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EXPERT OPINION

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Soft gel capsules improve melatonin's bioavailability in humans

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Objective: Oral bioavailability is one of the most important properties in drug design and development. A poor oral bioavailability can result in low efficacy and unpredictable response to a drug. Several dosages of melatonin have been used for various investigations to clarify its bioavailability in humans. Aiming to search for a pharmaceutical form, which is better absorbed, the pharmacokinetic (PK) profile of the new manufactured melatonin soft gelatin (soft gel) capsule form has been evaluated and compared with the commercially available melatonin powder.
Research design and methods: A total of 60 healthy volunteers received 1, 3 mg of melatonin powder and 1 mg of melatonin in soft gel capsules. PK profiles were obtained by analysis of melatonin plasma concentration, and the respective melatonin bioavailability was compared.
Results: Melatonin soft gel capsule form showed similar PK parameters compared with the highest doses of melatonin in powder form, but its bioavailability was improved.
Conclusions: Soft gel capsules improved the bioavailability of melatonin in humans even when administered dose was reduced. Considering the number of conditions in which melatonin supplementation is recommended, this evidence could support a broader use of melatonin in clinical practice.

Keywords: bioavailability, melatonin, pharmacokinetics, soft gelatin capsules

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1. Introduction

Oral bioavailability is one of the most important properties in drug design and development. A high oral bioavailability reduces the amount of an administered drug necessary to achieve a desired pharmacological effect reducing the risk of side effects and toxicity. A poor oral bioavailability can result in low efficacy, therefore, leading to unpredictable response to a drug [1]. Over the past 15 – 20 years, melatonin, a neuroendocrine hormone secreted nightly primarily by pineal gland, has become a very popular substance drawing attention in both the research setting and as a nutritional supplement. In the last two decades, a compelling body of evidence has outlined the relevance of melatonin in human physiology and pathology [2]. The role of endogenous melatonin in circadian rhythm disturbances and sleep disorders is well established [3]: melatonin in fact, corrects the sleep disturbances, mental inefficiency and daytime fatigue that occur after flights [4]. Melatonin is useful as a hypnotic for delayed sleep-phase syndrome [5], and it is used successfully to treat serious sleep disorders in hyperactive and neurologically compromised children such as those with attention-deficit/hyperactivity disorder [6]. It is clear that melatonin is a pleiotropic molecule playing a key role, either in a variety of important physiological functions [7,8] or in pathological conditions like cancer [9-12]. Compelling evidences have supported a role of melatonin also in human reproduction [13,14] by evidencing its beneficial effects in particular on fertility, pregnancy

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wellness and embryo development [15,16]. Many of melatonin's actions are mediated through interaction with the G-protein-coupled membrane-bound melatonin receptors type 1 and type 2 (MT1 and MT2, respectively) or, indirectly with nuclear orphan receptors from the RORα/RZR family [17]. Melatonin, due to its small size and amphiphilic nature, crosses cell membrane and acts through nonreceptor-mediated mechanisms, serving as a scavenger for reactive oxygen species and reactive nitrogen species, either by reducing concentration of highly reactive hydroxyl radical *in vitro* and *in vivo*, or by stimulating antioxidative enzymes, superoxide dismutase and glutathione peroxidase [18,19]. Melatonin is produced predominantly in the pineal gland, retina, and, in lesser amounts, in the brain and extracranial sites (gastrointestinal tract, the eye, the immune system and ovaries), but it can be produced also *de novo* and metabolized in the peripheral sites [20-22].

Considering these interesting results, many studies have examined the pharmacokinetics (PKs) of melatonin and in particular its bioavailability. It is important to note that bioavailability of melatonin varies depending on several factors. Perhaps only 33% gets absorbed through the average gastrointestinal tract when intravenous and oral data are compared, confirming that the low bioavailability of melatonin is a consequence of hepatic first-pass extraction, which converts melatonin to its metabolite before it enters the systemic circulation [23]. Several different oral melatonin formulations have been developed, including immediate release, controlled (sustained) release and surge-sustained release. A large range of doses have been used in clinical trials, with considerable debate regarding the role of low-dose (0.1 – 0.5 mg) and high-dose (2 – 10 mg) melatonin. Past studies have found bioavailability of exogenous melatonin to be highly variable, ranging from 1 to 74% [24-27], although this broad range may indicate formulation and/or dose input dependence. Endogenous melatonin is primarily metabolized in the liver by hydroxylation (~ 90%) to 6-hydroxymelatonin and excreted in the urine following conjugation with sulfuric or glucuronic acid [28,29]. Research in mice using radiolabeled melatonin found that after 48 h, 70% of the tracer was in urine and 15% in the feces [29]. Currently, the most popular melatonin's formula commercially available are tablets or drops, even if several attempts have been made to improve oral bioavailability, as the development of starch microspheres for intranasal administration to optimize preparation conditions [30]. In this regard, individualization of new pharmaceutical forms could help to obtain the optimal effects of melatonin treatment, considering that several factors as drug interactions and variations in pH within the gastrointestinal tract could influence the bioavailability of melatonin [31,32]. In this regard, the development of soft gelatin (soft gel) capsule form offers several advantages such as in particular protecting the encapsulated compound against oxygen, light (this is peculiar keeping in mind that melatonin is light-sensitive) and interference from other compounds. Moreover soft gel improves swallow ability, masks odors and unpleasant taste. Its use is ideal to deliver compounds with sufficient solubility in a pharmaceutically acceptable nonaqueous vehicle, like oil sunflower seeds, thereby removing any dissolution rate-limiting steps and yielding to a faster, uniform and enhanced absorption [33]. Based on this evidence, this study was undertaken to test a new melatonin soft gel capsule formulation by comparing its bioavailability with the commercially available powder form.

2. Materials and methods

2.1 Patients and methods

The study involved 60 healthy volunteers, men and women (30 male and 30 female), enrolled at the AGUNCO Obstetrics and Gynecology Center (Rome, Italy). Volunteers were aged between 20 and 37 years, with a body mass index ranging between 21 and 25 kg/m^2; additional demographic data were weighed in the range 58 – 87 kg and height 165 – 187 cm. Furthermore, subjects were evaluated on the basis of medical history, physical examination and laboratory screenings, and subjects who were found in poor general health were excluded. Volunteers were divided in three different groups, 20 subjects received 1 mg of melatonin powder, 20 subjects received 3 mg of melatonin powder, while the remaining 20 received 1 mg of melatonin in soft gel capsules (LO.LI. pharma Rome, Italy). Before entering the trial a written informed consent was obtained from the volunteers, and the study was approved by the ethical committee. PK parameters were evaluated based on the analysis of the melatonin plasma concentration. Blood samples were collected by venous puncture at pre-dose (0), and at 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, 240, 360 min post-administration.

The study consisted of three different phases:

- Phase I: 1 mg of melatonin powder was administrated.
- Phase II: 1 mg of melatonin soft gel was administrated.
- Phase III: 3 mg of melatonin powder was administrated.

In order to highlight the relative PK parameters, the melatonin plasma concentration was analyzed. Blood samples were collected by venous puncture at pre-dose (0), and at 10; 20; 40; 60; 80; 100; 120; 140; 160; 180; 240; 360 min post-administration. Blood samples were collected in heparinized tubes and stored at -20°C until analysis was performed. Melatonin was extracted from plasma and its quantification was performed using gas chromatography (GC)–mass spectrometry (GC Agilent 6890, Agilent Milano, Italy) analysis after extraction with organic solvents and derivatization as described in reference [34]. Briefly, a (1.0 µl) injection was performed on a splitless mode at 270°C and a capillary column Agilent 122-5532 DB-5 ms (0.25 mm × 30 m × 0.25 µm Agilent Milano, Italy) was used. The flow rate was fixed to 1.2 ml/min. Total run time was 15 min: oven at 70°C from 0 to 1 min; 20°C/min to 150°C; 10°C/min to 240°C;

Table 1. Pharmacokinetic parameters after oral administration of 1 mg powder, 1 mg soft gel or 3 mg powder of melatonin.

Mean ± SD			
T_{max} (min)	**1 mg powder**	**1 mg soft gel**	**3 mg powder**
	60 ± 6.15	**60 ± 5.20**	**40 ± 8.85**
ns 1 mg powder versus 1 mg soft gel ‡1 mg powder versus 3 mg powder ‡1 mg soft gel versus 3 mg powder			
C_{max} (pg/ml)	**1 mg powder**	**1 mg soft gel**	**3 mg powder**
	799.1 ± 39.03	**2620 ± 93.30**	**2405 ± 278.9**
*1 mg powder versus 1 mg soft gel *1 mg powder versus 3 mg powder ns 1 mg soft gel versus 3 mg powder			
AUC	**1 mg powder**	**1 mg soft gel**	**3 mg powder**
	90516 ± 6579	**283200 ± 11127**	**26911 ± 20291**
*1 mg powder versus 1 mg soft gel *1 mg powder versus 3 mg powder ns 1 mg soft gel versus 3 mg powder			

Data are tabled as mean ± SD.

*$p < 0.001$.

‡$p < 0.01$ by ANOVA followed by Bonferroni post-test.

$AUC_{(0 - 360)}$: (µmol•min/l) area under the plasma concentration–time curve to the last measured concentration; C_{max}: (µmol/l) maximum observed plasma concentration during the 0 – 360 min dosing interval; ns: Not significant; SD: Standard deviation.

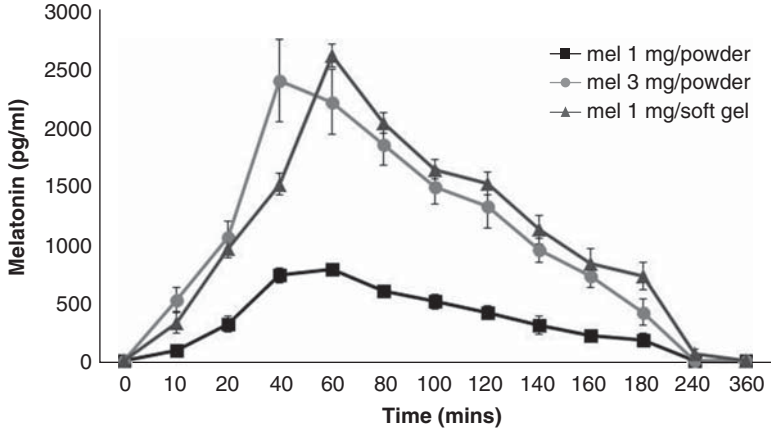

Figure 1. Comparison of the melatonin plasma concentration (pg/ml) after oral administration of 1 mg powder, 1 mg soft gel or 3 mg powder of melatonin.

4 min at 320°C in post-run. A MS 5973 Network Series detector (Agilent Milano, Italy) in sim mode was used to analyze the results.

2.2 PK parameters

PK parameters, C_{max} and T_{max}, were obtained directly from the plasma concentration data and the $AUC_{(0 - 360)}$ value was calculated by trapezoidal method between 0 and 360 min (**Table 1**). Data set were compared using one-way ANOVA with Bonferroni multi-comparison correction test.

3. Results

The analysis of melatonin plasma concentration allowed us to obtain interesting results about the PK parameters of the two formulations (powder and soft gel capsules) and their

bioavailability. There are no significant differences between T_{max} values of 1 mg of melatonin powder and 1 mg of melatonin soft gel capsule. Both are significantly higher than T_{max} values of 3 mg of melatonin powder.

A 1 mg powder has a significant low C_{max} value in respect to 3 mg of melatonin powder and 1 mg of melatonin soft gel capsule. The extent of absorption AUC $_{(0 - 360)}$ are similar between 1 mg of melatonin soft gel capsule and 3 mg of melatonin powder, in fact no significant differences are recorded. On the contrary, both groups have AUC values significantly higher than those of 1 mg powder (**Figure 1**).

4. Discussion

Melatonin is a pleiotropic molecule playing a key role both in several physiological and pathological conditions [2]. Several dosages of melatonin have been used for various investigations; nevertheless, it is unclear what dose is the optimal to elicit a pharmacological effect and what is its absolute bioavailability. The most popular melatonin's formula commercially available are tablet or drops and many factors can influence their efficacy. So this study was undertaken to analyze if a new pharmaceutical form (soft gels) could improve melatonin's bioavailability in humans.

It is reported that many compounds encapsulated in soft gels are better absorbed compared with other conventional oral pharmaceutical forms. Among all, diclofenac potassium liquid-filled soft gel capsules were demonstrated to be more rapidly and consistently absorbed than the commercially available diclofenac potassium tablets, resulting in a shorter and more consistent time to onset of analgesia [35]. Similarly, ibuprofen in soft gel is absorbed faster than both film-coated tablet and liquid (prepared from effervescent ibuprofen tablet) [36]. In addition, cyclosporine, when administrated in soft gel, reaches therapeutic blood levels that are not achievable from the oral solution form [37]. Another example is the soft gel capsule containing levothyroxine dissolved in glycerin, which showed the most consistent dissolution pattern when compared with two different tablet formulations (the generic levothyroxine sodium by Sandoz, Inc. and Synthroid by Abbott) [38].

In the present study, we compared the bioavailability of melatonin between the classical commercially available powder form and the new encapsulated soft gel form. Twenty healthy subjects received 1 mg of melatonin powder, 20 received 3 mg of melatonin powder and the remaining 20 received 1 mg of melatonin soft gel capsules. Results evidenced that 3 mg of melatonin powder and 1 mg melatonin soft gel capsules had the same PK, but comparing the absorption, 1 mg melatonin soft gel capsules was faster absorbed than 3 mg melatonin powder. 1 mg of melatonin powder had a low PK and was not well absorbed.

5. Conclusion

Development of soft gel capsules is of growing interest and several studies report the ability to perform a uniform, faster and enhanced absorption compared to other oral forms. The use of different pharmaceutical forms to improve the oral absorption represents one of the main challenges in the clinical practice. So, melatonin was manufactured in a new soft gel capsule form in order to compare its absorption characteristics with the commercially available melatonin powder.

Soft gel capsules showed an improved bioavailability, even with a low dosage of melatonin (1 mg), thereby representing a clinical advantage for the treatment of several physiological and pathological disorders in which the melatonin supplementation is recommended.

Declaration of interest

Lo.li. Pharma S.r.l. provided the medication for this study and Gianfranco Carlomagno is an employee of Lo.li. Pharma S.r.l. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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