A single pill combining bisoprolol and amlodipine: An open label, multi-dose, single-sequence study to investigate potential pharmacokinetic drug-drug interaction between the two compounds in healthy adult volunteers

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Introduction

Hypertension is a major risk factor for cardiovascular complications such as coronary artery disease, stroke, congestive heart failure and renal failure. It has been long- and widely-accepted that adequate treatment will reduce the incidence of these outcomes (1).

Several clinical trials have shown that the majority of hypertensive patients needed more than 1 drug to reach their individual target blood pressure (BP) (2).

This is reflected by the current European hypertension guidelines, which recommend the initiation of treatment in most patients with a single pill (SP) comprising two drugs, to improve the speed, efficiency, and predictability of BP control. (2) Preferred two drug combinations are renin angiotensin inhibitor (ACEI) with a calcium channel blocker (CCB) or a diuretic (3).

In the current guidelines, a beta-blocker (BB) in combination with a diuretic or any drug from the other major classes is defined as an alternative when there is a specific indication for a beta-blocker, e.g. angina, post myocardial infarction, heart failure, or heart rate control in hypertensive patients (3). The combination of a beta-blocker with a thiazide diuretic was previously criticized because of the increased risk of developing diabetes (4). Therefore, in patients for whom a beta-blocker is indicated, the combination with a dihydropyridine CCB is a probably better alternative.

Abstract

Single pill (SP) combinations support adherence to therapy and rule out clinically relevant interactions that could affect their efficacy or safety when administered in combination.

The primary objective of this single center, open-label, single-sequence three-period study was to assess the pharmacokinetic drug-drug interaction of bisoprolol and amlodipine given concomitantly at steady-state. The secondary objective was to determine the safety and tolerability of the combination in healthy volunteers.

A total of 22 healthy male subjects were enrolled and 20 of them were analysed for PK parameters. The geometric LS mean ratios for the AUC_{τ,ss}, C_{max,ss} and C_{min,ss} for amlodipine administered concomitantly with bisoprolol versus amlodipine alone were 527675.6/499657.8 pg*h/ml (105.61%), 28124.0/25778.3 pg/ml (109.10%) and 17862.9/16718.4 pg/ml (106.85%), respectively. On the other hand the geometric LS mean ratios for the AUC_{τ,ss}, C_{max,ss} and C_{min,ss} for bisoprolol administered concomitantly with amlodipine versus bisoprolol alone were 834.130/765.984 ng*h/ml (105.61%), 58.253/53.336 ng/ml (109.10%) and 16.996/14.646 ng/ml (106.85%), respectively. No significant difference was observed for T_{max,ss} between bisoprolol and amlodipine alone (p-value > 0.05). In general, the treatment with both substances was well tolerated.

Overall, the bioavailability of bisoprolol and amlodipine was considered equivalent when administered alone or concomitantly. Consequently, no DDI exists between these two molecules. An SP containing bisoprolol and amlodipine could therefore be a fundamental treatment option for hypertensive patients requiring this combination.
The requirements for the development of a single pill combination are defined in the “Guideline on clinical investigation of medicinal products in the treatment of hypertension” of the European Medicines Agency. There are several scenarios defined. One is the development of two antihypertensives in one tablet for patients adequately controlled with the individual products, given concurrently, but as separate tablets. The primary aim is to reduce the number of tablets the patient has to take. In this scenario, all substances need to be well known. In addition, the joint application of the components must already be in widespread use in the proposed dosage strengths and has to be safe (5). Following this guideline, we started the development of a single pill containing the BB bisoprolol and the CCB amlodipine.

Bisoprolol is a cardioselective β-1-adrenergic blocking agent. In pharmacological trials mean elimination half-lives of 11 hours (h) for the unchanged drug and 12 h for total radioactivity were observed after oral administration of bisoprolol. Bisoprolol was nearly complete enteral absorbed (6). Fifty percent were eliminated renally as unchanged bisoprolol and the other 50% metabolically, with subsequent renal excretion of the metabolites. Less than 2% of the dose were recovered from the feces. Total and renal clearance were calculated as 15.6 L/hr and 9.6 L/hr, respectively. The volume of distribution was 226 L. Concomitant food intake did not influence the bioavailability of bisoprolol (7).

Drug-drug interactions have been described for the concomitant use of phenytoin, phenobarbital, phenylbutazone, ranitidine, cyclosporine A (13). Amlodipine is slowly metabolized in the liver by CYP3A4 which may be the major course for interactions and excretion of drugs, or known to potentiate or predispose to undesired effects were excluded, as were subjects with a history of significant gastrointestinal, liver or kidney disease that may have affected drug bioavailability. Subjects were also excluded if they had significant cardiovascular, pulmonary, hematologic, neurological, psychiatric, endocrine, immunologic or dermatologic diseases, or any clinically significant illness in the 28 days prior to the first day of treatment of this study. Subjects who had taken any enzyme-modifying drugs, including strong inhibitors of cytochrome P450 enzymes, in the previous 28 days before day 1, or had taken bisoprolol or amlodipine or any investigational product in the previous 28 days before day 1 were also not eligible for study enrollment.

Materials and methods

This study was conducted in compliance with the study protocol, the ethical principles that have their origins in the Declaration of Helsinki, the ICH Guideline E6 for GCP, the Directive 2001/20/EC (Europe) and the Tri-Council Policy Statement (Canada). A letter of non-objection was obtained from the Canadian authorities.

Eligible subjects were healthy males, aged between 18 and 45 years, with a seated pulse rate of at least 66 beats per minute (bpm), and a seated BP of at least 110/60 mmHg at screening, and no history of significant hypersensitivity to bisoprolol, amlodipine, or any related products. Subjects with significant gastrointestinal, liver or kidney disease, or any other condition known to interfere with the absorption, distribution, metabolism or excretion of drugs, or known to potentiate or predispose to undesired effects were excluded, as were subjects with a history of significant gastrointestinal, liver or kidney disease that may have affected drug bioavailability. Subjects were also excluded if they had significant cardiovascular, pulmonary, hematologic, neurological, psychiatric, endocrine, immunologic or dermatologic diseases, or any clinically significant illness in the 28 days prior to the first day of treatment of this study. Subjects who had taken any enzyme-modifying drugs, including strong inhibitors of cytochrome P450 enzymes, in the previous 28 days before day 1, or had taken bisoprolol or amlodipine or any investigational product in the previous 28 days before day 1 were also not eligible for study enrollment.

Under use of amlodipine drug-drug interactions have been described for statins (10), clarithromycin (11), tacrolimus (12), and cyclosporine A (13). Amlodipine is slowly metabolized in the liver by CYP3A4 which may be the major course for interactions (10).

The primary objective of our study which was part of the development process of the single pill combination bisoprolol/amlodipine was to investigate the pharmacokinetic drug-drug interaction potential of bisoprolol and amlodipine. The secondary objective was to determine the safety and tolerability of the combination in healthy volunteers.

Materials and methods

This was a single center, open-label, single-sequence, three-period study conducted by Algorithme Pharma Inc, Canada.

The protocol and the informed consent forms were approved by the Quebec Institutional Review Board of IRB Services (Version 3.0 2015/09/28) on 29 Sep 2015.
following blood collection, samples were centrifuged at a temperature of 4°C nominal and at approximately 1500 g for 10 minutes. The plasma obtained was separated into duplicate polypropylene culture tubes, when feasible. The samples were frozen in an upright position and retained in the clinic’s freezers at a temperature of -20°C nominal until sent on dry ice to the bioanalytical facility for assay. The time from blood sample collection to plasma aliquot storage were within 90 minutes. Sample pre-treatment involved the protein precipitation extraction of bisoprolol and amlodipine from 0.100 mL of human plasma; Bisoprolol-D (deuterated chemical analog)5 and amlodipine-D4 were used as the internal standards (IS1 and IS2). The compounds were identified and quantified using reversed-phase HPLC with MS/MS detection over theoretical concentration ranges of 0.500 ng/mL to 75.000 ng/mL for bisoprolol, and 5.0 ng/mL to 30000.0 pg/mL for amlodipine.

Multiple stock solutions of bisoprolol and amlodipine were prepared and compared to verify the accuracy of reference standard weighing. Stock solutions were deemed acceptable if the percent difference between mean response ratios of at least two percent stock solutions was ≤±5.0%. Solutions of bisoprolol, amlodipine, bisoprolol-D5 and amlodipine-D4 were stored at 4°C nominal. Furthermore, stock solutions of bisoprolol, amlodipine, bisoprolol-D5 and amlodipine-D4 were screened for potential interference at the retention times and mass transitions of bisoprolol, amlodipine, bisoprolol-D5 and amlodipine-D4 and were free of significant interference. The stock solutions used for calibrators had a different lot number than the stock solutions used for QC samples. All calibrant and QC samples were stored at -20°C nominal for use during study sample analysis. For bisoprolol, calibrant concentrations ranged from 50.0 pg/mL to 30000.0 pg/mL for amlodipine.

The concentrations were calculated using peak area ratios and the linearity of the calibration curve was determined using least squares regression analysis employing a weighted (1/x²) linear \( y = mx + b \) for bisoprolol and amlodipine, respectively. For bisoprolol the between-run accuracy was 91.3% to 99.2% and the between-run precision 2.7% to 5.5%. Within-run accuracy using Liquid Handling System (LHS) was 88.2% to 101.7% and within-run precision using LHS 0.5% to 3.7%. For amlodipine the between-run accuracy was 91.3% to 99.2% and the between-run precision 2.7% to 5.5%. The within-run accuracy using LHS was 88.2% to 101.7% and within-run precision using LHS 0.5% to 3.7%.

Safety was evaluated through assessment of adverse events, standard laboratory evaluations, vital signs, BP, and ECG.

**Results**

A total of 22 subjects (mean age 32.4 years) were enrolled in this study. Demographic data are given in (Table 1). One subject dropped out right at the beginning of period 1 so that 21 subjects (95%) received 5 days treatment of bisoprolol 10 mg OD, 12 days treatment of amlodipine 10 mg OD and also 5 days treatment of bisoprolol 10 mg and amlodipine 10 mg concomitantly OD. Due to diarrhea one subject was excluded from statistical analysis of PK parameter. Thus, 20 subjects were eligible for statistical analysis. The geometric LS mean ratios for AUC(0-t), C(\text{max}) and C(\text{min}) for bisoprolol in treatment period 3 (bisoprolol and amlodipine) versus treatment period 1 (bisoprolol alone) were 108.9% (CI: 105.7-112.2), 109.2% (CI: 106.5-112.0), and 116.0% (CI: 109.0-123.6), respectively (Table 2). The geometric LS mean ratios for AUC(0-t), C(\text{max}) and C(\text{min}) for amlodipine in treatment period 3 (bisoprolol and amlodipine) versus treatment period 2 (amlodipine alone) were 105.6% (CI: 103.1-108.1), 109.1% (CI: 104.3-114.1) and 106.9% (CI: 103.6-110.2), respectively (Table 3). The 90% confidence intervals are contained within the general acceptance interval of 80.00-125.00% which indicates, that the slight difference observed will not be of clinical relevance. No significant difference was observed for T(\text{max}) between both treatments (p-value > 0.05) for bisoprolol and amlodipine. Although formally not required bioequivalence was impressively demonstrated (figure 2). A total of 46 Adverse Events (AEs) were reported for 15 of
the 22 (68%) subjects who participated in this study. All AEs were deemed mild (44/46; 96%) and moderate (2/46; 4%) in severity. No severe AEs were observed during the study. The most common AEs reported in this study were skin disorders (8 subjects), followed by headache, which was experienced by 7 subjects, somnolence (6 subjects), dizziness and pain (4 subjects). The incidence of AEs was the same for the subjects dosed with amlodipine given alone and amlodipine and bisoprolol given concomitantly (33%) and lower than that of subjects dosed with bisoprolol given alone (50%). The incidence of drug-related AEs was also similar between amlodipine given alone and amlodipine and bisoprolol given concomitantly (24% and 29 %, respectively) and lower than that of subjects dosed with bisoprolol given alone (50%).

All adverse events are presented in (Table 4). Generally, the subjects showed laboratory values within normal range in all treatment groups and all vital signs assessments and ECGs were judged not clinically significant. No relevant changes in BP were observed. Generally, the physical examination findings were considered normal or without any observed changes. One subject had an abnormal physical examination at the post-study visit that was judged not clinically significant.

No serious adverse events (SAE) and no deaths were reported for any of the subjects enrolled in this study. No subject was withdrawn by the investigator for safety reasons (Table 4).

**Discussion**

For amlodipine, a good efficacy and safety profile has been documented in the treatment of hypertension. In addition, there is a strong evidence from large randomized controlled trials for reduction of cardiovascular events (14). BB are used for the treatment of hypertension in patients with coronary artery disease, with heart failure and reduced ejection fraction (or with other comorbidities for which a beta-blocker is helpful), in patients younger than 60, and in patients in whom other classes of anti-hypertensives are unsuitable. The combination of bisoprolol and amlodipine is a reasonable combination for lowering BP according to current treatment recommendations. The different modes of action make a combination of amlodipine and bisoprolol suitable to reduce BP. CCBs dilate the peripheral vessels and thereby decrease the blood pressure. The decrease in blood pressure induces a reflex increase in heart rate which could counteract the decrease in blood pressure and diminish the decrease in rate pressure product; Therefore, the influence of a BB on heart rate is a reasonable effect to combine a BB with a CCB (15).
However, the combination of two substances increases the risk for drug-drug interactions (DDIs), which are the major concern among patients receiving multidrug therapy (16). DDIs are qualitative or quantitative modifications of the effect of a drug by the simultaneous or successive administration of a different one. They may result in the alteration of therapeutic effect and safety of either or both drugs and can be due to the mechanism of the administered drugs (pharmacodynamic) or a reduction or enhancement of drug elimination (pharmacokinetic). Pharmacokinetic interactions commonly occur at the level of drug metabolizing enzymes or at the level of drug transporter proteins (16).

DDIs are known to be a risk factor for the development of adverse drug reactions (ADR). Ganeva et al. performed a prospective observational study comprising all consecutive inpatients admitted to the Clinic of Dermatology and Venereology at the University Hospital Stara Zagora for a two years period. Systemic medication was screened for potential DDIs using an electronic drug interactions checker. Potential DDIs were frequent in hospitalized dermatology patients. The drug groups most commonly involved were cardiovascular drugs. Hypotension was the most common expected clinical presentation of the potential DDIs (17).

Patients with hypertension are particularly vulnerable to DDIs due to their advanced age, gender, and polypharmacy. Siva et al. performed a prospective observational study in a hospital setting in South India for a period of 9 months. Hypertensive inpatients who received more than 3 drugs per prescription staying at hospital for more than 24 hours were included in the study. The overall incidence of DDIs was found to be 21.14%. The most common drugs responsible for DDIs were Insulin, followed by Metoprolol, Torsemide, and Hydrochlorothiazide. The most common consequences of interacting pairs were reduced serum potassium levels and hyperglycemia (18).

Common DDIs described in the literature for BB are bradycardia and hepatic interactions for metoprolol, labetolol, propranolol, carvidolol, nebivolol, and bisoprolol. DDIs described for calcium-channel blockers are bradycardia and heart block, with heart rate-reducing agents for verapamil, and hepatic interactions with simvastatin and atorvastatin for amloidine and nifedipine (19).

Bisoprol is subject to moderate hepatic metabolism. Only oxidative pathways have been detected, with no subsequent conjugation. It is metabolized primarily by CYP3A4 to inactive metabolites and by CYP2D6, which is not expected to be clinically significant (20). Amlodipine is extensively metabolised in the liver via CYP3A4 isozyme. Firstly, it undergoes oxidation to the pyridine derivative, and then oxidative deamination or ester hydrolysis (21, 22).

Due to the metabolism characteristics, bisoprolol and amloidipine seem to be the ideal candidates for a single pill combination, when the concomitant use of a BB and a CCB is required for clinical reasons.

Our study was focused on detecting DDIs between bisoprol and amloidipine. DDIs in combination with other drugs are reported. However, the results of our study suggest that the steady-state bioavailability of bisoprolol and amloidipine was slightly increased when these products were administered concomitantly. However, the 90% CI for the ratios in the geometric means for steady state $C_{\text{max}}$ and steady-state AUC, between bisoprolol/amloidipine and its monocomponents are in range required by the regulatory authorities to show bioequivalence.

The AEs observed are in line with the safety information published for the two compounds. In addition, it has to be taken into account that this antihypertensive combination has been tested in healthy volunteers and therefore the AEs reported were not unexpected. The lower incidence of AEs for the combination

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Phase 1 – bisoprol (n=22)</th>
<th>Phase 2 – amloidipine (n=21)</th>
<th>Phase 3 – bisoprol/amloidipine (n=22)</th>
<th>Total (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin disorders*</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>8</td>
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<tr>
<td>Headache (incl. Head pressure)</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Somnolence</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>6</td>
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<tr>
<td>Pain**</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
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<tr>
<td>Dizziness</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>4</td>
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<tr>
<td>Hot flush</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Vessel puncture site pain / phlebitis</td>
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<td>0</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Diarrhoea</td>
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<td>1</td>
<td>2</td>
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<tr>
<td>Dry eyes</td>
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<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Injuries</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Sinus congestion</td>
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<td>Dry lips</td>
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<td>Tremor</td>
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<td>Thirst</td>
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<td>Insomnia</td>
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<tr>
<td>Nausea</td>
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</table>

*incl. rash, acne, erythema, dry skin
**(abdominal, back, extremity, neck)
and amlodipine alone compared to bisoprolol given alone might support the thesis, that a combination of two antihypertensive drugs could reduce adverse drug reactions.

A limitation of our study might be, that concomitant medication was excluded. In real life, patients suffering from hypertension will usually receive additional medication like anti-diabetics or statins. However, for agents combined in a single pill DDIs can be ruled out for these substances due to investigations that are part of the development process.

From our perspective, when patients require more than one drug to reach target BP, the use of SP formulations is preferable to the use of free combinations because the potential for significant pharmacokinetic interactions that could affect efficacy or safety has been investigated and reduced. This is an additional advantage for the use of SP formulations beside the improvement of patient’s adherence.

Conclusions

It is reasonable to assume that no pharmacokinetic DDIs between bisoprolol and amlodipine exist since the bioavailabilities in terms of $AUC_{ss}$, $C_{max,ss}$ and $C_{min,ss}$ of bisoprolol and amlodipine were equivalent when both drugs were administered alone or concomitantly. The combination of drugs tested were safe and well tolerated by the subjects included in this study. A SP containing bisoprolol and amlodipine could be an important treatment option for hypertensive patients requiring this combination.

References


