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RESEARCH PAPER

Development of a Sustained Release Dosage Form for α-Lipoic Acid. II. Evaluation in Human Volunteers

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ABSTRACT

Within this study an oral sustained release dosage form of α-lipoic acid (thioctic acid) has been generated and evaluated in healthy volunteers. A granulate comprising 56.8% α-lipoic acid and 43.2% chitosan acetate was compressed to tablets (weight: 0.45 g; diameter: 10.0 mm; thickness: 4 mm). Three of these tablets were administered at once orally to each volunteer. Prior to administration and then every hour for 12 hours blood samples were taken from the antebrachial vein. α-Lipoic acid concentrations in plasma were quantified via precolumn derivatization and reversed-phase high-performance liquid chromatography (HPLC). Results demonstrated that an increased plasma level of α-lipoic acid can be achieved by this formulation for at least 12 hours. Within this time period at least two maximum plasma concentrations were reached. The first one is based on the release of α-lipoic acid, which is not ionically and therefore only loosely bound to chitosan, whereas a second maximum is based on the release of the drug during the enzymatic degradation of the chitosan matrix in the colon. The $AUC_{0\rightarrow 12}$ was determined to be 183.8 ± 101.4 μg × min/mL (mean ± SD; n = 8). Because of the pulsed sustained release of α-lipoic acid, the dosage form described here seems to be highly beneficial in order to stimulate the glucose uptake in the case of diabetes type II.

Key Words: α-Lipoic acid; Chitosan; Controlled drug release; Diabetes; Oral drug delivery.

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INTRODUCTION

Although there have been numerous oral formulations for α-lipoic acid (thioctic acid) available on the market for many years, no sustained release dosage form for this therapeutic agent has been generated so far. The maintenance of a therapeutic concentration of α-lipoic acid over a whole day, however, might be highly beneficial to guarantee a permanent antioxidative and free radical scavenging effect,[1,2] to prevent early glomerular injury in diabetes mellitus,[3] and/or to stimulate glucose uptake in case of diabetes type II.[4,5] In order to provide a permanently raised level of α-lipoic acid in the systemic circulation, commercially available oral dosage forms have to be administered at least six times a day.[6] The development of a sustained release delivery system for α-lipoic acid should therefore minimize the frequency of dosing and improve the compliance.[7] In case of treatment of diabetes a pulsed sustained release system might be even more efficient given that it is leading to a greater stimulation in the glucose uptake from the circulatory system into the muscle. As shown by in vitro studies, a sustained release of α-lipoic acid can be easily achieved by the coadministration of the cationic polymer chitosan within the delivery system.[7] Based on ionic interactions, the more chitosan is added to α-lipoic acid, the stronger is the retardation of drug release. Furthermore, it was shown that at a comparatively high ratio of chitosan in the delivery system, a certain ratio of α-lipoic acid is no longer released from the dosage form. On the other hand, chitosan is well known to be enzymatically degraded in the colon by bacterial polysaccharidases.[8,9] Tozaki et al., for instance, generated a colon-specific delivery system for insulin by using chitosan capsules, which are degraded in the colon thereby releasing the therapeutic agent.[10] The use of both effects—the more or less immediate release of rather loosely bound α-lipoic acid as well as its controlled release following the degradation of chitosan in the colon—should allow the development of a twice-pulsed, sustained release system for the drug. In order to achieve that goal, half of the drug should be released via a simple diffusion process leading to a first α-lipoic acid maximum in the blood within the first 6 hours. The other half, being ionically bound to chitosan, should be released by the degradation of the polymer in the colon leading to the second maximum within the next 6 hours. A twice daily application of such a delivery system should consequently result in a four-times daily pulsed sustained release of α-lipoic acid. The design of such a dosage form as well as its evaluation in human volunteers focusing on the pharmacokinetic of α-lipoic acid provided by the new formulation and biofeedback studies of the glucose level were the aim of the present study.

MATERIALS AND METHODS

Preparation and In Vitro Characterization of the Delivery System

Chitosan was hydrated in acetic acid and demineralized water. The resulting gel was homogenized with α-lipoic acid. The amount of all compounds used is listed in Table 1. After wet granulation the granulate was dried at 37° C, grinded with a dry granulator (Dry Granulator TG 2000; Erweka GmbH, Germany), and compressed (Korsch, Type EKO-DMS, Berlin, Germany) to tablets (diameter: 10.0 mm; thickness: ~4 mm). The compaction force was kept constant during the preparation of all tablets.

The amount of α-lipoic acid in the tablets was determined via high-performnace liquid chromatography (HPLC) analysis. Tablets were grinded and aliquots of 50.0 mg dissolved in 10.0 mL of 50% (v/v) acetic acid for 1 hour at 40° C. Thereafter, 10.0 mL of tetrahydrofuran was added and the dissolution process allowed to proceed for 15 min at 40° C. After the addition of 20.0 mL of demineralized water, aliquots were centrifuged and 20 μL of the supernatant fluid was directly injected for HPLC analysis. Separation was achieved by using a C_{18}-column (Nucleosil 100-5C18, 250 mm × 4 mm) at 20° C. Elution was performed as follows: flow rate 0.8 mL/min, 0–15 min, eluent 10 mM phosphoric acid/acetonitrile (6:4; v/v). α-Lipoic acid was detected by absorbance at 200 nm with a diode array absorbance detector (Perkin-Elmer 235C, Vienna, Austria). Peak areas were directly proportional to mass of standards injected. Concentrations were determined by interpolation from a standard curve.

Table 1. Quantity of compounds used for the preparation of test tablets.

Compound	Quantity
α-Lipoic acid	48 g
Chitosan	32 g
Acetic acid (glacial)	64 mL
Demineralized water	320 mL

Table 2. AUC values after oral administration of chitosan/α-lipoic acid tablets in eight volunteers (f = female; m = male) calculated by the linear trapezoidal rule.

Volunteer	f/m Weight	$AUC_{0\rightarrow 6}$ (μg × min/mL)	$AUC_{6\rightarrow 12}$ (μg × min/mL)	$AUC_{0\rightarrow 12}$ (μg × min/mL)
1	f 56 kg	65.2	47.4	112.6
2	m 64 kg	99.5	209.3	308.8
3	f 52 kg	195.9	0.0	195.9
4	f 50 kg	79.7	0.0	79.7
5	f 55 kg	55.3	230.9	286.2
6	f 70 kg	208.7	93.4	302.1
7	m 85 kg	107.9	0.0	107.9
8	f 56 kg	52.4	24.5	76.9

The release profile of the dosage form was established by the paddle method according to the European Pharmacopeia. Tablets were placed in the dissolution apparatus filled with 800 mL of demineralized water. Release studies were performed at 37° C and an agitation of 50 rotations per minute. Aliquots (1.0 mL) were withdrawn from the release medium at predetermined time points and the amount of released α-lipoic acid was determined via HPLC analysis as described above.

Design of the In Vivo Study

Eight healthy volunteers (6 female, 2 male) with a mean age of 25.1 ± 4.4 years and a body weight as listed in Table 2 participated in the study. After explaining the nature and purpose of the study, consent was obtained from each subject. The trial protocol was approved by the Ethics Committee of the Medical Faculty, University of Vienna. After a fasting period of 12 hours, three chitosan/α-lipoic acid tablets (each 0.45 g) corresponding to a total amount of 766.8 mg α-lipoic acid were given orally at 8:00 a.m. to each volunteer. In order to analyze the volunteers' observations and experiences during the application of the dosage form, they were asked to fill out a questionnaire as shown in Table 3. Multiple blood samples (5 mL) were collected from the antebrachial vein before (baseline) and hourly after oral administration for 12 hours. During the study volunteers were allowed to drink water ad libitum.

Quantification of α-Lipoic Acid in Human Plasma

Synthesis and Preparation of the Internal Standard

The quantification of α-lipoic acid in human plasma was performed using 11-mercaptoundecanoic acid as the internal standard. The compound was synthesized by refluxing of 11-bromoundecanoic acid (Aldrich, Vienna, Austria) with thiourea (Sigma, Vienna, Austria) in ethanolic sodium hydroxide solution. The resulting

Table 3. Questionnaire. The number of volunteers who agreed with a proposed answer is given in each section.

How was the size of the tablet?				
too small: 0	well sized: 5	too big: 3		
How was the smell of the tablet?				
good: 0	no smell: 7	bad: 1		
How was the taste of the tablet?				
good: 0	no taste: 8	bad: 0		
How was the tablet to be swallowed?				
easy: 1	normal: 2	difficult: 5	very difficult: 0	not possible: 0
How was the feeling during swallowing?				
slips easily: 1	unsmooth surface: 2	non scratching: 0	scratching: 5	slips hardly: 0
Side effects				
none: 7	headache: 0	nausea: 0	irritation of the stomach: 0	irritation of the esophagus: 1

11-mercaptoundecanoic acid was precipitated by acidification and purified by recrystallization from light petroleum-ethanol mixtures.[11] The chemical structure of the isolated compound was confirmed via nuclear magnetic resonance (NMR) analysis (BRUKER Avance 200 MHz).

The internal standard solution was prepared by dissolving 10.0 mg of 11-mercaptoundecanoic acid in 10 mL methanol and by diluting this stock solution 1:20 with the same solvent.

Extraction of α-Lipoic Acid from Plasma

The quantification of α-lipoic acid in human plasma was performed in a slightly modified way as described previously, allowing a differentiation between the (+)R- and (−)S-form of the drug.[12] In brief, heparinized blood samples (5 mL) were centrifuged (Hermle, Z 323K, Gosheim, Germany) at 3000 rpm for 5 min. Then, 20 μL of the internal standard solution was added to the supernatant fluid (2 mL). For acidification 300 μL of 1 M HCl was added to each sample followed by extraction with 5 mL of n-heptane/n-butanol (99+1) for 10 min. After centrifugation (13,000 g; 5 min), 3 mL of the organic phase were back-extracted into 250 μL of 200 mM sodium borate buffer pH 9.2. Traces of remaining organic solvents were removed under nitrogen stream. To 150 μL of the aqueous phase 20 μL of an aqueous 100 mM $SnCl_2$/2 M NaOH solution was added in order to reduce the disulfide bond of α-lipoic acid. After an incubation period of 20 min at room temperature, 40 μL of 1 M phosphate buffer pH 3.9, 40 μL of 100 mM Na_2EDTA, and 40 μL of the derivatization mixture (methanolic 10 mM *o*-phthalaldehyde and 10 mM D-phenylalanine in 10 mM H_3PO_4 mixed in equal volumes 1 hour before use) were added. The reaction was terminated by acidification with 70 μL of 500 mM H_3PO_4. After incubation for an additional 20 minutes at room temperature, 50 μL of eluent was added and aliquots (50 μL) of the solution was injected for HPLC analysis.

HPLC Analysis

Samples were separated on a C_{18}-column (Nucleosil 100-5C18, 250 mm × 4 mm) at 35° C. Elution was performed as follows: flow rate, 1.1 mL/min, 0–25 min; eluent, 20 mM Na_2HPO_3/methanol/acetonitrile (55+22.5+22.5). The fluorescence-labeled derivatives of α-lipoic acid and the internal standard were detected by a fluorescence detector (Fluorescence Detector LS 40, Perkin Elmer) set at an excitation wavelength of 230 nm and equipped with an emission filter < 510 nm. The amount of α-lipoic acid was quantified from the integrated peak areas and calculated by interpolation from a corresponding standard calibration curve.

Determination of the Blood Glucose Level

In parallel to the quantification of α-lipoic acid concentration in plasma, blood glucose level was determined. Aliquots (32 μL) of the collected blood samples were applied on Reflotron® Glucose test strips and the glucose concentration was determined with a Reflotron (Roche Diagnostics, Mannheim, Germany). After a wash-out period of 14 days the blood glucose level was determined in the same volunteers in the same way but without prior oral administration of α-lipoic acid.

Pharmacokinetic Data Analysis

All plasma data used in calculations were corrected for the endogenous plasma lipoate concentration determined by quantifying the plasma α-lipoic acid concentration in the samples taken prior to administration. The area under the plasma concentration-time curve from 0 up to 12 hours ($AUC_{0\rightarrow12}$), 0 up to 6 hours ($AUC_{0\rightarrow6}$), and 6 up to 12 hours ($AUC_{6\rightarrow12}$) was calculated by the linear trapezoidal rule. The terminal elimination half-life of peak I and II was determined via log-linear regression. Statistical data analysis was performed using the *t*-test with $p < 0.05$ as the minimal level of significance.

RESULTS AND DISCUSSION

Preparation of the Drug Delivery System

Chitosan/α-lipoic acid tablets were prepared based on the information gained from in vitro evaluations of sustained release dosage forms for α-lipoic acid.[7] The weight of the final dosage form was 0.45 ± 0.02 g. The ratio of α-lipoic acid in the dosage form (diameter: 10.0 mm; thickness: 4 mm) was determined to be 56.8% ± 5.1% (m/m; mean ± SD; n = 5). The drug release profile of the tablets is shown in Figure 1, demonstrating the manifestation of a plateau phase after 3 hours when approximately 50% of the drug was released. An explanation for this retardation of drug release can be given by the strong ionic interactions between the cationic polymer chitosan and the anionic

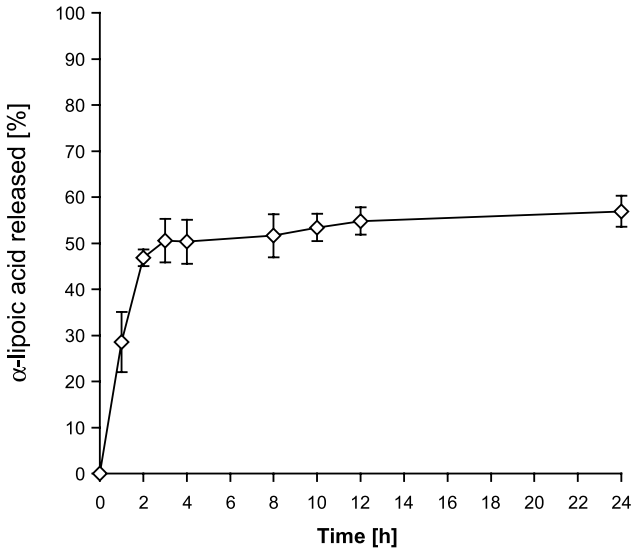

Figure 1. Dissolution profile of chitosan acetate/α-lipoic acid tablets.

group of α-lipoic acid. In contrast, the ratio of α-lipoic acid without a counter ion on the polymer was released from the delivery system quite rapidly. These in vitro studies, however, can only to a limited extent mimic the performance of the delivery system along the GI tract. In vivo, various further parameters such as the influence of colonic enzymes on drug release have to be taken into consideration.

Convenience of Administration of the Drug Delivery System

All volunteers were asked about their experiences during the oral intake of chitosan/α-lipoic acid tablets. The results of this study are summarized in Table 3. Apart from experiences covered by questions and proposed answers provided in the questionnaire, no additional experiences and/or observations were made by any volunteer during the study. As some volunteers had problems swallowing the tablets and felt scratching during the application process, an improvement in the formulation seems to be necessary. A reduction in the size of the tablet on the one hand, and a coating of the dosage form on the other, will certainly contribute to overcome these shortcomings.

Quantification of α-Lipoic Acid in the Plasma

The HPLC profiles of plasma samples after derivatization showed that all three analyte peaks were well separated and free from interfering matrix constituents. A representative HPLC chromatogram is shown in Figure 2. The average retention time for

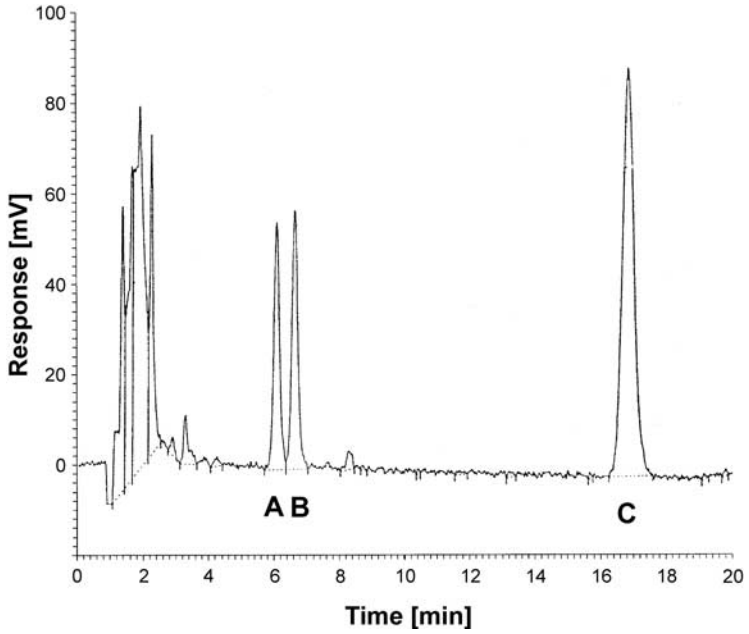

Figure 2. Representative HPLC profile of a plasma sample after derivatization. A: (+)R-α-lipoic acid derivative; B: (−)L-α-lipoic acid derivative; C: 11-mercaptoundecanoic acid derivative.

(+)R-α-lipoic acid derivative, the (−)S-α-lipoic acid derivative, and the 11-mercaptoundecanoic acid derivative was 6 min, 7 min, and 17 min, respectively. The limit of quantification for both enantiomers was 15 ng/mL plasma.

Pharmacokinetic Studies

The endogenous level of α-lipoic acid after 12 hours fasting was determined to be 112 ± 67 ng/mL. The plasma α-lipoic acid concentration determined in human volunteers after single oral administration of three chitosan/α-lipoic acid tablets is shown in Figure 3. The number of each volunteer corresponds to the number of each volunteer listed in Table 2, where the individual area under the curve (AUC) values after oral administration are provided. The mean values in plasma α-lipoic acid concentration of this study are shown in Figure 4. In most volunteers at least two release maxima (peak I and II) were reached within 12 hours. A first maximum was reached in most volunteers within an hour after application. It is likely based on the release of α-lipoic acid, which is not tightly bound to chitosan. A further concentration maximum following the first one after 4–10 hours seems to be based on the release of the drug when the chitosan matrix is enzymatically degraded in the colon. This result is in good agreement with the GI transit time of single unit dosage forms, which arrive in the colon 4.5±1.7 hours after administration,[13] taking additional time for enzymatic degradation by colonic bacteria and for absorption into consideration. Macleod et al., for instance, generated a selective colon drug delivery system based on chitosan demonstrating that more than 85% of the model compound was released from the tablets in the colon after 6.8 hours.[14] Studies focusing on the absorption of α-lipoic acid from different regions of the GI tract of the rat demonstrated that the uptake of the drug from the colon is as good as from the small intestine.[15] Furthermore, within the observation period the mean α-lipoic acid concentration was constantly above the endogenous level, underlying the sustained release effect of the delivery system. When commercially established oral formulations for α-lipoic acid are employed, more than 99% of the drug is eliminated from the blood within 4 hours after oral dosing.[6] In order to provide an increased plasma level of α-lipoic acid by such formulations, an oral administration becomes necessary at least six times a day. Taking compliance problems into consideration, the practical use of such formulations is therefore quite unrealistic. In contrast, a permanently increased plasma concentration of the drug can be achieved in an utmost simple fashion by a twice-a-day application of the sustained release delivery system described here. The pharmacokinetic parameters of the study are summarized in Table 4.

Recent studies demonstrated that chronic parenteral treatment with α-lipoic acid enhances insulin-stimulated glucose transport and nonoxidative and oxidative glucose metabolism in insulin-resistant rat skeletal muscle, with the R-(+) enantiomer being much more effective than the S-(−) enantiomer.[16] According to these findings, it was of interest to see whether there are

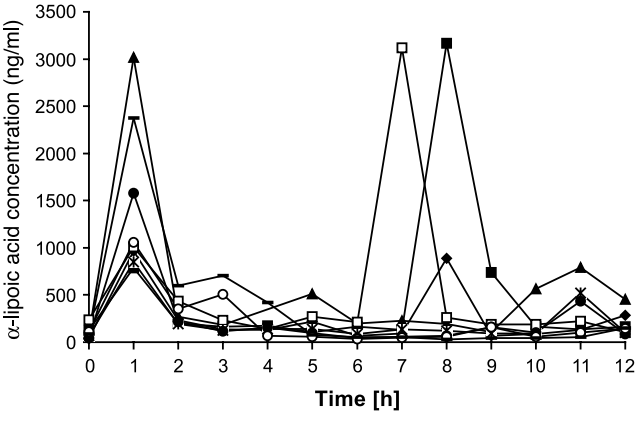

Figure 3. Plasma α-lipoic acid concentration in eight volunteers after single oral administration of three chitosan/α-lipoic acid tablets. Key: ◆, volunteer 1; □, volunteer 2; −, volunteer 3; ○, volunteer 4; ■, volunteer 5; ▲, volunteer 6; ●, volunteer 7; ★, volunteer 8.

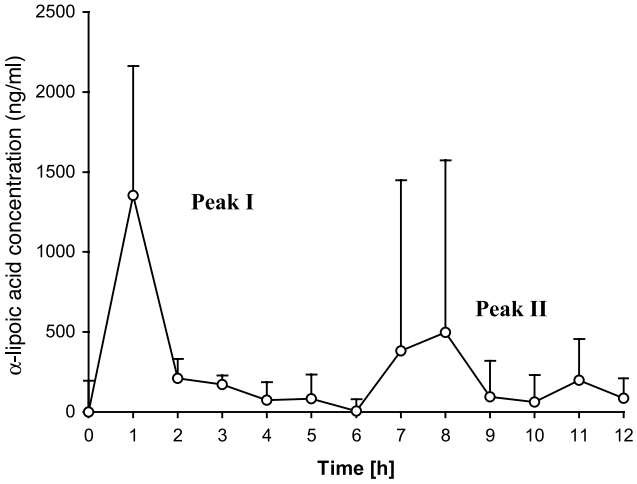

Figure 4. Pharmacokinetic of orally given α-lipoic acid: Mean α-lipoic acid concentration (±SD) in plasma of eight volunteers after single dosing of three chitosan/α-lipoic acid tablets. Indicated values were corrected for the endogenous plasma lipoate concentration.

Table 4. Pharmacokinetic parameters.

α-Lipoic acid	Arithmetic mean ± SD
$AUC_{0\rightarrow 12}$ (μg × min/mL)	183.8 ± 101.4
Peak I	
$AUC_{0\rightarrow 6}$ (μg × min/mL)	108.1 ± 61.5
C_{max} (ng/mL)	1354.5 ± 807.1
t_{max} (h)	1
$t_{1/2}$ (h)	1.2
Peak II	
$AUC_{6\rightarrow 12}$ (μg × min/mL)	75.7 ± 94.8
C_{max} (ng/mL)	497.5 ± 1074
t_{max} (h)	8
$t_{1/2}$ (h)	1.3

differences in the pharmacokinetic of the R-(+) enantiomer and the S-(−) enantiomer after oral dosing. The method of plasma work-up and HPLC analysis used allowed the separate quantification of the R- and S-forms of α-lipoic acid in the plasma. Results demonstrated an identical pharmacokinetic relationship of both enantiomers (data not shown). The information gained within this study might therefore provide the basis for the development of oral delivery systems for the R-(+) enantiomer.

Biofeedback Study

As it was demonstrated within this study that a desired pulsed sustained release of α-lipoic acid can be reached by chitosan/α-lipoic acid tablets that might be highly beneficial in treatment of diabetes type II, the influence on the blood glucose level was also evaluated during the study in human volunteers. Results of this study, however, demonstrated no significant reduction in the glucose level compared to untreated volunteers. A reason for this observation can be seen in the fact that the study was performed with healthy volunteers and not with diabetes type II patients. Studies carried out in healthy rats, for instance, also demonstrated no reduction in the blood glucose level, whereas in diabetic rats a significant reduction in the glucose level was achieved after 10 days of daily application.[17] In addition, the test period of merely 12 hours might be too short to see an effect. The daily administration of α-lipoic acid for 5 days in diabetic rats led to no significant glucose-lowering effect,[18] whereas a significant reduction in the blood glucose level was observed after 10 days by another research group.[17]

Detailed, long-term studies with the novel oral delivery system described here in diabetes type II patients will therefore be the subject of ongoing studies.

CONCLUSION

Because of a loose binding of α-lipoic acid to the matrix on the one hand, and the degradation of this chitosan matrix in the colon on the other, a pulsed sustained release system for α-lipoic acid was generated. A twice-daily application of this dosage form seems to provide a continuously increased level of the drug in the plasma and several release maxima per day. The novel dosage form should be highly beneficial in order to guarantee a permanent antioxidative and free radical scavenging effect, preventing early glomerular injury and stimulating glucose up-take in the case of diabetes type II.

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