humans, it is conservatively assigned as a BCS class III drug substance. Based on the available literature and experimental data generated in preparation of this monograph, the biowaiver procedure would be applicable for solid oral dosage forms containing levamisole hydrochloride as the only active ingredient. Due to the lack of data in the literature regarding excipient effects specifically related to the bioequivalence of solid oral dosage forms containing levamisole, it is currently recommended that the product complies with the ICH and WHO guidelines: the tested formulation should have the same qualitative composition as the comparator, contain very similar quantities of those excipients, and be very rapidly dissolving at pH 1.2, 4.5, and 6.8.

Conflict of interest

This article is a part of the project of the International Pharmaceutical Federation, Focus Group BCS & Biowaiver (www.fip.org/bcs). The contents of this monograph are based on the data available in the literature and represent the scientific opinion of the authors but not necessarily the policies of regulatory authorities or the International Pharmaceutical Federation.

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