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(R)-α-lipoic acid oral liquid formulation: pharmacokinetic parameters and therapeutic efficacy

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Summary. Despite its numerous potentials, oral administration of α-lipoic acid (ALA) is characterised by pharmacokinetic limitations that reduce its therapeutic efficacy. Indeed, phenomena such as reduced solubility, lack of gastric stability and hepatic degradation determine a bioavailability of around 30% and a short half-life of ALA. The innovative oral formulation has the potential to overcome these pharmacokinetic limitations as it uses only the R enantiomer of α-lipoic acid, the natural and more active form, whose solubility and stability in gastric environment are ensured by the patented liquid solution. Analysis of the pharmacokinetic profile showed that, with this formulation, the absorption of R-ALA is accelerated with high plasmatic concentrations and prolonged stability. Therefore, the bioavailability, which tends towards intravenous bioavailability, is markedly greater than that recorded with a solid R-ALA formulation. To overcome the pharmacokinetic limitations of current solid oral formulations, one potentiates the biological efficacy of ALA: indeed, in animal models, R-ALA in a liquid formulation amplifies the recovery of the conduction velocity of sensory and motor nerves altered by diabetic neuropathy. These results highlight that the characteristics of R-ALA in the liquid formulation lead to a more effective and faster biological response suggesting a new therapeutic scenario for numerous oxidative stress-dependent pathologies (diabetic complications, peripheral neuropathies, neurodegenerative and cardiovascular diseases, etc.) requiring chronic treatment.

Key words: α-lipoic acid, R-ALA, oral liquid formulation, solubility, pharmacokinetics, pharmacology.

Introduction

α-lipoic acid (ALA) represents a clear example of the thin line that exists between the world of dietary supplements and that of drugs (1, 2). ALA supplementation, which originally was introduced to increase the energy requirements of tissues and organs (3, 4), is today more in demand as a support for the treatment of all those diseases characterised by insufficient mitochondrial activity responsible for a reduced production of cellular energy and an uncontrolled formation of free radicals (5, 6).

Thanks to its particular chemical structure, ALA exerts its biological activity both in a hydrophilic (cytoplasm, extracellular matrix) and hydrophobic (plasma membranes) environment (6), features that allow us to consider it as an important ubiquitous factor.

Over the last decades, additional biochemical and cellular properties have been attributed to ALA, thus broadening the knowledge of its multiple actions. Beside being a well-known enzymatic co-factor (7) and powerful antioxidant (5, 8, 9), this compound regulates the transcription of numerous genes (9-11), participates in the glucidic (12, 13) and lipid (14, 15) me-

tabolism, increases sensitivity to insulin (16, 17) and behaves as a chelating agent of heavy metals (18). All these functions underline its pharmacological potential (19). Countries such as Germany, Hungary, Austria, Poland, Romania and the USA use ALA as an ethical drug (1, 2, 19, 20) encouraged by the beneficial effects highlighted in clinical studies carried out for the treatment of numerous diseases (21-23). Significant results have been obtained over a brief period of time in the case of intravenous administration of ALA (22, 23). This treatment modality allows to achieve the maximum plasma level, even if for a short period of time due to marked accumulation in various tissues (24-26) and to its rapid metabolism that influences its half-life (1, 27, 28). The degradation process that occurs via an oxidative mechanism, takes place especially during ALA's first passage through the liver. At the same time, this evidence heralds the limitations of the oral formulations of ALA, which are more appropriate for a long-term therapeutic schedule but limited by other factors that contribute to reduce its biological efficacy. Indeed, phenomena such as reduced solubility in an acidic environment, enzymatic degradation and lack of stability that characterise its gastric and hepatic passage when taken orally, limit the potential of ALA (1, 5). For this reason, over the past years, various chemical interventions and formulations have been tested to achieve greater plasma bioavailability of ALA even after oral administration and to ensure better therapeutic effects (29-31).

Overcoming the pharmacokinetic limitations of solid oral formulations currently available (bioavailability of around 30% and short half-life) (1, 32), one potentiates the biological efficacy of ALA in the daily treatment of oxidative stress-dependent diseases (e.g. diabetic complications, mechanical compression neuropathies, neurodegenerative and cardiovascular pathologies, physical and mental impairment, obesity etc.) (1, 5, 6, 32-35). Moreover, an efficient and rapid supplementation of ALA can be a valid support even in those physiological situations, such as ageing, that are characterised by a drastic reduction of the endogenous antioxidant defence systems (36, 37).

In this context, there is a new patented oral liquid formulation (Liponax sol) that represents a valid alternative to improve the pharmacokinetic parameters of α–lipoic acid thus potentiating its therapeutic efficacy (38).

To date, the various strategies aiming at increasing the bioavailability of ALA after oral administration have not yet resulted in a definitive solution.

ALA supplements are recommended to be taken on an empty stomach in order to exploit the acidic pH of the stomach that is necessary to favour the gastric absorption of a weak acid like ALA and to reduce competition from other nutrients during enteric absorption (1, 2, 39). Despite this and the progress achieved with the various solid pharmaceutical formulations (tablets, coated granules, retarded release and rapid release) (30, 31, 40), significantly increasing the plasma concentration and stability of ALA remains an objective to be achieved.

In most cases, oral formulations of ALA imply the use of racemic mixtures (R, S-ALA) (1, 40) that can be made more easily synthetically and are chemically stable. Indeed, due to the presence of an asymmetric carbon, ALA is present in the enantiomeric R (R-ALA) and S (S-ALA) forms (1, 2, 32). Stereoselectivity processes significantly affect the characteristics of the two enantiomers that turn out to be different both in the absorption and tissue distribution as well as in the degradation and elimination phases (6, 26). It has been demonstrated that independently from the R, S-ALA formulations used and from the administration modality (intravenous, oral), the bioavailability of R-ALA is higher than that observed for S-ALA (1, 2, 26). In addition, R-ALA represents the natural (1, 32), less toxic (41, 42) and biologically more efficient form compared to both S-ALA and to the racemic form (12, 32, 37, 43). Finally, series of clinical studies on mechanical neuropathies have documented the greater efficacy of R-ALA compared to the racemic form, especially in terms of rapidity and intensity of the therapeutic effect on the symptoms of pain with good effects on the quality of life of patients (44, 45). Using the enantiomeric R form instead of the racemic mixtures represents a concrete step forward in the therapeutic action of α–lipoic acid as it acts at two levels at the same time: 1) plasma bioavailability of the compound is increased and 2) only the natural and most effective form is supplied.

R-ALA bioavailability in oral liquid formulations

The evidence accumulated over the past years has underlined the fact that lack of ALA solubility in an aqueous and acidic environment such as that of the stomach has an impact on the fraction available for absorption and therefore, represents an important concomitant cause of the low bioavailability after oral administration. Moreover, the first section of the intestine, even if there is subject variability, takes part in the absorption of the residual fraction of ALA through specific carrier proteins (6, 24, 25). Whilst at low concentrations in the intestine there is active transport mediated by carriers, most certainly in competition with the short chain fatty acids, at high concentrations a diffusion mechanism is also favoured that is more rapid and less consuming from an energetic point of view (24). As a result, by ensuring an increase in the quantity of oral ALA available upstream for gastric and enteric absorption, one favours an increase in plasma bioavailability.

In light of this, salification of R-ALA allows to increase solubility in the gastric environment and ensure a greater fraction available for absorption (46). Moreover, it has already been documented that the oral administration of a defined drinkable solution, identical to the saline one injected intravenously, reaches a C_{max} (maximum plasma concentration) value that is 4.5 times higher than that obtained with an equal dose of a solid formulation (2, 27). This data confirms that the partial dissolution of tablets represents a crucial passage in absorption and moreover, allows an intense epatic metabolism of the absorbed fraction of ALA (1, 6). In contrast, immediate availability of the drinkable formulation allows greater absorption that is responsible for the transient saturation of the effect during its first passage through the liver and therefore, determines greater quantities of ALA in the plasma (2, 27).

These assumptions are at the basis of the innovative liquid formulation that combines the solubility of salified R-ALA with the greater stability in the gastric environment favoured by the co-solvent propylenic glycol (38). Moreover, as already documented for other substances (i.e. phenylbutazone, melatonin, rapamycin), a direct involvement of the co-solvent cannot be excluded in increasing the bioavailability of R-ALA (47-49) by exerting an active role in the absorption phase.

Analysis of the plasmatic profile of R-ALA carried out after oral administration of the liquid formulation in an animal model has highlighted an improvement of the pharmacokinetic parameters compared to what has been recorded in a parallel treatment with a solid formulation.

For this pharmacokinetic study, R-ALA in a solid (tablet) or liquid formulation was administered orally to male Sprague-Dawley rats that had been previously deprived of food for 18-20 hours.

Following a single administration of R-ALA (10 or 50 mg/kg), blood samples were collected in test tubes containing the anticoagulant heparin and taken at different time intervals (0, 5, 30, 60, 120 and 240 min). Concentrations of ALA in the plasmatic fraction obtained after centrifugation at 13.000 rpm for 5 minutes were recorded using liquid chromatography-mass spectrometry. Finally, using analysis software (Kinetica™, PK/PD Analysis) the following pharmacokinetic parameters were calculated: C_{max}, T_{max} (time necessary to reach maximum concentration), AUC $_{0\text{-t (finale)}}$ (*area under the curve* relative to the plasmatic profile from time zero to *t*, last measurable value).

Confirming other published data in the literature but relative to pharmacokinetic profiles after administration of R, S-ALA to mice (50) or human subjects (51), the comparative study, summarised in Figure 1, showed that the plasma bioavailability of R-ALA

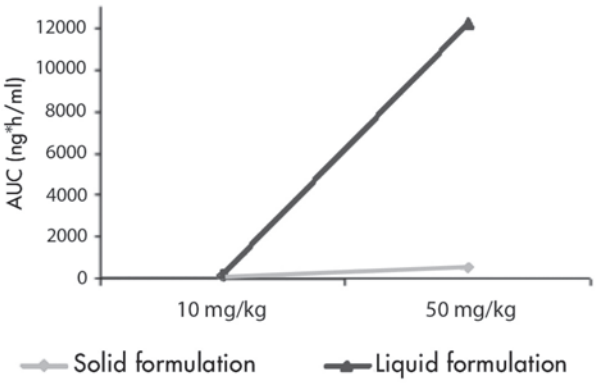


Figure 1. Dose-dependence of R-ALA bioavailability when administered orally.
Plasma bioavailability (AUC) in Sprague Dawley rats in relation to the oral administration of a dose of 10 or 50 mg/kg of R-ALA in solid or liquid formulations.

(represented by the AUC value) is directly proportional to the administered dose, independently of the type of oral formulation (solid or liquid). However, the highest plasma values are always recorded in the case of liquid formulations.

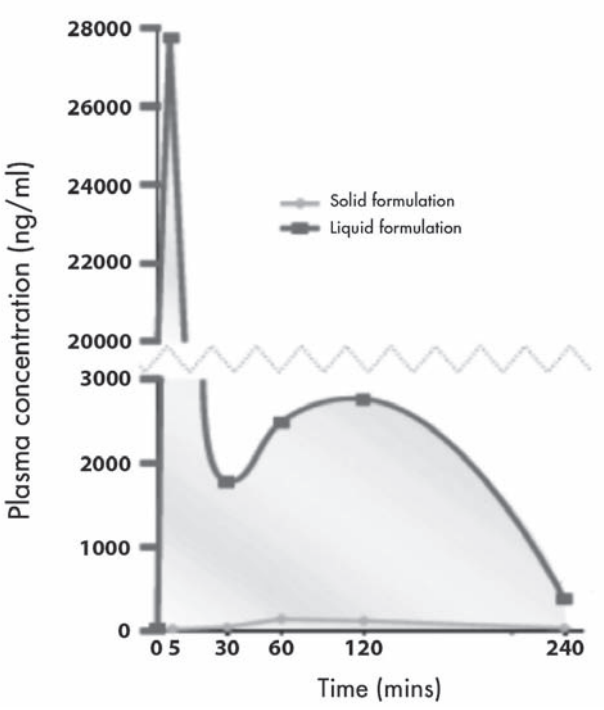
The slopes of the lines in Figure 1 depend on the physiological and biochemical processes that are at the basis of every pharmacokinetic profile.

In the case of a liquid formulation, at the highest considered dose (50 mg/kg), one obtains a plasma bioavailability of R-ALA that is significantly higher than that achieved after the administration of the same dose but in a solid form. This increase in bioavailability is reasonably due to various phenomena enhanced by the liquid formulation of R-ALA: increase of the absorbed fraction, saturation of the binding plasma proteins and slowing down of the degradation process. A synergy among these factors determines a greater quantity of free plasmatic R-ALA capable of reaching tissues and organs where it can exert its own biological function.

Analysing more in detail the plasmatic profiles of R-ALA after a single oral administration in the rat of a dose of 50 mg/kg (Fig. 2) one can see that the liquid formulation ensures a significantly greater bioavailability value compared to what is recorded after treatment with a solid formulation of R-ALA ($AUC_{liquid\ R\text{-}ALA}$ = 12231.90 $ng*ml^{-1}*h$ *vs* $AUC_{solid\ R\text{-}ALA}$ 512.33 $ng*ml^{-1}*h$).

In fact, the liquid formulation

- Accelerates absorption of R-ALA as highlighted by the maximum peak recorded more rapidly compared to that attributed to the solid formulation ($T_{max\ liquid\ R\text{-}ALA}$ = 5min *vs* $T_{max\ solid\ R\text{-}ALA}$ = 60 min): greater solubility due to the salified form of R-ALA and to the presence of a co-solvent favours a rapid absorption mainly at a gastric level;
- Allows a higher plasma concentrations of R-ALA ($C_{max\ liquid\ R\text{-}ALA}$ = 27600ng/ml *vs* $C_{max\ solid\ R\text{-}ALA}$ 195ng/ml): greater solubility and stability in the gastric milieu increase the quantity of R-ALA available for absorption;
- Promotes a persistent stability of R-ALA in the plasma as demonstrated by the concentrations recorded in the plasma samples taken at later time intervals ($C_{240\ min\ liquid\ R\text{-}ALA}$ =353.1 ng/ml) that are even higher than the maximum peak of the solid formulation ($C_{max\ solid\ R\text{-}ALA}$ 195 ng/ml). The degradation mechanisms of R-ALA are most certainly active and efficient as confirmed by the rapid decline of the pharmacokinetic profile but the significant amount of R-ALA absorbed by the liquid formulation contributes to the saturation of the degradation mechanisms slowing down kinetics. Moreover, at later time intervals not only further intestinal absorption but also a release of R-ALA weakly sequestered by plasmatic proteins or accumulated as a reserve in various tissues cannot be excluded.



	Solid formulation	Liquid formulation
$AUC_{0\text{-}t(final)}$ ($ng*ml^{-1}*h$)	512.33	12231.90
T_{max} (min)	60	5
C_{max} ($ng*ml^{-1}$)	195	27600
C_{120min} ($ng*ml^{-1}$)	150.7	2752
C_{240min} ($ng*ml^{-1}$)	28.08	353.1

Figure 2. Pharmacokinetic profiles of R-ALA after oral administration.
Blood tests for the analysis of plasma concentrations were carried out at 0, 5, 30, 60, 120, 240 minutes after the oral administration to male Sprague-Dawley rats, of a 50 mg/kg dose of R-ALA in solid or in liquid formulation.

Therapeutic efficacy and safety of R-ALA in the oral liquid formulation

To check whether the improved bioavailability of the innovative liquid formulation translates into an enhancement of its biological efficacy, certain effects of R-ALA were assessed in an experimental model of diabetes induced in Sprague Dawley rats after a single intraperitoneal injection of a 60 mg/kg dose of streptozotocin (52). This antibiotic develops selective toxicity for the pancreatic cells responsible for the production of insulin. After 3-4 weeks from induction of the damage, the animals present typical characteristics of diabetic subjects such as hyperglycemia, hypertriglyceridemia and weight loss; whilst after 5-6 weeks, also other complications become more evident including loss of peripheral neuronal function typical of diabetic neuropathy (52, 53).

To assess the efficacy in the recovery of certain parameters altered by the pathology (nerve conduction velocity, hyperglycaemia, hypertriglyceridemia), R-ALA was administered at 6 weeks after induction of the damage when manifest diabetes was evident. In this experimental design, liquid and solid R-ALA formulations were compared in control animals, as well as in healthy rats or rats made diabetic but not subjected to treatment, necessary in order to carry out a correct analysis of the results.

R-ALA in tablets allows the partial recovery exclusively of the conduction velocity of the sensory digital nerve (Fig. 3). In contrast, R-ALA in liquid formulation amplifies these positive effects as it favours greater recovery of the digital nerve function and also a more modest recovery of the caudal and sciatic motor nerve function (Fig. 3).

The action of R-ALA on the recovery of neuronal function is mainly due to its antioxidant properties capable of contrasting the hyperproduction of reactive oxygen species (ROS) triggered by hyperglycaemia (56, 57). A similar situation, due once again to oxidative stress, also takes place in other neuropathic forms, from mechanical ones (45) to those caused by chemotherapy treatments. (58). Hyperproduction of ROS, irrespective from the cause, has a negative impact on neuronal function as on the one hand it reduces the supply of nutritional factors and oxygen and on the other, it damages the membranes of the neurons responsible for nerve conduction (57). As a result, the faster and more efficient the antioxidant activity, the less the cellular damage thus contributing to recovery of neuronal function. In light of this, the liquid formulation of R-ALA that, as observed in the animal model, has greater therapeutic potential than the solid formulations, can be promoted as an efficient alternative for oral supplementation.

Moreover, as underlined by other recent observations (58) and by clinical studies carried out in diabetic patients (21, 59), the results which have just been presented confirm the differential impact of ALA on the various nerve fibre populations. The sensory fibres are more vulnerable to the toxic effects induced by oxidative stress and therefore, are more sensitive to the action of various antioxidants. Symptoms such as loss of sensitivity, numbness of the limbs, painful tingling or burning sensations that are all linked to the biochemical and structural alteration of the sensory fibres are the first to benefit from the administration of R-ALA. However, with the liquid formulation, one reaches a plasma concentration threshold that extends the protective and reparatory effects also to the larger nerve fibres such as the motor nerves.

Moreover, in the same experimental animal model of diabetes the action of oral R-ALA on hematic pa-

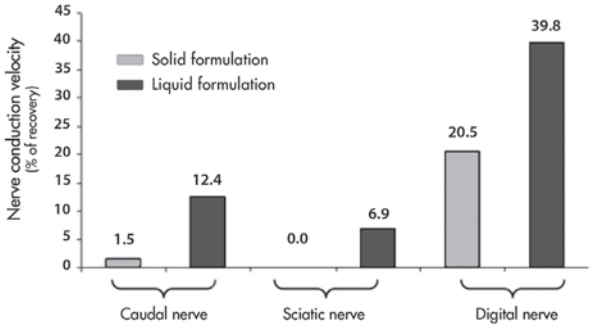


Figure 3. Percentage of recovery of conduction velocity of the caudal, sciatic and digital nerves after oral treatment with solid or liquid R-ALA formulations.
The conduction velocity of the caudal nerve, of the sciatic motor nerve and of the sensory digital nerves were analysed at the start and end of treatment with the two R-ALA formulations according to the methods described by Cavaletti et al. (54) and Tredici et al. (55). The values are shown as a percentage of recovery of nerve conduction velocity, following treatment with R-ALA and calculated on the basis of what was recorded in healthy animals and in those made diabetic but not treated.

rameters such as hypertriglyceridemia and glycaemia commonly altered in diabetic patients, were also evaluated. R-ALA in liquid formulation led to a significant reduction in the quantity of triglycerides and also even if moderately, of glucose in the blood. As a result, the increased solubility and greater stability of R-ALA in the liquid formulation leads to an increase in plasma bioavailability, a necessary prerequisite for a faster and broader biological response.

To date, the intravenous administration of ALA represents the formulation with the greatest clinical efficacy (21, 23, 59) as it allows to reach maximum bioavailability in the plasma.

Clinical studies carried out in the treatment of diabetic neuropathy in patients fixed a daily dose of R,S-ALA of 600 mg (300 mg R-ALA + 300 mg S-ALA), administered intravenously or orally as the recommended dose for biological efficacy in which tolerance and undesirable effects are identical to those observed with placebo (59, 64). Overcoming the limits attributable to gastrointestinal absorption, the innovative liquid formulation of R-ALA aims to achieve better bioavailability, close to intravenous bioavailability, with a good safety profile.

In the literature, there are therapeutic schedule that use higher doses for the treatment of more complex pathologies such as multiple sclerosis (21). On the other hand, sporadic cases of intoxication due to overdose of ALA have been published (e.g. 1200-1800mg/die) characterised by allergic reactions of the skin, transient gastrointestinal disorders (nausea, vomiting, abdominal pain etc.) (5, 21, 59, 64) or some rare cases of cholestasis (itching, alteration of hepatic enzymes); all these symptoms however, disappear upon interruption of ALA supplementation (65).

Numerous studies available today underline that ALA and even more so R-ALA, do not have genotoxic and mutagenic effects (60-62) and as documented by the toxicological responses obtained in various animals, it has an excellent safety profile (6, 60, 61, 63).

Conclusions

The oral liquid formulation of R-ALA (Liponax sol), by favouring greater and faster absorption, allows high bioavailability of R-ALA and prolonged stability in the plasma. By proposing R-ALA, which is the natural, biologically more active enantiomer, in a formulation capable of improving its solubility and ensuring stability in the gastric milieu, various pharmacokinetic limitations that have been attributed to the oral supplementation of lipoic acid can be overcome. The results obtained highlight that these characteristics lead to a more efficient and faster biological response, thus suggesting a new therapeutic scenario for numerous oxidative stress-dependent diseases that like in the case of peripheral neuropathies require chronic treatments.

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