Human Bioavailability and Pharmacokinetic Profile of Different Formulations Delivering Alpha Lipoic Acid

Fiorenzo Mignini1*, Cinzia Nasuti1, Giorgio Gioventu1, Valerio Napolioni2 and Piera Di Martino1

1School of Farmacy and Health product, University of Camerino, Camerino, Italy
2School of Biosciences and Biotechnologies, University of Camerino, Camerino, Italy

Abstract

Alpha-lipoic acid (ALA) is an important micronutrient with several pharmacologic as well as antioxidant properties. Oral formulations present problems due to characteristics of the molecule and of the pharmaceutical forms. It is known that ALA is poorly soluble; therefore to increase the solubility was reticulated in an amphiphilic matrix like lecithin. The goal of the present study was to characterize the bioavailability of new formulation and to compare the human pharmacokinetics profiles of two different pharmaceutical form: tablets and soft gel capsules following single oral administration of a ALA 600 mg. Blood samples were collected up to 8 h post dosing, and plasma α-lipoic acid concentrations were determined by Liquid Chromatography Mass Spectrometry (LC/MS/MS) detection. The results revealed that after rapid dissolution there is a good solubilisation by lecithin and that the two formulations show the same human pharmacokinetic profile. Tablet formulation is more versatile than soft capsules because it makes it possible to administer 600 mg of ALA with good compliance. The properties of the new formulation ensure rapid release in vivo and good bioavailability with high ALA content per unit dose and excellent homogeneity of release.

Keywords: α-Lipoic acid; Bioavailability; Human pharmacokinetic; New oral formulation; Amphiphilic matrix; Surfactants; Disaggregating agents

Abbreviations: Cmax: maximum plasma concentration; AUC: Area Under the plasma concentration-time Curve from time zero up to time t; Tmax: time of maximum plasma concentration

Introduction

Alpha-lipoic acid (1,2-dithiolane-3-valeric acid) (ALA), also known as thiotic acid, was discovered in 1937. Unlike antioxidant vitamins, ALA exhibits this activity in both the reduced and oxidated forms. Lipoic acid is a racemic mixture. The redox couple α-lipoic/dihydrolipoic acid containing the R-enantiomer is covalently bound to a lysine residue, forming an essential lipoamide, which functions as a co-enzyme for the E2 subunit of four multi-enzymatic mitochondrial complexes, for example pyruvate dehydrogenase [1-6]. After administration, ALA is reduced at the intracellular level by various enzymes and released into the extracellular environment as its principal metabolite, dihydrolipoic acid (DHLA) [7,8].

Oral formulations of α-lipoic acid present problems due to characteristics of the molecule (short blood half life, high presystemic elimination and hepatic first pass effect) and characteristics of the pharmaceutical forms. ALA is a poorly soluble molecule; the techniques used to increase its solubility in the gastrointestinal environment, such as micronisation and salification, have some drawbacks and disadvantages. Complexes and composites based on cyclodextrins or other polymers require costly processes that are often difficult to carry out and do not ensure complete complexation of the active ingredient. In addition, the active ingredient to polymer ratio is often a limiting factor in the preparation of an easy-to-administer pharmaceutical form. Micronisation processes do not always ensure significant increases of capsules, tablets and granulates. Emulsions and/or microemulsions, either simple or multiple, are often unstable and cannot carry pharmacologically active amounts of the medicament. Salification and/or solubilisation processes of conventional pharmaceutical forms sometime cannot improve the bioavailability of medicaments that are only slightly permeable and absorbable, or lipophilic ones, due to re-precipitation of the active ingredient in biological fluids, thus removing the advantage of a technological process to dissolve the medicament in the pharmaceutical formulation.

A new oral formulation of ALA, called ALA 600, uses a patented technology (EP1401405 B 1) to deliver significantly bio available and safe quantities of α-lipoic acid to the bloodstream. An amphiphilic matrix with surfactants coupled with super disaggregating agent’s increases the dissolution rate in vitro with improved bioavailability and less variability in absorption. The compositions can be obtained with a process which comprises the following steps: 1) adding surfactants to the amphiphilic matrix, to obtain a homogeneous solution or dispersion (preferably in amounts from 0.1% to 5%); 2) solubilising, suspending, dispersing, totally or partly embedding one or more active principles (preferably from 0.1% to 4.9%); 3) adding cyclodextrins and/or superdisaggregating agents; (preferably from 0.1% to 2.5%, to obtain a homogeneous dispersion); 4) optionally adding excipients; 5) optionally film-coaling with cellulose derivatives or methacrylic acid polymers. In a pilot study, we have observed that, respect to the recent published data, the new ALA formulation tends to show an improvement of Cmax and AUC values [9]. The goal of the present study was to characterize the bioavailability of new formulation and to compare the human pharmacokinetics profiles of two different pharmaceutical forms: tablets and soft gel capsules following single oral administration of an ALA 600 mg.

*Corresponding author: Fiorenzo Mignini, Professor, School of Farmacy and Health Product Experimental Medicine Unit University of Camerino, Via Madonna delle Carceri, Camerino (MC), Italy, Tel: +39 0737 403304; Fax: +39 0737 403325; E-mail: fiorenzo.mignini@unicam.it

Received April 04, 2012; Published October 31, 2012


Copyright: © 2012 Mignini F, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Materials and Methods

Pharmaceutical section

Investigational products: Both test preparations, the nutritional supplements soft gel capsules 300 mg (ALAnerv) and tablets 600 mg (ALA600), were supplied by Alfa Wassermann.

One soft gel capsule contains: active principles a-lipoic acid 300, polyunsaturated fatty acids (linoleic acid, α-linolenic acid) 180, natural vitamin E/d-alpha-tocopherol 7.5 mg, vitamins of the B complex (B1/thiamine mononhydrate, 1.05 mg; B2/riboflavin, 1.20 mg; B5/calcium d-pantothenate, 4.5 mg; B6/pyridoxine chloride hydrate, 1.5 mg) and selenium methionine 25 mg. Excipients: alimentary gelatine, glycerol, fatty acid triglycerides, magnesium stearate, polyglycerol oleate, soya oil, soya lecithin, titanium dioxide, red iron oxide.

One tablet of ALA600 contains: Active principles a-lipoic acid 600 mg and vitamins of the B complex (B1/thiamine mononhydrate, 2.1 mg; B2/riboflavin, 2.4 mg; B5/calcium d-pantothenate, 9 mg; B6/pyridoxine chloride hydrate, 3 mg). Excipients: di-calcium phosphate, cellulose microcrystalline, maltodextrins, polyvinylpyrrolidone, povidonpyrrolidone, colloidial silica, magnesium stearate, croscarmelllose sodium, talcum powder, soya lecithin, hydroxypropylmethylcellulose, stearic acid, titanium dioxide, red iron oxide.

Pharmacological process

The patent of oral formulation (EP 1401405 B1) was developed to improve the bioavailability of active ingredients added to the amphiphilic matrix, which are poorly absorbed via the oral route due to high variability of absorption in the gastrointestinal tract. This technology, which was originally used to deliver drug substances, has been applied in other fields, such as alimentary supply to obtain prompt-release and fast-absorption, and to enhance the bioavailability of a-lipoic acid.

In the step 1) the amphiphilic matrix is prepared. Any amphiphilic semisolid excipients or mixtures thereof are melted above 60°C, or solubilised or suspended in solvent (preferably water) to obtain a homogeneous solution/dispersion, which becomes again semisolid or solid at room temperature with eutectic properties at temperatures ranging from 35°C to 37°C (body temperature) or able to be used as granulating system. Afterwards, said excipients, which have become liquid upon melting or are already liquid at room temperature, are added with surfactants to obtain a homogeneous dispersion. In step 2), the active ingredient is solubilised, dispersed and/or embedded in the surface-activated amphiphilic matrix from step 1) to obtain a homogeneous solution, or dispersion, or granules. In step 3), the system from step 2) is added with different amounts of cyclodextrins and/or superdisintegrants until homogeneous dispersion. The resulting system can be distributed into soft- or hard-gelatine capsules to obtain a liquid, semisolid or solid pharmaceutical form inside the capsule. Alternatively, the system from step 2) can be loaded onto cyclodextrin and/or superdisintegrants and/or mixtures thereof to obtain powder, microgranules or granules having good free-flowing and/or lab letting characteristics. In step 4), excipients with different functions may be added to transform liquid or semisolid formulations into solid ones for the preparation of capsules, tablets, granulates, microgranules, minitablets, sachets, said excipients being, for example, silica, celluloses, starches, sugars, polyvinyl pyrrolidines, methacrylates, glidants, antiaggregants, lubricants such as magnesium stearate, stearic acid, talc, or the liquid semisolid formulations can be added with other liquid cosolubilizers such as, water, polyethylene glycols, glycerin, sorbitol. Amphiphilic compounds could be polar lipids (lecitins, phosphatidyl choline, phosphatidyl diethanolamine), glycol alkyl ethers such as diethylene glycol monooethyl ether (Transcutol®). Surfactants comprise phosphatides and lecithins (phosphatidyl cholines, phosphatidyl diethanolamines, sphingomyelins), anionic and non-ionic emulsifying waxes, sodium lauryl sulfate, sodium dodecyl sulfate, polysorbales, cholic acids, poloxamer, sodium sulfoctane, sodium lauryl sarcosinate. Superdisintegrants comprise sodium starch glycode (Explotab®), croscarmelllose sodium (Acdisol®) crosslinked polyvinylpyrrolidone (Amberlites® (IRP SS).

Pharmacokinetics section

Ethics: The trial protocol, informed consent form and subject recruitment procedures were approved by the Joint Ethical Committee (JEC) Universita di Camerino-Azienda ASUR Marche ZT-10 of Camerino. Before signing the informed consent form the volunteer were informed in detail by a physician. The subject was given ample time to enquire about the details of the trial. The consent form was signed by the informing physician and by the volunteer. The study was conducted in accordance with the Declaration of Helsinki in its revised edition, the Guidelines of Good Clinical Practice (CPMP/ICH/135/95) and the Directives 2001/20/EC and 2005/28/EC and with international and local regulatory requirements.

Subjects: demographic and other baseline characteristics: A sample size of 18 subjects was considered adequate to accurately assess the bioavailability and the pharmacokinetics parameters of the investigational drug. The 18 healthy volunteers were 9 males and 9 females. Their demographic and baseline characteristics are summarized in (Tables 1 and 2); the vital signs are summarized in (Table 3). Three of the female volunteers have a Body Mass Index (BMI) lower than the limit 20, but they were healthy considering all the others parameters, so it was evaluated the ratio lean mass/fat mass by bioelectric impedance technique and they have values similar to the other volunteers, considering this they were enrolled.

Presence of cardia, pulmonary, gastrointestinal, endocrine, musculoskeletal, neurological, haematological, hepatic or renal disease, unless deemed not clinically significant by the investigator; presence of any significant physical or organ abnormality; history or evidence of psychiatric or psychological disease (including depression) unless deemed not clinically significant by the investigator; any clinically significant illness during the 4 weeks before this study; pregnancy, lactation period; smoking; history of alcohol and drug abuse; known history of hypersensitivity to a-lipoic acid and to other sulphur molecules; use of any prescription medication in the 14 days preceding this study; use of over-the-counter (OTC), homeopathic and herbal medicines in the 14 days preceding this study; use of preparations containing a-lipoic acid in the 30 days preceding this study; participation in a clinical trial

<table>
<thead>
<tr>
<th>Variable/Characteristics</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, males (N, %)</td>
<td>10 (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>38 ± 6</td>
<td>30</td>
<td>47</td>
</tr>
<tr>
<td>Height, cm (mean ± SD)</td>
<td>181 ± 5</td>
<td>174</td>
<td>188</td>
</tr>
<tr>
<td>Body weight, kg (mean ± SD)</td>
<td>81 ± 3</td>
<td>66</td>
<td>105</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>24.8 ± 2.7</td>
<td>21.3</td>
<td>29.7</td>
</tr>
<tr>
<td>Systolic blood pressure (mean ± SD)</td>
<td>123.8 ± 7.7</td>
<td>110</td>
<td>140</td>
</tr>
<tr>
<td>Diastolic blood pressure (mean ± SD)</td>
<td>73 ± 3.9</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Heart rate (mean ± SD)</td>
<td>75 ± 8.8</td>
<td>55</td>
<td>90</td>
</tr>
<tr>
<td>Temperature (mean ± SD)</td>
<td>36.1 ± 0.3</td>
<td>35.5</td>
<td>36.9</td>
</tr>
</tbody>
</table>

Table 1: Demographic Data and Baseline Characteristics – Males.
ALA plasma levels were determined by high-performance liquid chromatography HPLC (Agilent 1200 SL) equipped with triple quadrupole mass detector (Agilent 6410) and column Acquity UPLC BEH C18, 50 mm × 2.1 mm × 1.7 µm. The mobile phase was made up of 5 mM of ammonium acetate solution for LC/MS (0.3854 g/L) in water.

The stock solutions of lipoic acid (SSLA, Sigma 117K0679) and internal standard (IS) were prepared in methanol. The internal reference was cyclohexanobutyric acid (Aldrich, 00105LA). Calibration curves were prepared for both solutions by spiking each plasma blank samples with proper volume of the standard solutions to obtain the calibration curve points of ALA. The standard calibration curves for ALA were constructed using the analyte/IS peak area ratios versus the nominal concentrations of the analytes. Chromatographic separations were carried out using acetonitrile as mobile phase and acetic acid 0.1% (pH 4.0 adjusted with ammonia solution) (65:35, v/v); the flow rate was 0.2 ml/min. The analytical column was kept at 30°C and the effluent was connected to an electrospray ionization MS interface without splitting. Electrospray ionization was performed using nitrogen at 10 l/min flow rate, 40 psi nebulizing pressure, and 350°C drying gas temperature. Capillary voltage was set at 3000 V. Fragment voltage was applied between the capillary outlet and the first skimmer-produced fragment ions by in-source collision-induced dissociation by nitroson. 70 V optimum fragment voltage was selected (varying 50-150 V). The Limit of Determination (LoD) was 5 µg/ml; the Lower Limit of Quantitation (LoQ) was 20 µg/ml; the limit used for data certification was 50 µg/ml.

Safety Assessment: Safety was assessed at screening (Visit 1) and at the end of study (Visit 4) by vital signs, a physical examination (general appearance, skin, eyes, ENT, chest/lungs, heart/cardiovascular, muscular-skeletal system, abdomen, kidney, lymph nodes, nervous system, genitourinary and endocrine system) and routine laboratory tests: haematology; erythrosedimentation rate (ESR), red blood cell count (RBC), white blood cells total and differential count (WBC), haemoglobin, haematocrit, platelet count, prothrombin time; clinical chemistry: plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), glucose, creatinine, total serum proteins, sodium and potassium; urinalysis: specific weight, pH, glucose, ketones, haemoglobin, protein, bilirubin, urobilinogen, nitrates. At screening (Visit 1) only virology: hepatitis B antigen (HBsAg), hepatitis C antibody (HCV-Ab), human immunodeficiency virus 1 and 2 antibodies (HIV-1 and -2 Ab), pregnancy test. Adverse events were monitored throughout the study.

Statistical analysis: Statistical analyses were performed using the commercially available computer program Origin (version 7.0). All data are presented as mean ± standard error (SE). Descriptive statistics of the concentration-time data of the two formulation of α-lipoic acid were carried out. Pharmacokinetic data are presented as mean ± SEM. To compare the means of continuous parameter one-way ANOVA test was used, while for non-continuous parameters such as T_{max} and half life, a two sided t-test was performed.

Results Pharmacokinetic Analysis

All the data of the plasma concentration of the two treatments were collected and the pharmacokinetic parameters were calculated assuming the value of 0 for the data under the limit of revelation (50µg/ml). The mean concentrations of the two drugs in the healthy volunteers were calculated for each sampling point and the concentration curves were built (Figure 1).
The values of the pharmacokinetics parameter $C_{\text{max}}$ were $6.66 \pm 1.17$ µg/ml for treatment A and $7.00 \pm 1.32$ µg/ml for treatment B with a coefficient of variation percent (CV%) respectively of 74.4% and 80.0%. AUC$_{0-\text{inf}}$ was $5.23 \pm 7.44$ mg/ml*h for treatment A and $5.77 \pm 0.81$ µg/ml*h for treatment B (CV of 60.3% and 59.9%); AUC$_{\text{inf}}$ were $5.65 \pm 7.45$ µg/ml*h for treatment A and $6.06 \pm 0.79$ µg/ml*h for treatment B (CV of 55.9% and 55.5%). $T_{\text{max}}$ parameter were 45.0 ± 4.4 min for the treatment A and 51.8 ± 4.3 for treatment B (CV of 41.2% and 35.4%), while half-life was 46.2 ± 15.1 min for treatment A and 40.2 ± 6.3 for treatment B (CV of 138.8% and 66.4%).

Safety evaluation

No adverse events were observed during the study. All the subjects examined showed normal values in both screening and final visit. Vital signs, clinical examination and objective laboratory tests were normal at the beginning and the end of the study.

No subject left the study due to adverse events or the investigator’s decision.

Statistical analysis

The statistical analysis of the pharmacokinetics parameter showed no significant difference between the two formulations (Table 4).

Discussion

Even though a normal diet contains ALA, food sources fail to provide a pharmacologically active quantity of antioxidant as only small quantities present in food can be absorbed as free ALA [10]. Furthermore, pharmacokinetic analysis of an endogenous substance (as in the case of ALA) is complicated by the interference of basal levels and/or metabolism of the substance. These represent limiting factors in obtaining precise and reliable analyses of plasma levels of lipoic acid or its metabolites, and help to explain the incongruities between published data. Therefore, the analysis of ALA in biological samples has demanded new and complex methodological approaches [11,12]. The analytical procedure used in this study is one of the most reliable methods for identifying ALA plasma levels. In clinical practice, lipoic acid is administered in racemic form (rac-ALA), the pharmacokinetic characteristics of which have already been studied in man after single dose administration [13,14,11,15]. Some studies have examined how food intake and severe renal damage influence the pharmacokinetic parameters of rac-ALA [13,16].

In this study, the $C_{\text{max}}$ of ALA new formulation was higher and was reached about 15 minutes later than reported in healthy volunteers after a single oral administration of rac-ALA 600 mg [17-20], implying that the galenic formulation influences absorption and absorption time. For example the rate of absorption of lipoic acid is not substantially influenced by the time of gastric emptying, as demonstrated in studies in insulin-dependent diabetics with habitual delayed gastric emptying, in which no important influence on ALA bioavailability was observed [14]. A clear relationship between bioavailability and the antioxidative pharmacological effects has not yet been established. The good $C_{\text{max}}$ indicates that new formulation positively affects absorption and absorption time regardless of the pharmaceutical form.

The AUCinf values of new formulation are not high, in accordance with the half-life value, which does not depend on the dose but is correlated to the general conditions of the organs and systems. Taking into consideration the fact that the present is a cross-over study, it evident that ALA in softgel capsules and tablets is absorbed more consistently and rapidly and is eliminated efficiently. The AUC value of two treatments is a more reliable measure of ALA bioavailability, since it is directly related to the total amount of non-modified drug which reaches systemic circulation. Careful assay and complete observation of drug elimination were made possible in this study through blood sample collection.

Some studies report that the average plasma concentrations at which the therapeutic effects of lipoic acid begin to be seen correspond to $C_{\text{max}}$ and AUC values equivalent to 4-5 µg/ml and 2.85 µg/ml*h, respectively [12,15,19,21]. Nevertheless, a clear relationship between bioavailability and the antioxidative pharmacological effects has not yet been established.

The dose-independent half-life correlated to the general conditions of the organs and systems, further indicating that new formulation is absorbed consistently and rapidly and is eliminated efficiently.

Examining the correlation between pharmacokinetic parameters and therapeutic efficacy makes it possible to acquire important information for designing preclinical and clinical studies. The pharmacokinetic characteristics of a compound can significantly limit its clinical use if pharmacologically active concentrations are not reached and/or maintained for the time necessary to evoke therapeutic effects. Since it is not possible to correlate the pharmacokinetics and pharmacodynamics of ALA [21], the therapeutic effects depend preponderantly on the $C_{\text{max}}$ and AUC values rather than on the time values for reaching maximum concentration ($T_{\text{max}}$), elimination half-life ($T_{1/2}$) or median retention time (MRT). This opinion is supported by pharmacokinetic studies conducted in patients, which demonstrated that therapeutic response is positively correlated to the $C_{\text{max}}$ value [22-24].

The antioxidant capacity of ALA depends fundamentally on the induction of glutathione regeneration at the cellular level. The increase of glutathione after ALA administration is probably due to an adaptive cellular response induced by improved employment of the cysteine accumulated in the cell [25]. It has recently been reported that the cellular kinetics of ALA are proportional to the plasma kinetics of the compound [18,20]. Considering the time needed to regenerate cellular glutathione after administration of ALA, new formulation in two different forms could prove to be an able to reach its target quickly at active concentrations and could serve as an excellent point of departure for pursuing further interesting pharmacological and therapeutic developments. The properties of the new formulation ensure rapid

![Figure 1: Concentration/Time Curves of the two formulation of a-lipoic acid. Values are the mean ± Standard Error](image-url)
release in vivo with high ALA content per unit dose and excellent homogeneity of release.

ALA solubility may be increased by vehicle in an amphiphilic matrix like lecithin, a good emulsifier. After rapid dissolution there is a good solubilization by lecithin: an inexpensive method that is very simple to use. The pharmacokinetic characteristics of tablets and softgel capsules, both delivering new ALA formulation, show the same human pharmacokinetic profile. Rapid release of ALA is achieved through the high speed of solubilization of the soft gelatin and tablets by the action of the superdisaggregating agents. The results should prove useful in treating inflammatory processes and osteoarticular neuropathies, in particular canalicul syndromes, sensory or motor radiculopathies, diabetic polyneuropathies, and peripheral neuropathies of various origins.

Acknowledgments

The authors thank the Chelab Laboratory for their help in determining α-lipoic acid; a catalytic agent associated with pyruvate dehydrogenase. Biochem Pharmacol 50: 513-514.

References


