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Potential drug interactions with melatonin

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HIGHLIGHTS

- 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.

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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 not only 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 radioimmunoassay 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-Ethinhyloestradiol 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.

1. 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 cytochrome 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].

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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 also 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, it 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.

2. 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 nmol) was added into an incubation system comprising a NADPH-generating system, adenosine 3′-phosphate 5′-phosphosulphate (PAPS, 50 nmol) and 50 μl of hepatic post-mitochondrial fraction. Incubation was carried out at 37 °C for 20 min. Reaction was terminated by the addition of 0.2 M perchloric acid (250 μl) and protein was precipitated by centrifugation at 2500 ×*g* for 15 min. 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 dimethylsulphoxide 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 ± 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.

3. Results

The formation by human hepatic S9 of 6-sulphatoxymelatonin from both melatonin and 6-hydroxymelatonin was linear with incubation time for at least 1 h. 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 μM or higher (Fig. 1A); no such inhibition was noted when melatonin was replaced with 6-hydroxymelatonin (Fig. 1B).

5-Methoxypsoralen at a concentration as low as 5 μM, significantly suppressed the conversion of melatonin to 6-sulphatoxymelatonin by rat liver S9 (Fig. 2A). At the highest concentrations (>500 μM), 5-methoxypsoralen also caused a significant inhibition in the generation of 6-sulphatoxymelatonin from 6-hydroxymelatonin (Fig. 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 μM, significantly decreased the production of 6-sulphatoxymelatonin from melatonin (Fig. 2C). On the other hand, the inhibitory effect on the formation of 6-sulphatoxymelatonin from 6-hydroxymelatonin was evident only at 50 μM concentration, and was relatively modest (Fig. 2D).

Diazepam at concentrations of 50 μM and higher caused a significant concentration-dependent inhibition of the production of 6-