Accepted Manuscript

Potential drug interactions with melatonin

Eleni Papagiannidou, Debra J. Skene, Costas Ioannides

PII: S0031-9384(14)00207-8
DOI: doi: [10.1016/j.physbeh.2014.04.016](https://doi.org/10.1016/j.physbeh.2014.04.016)
Reference: PHB 10379

To appear in: *Physiology & Behavior*

Received date: 9 October 2013
Revised date: 31 March 2014
Accepted date: 4 April 2014

Please cite this article as: Papagiannidou Eleni, Skene Debra J., Ioannides Costas, Potential drug interactions with melatonin, *Physiology & Behavior* (2014), doi: [10.1016/j.physbeh.2014.04.016](https://doi.org/10.1016/j.physbeh.2014.04.016)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Eleni Papagiannidou, Debra J Skene and Costas Ioannides

Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH

UK

Running title: Drug interactions with melatonin

Keywords: melatonin-drug interactions-17-Ethinylestradiol-5-Methoxypsoralen-CYP1A2

Address for correspondence:

Professor Debra J Skene

Faculty of Health and Medical Sciences

University of Surrey,

Guildford, Surrey

GU2 7XH, UK

Telephone No: +44-1483-689706

Email: d.skene@surrey.ac.uk

Abstract

Possible interactions of melatonin with concurrently administered drugs were investigated in *in vitro* studies utilising human hepatic post-mitochondrial preparations; similar studies were conducted with rat preparations to ascertain whether rat is a suitable surrogate for human. Drugs were selected based on the knowledge that the 6-hydroxylation of exogenous melatonin, its principal pathway of metabolism, is mainly mediated by hepatic CYP1A2, but also on the likelihood of the drug being concurrently administered with melatonin. Hepatic preparations were incubated with either melatonin or 6-hydroxymelatonin in the presence and absence of a range of concentrations of interacting drug, and the production of 6-sulphatoxymelatonin monitored using a radio-immunoassay procedure. Of the drugs screened, only the potent CYP1A2 inhibitor 5-methoxypsoralen impaired the 6-melatonin hydroxylation at pharmacologically relevant concentrations, and is likely to lead to clinical interactions; diazepam, tamoxifen and acetaminophen (paracetamol) did not impair the metabolic conversion of melatonin to 6-sulphatoxymelatonin at concentrations attained following therapeutic administration. 17-Ethinylloestradiol appeared not to suppress the 6-hydroxylation of melatonin but inhibited the sulphation of 6-hydroxymelatonin, but this is unlikely to result in an interaction following therapeutic intake of the steroid. Species differences in the inhibition of melatonin metabolism in human and rat hepatic post-mitochondrial preparations were evident implying that the rat may not be an appropriate surrogate of human in such studies

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a versatile pineal hormone secreted during darkness that has been implicated in a wide variety of physiological functions, including regulation of circadian rhythms [1], control of seasonal reproduction [2], modulation of insulin secretion [3], immune function [4,5], retinal function [6] and neuroprotection [7].

Melatonin is extensively metabolised primarily through hydroxylation at the 6-position, catalysed selectively by the microsomal CYP1A2 enzyme of the cytochrome P450 superfamily, which is localised in the liver, with minor contributions from CYP2C19, CYP1A1 and CYP1B1, the latter two enzymes being largely extrahepatic [8-10]. The generated 6-hydroxymelatonin is subsequently conjugated with sulphate or glucuronide and excreted in the urine. Another important metabolite is *N*-acetylserotonin which is formed by *O*-demethylation, and may represent as much as 20% of the dose [11]. At least in the rat, mitochondrial cytochromes P450 can also metabolise melatonin, once again the primary metabolite being 6-hydroxymelatonin; the main contributors to the mitochondrial metabolism of melatonin are CYP3A and CYP2C6 [12]. Because of its rapid metabolism, melatonin is characterised by a short half-life of about an hour that limits its use. However, there is a marked inter-individual variation in plasma levels of melatonin following oral administration which can be as extensive as 25-fold [13-15]. It has been proposed that in genetically poor CYP1A2 metabolisers the plasma levels are much higher as a result of suppressed metabolism, giving rise to a longer half-life [16]. High plasma levels may explain the loss of pharmacological response to exogenous melatonin following long-term intake [17].

Melatonin is an effective phase shifting agent or 'chronobiotic' and has been used successfully in the treatment of circadian rhythm disorders such as jet lag, shift work, delayed sleep phase insomnia [18-20] and non 24 h sleep/wake disorder suffered by the totally blind [21]. Its ability to reduce sleep latency has led to its use in primary insomnia [22,23]. Moreover, it is also an established potent antioxidant acting not only by scavenging reactive oxygen/nitrogen species [24] but by additionally up-regulating the synthesis of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase, and by increasing the

cellular concentration of the nucleophilic tripeptide glutathione [25, 26]. By virtue of its antioxidant activity, melatonin has been shown to protect against DNA damage and suppress cellular proliferation, and may function as an anticancer agent capable of suppressing all three stages of carcinogenesis, namely initiation, promotion and progression [27-29]. Moreover, melatonin has been proposed as a possible neuroprotective drug in the treatment of conditions such as Parkinson's disease and Alzheimer's disease [30]. Finally, melatonin has been shown to enhance the effectiveness and attenuate the toxicity of anticancer cytotoxic drugs [31-33].

As a result of the diversity of its biological activities, and in particular its sleep-promoting potential, melatonin use is extensive. In many countries, such as the USA, it is available over the counter whereas in others, including the United Kingdom, is only available on prescription. The increased use of melatonin raises the possibility of interactions with other co-administered drugs following modulation of CYP1A2. Pharmacological plasma levels of melatonin may be influenced as a result of concurrent exposure to chemicals, including drugs that modulate the expression of CYP1A2, the principal catalyst of its metabolic degradation. Increased CYP1A2 activity will lead to lower plasma levels and vice versa. For example, plasma melatonin levels were increased following fluvoxamine administration [34], presumably by impairing its cytochrome P450-mediated metabolism [35, 36]; fluvoxamine is a potent inhibitor of CYP1A2 [37] and to a lesser extent of CYP2C19 [38]. Exogenous serum melatonin levels were suppressed by smoking, especially when the levels of the hormone were high [39]. Polycyclic aromatic hydrocarbons, a class of carcinogenic compounds present in tobacco, up-regulate CYP1A2 expression leading to accelerated melatonin metabolism. Similarly, concomitant consumption of caffeine whose metabolism is principally catalysed by CYP1A2, more than doubled plasma levels and increased the bioavailability of melatonin, by impairing its presystemic metabolism [40]. Moreover, drugs that are CYP2C19 substrates such as omeprazole, lansoprazole and citalopram, increased the urinary excretion of 6-sulphatoxymelatonin in individuals taking

exogenous melatonin; presumably these compounds decrease the CYP219-mediated metabolism of melatonin to acetylserotonin [41].

As the consumption of melatonin continues to rise, the likelihood of interactions with other drugs as a consequence of cytochrome P450 modulation increases. In the present study we investigated the potential of drugs co-administered with melatonin to influence its 6-hydroxylation and subsequent conjugation in human hepatic post-mitochondrial preparations. As melatonin is available in many countries without prescription, it is likely to be consumed concurrently with a wide array of other drugs. . In the current study, the first to address this issue, it was considered prudent to select drugs based on two criteria. Firstly drugs that may be taken with melatonin on a long-term basis, e.g. 17α -ethinyloestradiol present in the contraceptive pill, and secondly drugs that are known to interact with CYP1A2 as substrates or inhibitors, e.g. 5-methoxypsoralen, this being the principal catalyst of melatonin 6-hydroxylation both in rats and human [9,10], the major pathway of its metabolism.

Materials and methods

Acetaminophen (paracetamol), diazepam, 17α -ethinyloestradiol, 5-methoxypsoralen, tamoxifen, melatonin, 6-hydroxymelatonin, fluvoxamine maleate and all cofactors were purchased from Sigma-Aldrich (Poole, Dorset, UK). The antibody to 6-sulphatoxymelatonin, raised in sheep, was a generous gift from Stockgrand Ltd., University of Surrey, Guildford.

Three male Wistar albino rats (200-250 g) were purchased from B&K Universal Ltd. (Hull, East Yorkshire, UK), and housed in a 12:12 hour light: dark cycle (LD; lights on at 06.00 h). Animals were killed by cervical dislocation, livers were removed and postmitochondrial supernatant (S9) was prepared by differential centrifugation and stored in 1 ml aliquots at -20°C until use. The protein concentration was determined by the method of Lowry et al. [42]. Human liver from a 47-year old male Caucasian who died as a result of subarachnoid haemorrhage was obtained from the Peterborough Hospital Human Research Tissue Bank; he was a smoker and drank alcohol. The tissue was delivered snap-frozen in

ice, and was stored at -80°C . Post-mitochondrial supernatant (S9) was prepared by differential centrifugation as for the rat. The study received approval from the University of Surrey Ethics Committee.

Determination of melatonin 6-hydroxylase activity in hepatic preparations was achieved by measuring its sulphate conjugate, as we have previously described [10]. Essentially melatonin (25 nmole) was added into an incubation system comprising a NADPH-generating system, adenosine 3'-phosphate 5'-phosphosulphate (PAPS, 50 nmole) and 50 μl of hepatic post-mitochondrial fraction. Incubation was carried out at 37°C for 20 minutes. Reaction was terminated by the addition of 0.2 M perchloric acid (250 μl) and protein was precipitated by centrifugation at $2500 \times g$ for 15 minutes. The levels of 6-sulphatoxymelatonin were determined by a validated radioimmunoassay procedure [43]. The sulphate conjugation of 6-hydroxymelatonin was determined using the above incubation procedure, except that melatonin was replaced with its 6-hydroxy metabolite. Drugs that were not water soluble were dissolved in either dimethylsulfoxide or ethanol at a final concentration of 1% (v/v). In preliminary studies (results not shown), the generation of 6-sulphatoxymelatonin from either melatonin or 6-hydroxymelatonin was not modulated by these solvents at the concentrations used.

Results are expressed as mean $\pm$ SEM. One-way ANOVA (SPSS, version 10) was performed to test for statistically significant differences between the groups, followed by Tukey post-hoc when significance was found.

Results

The formation by human hepatic S9 of 6-sulphatoxymelatonin from both melatonin and 6-hydroxymelatonin was linear with incubation time for at least one hour. Similarly, with both substrates, formation of 6-sulphatoxymelatonin was linear with S9 concentration at least up to 200 μl per incubation (results not shown). The 6-sulphatoxymelatonin generation by rat S9 has already been validated [10].

Fluvoxamine suppressed the formation of 6-sulphatoxymelatonin from melatonin by rat S9 in a concentration-dependent manner, statistical significance being achieved at concentrations of 50 μ M or higher (Figure 1A); no such inhibition was noted when melatonin was replaced with 6-hydroxymelatonin (Figure 1B).

5-Methoxypsoralen at a concentration as low as 5 μ M, significantly suppressed the conversion of melatonin to 6-sulphatoxymelatonin by rat liver S9 (Figure 2A). At the highest concentrations (> 500 μ M), 5-methoxypsoralen also caused a significant inhibition in the generation of 6-sulphatoxymelatonin from 6-hydroxymelatonin (Figure 2B). When rat liver S9 was substituted by human liver S9 the inhibition of 6-sulphatoxymelatonin formation from melatonin was more markedly impaired. It was observed that 5-methoxypsoralen, at concentrations as low as 0.01 μ M, significantly decreased the production of 6-sulphatoxymelatonin from melatonin (Figure 2C). On the other hand, the inhibitory effect on the formation of 6-sulphatoxymelatonin from 6-hydroxymelatonin was evident only at 50 μ M concentration, and was relatively modest (Figure 2D).

Diazepam at concentrations of 50 μ M and higher caused a significant concentration-dependent inhibition of the production of 6-sulphatoxymelatonin from melatonin in rat liver postmitochondrial fractions (Figure 3A). In contrast, when 6-hydroxymelatonin was used as substrate, diazepam significantly inhibited the generation of 6-sulphatoxymelatonin only at the 500 μ M concentration (Figure 3B). When human liver postmitochondrial fractions replaced the rat fractions, diazepam at the highest concentrations used, namely 100 μ M and 500 μ M, impaired the formation of 6-sulphatoxymelatonin from melatonin (Figure 3C). When 6-hydroxymelatonin served as substrate, the production of 6-sulphatoxymelatonin was also significantly decreased, but only at the highest concentration (500 μ M) of diazepam used (Figure 3D). The effect, however, appeared less pronounced than when melatonin was used as substrate.

Tamoxifen at the two highest concentrations tested (500 μ M and 1000 μ M) significantly suppressed the generation of 6-sulphatoxymelatonin from melatonin when rat postmitochondrial fractions were utilised (Figure 4A) but no inhibition was evident when 6-

hydroxymelatonin was used as substrate (Figure 4B). Following incubation with human hepatic postmitochondrial preparations, tamoxifen had no effect on the amount of 6-sulphatoxymelatonin produced from either melatonin (Figure 4C) or 6-hydroxymelatonin (Figure 4D).

Acetaminophen, at high concentrations (50 μ M), decreased the amount of 6-sulphatoxymelatonin generated by rat liver from either melatonin (Figure 5A) or 6-hydroxymelatonin (Figure 5B). In contrast, in human liver acetaminophen failed to show any significant inhibitory effect on the generation of 6-sulphatoxymelatonin from either melatonin (Figure 5C) or 6-hydroxymelatonin (Figure 5D).

17 α -Ethinylestradiol potently inhibited the metabolism of melatonin to 6-sulphatoxymelatonin, significant inhibition being evident at concentrations as low as 1 μ M, the lowest concentration employed in the current studies; the effect of 17 α -ethinylestradiol was concentration dependent (Figure 6A). In contrast, no inhibitory effect was observed when 6-hydroxymelatonin served as substrate (Figure 6B). In human liver, 17 α -ethinylestradiol at concentrations of 5 μ M and higher gave rise to a marked and concentration-dependent inhibition of the generation of 6-sulphatoxymelatonin from melatonin (Figure 6C). In contrast to the rat, however, 17 α -ethinylestradiol also impaired the sulphation of 6-hydroxymelatonin (Figure 6D).

Discussion

The increasing use of melatonin co-administered with other drugs raises the possibility of interactions leading to elevated melatonin levels. The selection of drugs to be studied that could potentially interact with melatonin was based on the knowledge that the 6-hydroxylation of exogenous melatonin, its principal pathway of metabolism, is mainly mediated by hepatic CYP1A2 in both rats and humans [9, 10], but also on the likelihood of the drug being concurrently administered with melatonin. The metabolism of melatonin was determined using a validated, sensitive radio-immunoassay procedure that monitors the

generation of the sulphate conjugate of 6-hydroxymelatonin, 6-sulphatoxymelatonin [10, 43]. In order to discern the effects of the drugs on the cytochrome P450-mediated 6-hydroxylation of melatonin from its subsequent sulphate conjugation, the effects of the drugs on the generation of 6-sulphatoxymelatonin from 6-hydroxymelatonin were also investigated. Moreover, to ascertain whether rat is a suitable surrogate for human in investigating drug interactions resulting from cytochrome P450 modulation studies were also carried out in this species; this is based on the premise that CYP1A2, responsible for the 6-hydroxylation of melatonin in both species, is phylogenetically conserved so that the human protein shares extensive structural similarity and displays similar substrate specificity to the rat protein [44].

In preliminary studies, it was established that the rate of formation of 6-sulphatoxymelatonin by human post-mitochondrial preparations from 6-hydroxymelatonin was orders of magnitude higher compared with melatonin indicating that the sulphation step is not rate-limiting, in agreement with our previous studies using rat preparations [10].

The antidepressant fluvoxamine was used as a positive control; it is an established potent inhibitor of CYP1A2 [36], and early work showed that a single dose of fluvoxamine increased the nocturnal production of endogenous melatonin in humans indicating for the first time that fluvoxamine and melatonin may interact [34]. Moreover, there is experimental evidence that its co-administration with melatonin increases human plasma melatonin levels by 6-fold and the area under the curve (AUC) by 9-fold, most likely due to inhibition of its metabolism [36]. Inhibition of the 6-hydroxylation was confirmed in the present study using rat hepatic preparations and a 50 μ M melatonin concentration, but the effect was evident only at concentrations of 50 μ M or higher. In a clinical setting the concentration of both drugs would be expected to be <0.1 μ M [45] [37], so that the metabolism of melatonin may be impaired leading to a rise in plasma levels.

Of the drugs employed in the present study, 5-methoxypsoralen, a drug employed in the treatment of psoriasis, was clearly the most potent causing almost complete impairment of melatonin metabolism at a concentration of 0.1 μ M, with inhibition being observed even at 0.01 μ M, the lowest concentration studied. The sulphate conjugation of 6-hydroxymelatonin,

however, was not modulated at these 5-methoxypsoralen concentrations allowing us to conclude that the cytochrome P450-mediated hydroxylation is the susceptible pathway. It has already been reported that exposure to 5-methoxypsoralen elevates plasma levels of endogenous and exogenous melatonin [46-48]. A very interesting observation was that the rat necessitated a far higher concentration (5 μM) of 5-methoxypsoralen for inhibition to be manifested. As no such difference was noted when 6-hydroxymelatonin served as the substrate, the difference may be attributed to the cytochrome P450-catalysed hydroxylation of melatonin. It is thus apparent that human CYP1A2 may be more sensitive to 5-methoxypsoralen-inhibition compared with the rat orthologous protein. The potent inhibition of melatonin metabolism by 5-methoxypsoralen may reflect the fact that it is a mechanism-based inhibitor in both rat and human liver microsomes, i.e. it is first metabolically converted to a reactive metabolite that interacts covalently with the haem and/or protein moiety of cytochrome P450 resulting in loss of activity [49]. It is conceivable that the higher sensitivity of human melatonin metabolism compared with rat probably represents more effective generation of the metabolite by the former. Since 5-methoxypsoralen, at therapeutic doses, is able to inhibit the CYP1A2-mediated caffeine metabolism in psoriasis patients [50], it is likely that the 6-hydroxylation of exogenous melatonin is similarly suppressed. It is pertinent to point out that the mean 5-methoxypsoralen plasma concentration after a standard oral dose reaches 1.75 μM [51]; this concentration is markedly higher than the concentration of 0.01 μM that elicited inhibition of melatonin 6-hydroxylase activity in the current study.

Marked, concentration-dependent, inhibition of melatonin metabolism to 6-sulphatoxymelatonin was also observed with the oral contraceptive steroid 17 α -ethinyloestradiol, in both rat and human post-mitochondrial preparations. However, a species difference was evident when 6-hydroxymelatonin served as the substrate, with only the human activity being impaired; thus the suppression of melatonin metabolism to 6-sulphatoxymelatonin in the rat may be attributed to impairment of the initial hydroxylation reaction. However, in human it appears that the conjugation reaction accounts for the marked drop in the formation of 6-sulphatoxymelatonin from melatonin. 17 α -ethinyloestradiol is

extensively sulphated [52] and the sulphate is primarily a storage form of this oestrogen, thus it is feasible that the steroid may competitively inhibit sulphation of 6-hydroxymelatonin in human post-mitochondrial preparations. Mean serum ethinyloestradiol levels following 30 mg administration were reported to be about 0.5 nM [53], which is much lower than the concentration that caused impairment in the metabolism of melatonin (1 μ M) in the current study so that at clinically relevant concentrations (< 10 nM) it is unlikely to impair the sulphate conjugation of exogenous melatonin. However, the much lower endogenous levels of melatonin may be impaired by 17 α -ethinyloestradiol and such a mechanism may explain to some extent why melatonin levels during the night are higher in females taking oral contraceptives [54-56]. Since 17 α -ethinyloestradiol appeared to decrease the sulphate conjugation of 6-hydroxymelatonin, it would be advisable in studies assessing melatonin treatment to exclude volunteers that use oral contraceptives as it could influence their melatonin profiles.

Diazepam suppressed the metabolism of melatonin to 6-sulphatoxymelatonin at concentrations higher than 50 and 100 μ M in rat and human liver microsomes respectively. Since in both rat and human liver high concentrations of diazepam impaired the sulphation of 6-hydroxymelatonin, it may be inferred that the decrease in the conversion of melatonin to 6-sulphatoxymelatonin is largely a consequence of a less efficient sulphate conjugation. It is pertinent to point out that CYP3A4 is the principal catalyst of diazepam metabolism in humans [57], so that competitive inhibition is unlikely. Steady-state plasma concentrations of diazepam are about 1.2 μ M [58] which is much lower than the required concentration to impair the sulphate conjugation of 6-hydroxymelatonin, thus concomitant use of diazepam with melatonin will not impair the metabolism of the hormone.

A species difference was evident when tamoxifen, a widely used drug in the treatment of breast cancer, was investigated, as it impaired rat, but not human, melatonin 6-hydroxylase activity. This lack of effect in human liver could possibly be attributed to the minor contribution of CYP1A2 in human hepatic tamoxifen metabolism [59]; to our knowledge, the contribution of CYP1A2 in tamoxifen metabolism in the rat has not been reported. On the

basis that the inhibition occurred only in the rat liver and at high concentrations, it may be predicted that administration of tamoxifen would be unlikely to impair the metabolism of melatonin when administered concomitantly. It is of interest that melatonin has been used in combination with tamoxifen in order to increase the effectiveness of the anticancer agent [60].

Similar to tamoxifen, acetaminophen (paracetamol) inhibited the conversion of melatonin to 6-sulphatoxymelatonin only in the rat, as a result of impaired sulphate conjugation of 6-hydroxymelatonin. It is possible that acetaminophen impairs melatonin metabolism in the rat due to the formation of the reactive N-acetyl-*p*-benzoquinone imine, which covalently binds to proteins. This is possibly the reason why inhibition was observed, at both the oxidation and sulphation pathways of melatonin metabolism, and only at high concentrations, as at low concentrations the reactive intermediate is probably detoxified by glutathione conjugation [61]. The lack of activity of human liver may represent poor cytochrome P450-generation of the reactive imine by the human liver, and/ or that the detoxification of the reactive metabolite by glutathione may be more efficient in human liver compared with rat.

Species differences in the inhibition of melatonin metabolism in human and rat hepatic post-mitochondrial preparations was evident implying that the rat may not be an appropriate surrogate of human in such studies. Of all the drugs screened for their potential to inhibit melatonin 6-hydroxylation in the present study, only the potent CYP1A2 inhibitor 5-methoxypsoralen is likely to impair this metabolic pathway in humans, whereas diazepam, tamoxifen and acetaminophen are unlikely to display such an effect. 17 α -Ethinylestradiol appeared not to impair the 6-hydroxylation but to inhibit the sulphation of exogenous melatonin in human liver, although this is unlikely to be manifested *in vivo* since the plasma concentration following therapeutic intake is not sufficient to impair the sulphation of melatonin. This inhibitory effect of 17 α -ethinylestradiol could be beneficial, especially in individuals with low plasma melatonin levels, leading to a prolonged pharmacological of the hormone.

Acknowledgements

The authors thank Stockgrand Ltd (University of Surrey, Guildford) for partial funding of this study and for 6-sulphatoxymelatonin radioimmunoassay reagents. D.J.S. is a Royal Society Wolfson Research Merit Award holder.

Declaration of interest

This was not an industry-supported study. D.J.S is a co-director of Stockgrand Ltd. The other authors have indicated no actual or potential conflict of interest.

Author contributions

Concept/Design: EP, DJS, CI

Acquisition of data: EP

Data analysis/Interpretation: EP, DJS, CI

Drafting of Manuscript: DJS, CI

Critical revision and approval of manuscript: EP, DJS, CI

References

- [1] CARDINALI DP, SRINIVASAN V, BRZEZINSKI A, et al. Melatonin and its analogs in insomnia and depression. *J Pineal Res* 2012;52:365-375.
- [2] DARDENTE H. Melatonin-dependent timing of seasonal reproduction by the pars tubularis: pivotal role for timelengths and thyroid hormones. *J Neuroendocrinol* 2012;24:249-266.
- [3] PESCHKE E, BÄHR I, MÜHLBAUER E. Melatonin and pancreatic islets: Interrelationships between melatonin, insulin and glucagon. *Int J Mol Sci* 2013;14:6981-7015.
- [4] SKWARLO-SONTA K. Melatonin in immunity: Comparative aspects. *Neuro Endocrinol Lett* 2002;2:61-66.
- [5] REDOGNA F, DIEDERCH M, GHIBELLI L. Melatonin: a pleiotropic molecule regulating inflammation. *Biochem Pharmacol* 2010;80:1844-1852.
- [6] GUIDO ME, GARBARINO-PICO E, CONTIN MA, et al. Inner retinal circadian clocks and non-visual photoreceptors: novel players in the circadian system. *Prog Neurobiol* 2010;92:484-504.
- [7] GIUSTI P, FRANCESCHINI D, PETRONE M, et al. In vivo and in vitro protection against kainite-induced excitotoxicity by melatonin. *J Pineal Res* 1996;20:226-231.
- [8] MA X, IDLE JR, KRAUSZ KW, et al. Metabolism of melatonin by cytochromes cytochrome P450.
- [9] FACCIOLI G, HIDESTRAND M, VON BAHR C, et al. Cytochrome P450 isoforms involved in melatonin metabolism in human liver microsomes. *Eur J Clin Pharmacol* 2001;56:881-888.
- [10] SKENE DJ, PAPAGIANIDOU E, HASHEMI E, et al. Contribution of CYP1A2 in the hepatic metabolism of melatonin: studies with isolated microsomal preparations and liver slices. *J Pineal Res* 2001;31:333-342.

- [11] YOUNG IM, LEONE RM, FRANCIS P, et al. Melatonin is metabolised to N-acetyl serotonin and 6-hydroxymelatonin in man. *J Clin Endocrinol Metab* 1985;60:114-119.
- [12] SEMAK I, KORIK E, ANTONOVA M, et al. Metabolism of melatonin by cytochrome cytochromes P450 in rat liver mitochondria and microsomes. *J Pineal Res* 2008;45:515-523.
- [13] WALDHAUSER FM, WALDHAUSER HR, LIEBERMAN M-H, et al. Bioavailability of oral melatonin in humans. *Neuroendocrinology* 1984;39:307-313.
- [14] ALDHOUS M, FRANEY C, WRIGHT J, et al. Plasma concentrations of melatonin in man following oral absorption of different preparations. *Br J Clin Pharmacol* 1985;19:517-521.
- [15] FOURTILLAN JB, BRISSON AM, GOBIN P, et al. Bioavailability of melatonin in humans after day-time administration of D₇ melatonin. *Biopharmac Drug Dispos* 2000;21:15-22.
- [16] BRAAM W, VAN GEIJSWIJK I, KEIJZER H, et al. Loss of response to melatonin treatment is associated with slow melatonin metabolism. *J Intell Disab Res* 2010;54:547-555.
- [17] LOCKLEY SW, SKENE DJ, JAMES K, et al. Melatonin administration can entrain the free-running circadian system of blind subjects. *J Endocrinol* 2000;164:R1-R6.
- [18] LEWY AJ, AHMED S, JACKSON JM, et al. Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiol Int* 1992;9:380-392.
- [19] ARENDT J, SKENE DJ. Melatonin as a chronobiotic. *Sleep Med Rev* 2005;9:25-39.
- [20] BURGESS HJ, REVELL VL, MOLINA TA, et al. Human phase response curves to three days of daily melatonin : 0.5 mg versus 3.0 mg. *J Clin Endocrinol Metab* 2010;95:1-7.
- [21] SKENE DJ, ARENDT J. Circadian rhythm sleep disorders in the blind and their treatment with melatonin. *Sleep Med* 2007;8:651-655.

- [22] BENDZ LM, SCATES AC. Melatonin treatment for insomnia in pediatric patients with attention-deficit hyperactivity disorder. *Ann Pharmacother* 2010;44:185-191.
- [23] FERRACIOLI-ODA E, QAWASMI A, BLOCH MH. Meta-analysis: Melatonin for the treatment of primary sleep disorders. *Plos ONE* 2013;;8:e63773
- [24] TAN DX, MANCHESTER LC, TERRON MP, et al. One molecule, many derivatives; a never-ending interaction of melatonin reactive oxygen and nitrogen species. *J Pineal Res* 2007;42:28-42.
- [25] GALANO A, TAN DX, REITER RJ. Melatonin as a natural ally against oxidative stress: a physicochemical examination. *J Pineal Res* 2011;51:1-16.
- [26] RODRIGUEZ C, MAYO JC, SAINZ RM, et al. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 2004;36:1-9.
- [27] DAUCHY T, BLASK TE, DAUCHY EM, et al. Antineoplastic effects of melatonin on a rare malignancy of mesenchymal origin: melatonin receptor-mediated inhibition of signal transduction , linoleic acid metabolism and growth in tissue-isolated human leiomyosarcoma xenografts. *J Pineal Res* 2009;47:32042.
- [28] MEDIAVILLA MD, SANCHEZ-BARCELO EJ, TAN DX, et al. Basic mechanisms involved in the anti-cancer effects of melatonin. *Curr Med Chem* 2010;17:4462-4481.
- [29] HILL SM, FRASCH T, XIANG S, et al. Molecular mechanisms of melatonin anticancer effects. *Integr Cancer Ther* 2009;8:337-346.
- [30] PANDI-PERUMAL SR, BAHAMMAM AS, BROWN GM, et al. Melatonin antioxidative defence: Therapeutical implications for aging and neurodegenerative processes. *Neurotox Res* 2013;23:267-300.
- [31] LISSONI P. Biochemotherapy with standard chemotherapies plus the pineal hormone melatonin in the treatment of advanced solid neoplasms. *Pathol Biol* 2007;55:201-204.

- [32] REITER RJ, TAN DX, SAINZ RM, et al. Melatonin: reducing the toxicity and increasing the efficacy of drugs. *J Pharm Pharmacol* 2002;54:1299-1321.
- [33] TRIPATHI DN, JENA GR. Effect of melatonin on the expression of Nrf2 and NF-KappaB during cyclophosphamide-induced urinary bladder injury in rat. *J Pineal Res* 2010;48:324-331.
- [34] SKENE DJ, BOJKOWSKI CJ, ARENDT J. Comparison of the effects of acute fluvoxamine and desipramine administration on melatonin and cortisol production in humans. *Br J Clin Pharmacol* 1994;37:181-186.
- [35] VON BAHR C, URSING C, YASUI N, et al. Fluvoxamine but not citalopram increases serum melatonin in healthy subjects – an indication that cytochrome P450 CYP1A2 and CYP2C19 hydroxylate melatonin. *Eur J Clin Pharmacol* 2000;56:123-127.
- [36] HÄRTTER S, GRÖZINGER M, WEIGMANN H, et al. Increased bioavailability of oral melatonin after fluvoxamine coadministration. *Clin Pharmacol Ther* 2000;67:1-6.
- [37] BRØSEN K, SKJELBO E, RASMUSSEN BB, et al. Fluvoxamine is a potent inhibitor of cytochrome P4501A2. *Biochem Pharmacol* 1993;24:1211-1214.
- [38] YAO C, KUNZE KL, TRAGER WF, et al. Comparison of in vitro and in vivo inhibition potencies of fluvoxamine toward CYP2C19. *Drug Metab Dispos* 2003;31:565-571.
- [39] URSING C, VON BAHR C, BRISMAR K, et al. Influence of cigarette smoking on melatonin levels in man. *Eur J Clin Pharmacol* 2005;61:197-201.
- [40] HÄRTTER S, NORDMARK A, DIRK-MATTHIAS R, et al. Effects of caffeine intake on the pharmacokinetics of melatonin, a probe drug for CYP1 A2 activity. *Br J Clin Pharmacol* 2003;56:679-682.
- [41] HUUEKA K, RIUTTA A, HAATAJA R, et al. The effect of the CYP2C19 substrate on the metabolism of melatonin in the elderly: A randomized, double-blind, placebo-controlled study. *Methods Find Exp Clin Pharmacol* 2006;28:447-450.
- [42] LOWRY OH, ROSEBROUGH NJ, FARR AL, et al. Protein determination with the Folin phenol reagent. *J Biol Che.* 1951;193:265-271.

- [43] ALDHOUS M., ARENDT J. Radioimmunoassay for 6-sulphatoxymelatonin in urine using an iodinated tracer. *Ann Clin Biochem* 1988;25:298-303.
- [44] GUENGERICH FP. Comparisons of catalytic selectivity of cytochrome P450 subfamily enzymes of different species. *Chem-Biol Inter* 1997;106:161-182.
- [45] NATHAN RS, PEREZ JM, POLLOCK BG, et al. The role of neuropharmacologic selectivity in antidepressant action; fluvoxamine versus desipramine. The role of neuropharmacologic selectivity in antidepressant action: fluvoxamine versus desipramine. *J Clin Psychiatry* 1990 ;51 :367-372.
- [46] SOUETRE E, SALVATI E, BELUGOU JL, et al. 5-Methoxypsoralen increases the plasma melatonin levels in humans. *J. Invest Dermatol.* 1987;89:152-155.
- [47] GARDE E, MICIC S, KNUDSEN K, Angelo, H.R., et al. 8-methoxypsoralen increases daytime plasma melatonin levels in humans through inhibition of metabolism. *Photochem Photobiol* 1994;60:475-480.
- [48] MAUVIARD F, RAYNAUD F, GEOFFRIAU M, et al. 5-Methoxypsoralen inhibits 6-hydroxylation of melatonin in the rat. *Biol Signals* 1995;4:32-41.
- [49] FOUIN-FORTUNET H, TINEL M. DESCATOIRE V, et al. Inactivation of cytochrome P-450 by the drug methoxsalen. . *Pharmacol Exp Ther* 1986; 236:237-247.
- [50] BENDRISS EK, BECHTE Y, BENDRISS A. et al. Inhibition of caffeine metabolism by 5-methoxypsoralen in patients with psoriasis. *Br J Clin Pharmacol* 1996;41:421-424.
- [51] TREFFEL P, MAKKI S, HUMBERT P. et al. A new micronized 5-methoxypsoralen preparation. Higher bioavailability and lower UVA dose requirement. *Acta Derm Venereol* 1992;72:65-67.
- [52] PACIFICI GM, BACK DJ. Sulphation and glucuronidation of ethinyloestradiol in human liver in vitro. *J Steroid Biochem* 1988;31:345-349.

- [53] BAISINI O, BENINI F, PETRAGLIA F. et al. Ursodeoxycholic acid does not affect ethinylestradiol bioavailability in women taking oral contraceptives. *Eur J Clin Pharmacol* 2004;60:481-487.
- [54] BRUN J, CLAUSTRAT B, DAVID M. Urinary melatonin, LH, oestradiol, progesterone excretion during the menstrual cycle or in women taking oral contraceptives. *Acta Endocrinol* 1987; 116:145-149.
- [55] WEBLEY GE, LEIDENBERGER F. The circadian pattern of melatonin and its positive relationship with progesterone in women. *J Clin Endocrinol Metab* 1986;63:323-328.
- [56] WRIGHT KP JR, BADIA P. Effects of menstrual cycle phase and oral contraceptives on alertness, cognitive performance, and circadian rhythms during sleep deprivation. *Behav Brain Res* 1999;103:185-194.
- [57] ONO S, HATANAKA T, MIYAZAWA S. et al. Human liver microsomal diazepam metabolism using cDNA-expressed cytochrome P450s: role of CYP2B6, 2C19 and the 3A subfamily. *Xenobiotica* 1996;26:1155-1166.
- [58] GREENBLATT DJ, LAUGHREN TP, ALLEN MD. et al. Plasma diazepam and desmethyldiazepam concentrations during long-term diazepam therapy. *Br J Clin Pharmacol* 1981;11:35-40.
- [59] CREWE HK, NOTLEY LM., WUNSCH RM. et al. Metabolism of tamoxifen by recombinant human cytochrome P450 enzymes: formation of the 4-hydroxy, 4'-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen. *Drug Metab Dispos* 2002;30:869-874.
- [60] GARCIA JJ, REITER RJ, ORTIZ GG. et al. Melatonin enhances tamoxifen's ability to prevent the reduction in microsomal membrane fluidity induced by lipid peroxidation. *J Membr Biol* 1998;162:59-65.

[61] ALLAMEH A, VANSOUN EY, ZARGHI A. Role of glutathione conjugation in protection of weanling rat liver against acetaminophen-induced hepatotoxicity. *Mech Ageing Dev* 1997;95: 71-79.

Legends to figures

Figure 1: Effect of fluvoxamine on the generation of 6-sulphatoxymelatonin from (A) melatonin and (B) 6-hydroxymelatonin by rat liver post-mitochondrial supernatant.

Results are presented as mean $\pm$ SEM for triplicate determinations. *** $p < 0.001$ compared with vehicle control.

Figure 2: Effect of 5-methoxypsoralen on the generation of 6-sulphatoxymelatonin from melatonin and 6-hydroxymelatonin by rat and human liver post-mitochondrial supernatant.

Rat liver post-mitochondrial preparations were incubated with (A) melatonin and (B) 6-hydroxymelatonin. Human liver post-mitochondrial preparations were incubated with (C) melatonin and (D) 6-hydroxymelatonin. Results are presented as mean $\pm$ SEM for triplicate determinations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with vehicle control (Veh).

Figure 3: Effect of diazepam on the generation of 6-sulphatoxymelatonin from melatonin and 6-hydroxymelatonin by rat and human liver post-mitochondrial supernatant.

Rat liver post-mitochondrial preparations were incubated with (A) melatonin and (B) 6-hydroxymelatonin. Human liver post-mitochondrial preparations were incubated with (C) melatonin and (D) 6-hydroxymelatonin. Results are presented as mean $\pm$ SEM for triplicate determinations. * $p < 0.05$; *** $p < 0.001$ compared with vehicle control (Veh).

Figure 4: Effect of tamoxifen on the generation of 6-sulphatoxymelatonin from melatonin and 6-hydroxymelatonin by rat and human liver post-mitochondrial supernatant.

Rat liver post-mitochondrial preparations were incubated with (A) melatonin and (B) 6-hydroxymelatonin. Human liver post-mitochondrial preparations were incubated with (C)

melatonin and (D) 6-hydroxymelatonin. Results are presented as mean $\pm$ SEM for triplicate determinations. * $p < 0.05$ compared with vehicle control (Veh)

Figure 5: Effect of acetaminophen on the generation of 6-sulphatoxymelatonin from melatonin and 6-hydroxymelatonin by rat and human liver post-mitochondrial supernatant.

Rat liver post-mitochondrial preparations were incubated with (A) melatonin and (B) 6-hydroxymelatonin. Human liver post-mitochondrial preparations were incubated with (C) melatonin and (D) 6-hydroxymelatonin. Results are presented as mean $\pm$ SEM for triplicate determinations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with vehicle control (Veh)

Figure 6: Effect of 17 α -ethinyloestradiol on the generation of 6-sulphatoxymelatonin from melatonin and 6-hydroxymelatonin by rat and human liver post-mitochondrial supernatant.

Rat liver post-mitochondrial preparations were incubated with (A) melatonin and (B) 6-hydroxymelatonin. Human liver post-mitochondrial preparations were incubated with (C) melatonin and (D) 6-hydroxymelatonin. Results are presented as mean $\pm$ SEM for triplicate determinations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with vehicle control (Veh)

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

- The potential of melatonin to interact with other drugs was investigated in human liver microsomes
- After incubation with melatonin/6-hydroxymelatonin, 6-sulphatoxymelatonin was monitored
- 5-Methoxypsoralen impaired the 6- hydroxylation of melatonin
- 17-Ethinhyloestradiol inhibited the sulphation of 6-hydroxymelatonin