UNIVERSIDADE D
COIMBRA

Marco António Rios dos Santos

Relatório de Estágio e Monografia intitulada "Melatonin: New avenues for an old molecule" referentes à Unidade Curricular "Estágio", sob a orientação da Dra. Ana Cristina Gonçalves Martins Pimentel de Oliveira e da Professora Doutora Maria Manuel da Cruz Silva, apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

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Eu, Marco António Rios dos Santos, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o n.º 2015240280, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatório de Estágio e Monografia intitulada "Melatonin: New avenues for an old molecule" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

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Coimbra, 1 de outubro de 2021.

(Marco Rios Santos)

PARTE I

Relatório de Estágio

Farmácia São Sebastião

Estágio sobre a orientação da Dra. Ana Cristina Gonçalves Martins Pimentel de Oliveira

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Abreviaturas

ANF	Associação Nacional das Farmácias
COVID-19	Doença do coronavírus 2019
FFUC	Faculdade de Farmácia da Universidade de Coimbra
MICF	Mestrado Integrado em Ciências Farmacêuticas
MNSRM	Medicamentos não sujeitos a receita médica
MSRM	Medicamentos sujeitos a receita médica
PIM	Preparação individualizada da medicação
SARS-CoV-2	Coronavírus da síndrome respiratória aguda grave 2
SWOT	*Strengths*, *Weaknesses*, *Opportunities*, *Threats*

1. Introdução

O Mestrado Integrado em Ciências Farmacêuticas (MICF) lecionado na Faculdade de Farmácia da Universidade de Coimbra (FFUC) durante os anos letivos 2015/2016 a 2020/2021 apresenta a unidade de Estágio Curricular, para a qual o segundo semestre do quinto ano de estudo é exclusivo. A FFUC permite a concretização de estágios, no âmbito desta unidade curricular, em diversas áreas contextualizadas pelo Ato Farmacêutico, nos termos do Estatuto da Ordem dos Farmacêuticos, mas tendo em conta o percurso em questão, o presente relatório concentra-se apenas na área de farmácia comunitária.

O único estágio realizado no âmbito da unidade curricular decorreu na Farmácia São Sebastião, sita na Rua Vitorino Nemésio, em Coimbra, que funciona sob direção técnica da Dra. Ana Cristina Gonçalves Martins Pimentel de Oliveira; com início a 11 de janeiro de 2021, término a 19 de junho de 2021 e duração equivalente às 810 horas estipuladas. O motivo da equivalência deve-se a ajustes consequentes do contexto epidemiológico associado à doença do coronavírus 2019 (COVID-19).

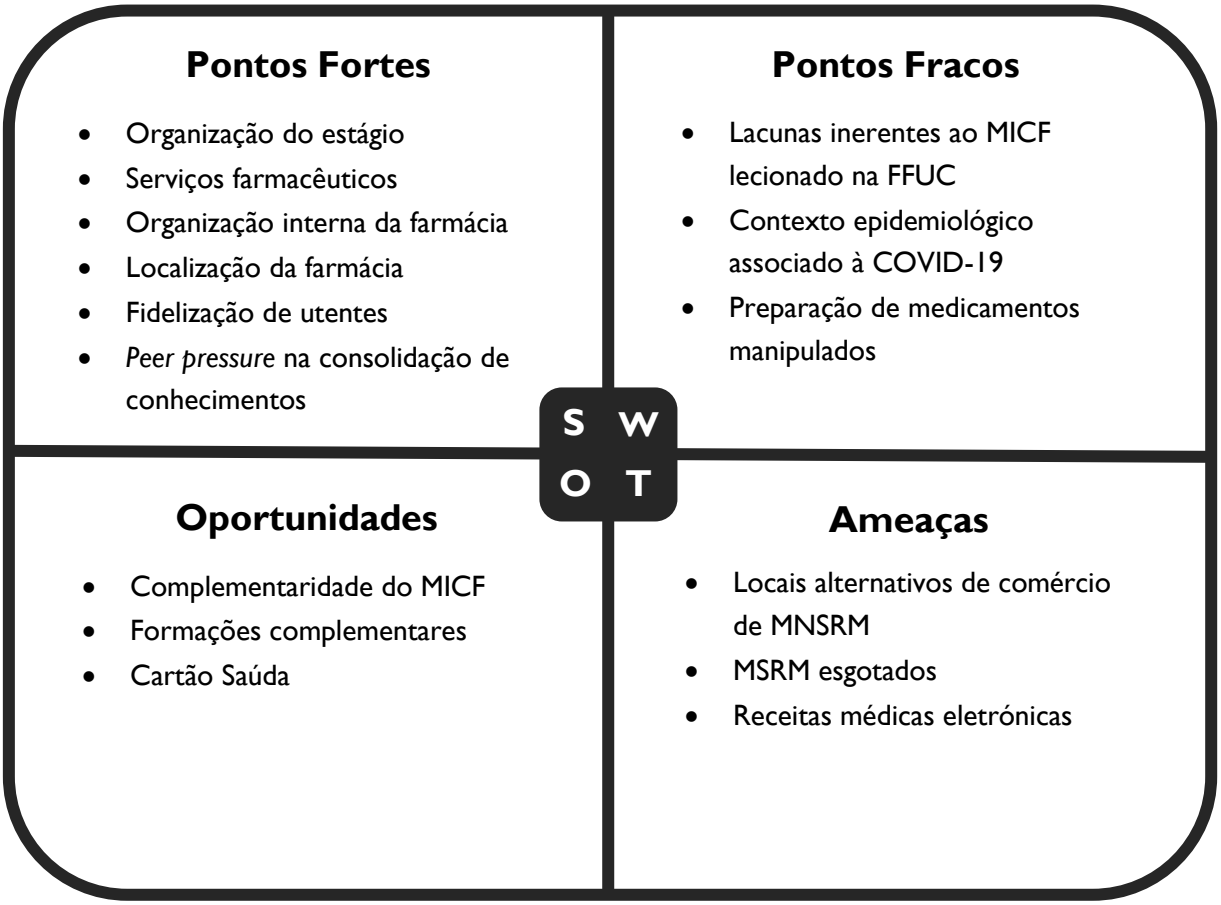
No contexto do Sistema Nacional de Saúde, as farmácias distribuídas por todo o território nacional constituem unidades de saúde de primeira linha, na medida em que delas constam farmacêuticos e técnicos auxiliares de farmácia que promovem a literacia em saúde junto das populações a custo zero, aliviando sobrecargas de hospitais e centros de saúde nalguns cuidados de saúde imediatos e evitando encargos financeiros desnecessários para os utentes.

Apesar da transversalidade de todas as unidades curriculares do MICF no âmbito da farmácia comunitária, destacam-se os conhecimentos consolidados nas unidades curriculares de Bacteriologia e Análises Bacteriológicas, Dermofarmácia e Cosmética, Farmácia Clínica, Farmacologia Geral, Farmacologia I, Farmacologia II, Farmacoterapia, Indicação Farmacêutica, Parasitologia e Análises Parasitológicas e Virologia para o cumprimento de requisitos mínimos da figura de estagiário.

O presente relatório de estágio inclui uma análise SWOT que contempla observações associadas às competências adquiridas durante o mesmo. A mesma apresenta pontos fortes (S – *Strengths*) e fracos (W – *Weaknesses*) intrínsecos ao funcionamento do estágio, assim como oportunidades (O – *Opportunities*) e ameaças (T – *Threats*) que, apesar da sua natureza extrínseca, não deixam de influenciar o rendimento do mesmo. Em segundo lugar, este relatório admite três casos práticos de atendimento ao utente. Estes elementos reiteram a importância do farmacêutico comunitário na condição de profissional de saúde e, ainda mais importante para a sociedade portuguesa, enquanto agente de saúde pública.

2. Análise SWOT

Tabela I – Representação esquemática dos tópicos abordados na presente análise SWOT, com vista a incluir conhecimentos adquiridos tanto ao longo do estágio curricular, como na articulação do mesmo com a propriedade intelectual assimilada ao longo do MICF.



2.1. S – *Strengths* / Pontos Fortes

2.1.1. Organização do estágio. Serviços farmacêuticos

Desde o início do estágio até ao dia 19 de fevereiro de 2021, o atendimento ao público por parte dos estagiários foi desaconselhado, no sentido de dar lugar a um período de tempo necessário para os mesmos se familiarizarem com as marcas comerciais associadas aos medicamentos sujeitos a receita médica (MSRM), tanto marcas registadas ou marcas de medicamentos genéricos) e respetivos grupos homogéneos; medicamentos não sujeitos a receita médica (MNSRM), suplementos alimentares, dispositivos médicos, produtos cosméticos e outros produtos de saúde e bem-estar que a farmácia disponibilize para venda. Não obstante, surgiu a oportunidade de os estagiários procederem a validações de *stock* existente dos mesmos. Além da espontânea exposição visual aos deslizantes, frigorífico, lineares e outras estruturas de armazenamento dos produtos da farmácia, a imposição da

tarefa de receção de encomendas aos estagiários revelou-se uma ferramenta inesperada na contextualização dos mesmos à dinâmica da farmácia.

Quando foi normalizado o atendimento ao público para os estagiários, com a supervisão por um farmacêutico da equipa da Farmácia São Sebastião, a dispensa de medicamentos constantes de receitas médicas prescritas através do Serviço Nacional de Saúde terá sido o procedimento mais comum nesta vertente do estágio. Por outro lado, os estagiários terão sido instruídos nalguns serviços especializados que a farmácia providencia, nomeadamente na quantificação de parâmetros antropométricos (altura, massa corporal e índice de massa corporal), clínicos e bioquímicos (glicose, colesterol total, triglicerídeos, saturação de oxigénio, tensão arterial e frequência cardíaca). No mesmo gabinete onde decorrem a maioria destas análises com os utentes, também decorrem consultas de podologia, às quais os utentes idosos são mais frequentes de ingressar, assim como terão começado a existir consultas de nutrição nas últimas semanas do estágio.

Apesar dos estagiários não participarem na iniciativa, os farmacêuticos da Farmácia São Sebastião admitem competências na administração de medicamentos injetáveis e vacinas não constantes do Plano Nacional de Vacinação, constituindo outro aspeto apologista da figura de Farmacêutico enquanto prestador de cuidados de saúde.

Além das competências supramencionadas, a Farmácia São Sebastião dispõe do serviço de preparação individualizada da medicação (PIM) ao qual, tal como na preparação de medicamentos manipulados, os utentes manifestam a sua intenção *a priori* e os medicamentos são, no ato da preparação, acondicionados nos *blisters* organizados em semanas destinados à PIM no laboratório da farmácia. O serviço de PIM é um adjuvante consistente no cumprimento da posologia dos medicamentos utilizados pela população idosa, especialmente dado o facto de que a generalidade dos utentes desta faixa etária que se deslocam frequentemente à farmácia se encontra em situações de polimedicação, com probabilidade aumentada de sujeição em erros no cumprimento das posologias indicadas.

Nas situações em que o utente recorre à farmácia no sentido do tratamento de enfermidades menores, além das eventuais medidas farmacológicas indicadas, tomou-se a iniciativa de indicar medidas não farmacológicas complementares ao seu tratamento e no sentido da prevenção de recidivas. No âmbito do contexto epidemiológico derivado da COVID-19, a Farmácia São Sebastião, além de vender testes rápidos de antigénio de coronavírus da síndrome respiratória aguda grave 2 (SARS-CoV-2, agente etiológico da COVID-19), contou com os seus farmacêuticos e estagiários na indicação do modo de utilização a cada atendimento associado.

2.1.2. Organização interna da farmácia

A zona comum aos utentes e *staff* da Farmácia São Sebastião dispõe de três balcões de atendimento ao utente, múltiplos expositores com gamas específicas de produtos, nomeadamente protetores solares (tendo em conta a proximidade do verão), produtos de uso veterinário, produtos ortopédicos e de geriatria, entre outros; e lineares com alguns dos produtos de venda livre mais consumidos pelos seus utentes, desde produtos de alimentação infantil, gamas de diversas marcas de produtos cosméticos, máscaras, artigos bucodentários, suplementos alimentares e medicamentos e produtos indicados para os tratos gastrointestinal e respiratório, etc.. Por outro lado, existem gavetas que incluem outros suplementos alimentares, nomeadamente aqueles indicados para o incremento da condição dos ossos e articulações; produtos de higiene íntima, materiais de penso, cremes hidratantes, emplastros, medicamentos e outros produtos de uso veterinário, etc.. Numa divisão separada da zona comum, existe um gabinete onde decorre o acompanhamento ao utente ainda mais individualizado e no qual decorrem as análises anteriormente mencionadas, assim como a administração de medicamentos injetáveis e vacinas e consultas de podologia.

Na área exclusiva ao *staff* da Farmácia São Sebastião, encontra-se o laboratório no qual se procede à preparação de medicamentos manipulados, PIM e preparação de suspensões prescritas por médicos e que se encontravam sob a forma de pó dentro da embalagem, assim como à preparação de *kits* de testes rápidos de antigénio de SARS-CoV-2 para venda ao público. No *backoffice*, decorre a receção de encomendas de todos os medicamentos e artigos da farmácia, e nele estão presentes os armários com deslizantes onde se encontram todos os MSRM, alguns MNSRM e outros produtos indicados para a terapêutica associada aos medicamentos que neles constam, ao dispor da farmácia. Em regra, os medicamentos são organizados ao longo dos deslizantes por ordem alfabética, e há lugares distintos para antibióticos, medicamentos compostos por saquetas, medicamentos e produtos oftálmicos e pomadas, cremes e loções análogas.

Tal organização interna permite a associação facilitada dos artigos pretendidos aquando do atendimento aos utentes à sua localização, agilizando esse mesmo processo e aprimorando a qualidade do atendimento em geral.

2.1.3. Localização da farmácia. Fidelização de utentes

Em maio de 2020, a Farmácia São Sebastião partiu de uma infraestrutura localizada em plena Avenida Elísio de Moura, em Coimbra, para outra localizada no início na localidade de

Tovim. Apesar da mudança, a farmácia não deixou de se localizar perto do Bairro de São Sebastião, e não deixou de ocorrer um *upgrade* no destaque da mesma.

O número consistente de utentes fidelizados à Farmácia São Sebastião, de diversas faixas etárias e alguns até comuns às mesmas famílias, representa uma continuidade no acompanhamento do perfil farmacoterapêutico por parte dos seus farmacêuticos e estagiários, assim como de um aconselhamento, além de fidedigno (independentemente de a quem ela recorre), personalizado. Mais importante ainda, os utentes frequentes da Farmácia São Sebastião confiam nos farmacêuticos e nos estagiários por eles orientados, contribuindo para o fomento da figura de Farmacêutico em geral.

2.1.4. *Peer pressure* na consolidação de conhecimentos

Mesmo que o MICF lecionado na FFUC seja um curso apto na formação de futuros farmacêuticos comunitários, os incentivos por parte da equipa da Farmácia São Sebastião do sentido de responsabilidade associado à figura de Farmacêutico foram notáveis para a simplificação e revisão de conhecimentos a título individual e a transmitir aos utentes. Tais esforços foram complementares com a leitura de fluxogramas das patologias menores mais comuns dos utentes e artigos científicos concernentes aos medicamentos manipulados preparados na farmácia.

2.2. W – *Weaknesses* / Pontos Fracos

2.2.1. Lacunas inerentes ao MICF lecionado na FFUC

Por mais apto que possa ser o MICF no contexto da farmácia comunitária, houve lacunas que influenciaram negativamente o desempenho enquanto estagiário.

Nas duas vezes em que se ingressou na unidade curricular de Farmacologia I, estava prevista a abordagem a fármacos que atuam no trato gastrointestinal. Contudo, tal não foi possível por falta de tempo na calendarização prevista para o programa da unidade curricular. Em segundo lugar, a unidade curricular de Preparações de Uso Veterinário não contemplou as formas farmacêuticas dos medicamentos e produtos expostos na farmácia. Também não houve abordagem ao longo do MICF de produtos de geriatria (espessantes alimentares, géneros alimentícios enriquecidos, etc.) e medicamentos e produtos oftálmicos.

Em último lugar, quando os utentes que aderem à farmácia vêm munidos de receitas médicas com as posologias e dosagens associadas a cada medicamento, não surge a necessidade imediata do farmacêutico ou estagiário que os atende de sabê-las *a priori*. Este acontecimento pode justificar o facto de não serem abordadas posologias ao longo do MICF,

exceto na análise e resolução de casos práticos, no âmbito das unidades curriculares de Farmacologia I, Farmacologia II, Farmacoterapia, Farmácia Clínica e Indicação Farmacêutica, o que representa uma minoria de todas as substâncias ativas e respetivas formas farmacêuticas lecionadas.

2.2.2. Contexto epidemiológico associado à COVID-19

Na semana de 11 a 15 de janeiro de 2021, altura em que se registavam milhares de novos casos de infeções por COVID-19, a diretora técnica da Farmácia São Sebastião tomou a decisão de dispensar os estagiários na semana seguinte. Numa segunda instância, uma estagiária obteve um resultado positivo num teste de diagnóstico de SARS-CoV-2. Por este motivo, o *staff* da farmácia que esteve em contacto com a estagiária foi convidado a cumprir isolamento profilático, de acordo com as diretrizes da Direção Geral da Saúde. Ambas as situações prejudicaram o normal funcionamento da farmácia, tanto pelas ocorrências propriamente ditas como pelo caráter repentino das mesmas.

2.2.3. Preparação de medicamentos manipulados

No sentido de colmatar falhas na produção de algumas formas farmacêuticas à escala industrial, os farmacêuticos destacam-se no âmbito dos medicamentos manipulados, na medida em que são os profissionais de saúde reconhecidos pela República Portuguesa para a sua preparação [1].

A equipa da Farmácia São Sebastião executou a produção de diversas fórmulas magistrais, enfatizando-se cápsulas de ivermectina e creme de permetrina como as mais produzidas em laboratório durante o estágio. Apesar da Farmácia São Sebastião ter sido requisitada por utentes e farmácias na preparação de medicamentos manipulados, este procedimento cai em desuso face à crescente industrialização da conceção das formas farmacêuticas em geral; já sendo poucas as formas farmacêuticas que requerem preparação em farmácia, não se sentiu a necessidade de os estagiários participarem nesse processo além da elaboração de documentação preliminar.

2.3. O – *Opportunities* / Oportunidades

2.3.1. Complementaridade do MICF

Não se equacionaria a possibilidade de realizar este estágio com aproveitamento se não fosse precedido da maioria das unidades curriculares do MICF ingressadas ao longo dos

últimos 6 anos letivos. A versatilidade de conteúdos das unidades curriculares contempladas no MICF lecionado na FFUC corresponde à versatilidade de categorias de produtos que a Farmácia São Sebastião dispõe. Por outro lado, as competências de comunicação no atendimento ao utente adquiridas ao longo do estágio são exclusivas a este, assim como as marcas comerciais de medicamentos e outros produtos que a Farmácia São Sebastião dispõe, pelo que o MICF depende também da realização do estágio curricular para ter nexo na formação de farmacêuticos comunitários, que constituem a maioria da classe farmacêutica. Infere-se que exista uma coordenação entre as unidades curriculares e o estágio previsto no MICF.

2.3.2. Formações complementares

Enquanto membros da equipa da Farmácia São Sebastião, os estagiários foram convidados a participar em dois *webinars*, promovidos pela Pierre Fabre, relativos à gama de colutórios Eludril e à gama de produtos de proteção solar da Avène. Ambas as iniciativas desempenharam um papel relevante na performance do atendimento ao utente, no tratamento de patologias do âmbito da estomatologia e medicina dentária e prevenção de enfermidades associadas à época de verão, respetivamente.

2.3.3. Cartão Saúda

O Cartão Saúda é uma iniciativa da Associação Nacional das Farmácias (ANF) que funciona num regime de acumulação de pontos, os quais poderão ser rebatidos por descontos imediatos nas compras realizadas na farmácia pelos utentes, assim como por produtos constantes de catálogos sazonais elaborados pela ANF. O Cartão Saúda sugere a fidelização de utentes à rede Farmácias Portuguesas da ANF, tendo em conta a adesão da Farmácia São Sebastião à mesma.

2.4. T – *Threats* / Ameaças

2.4.1. Locais alternativos de comércio de MNSRM

Paralelamente à atividade das farmácias, existem postos de venda de MNSRM em diversas superfícies comerciais, concretamente centros comerciais e áreas de serviço das autoestradas portuguesas. Tal aval foi garantido pelo INFARMED, I.P. pelo Decreto-Lei n.º 134/2005, de 16 de agosto, com fundamento na "redução dos preços dos MNSRM" e na "concorrência efetiva entre os vários canais de distribuição e comercialização" [2].

Apesar destes motivos serem favoráveis aos utentes, aludindo ao fator de erro humano como risco consistente associado à automedicação, a medida em questão conduz à desvalorização da automedicação, derivada da ausência ou deficiência em farmacêuticos na indicação farmacêutica inerente a estes pontos de venda.

2.4.2. MSRM esgotados

A Farmácia São Sebastião, nos meses em que decorreu o estágio, padeceu de ruturas de *stock* de fornecedores de medicamentos com diazepam (Valium® e outros medicamentos genéricos), liraglutido (Victoza®) e levotiroxina sódica (Eutirox®) como substâncias ativas, entre outros medicamentos e produtos vendidos na farmácia sujeitos a rateio.

Tais lacunas na encomenda destes itens são prejudiciais para o modelo empresarial da farmácia, na medida em que se perde a oportunidade de comercializar os medicamentos e produtos de saúde que o utente pretende eficientemente. Além disso, o próprio utente também fica a perder, nomeadamente em situações de dificuldade de deslocação à farmácia, colocando em risco a continuidade de uma eventual terapêutica indicada ou prescrita.

2.4.3. Receitas médicas eletrónicas

No que concerne às receitas médicas eletrónicas materializadas, delas consta uma tabela com a coluna Encargos, que respeita a seguinte frase para cada medicamento prescrito: "Esta prescrição custa-lhe, no máximo, [montante em euros], a não ser que opte por um medicamento mais caro.". Contudo, o montante citado para um dado medicamento pode variar entre a data de emissão da receita médica e o dia da dispensa do medicamento, havendo potencial de indução em erro no utente. Enquanto estagiário, surgiu a necessidade de enfatizar essa discrepância em momentos de atendimento aos utentes.

Por outro lado, as receitas médicas eletrónicas desmaterializadas não enumeram os medicamentos que delas constam quando são emitidas e enviadas aos utentes, o que gera um sentimento de incerteza quando esta é apresentada ao farmacêutico ou estagiário que ela processou, nomeadamente em utentes de terceira idade.

3. Casos Práticos

3.1. Dermatite seborreica

Uma utente do sexo feminino com 41 anos de idade pretendia uma embalagem de 3 x 21 comprimidos revestidos Aranka®. Antes da dispensa do medicamento, queixava-se da formação de crostas amareladas no couro cabeludo. Após análise das mesmas, inferiu-se que se tratava de uma dermatite seborreica. A utente confirmou que sofre de "dermatite nervosa" que afetava também as pálpebras, além do couro cabeludo. Além disso, constatou que lhe aconselharam o champô Nizoral® no tratamento da dermatite; contudo, acabou por ser indicado o champô Tedol®. Apesar de ambos os champôs apresentarem cetoconazol como substância ativa, na dosagem de 20 mg/g, o champô Tedol® disponível na farmácia apresentava 200 mL de volume, enquanto o champô Nizoral® apresentava apenas 100 mL. Tendo em conta a recorrência da dermatite seborreica, indicou-se o champô com maior volume, assim como se mencionou a posologia de aplicação duas a três vezes por semana, em alternância com o champô normal.

3.2. Infeção do trato urinário

Uma utente do sexo feminino, com 37 anos de idade, recorreu a uma casa de banho pública e apresentou sintomatologia associada a uma infeção do trato urinário. No dia seguinte, a mãe da utente desloca-se à farmácia e pede aconselhamento para o tratamento dessa sintomatologia. Terá sido indicado um suplemento alimentar Fairline à base de extrato seco de arando vermelho (*Vaccinium macrocarpon*), ácido L-ascórbico e D-manose, funcionando os três componentes no sentido da prevenção da infeção urinária [3 – 5]. No que diz respeito à posologia, foi indicado à utente a administração oral de duas cápsulas por dia (uma de manhã e outra à noite), durante três dias, e uma cápsula por dia a partir do quarto dia, até ao fim da embalagem.

3.3. Úlcera labial

A mãe de uma criança com 14 anos de idade entra na Farmácia São Sebastião e pede uma embalagem de cápsulas Furadantina MC® 100 mg, sem prescrição médica, com o objetivo de tratar um lábio ferido e purulento da criança. O medicamento sugerido não foi reconhecido como uma opção de tratamento da patologia em questão, tanto por se tratar de um medicamento sujeito a receita médica, como pelo facto de ser um medicamento primariamente indicado para o tratamento de infeções do trato urinário inferior. Em

alternativa, foi dispensada uma embalagem de Fucidine® 20 mg/g creme, que está relacionado com uma administração tópica, mas é principalmente indicado para o tratamento de infeções cutâneas localizadas, a ser administrado na zona da úlcera duas vezes por dia.

4. Considerações Finais

Findo o estágio curricular na Farmácia São Sebastião, confirma-se a perceção de que a figura de Farmacêutico desempenha um papel importante na sociedade portuguesa como é conhecida atualmente, principalmente por garantir que a ciência que concerne os medicamentos é transmitida aos utentes a custo zero e de forma personalizada. Por outro lado, termina-se o estágio com a impressão de que ficou mais por saber dos medicamentos e produtos que uma dada farmácia pode vender, revendo-se este exemplo na versatilidade de soluções terapêuticas que a farmácia pode providenciar à população.

É certo que houve erros cometidos ao longo do estágio – alguns mais do que uma vez –, mas o estágio curricular no contexto do MICF constitui uma oportunidade de aprendizagem complementar ao curso. O balanço do estágio não deixa de ser positivo, porque o impacto dos erros sistemáticos cometidos no início decresceu.

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PARTE II

Monografia

"Melatonin: New avenues for an old molecule"

Sobre a orientação da Professora Doutora Maria Manuel da Cruz Silva

Abbreviations

3-OHM 3-Hydroxymelatonin

6-OHM 6-Hydroxymelatonin

8-OHdG 8-Hydroxy-2'-deoxyguanosine

AchE Acetylcholinesterase

AD Alzheimer's disease

AFMK Acetyl-*N*-formyl-5-methoxykynurenamine

Akt Protein kinase B

Al Aluminum

AMK *N*1-Acetyl-5-methoxykynuramine

AMPK 5' Adenosine monophosphate-activated protein kinase

APP Amyloid precursor protein

ASMT *N*-Acetylserotonin *O*-methyltransferase

ATP Adenosine triphosphate

Aβ Amyloid-beta

BACE1 Beta-secretase 1

BaP Benzo(a)pyrene

Bax B-Cell lymphoma-associated X protein

Bcl-2 B-cell lymphoma 2

BDNF Brain-derived neurotrophic factor

BMSC Brain mesenchymal stem cell

BRB Blood-retinal barrier

BRET Bioluminescence resonance energy transfer

CaMKII Calcium ion/calmodulin-dependent protein kinase II

cAMP Cyclic adenosine monophosphate

CASP Caspase

CAT Catalase

CHOP CCAAT/enhancer-binding protein homologous protein

ColIV Collagen IV

COPD Chronic obstructive pulmonar disease

COX-2 Cycloxygenase-2

CREB1 cAMP-response element binding protein 1

CTFβ Beta-secretase-generated C-terminal fragment

DN Diabetic nephropathy

DNA Deoxyribonucleic acid

DR Diabetic retinopathy

ER Endoplasmic reticulum

Fn Fibronectin

G6PD Glucose-6-phosphate dehydrogenase

GDNF Glial cell-derived neurotrophic factor

GI Gastrointestinal

GSH Reduced glutathione

GSH-Px Glutathione peroxidase

GSSG Oxidized glutathione

HO-1 Hemoxygenase-1

HPMC Hydroxypropylmethylcellulose

I/R Ischemia-Reperfusion

IFN-γ Interferon-gamma

IL Interleukin

iNOS Inducible nitric oxide synthase

LC3 Microtubule-associated proteins 1A/1B light chain 3B

LDH Lactate dehydrogenase

LPS Lipopolysaccharide

LRP1 Low-density lipoprotein receptor-related protein-1

MAO Monoamine oxidase

MAPK Mitogen activated protein kinases

MDA Malondialdehyde

MEG3 Maternally expressed gene 3

Mfn Mitofusin

MOF Multiple organ failure

mPTP Mitochondrial permeability transition pore

mTOR Mechanistic target of rapamycin

MYD88 Myeloid differentiation primary response 88

NDDs Neurodegenerative diseases

NEP Neprilysin

NF-κB Nuclear factor kappa B

NLRP3 NOD-like receptor family pyrin domain containing 3

NMDA *N*-methyl-D-aspartate receptor

NQO1 NAD(P)H: quinone oxidoreductase 1

Nrf2 Nuclear factor erythroid 2-related factor 2

PD Parkinson's disease

PERK Protein kinase R-like endoplasmic reticulum kinase

PGC 1α Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

PI3K Phosphatidylinositol 3-kinase

PINK1 Phosphatase and tensin homolog-induced putative kinase 1

PKA Protein kinase A

PPARγ Peroxisome proliferator-activated receptor gamma

RAGE Receptor advanced glycation end productions

RNS Reactive nitrogen species

ROS Reactive oxygen species

SCN Suprachiasmatic nucleus

SIRT Sirtuin

Smad3 Mothers against decapentaplegic homolog 3

SNAT Serotonin *N*-acetyltransferase

SOD2 Superoxide dismutase 2

STAT3 Signal transducers and activators of transcription 3

TLR Toll-like receptor

TNF-α Tumor necrosis factor alpha

TRX-1 Thioredoxin-1

TXNIP Thioredoxin-interacting protein

VEGF Vascular endothelial growth factor

Resumo

Melatonina, também conhecida como *N*-acetil-5-metoxitriptamina, é uma hormona derivada do triptofano produzida principalmente na glândula pineal em humanos, apesar de ser sintetizada localmente em vários tecidos e órgãos. O seu uso clínico é mais prevalente para o tratamento da insónia e outros distúrbios do sono, mas pode também contribuir para o tratamento de processos inflamatórios e doenças neurodegenerativas. Esta revisão pretende providenciar uma atualização em investigação atual concernente ao papel da melatonina, tanto enquanto molécula endógena ou fármaco, na regulação de ciclos de sono, ritmos circadianos, processos inflamatórios que podem comprometer função cardiovascular, respiratória, gastrointestinal ou renal, doença de Alzheimer e doença de Parkinson. A revisão encerra com um capítulo que aborda alguma da investigação respeitante aos análogos sintéticos da melatonina agomelatina e ramelteon, assim como novos compostos com atividade farmacológica promissora.

Palavras-chave: Agomelatina, Doença de Alzheimer, Ritmo Circadiano, Inflamação, Melatonina, Doença de Parkinson, Ramelteon

Abstract

Melatonin, also known as *N*-acetyl-5-methoxytryptamine, is a tryptophan-derived hormone mostly produced in the pineal gland in humans, despite being synthesized locally at several tissues and organs. Its clinical use is more prevalent for treatment of insomnia and other sleep disturbances, but it may also contribute to treatment of inflammatory processes and neurodegenerative diseases. This review aims to provide an update on current research concerning the role of melatonin, either as an endogenous molecule or as a drug, in sleep-wake cycle regulation, circadian rhythms, inflammatory processes that may compromise cardiovascular, respiratory, gastrointestinal and renal function, Alzheimer's disease and Parkinson's disease. The review ends with a chapter revealing some of the research concerning the melatonin synthetic analogs agomelatine and ramelteon, as well as hint new compounds with promising pharmacological activity.

Keywords: Agomelatine, Alzheimer's disease, Circadian rhythm, Inflammation, Melatonin, Parkinson's disease, Ramelteon

1. Introduction

Melatonin (Figure 1), whose name in accordance with chemical nomenclature is *N*-acetyl-5-methoxytryptamine, is a tryptophan-derived hormone which, in humans, is produced and released by the pineal gland, primarily at night, and regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus. While there have been discovered different pathways regarding melatonin biosynthesis in plants, humans and other animals, the amount of tryptophan necessary for such biochemical reactions is released and acquired from diet in humans [1, 2].

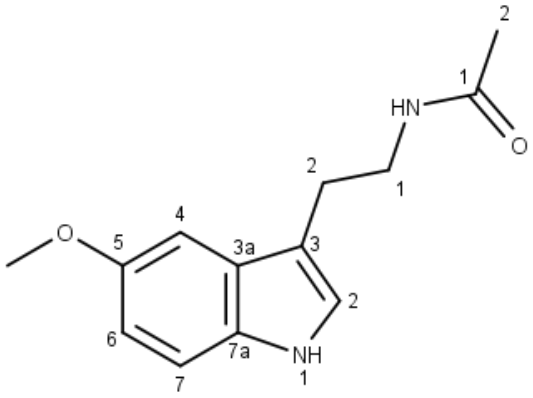

Figure 1 – Chemical structure of melatonin. As it presents both lipophilic and hydrophilic properties, due to its 5-methoxy group and its *N*-acetyl side chain, this compound permeates into the cerebrospinal fluid and blood with ease and can influence the activity of all human cells and interact with other compounds, making it a potential medical adjuvant [1, 2].

In order to obtain melatonin, L-tryptophan is hydroxylated into 5-hydroxy-tryptophan by the enzyme tryptophan hydroxylase. 5-Hydroxytryptophan, then, suffers decarboxylation by the enzyme 5-hydroxytryptophan decarboxylase, which leads to the production of serotonin if vitamin B6 is present. On a second phase, serotonin is acetylated into *N*-acetylserotonin by the enzyme serotonin *N*-acetyltransferase (SNAT, also known as AANAT), where protein kinase A (PKA) must be present for this reaction to occur. Then, *N*-acetylserotonin suffers methylation to melatonin through the activity of *N*-acetylserotonin *O*-methyltransferase (ASMT). There is evidence supporting mitochondria as the main sites for melatonin production, due to the presence of ASMT, SNAT and 14-3-3 chaperone that prevents SNAT degradation and increases its affinity to serotonin. Given that the availability of SNAT, aside from tryptophan concentration, is a limiting factor in melatonin production, 14-3-3 plays an essential role in melatonin synthesis [2, 3].

While melatonin is mainly synthesized in the pinealocytes that constitute the pineal gland, it is also produced in areas such as bone marrow, skin, gastrointestinal (GI) tract, heart, kidney and placenta. Its concentration varies between 5-200 pg/mL throughout the day and has a half-life that varies between 20 and 40 minutes [2 – 4].

Melatonin synthesis begins at early stages of human life, with evidence pointing at melatonin production by the human fetus (even though maternal melatonin would be dominant), but it is generally established that it starts at 3 months after birth, peaks at 6 years old and decreases to adult levels at 12 years old. When a human being achieves 16 years of age, its pineal gland tends to calcify and melatonin production decreases as their age increases [2, 5].

In humans, melatonin is a full agonist of transmembrane melatonin receptors 1 (MT1) and 2 (MT2); both being G protein-coupled receptors, where MT1 is the most expressed subtype in the human brain and MT2 has increased binding affinity toward melatonin. Both subtypes are present in cell membranes and may be expressed by mitochondria as well. Moreover, proteins such as glucose transporters Glut/SLC2A and proton-driven oligopeptides PEPT1/2 carry melatonin across cell membranes and mitochondria, even though melatonin can be synthesized within mitochondria [1, 2, 4, 6].

MT1 activation translates into the inhibition of adenylate cyclase/cyclic adenosine monophosphate (cAMP)/PKA pathway, and the activation of MT2 leads to the inhibition of the same pathway, in addition to the guanylate cyclase/cyclic guanosine monophosphate/protein kinase G pathway. The activation of either subtype of melatonin receptors leads to the activation of the phospholipase C pathway and the increase of levels of inositol triphosphate and 1,2-diacylglycerol. On a nuclear level, melatonin can also bind to retinoid-related orphan receptors (RZRα/ROR). These pathways can influence cell proliferation, integration of circadian outputs, inflammation, immunity and the regulation of antioxidant enzymes [1 – 3].

The activation of MT1 or MT2 also inhibits wake-promoting signals. Considering normal physiological status, the levels of melatonin oscillate throughout the day: melatonin secretion increases at approximately 21:00 (due to the activation of β_1-adrenergic receptors located in the pinealocytes by norepinephrine, which activate the cAMP/PKA pathway), peaks during overnight hours and decreases in the next morning (when a lack of norepinephrine stimulation is observed). The recurrence of these daily variations in melatonin production is due to the daylight variations to which the retinal ganglion cells of the human body are exposed, and as such, melatonin plays a consistent role in determining timings for biomolecules and their respective pathways, contributing to the establishment of biological rhythms. Environmental factors such as seasonal changes in temperature and photoperiods can influence

melatonin synthesis as well. On a preventive note, there are groups of clock genes which determine circadian rhythms for most human cells and seem to attenuate circadian disturbances [1 – 3, 5, 6].

Elderly people are vulnerable to biological rhythm disorders, which reflect upon low-quality sleep at night. There is also scientific evidence supporting a decline in melatonin secretion (but not in melatonin production) as age increases, which could lead to hypertension, stress, decreased management of body assets and development of neurodegenerative diseases (NDDs), such as Alzheimer's disease (AD) and Parkinson's disease (PD). The intake of exogenous melatonin could help elderly people in sleep quality improvement and next day alertness and retard myocardial aging. Night shift workers also tend to have relatively lower melatonin levels, with data pointing at a 33,8% decrease in melatonin production over 24 hours [2, 4, 5].

Melatonin is considered to possess a reliable safety profile. Taken as a supplement, melatonin intake presents little to no side effects, regardless of dosage and intake period, with daytime sleepiness, nausea, drowsiness, dizziness, agitation, fatigue and headaches among the most reported side effects [3 – 5]. Melatonin administration does not apply as a primary therapeutic approach, as it yet lacks scientific evidence supporting clinical efficacy for that standard, except in some cases regarding sleep disorders – for example, in the case of individuals with autism spectrum disorder, who can present lower melatonin levels. Even though the literature is incomplete on this subject, as melatonin plays a relevant role in circadian rhythm and sleep disorders, the treatment of circadian disruptions could prove to be useful in a decline of risk for cardiovascular, metabolic or degenerative diseases [1, 2, 4].

As aforementioned, there is an association between light exposure and melatonin production and secretion. While melatonin secretion can adapt to the length of each night, a certain balance is ideal, as exposure to light during nightly hours (for instance, due to usage of electronic devices such as computers, tablets or smartphones), emotional stress or jet lag disorder could influence melatonin levels and lead to hormonal dysregulation, disruptions in sleep-wake cycles and overall sleep quality, as well as increased risk for cancer. Melatonin has been consistently used to improve sleep quality and/or the discrepancies associated with jet lag disorder [1, 2, 5].

When it comes to the administration of exogenous melatonin, it usually happens through the oral route, but presents transdermal, transmucosal, intranasal and intravenous routes as alternatives. A wider effort to determine ideal long-term dosages for patients and to address these alternative routes is required. Circulating levels of melatonin can increase with the presence of other drugs, such as oral contraceptives or caffeine, because they

compete with melatonin for the hepatic enzyme cytochrome P-450, originating 6-hydroxymelatonin (6-OHM). 6-Sulfatoxymelatonin (aMT6s), the most abundant melatonin metabolite in urine, as well as 6-OHM, can be used as markers that represent the levels of melatonin in the blood, namely in the contexts of asthma and the validation of circadian rhythms in humans, respectively [1, 2, 4, 29].

Generally, aside from its capacity to bind into intracellular proteins, melatonin exerts its antioxidant activity due to its chemical characteristics, as it can work as an antioxidant and scavenger of free radicals based on oxygen (reactive oxygen species (ROS)) and nitrogen (reactive nitrogen species (RNS)) – mostly because of its indole aromatic ring and 5-methoxy group –, or as a promoter of the activity of antioxidant enzymes, such as superoxide dismutase, catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase, as well as the first enzyme in the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (G6PD); and as an inhibitor in prooxidative enzymes, such as xanthine oxidase or eosinophil peroxidase. The metabolites associated with these biochemical reactions can present antioxidant activity, yielding radical scavenger products through an antioxidant cascade, such as acetyl-N-formyl-5-methoxykynurenamine (AFMK), *N*1-acetyl-5-methoxykynuramine (AMK) or 3-hydroxymelatonin (3-OHM). Moreover, through epigenetic activation of nuclear factor erythroid 2-related factor 2 (Nrf2), melatonin improves the activity of antioxidative enzymes, such as heme oxygenase-1 (HO-1) and superoxide dismutase 2 (SOD2) [1 – 4, 21, 29, 34].

The antioxidant efforts regarding mitochondria are based on stable membrane potential and ionic balance, and could lead to an improvement in adenosine triphosphate (ATP) production. It becomes useful for mitochondria to be rich on melatonin, given that they are also abundant in ROS production associated with the electron transportation chain of aerobic respiration and in of ischemia-reperfusion injuries, as the accumulation of ROS cannot be sustained by antioxidant enzymes alone and can lead to structural damage in deoxyribonucleic acid (DNA), lipids and proteins. The ability of mitochondria to maintain stable the membrane potentials leads to an expedition in antioxidant processes involving electron transfer, protecting mitochondria against oxidative stress. Moreover, melatonin blocks permeation through mitochondrial permeability transition pores (mPTP), by decreasing mitochondrial membrane potential, and prevents alterations in mitochondrial DNA. The inhibition of the synthesis of pro-apoptotic proteins caused by mitochondrial melatonin leads to a blockage in cytochrome C (Cyt-C) release and, as a consequence, the annulation of mitochondrial apoptotic activity. The expression of anti-apoptotic factors can also be improved by melatonin, where they can be useful against pathologies involving inflammation, cancer and NDDs. However, melatonin is also known for inducing pro-apoptotic efforts in pathologies such as

hepatic carcinoma. It is then inferred melatonin preserves homeostasis through the establishment of a balance between local pro- and anti-apoptotic efforts [3, 4].

Melatonin plays a role in both chronic and acute inflammation and antiproliferative efforts. While it is also associated with nuclear receptors and receptor-independent pathways, through MT1 and MT2, melatonin is related to the suppression of the production of C-reactive protein and pro- and anti-inflammatory cytokines, leukotrienes, prostanoids and adhesion molecules [1, 2].

The nuclear factor kappa B (NF-κB) is related to the ratio between reduced and oxidized glutathione (GSH/GSSG ratio) and regulates multiple pathways regarding inflammation, cell proliferation and immunity, as well as gene expression of inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β). Mitochondrial ROS originated by TNF-α can further expedite the activation of NF-κB, hence creating a positive feedback loop in inflammatory response. There is evidence supporting a decrease in TNF-α and IL-6 release with melatonin supplementation, as well as a decrease in interferon-gamma (IFN-γ) and IL-1, through inhibition of NF-κB signaling, and an increase in levels of anti-inflammatory cytokines IL-4, IL-10 and IL-27. An association between NF-κB inhibition and the suppression of lipopolysaccharide (LPS)-induced inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression may occur, as well as between NF-κB inhibition and the inhibition of inflammasome activation, which in turn leads to the decrease of caspase-1 (CASP1) activity and the maturation of IL-1β. The NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome can also be activated through ROS, as it can be responsible for chemical changes in cellular targets. The NLRP3 inflammasome encompasses NLRP3, CASP1 and apoptosis-associated speck-like protein (ASC), where ASC can promote the maturation of IL-1β (from pro-IL-1β) and IL-18 [1, 2, 19, 31].

While each NDD presents its own set of pathological characteristics, many share similar signals and molecular patterns. Given that the prevalence of NDDs increases with aging, as well as genetic mutations and environmental factors (such as smoking or recreative alcohol consumption), a diminishment in melatonin production becomes a consistent factor in those conditions. When it comes to NDDs, changes in turnover of neurotransmitters can lead to inflammation, oxidative stress, endoplasmic reticulum (ER) stress, autophagy and dopamine loss. Circadian rhythm disturbances, glutamate-induced excitotoxicity, mitochondrial dysfunction and neuronal loss are also in the range of NDDs, partly thanks to the production of abnormal and toxic protein aggregates [1 – 5].

Melatonin, as well as metabolites AFMK, AMK and 3-OHM, play a protective role in brain tissue through their antioxidant properties, as they can inhibit mitochondrial damage and dampen the expression of detrimental proteins, preserving normal neurons. Microglial activation is also discouraged by melatonin, which would otherwise lead to cytokine release and oxidative damage. Neurodegeneration is ameliorated by melatonin through inhibition of Cyt-C leakage and suppression of CASP and pro-inflammatory cytokine activation. Melatonin may also play a role in neurogenesis in adults by promoting synaptic plasticity in neutral stem cells mediated by brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF). A restoration or regulation of neuronal cell cytoskeleton by melatonin may also improve the physiopathological consequences brought by NDDs [2, 4, 7].

Considering mutations in AD-related genes and suppressed autophagy, the causes of AD are associated with the build-up of extracellular senile plaques that result from amyloid-beta (Aβ) accumulation, the occurrence of intracellular neurofibrillary tangles rich on aggregated and hyperphosphorylated tau protein and a decline in function and number of neurons located in the hippocampus and cerebral cortex. This leads to a decline in neuron count and a suppression of neuronal maturation as the pathology progresses, as neurons are unable to get rid of these proteins in an aggregated fashion. The development of AD can lead to abnormalities in the cholinergic system, with irregular acetylcholine and acetylcholinesterase (AchE) levels [3, 5, 7, 42].

Aβ peptides are obtained if transmembrane amyloid precursor protein (APP) is subject to sequential cleavages by β- and γ-secretases. Aβ build-up can inhibit neural stem cell proliferation and neuronal differentiation in mice, as the generated amyloid plaques can reach extracellular areas and contribute to cytotoxicity, triggering neuroinflammation, mitogen activated protein kinases (MAPK) activation and phosphorylation of tau protein [5, 7, 35].

The hyperphosphorylation of tau protein results in irregularities in neuronal microtubule morphology, compromising cytoskeleton structure and impairing neurogenesis in AD patients. Neuroinflammation, characterized by oxidative stress, partly caused by the oxidant activity of Aβ, can lead to cell death and hinder neurogenesis in adults. AD manifests through a progressive cognitive decline resulting in memory loss and alterations in human behavior. Early AD diagnosis, as difficult as it may be for clinicians, opens the window to an opportunity for amelioration of symptoms and inhibition of the progression of AD before irreversible cognitive decline occurs [7].

Melatonin synthesis appears to be lower in patients with AD, which may be associated with pineal gland shrinking and calcification. Such a suppression may justify wake-sleep cycle abnormalities, lower sleep quality, sundowning and agitated nightly behavior in patients with

AD. MT1 upregulation may occur as a result of the decline in melatonin production. If the gene that encodes MT1 is silenced, an increase of APP processing may occur, and further aggravation AD symptoms may happen due to the lack of circadian regulation. Nevertheless, during sleep, melatonin can suppress Aβ production and aggregation, by promoting non-amyloidogenic processing and suppressing amyloidogenic processing of APP, and tau filament synthesis, avoiding Aβ toxicity in neurons, and exogenous melatonin administration may aid in circadian disturbances (as aforementioned) and cognitive performance in AD patients [2, 4, 7].

Considering gene mutations, again – while the etiological cause of PD is yet to be elucidated, it is known to be related to a progressive diminishment of dopaminergic neurons, which are responsible for autonomous movement and posture regulation, in the nigrostriatal pathway, resulting in rarefied dopamine. The presence of Lewy bodies comprised of aggregated α-synuclein (as well as its oligomers) in neurofilaments is also associated with PD. This phenomenon may lead to sleep, cognitive and mental disorders, aside from bradykinesia, posture disturbances, resting tremors and muscle stiffness [3, 5].

While under normal physiological conditions, α-synuclein favors dopaminergic regulation, neuroplasticity and synaptic transmissions, oxidative stress can favor aggregation of α-synuclein-related oligomers. Neurotoxicity alleviation provided by melatonin activity encompasses suppression of autophagy and α-synuclein aggregation. Through a shift towards increase in dopamine levels and maintenance of dopaminergic neurons, melatonin is able to dampen oxidative stress and apoptosis in animal PD models [3, 5].

Melatonin averts neurodegeneration-related PD through its activity towards free radical scavenging and against excitotoxicity. α-Synuclein cytotoxicity and related fibril production is suppressed by melatonin. In addition, melatonin affords increased CAT and SOD activity and GSH levels at neuronal cell soma and the nigrostriatal area in animal PD models [2, 4].

Although not encompassed in this review, recent research shows melatonin may also present roles in NDDs such as Huntington's disease, amyotrophic lateral sclerosis, multiple sclerosis, or vascular dementia [3].

Historically, the interest in the synthesis of new melatonin analogs has resided mostly in MT1 and MT2 agonists, to detriment of antagonists. Melatonin receptor agonism associated to analogs such as agomelatine (Figure 3) and ramelteon (Figure 4) may be attributed to their methoxy groups and amide moieties. MT2-selective agonists have also been easier to find due to the structural differences when compared to MT1 – for example, the binding site of MT2 is larger. The assumption that residues near the binding site might work as binding

contributors supports the idea that an analog design based on structure is more effective than a ligand-based design [6].

Synthetic melatonin analogs such as agomelatine and ramelteon have clinical purpose in reducing sleep latency. Ramelteon presents a half-life of 1,5 to 2 hours, and has higher affinity for melatonin receptors than melatonin itself. While both analogs are adequate for treatment of sleep disorders, agomelatine emphasizes in sleep disturbances associated with major depressive disorder. Agomelatine concentration peaks after 1 to 2 hours of intake [3, 4].

While there is yet the need for more research regarding the roles of melatonin in the human body, the present review aims to offer an update in recent clinical efforts to further establish the roles of melatonin in the regulation of circadian rhythms and sleep-wake cycles, inflammation-related processes such as oxidative stress and apoptosis, and NDDs, with emphasis on AD and PD. In addition, this review encompasses a final chapter presenting some of the most recent studies regarding melatonin synthetic analogs, such as agomelatine, ramelteon, among others.

2. Roles of melatonin

2.1. Role in sleep-wake cycle regulation and circadian rhythms

Under normal physiological conditions, melatonin levels fluctuate similarly between 24-hour cycles. In a study encompassing patients with idiopathic rapid eye movement sleep behavior disorder (RBD), a potential precursor of PD and other NDDs, there was a characterization of melatonin profiles through blood samples (Figure 2), as well as an assessment of the expression of clock genes PER1, PER2, PER3, BMAL1 and NR1D1, in support of a combination of melatonin supplementation and chronotherapy as treatment for RBD [8].

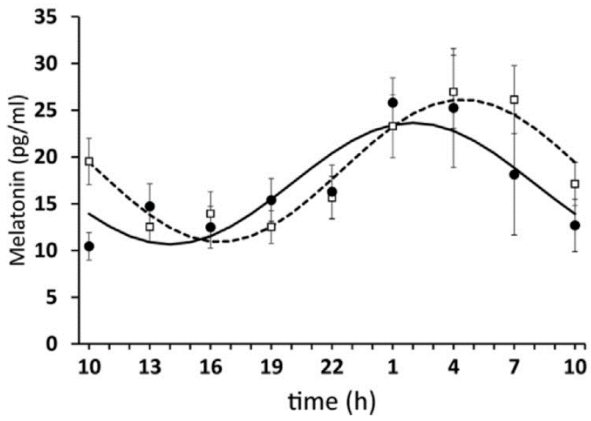

Figure 2 – Variations in melatonin concentrations among control patients (continuous line) and RBD patients (dashed line). Considering a 24-hour cycle for control patients, melatonin production increased at around 19:00, with an acrophase between 02:00 and 04:00 and a decline in the next morning. A 2-hour lag in RBD acrophase patients has been reported [8].

Another cause to justify a delay in endogenous melatonin production would be chronic sleep onset insomnia. Exogenous melatonin intake, as well as the introduction of adequate sleep hygiene practices, could accomplish a physiological improvement on several counts: longer total sleep and actual sleep durations, smaller sleep onset latency and number of night awakenings, increase in melatonin levels and a righteous shift in acrophase timings, resulting in a recovery of circadian secretion of melatonin to normal parameters in children. However, the information available on multiple melatonin supplementation dosages is not ideal, as melatonin supplementation may be more effective in some pathologies than others and there may be a lack in long-term clinical trials which evaluate long-term sleep problems in regard to various exogenous melatonin dosages [9].

Nevertheless, tablets of prolonged release of melatonin, at a 2 mg dosage, can imitate melatonin release at a physiological capacity, hence their approval for treatment of chronic insomnia described by poor sleep quality in individuals with 55 or more years of age. In a randomized, double-blind clinical trial, participants aged between 45 and 60 years old were subject to the administration of melatonin supplementation, at a 3 mg dosage, and an improvement in objective sleep disturbances related to primary insomnia, such as a decrease in early awakenings (which could lead to a reduction in total sleep time) and an increase in the percentage of N2 sleep (part of non-rapid eye movement sleep), was noted, but not in subjective efficacy [10].

In a study which included three groups of healthy people and diabetic people with and without diabetic retinopathy (DR) (which can lead to sight loss and blindness), diabetic patients overall presented lower melatonin levels in saliva at night and only those with DR seemed to

have inconsistent circadian rhythms. These disturbances may be caused by neuronal injury presented in the retina (which would lead to a lack of entrainment of SCN to light exposure), SCN and the hypothalamic tract. There were no significant differences in sleep duration or efficiency among the three groups and mean daytime melatonin levels were not significantly different, which leads to the conclusion that exposure to light at night did not lead to variations in melatonin concentration among the three groups. A lower circadian regulation of gene expression in diabetic patients is also reported. On the same note, circadian rhythm irregularities also affect night shift workers. However, individuals under longer periods of night shift work may adapt in a physiological context to night shift work. A study comprising 60 male participants concluded that those who had experienced 7 days of night shift work had increased cognitive performance and sleep quality than those who worked in night shifts for 4 days, as the former group had more structured and steadily increased melatonin production and sleep schedules, in accordance with night shift work. It is inferred melatonin supplementation could aid in circadian rhythm regulation on both conditions – in the case of night shift workers, the intake of melatonin supplementation could aid in sleep quality improvement and night shift work adaptation [11, 12].

Melatonin can ameliorate mild cognitive impairment and circadian disturbances caused by other environmental factors, such as prolonged light exposure, which leads to the disruption of normal circadian rhythms in humans and zebrafish, as well as increased cortisol levels if it reaches 24 hours of duration. In the case of zebrafish exposed to light for 14 hours (followed by 10 hours of darkness), melatonin had no effect in cortisol level variation when compared to untreated zebrafish, which leads to conclude melatonin activity was compromised in zebrafish exposed to light for 24 hours and exogenous melatonin administration was adequate in solving that issue [13].

An increase in cortisol production is a physiological consequence associated with stress and can be used as a biomarker for the study of that condition. In another study regarding zebrafish, melatonin was able to suppress an increase in cortisol levels, reduce locomotor activity and induce sleep states, supporting the recognition of its hypnotic and anti-anxiogenic properties [14].

To address the inconveniences associated with jet lag disorder, the study of a Dome matrix® (a cylinder-shaped tablet) comprised of sustained-release melatonin and delayed-release of caffeine has been developed, with melatonin being used for its hypnotic properties and caffeine for its ability to stimulate the central nervous system and promote wakefulness. Sustained-release melatonin within 8 hours was accomplished when low-viscosity hydroxypropylmethylcellulose (HPMC) was added to pellets prepared beforehand. Delayed-

release caffeine efforts were obtained through an association of high-viscosity HPMC (which acted as a cushioning agent in the early dissolution phase) and high-viscosity ethylcellulose (which exerted its activity in the prolonged dissolution phase) with caffeine-loaded alginate-chitosan particulates [15].

2.2. Roles in inflammatory processes

2.2.1. Roles in cardioprotection

Myocardial ischemia-reperfusion (I/R) is characterized by the damage associated with the replacement of blood supply to heart tissue and may be a consequence of myocardial infarction. The accumulation of radical species leads to cardiomyocyte death, cardiac mitochondrial disturbances, aggravation of myocardial infarct and an increase in mitophagy (removal of damaged mitochondria), resulting in deficient ATP production, excessive autophagy and cell apoptosis. Not only is melatonin proven to promote antioxidant enzyme activity but can also prevent changes to their structure and function in mitochondria. Protection against Krebs' cycle enzyme alterations is also assured by melatonin. Regarding its role against myocardial I/R, there is evidence proving a selective upregulation of MT2, in comparison to MT1, with both melatonin receptor subtypes present in murine myocardium. Moreover, melatonin administration or MT2 overexpression led to a decrease in cardiac troponin I, creatine kinase myocardial band and lactate dehydrogenase (LDH) levels, inhibition of apoptotic nuclei positivity and CASP3, CASP9 and CASP12 activity (with no influence in CASP8 activity), dampenage of Cyt-C and CCAAT/enhancer-binding protein homologous protein (CHOP) expression (the latter being associated with ER stress-related apoptosis) and an improvement in cardiac function parameters. There was also a decrease in tissue nitrotyrosine, 8-hydroxy-2'-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) (which work as indexes for nitrative stress, DNA and lipid oxidation, respectively) and a decrease in the expression of NADPH oxidase (leading to a decline in ROS production) and iNOS. These effects were not evident in MT2-silenced mice, resulting in a suppression of cardioprotective effects associated with melatonin on this group. The initiation of cardioprotective efforts in neonatal cardiomyocytes can also be triggered by Notch1/Hes1/RORα signaling, independently from melatonin activity, as a result of MT2 overexpression. Melatonin is also capable of stabilizing the calcium overload related to cell apoptosis and to myocardial I/R. Melatonin administration reduces the severity and incidence of myocardial infarcts and arrhythmias (possibly due to its ROS scavenging capacity, as ROS can depolarize mitochondria, and the phosphorylation of Cx43, which is a ventricular gap junction-related protein), as well as

achieve a balance in mitochondrial activity; the latter aspect is partially due to the activation of MAPK3/1 pathway. The ability of melatonin to block ROS permeation through mPTPs could help avoid cardiac mitochondrial depolarization and swelling/rupture as well [16 – 19].

Cardioprotection against myocardial I/R granted by melatonin seems to be similarly effective when administered as pretreatment, in the ischemic period or at the onset phase of reperfusion. Melatonin can diminish infarct size through the inhibition of apoptosis and the expression of autophagy-related proteins, such as CASP3 or B-cell lymphoma-associated X protein (Bax). In addition, there is evidence suggesting the potential for melatonin in the improvement of the expression of B-cell lymphoma 2 (Bcl-2), an anti-apoptotic protein [17].

Melatonin can also attenuate excessive mitophagy in myocardial I/R by decreasing beclin-1 and microtubule-associated proteins 1A/1B light chain 3B (LC3) levels (which, would otherwise create and expand phagosomes, respectively) and increasing protein p62 levels (associated with autophagic degradation). In addition, thanks to melatonin activity, mitochondrial fission is also attenuated, highlighted by the decrease of the levels of factors Drp1 and p-Drp1^{ser616}, and mitochondrial fusion is stimulated, evidenced by the increase of the levels of mitofusin-1 (Mfn1), Mfn2 and OPA1 [17].

Myocardial injury can result in the increase of levels of pro-inflammatory cytokines IL-1β, TNF-α and IL-6, as well as a decrease in anti-inflammatory cytokine IL-10. Melatonin is able to reduce levels of these pro-inflammatory cytokines and preserve IL-10 levels, and to bind to CAT and saturate all its binding sites without opening new ones, protecting its structure against foreign agents such as isoproterenol bitartrate, which has the capacity to cause mitochondrial dysfunction *in vivo* [18].

Sirtuins 1 and 3 (SIRT1/3), which are proteins mainly present in mitochondria, as well as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC 1α), also seem to present a dependency for melatonin activity. Through the inhibition of NF-κB and, consequently, of the SIRT1/PGC 1α/SIRT3/SOD2 pathway, the GSH/GSSG ratio will lower in value and excessive mitophagy in anoxia/reoxygenation injury will cease. The absence of SIRT3 expression has also proven to be harmful for the aforementioned melatonin cardioprotection efforts [18, 19].

In the matter of cardiac hypertrophy, which presents itself as the accumulation of dysfunctional mitochondria and misfolded proteins in the context of heart failure, autophagy protein 5 (Atg-5) is essential in autophagic processes, which are regulated by the protein kinase B (Akt)/mechanistic target of rapamycin (mTOR) pathway, among others. By dampening Atg-5-dependent autophagy dysfunction and regulating the Akt/mTOR pathway, melatonin works against pressure overload-induced cardiac hypertrophy [20].

Melatonin plays a role in acute kidney injury, which presents I/R injury as its most common cause. Melatonin administration can ameliorate the implications related to renal I/R injury in patients who underwent kidney transplant surgeries, and improve kidney function, as patients who went through melatonin intake saw a decrease in levels of oxidative stress biomarkers 8-OHdG, MDA and carbonyl protein (the latter being associated with protein oxidation), as well as a decrease in TNF-α [21].

2.2.2. Roles in intestinal inflammation

In the sequence of intestinal I/R, which can lead do multiple organ failure (MOF) (especially the human brain, and with it, a decline in cognitive function), the intestinal flora is dysfunctional and there is a release of pro-inflammatory entities which can compromise normal physiological activity. The release of TNF-α, IL-1β and IL-6 may lead to the production of ROS and the inflammatory response may be elevated. Melatonin administration in rats was successful in dampening the inflammatory response associated with intestinal I/R by inhibiting the toll-like receptor 4 (TLR4)/myeloid differentiation primary response 88 (MYD88) pathway in microglia, which would otherwise aggravate the inflammatory response through downstream NF-κB signaling and ROS release, and frontal cortex and hippocampus-related neuronal apoptosis. The inhibition of TLR4 signaling by melatonin has also proven to be useful in attenuating LPS-mediated duodenal inflammation in chicken [22, 23].

On the other hand, in the case of colitis (inflammation of the colon), there seem to be other factors at play. In a study involving dextran sodium sulfate-provoked colitis in mice, it was suggested melatonin, when associated with MT2 upregulation, could indirectly reduce inflammatory and apoptotic processes, by promoting the phosphorylation of phosphatidylinositol 3-kinase (PI3K)/Akt and activate Nrf2/SIRT1/RORα signalling, which would lead to dampenage of NF-κB signalling [24].

Another aspect favoring exogenous melatonin administration seems to be the maintenance of small intestine vasculature in mice. In a context of mild systemic inflammation, without MOF, melatonin was able to ameliorate LPS-promoted alterations to small intestine microvessels (as it favored the reduction of local mast cell and granulocyte count), although the results obtained were not ideal in the case of hepatic microvasculature. The results of this study favor a role for melatonin production in the GI tract, as its production occurs regardless of exposure to light [25].

2.2.3. Roles in diabetic retinopathy and nephropathy

DR is among one of the usual adversities subsequent to diabetes. As it encompasses the dysfunction of neurons and glial cells, DR can affect the blood-retinal barrier (BRB) in the same regard through Müller cells, which play a role in retinal neuron linkage. Under the context of high glucose concentrations that may be associated with diabetes, Müller cells suffer inflammatory processes and oxidative stress, further advancing the complications related to DR. Melatonin administration has been effective in ameliorating BRB injury through: (1) dampenage of the diminishment of tight junction proteins in mice with DR; (2) suppression of inflammatory activity and angiogenesis, evidenced by the reduction of TNF-α, IL-6, IL-8 and vascular endothelial growth factor (VEGF); (3) dampenage of oxidative stress, evidenced by the decrease in MDA and ROS levels and the increase in CAT and SOD levels. The last two accomplishments may be possible due to the activation of SIRT1 expression caused by melatonin, which can in turn lead to the deacetylation of forkhead box protein 1 (FOXO1) and the NF-κB p65 subunit. SIRT1 expression is also hindered by maternally expressed gene 3 (MEG3)/miRNA-204 signalling, which can be inhibited by melatonin. Another occurrence in support of these consequences may be the inhibition of Wnt/β-catenin signalling caused by melatonin, which would otherwise contribute to angiogenesis and BRB abnormalities through inflammation and autophagy disruptions, under the same context of high glucose levels [26, 27].

Melatonin administration plays a role against the development of diabetic nephropathy (DN). For example, under high glucose levels, melatonin was able to suppress NF-κB activation caused by high glucose levels, as the nuclear translocation of its p65 subunit was inhibited, and regulate inflammatory genes in mesangial cells of diabetic mice for cytokines such as TNF-α. Moreover, melatonin inhibited the expression of transforming growth factor beta 1 (TGF-β1), which is a profibrotic cytokine capable of downstream Smad3 pathway activation and the release of profibrotic factors fibronectin (Fn) and collagen IV (ColIV) present in the extracellular matrix. Melatonin suppressed the expression of p-Smad3, Fn and ColIV. Were it not for melatonin, these pathways could otherwise lead to extracellular matrix build-up and cell proliferation, and the further development of DN inflammation and renal fibrosis in mesangial cells, culminating in the loss of renal function [28].

2.2.4. Roles in pulmonary inflammation caused by environmental factors

ROS-stimulated endogenous melatonin production may play a role in ameliorating inflammatory processes associated with this chronic pathology. The aggravation of asthma can

be triggered by air pollutants, namely ozone and airborne fine particles. In a cohort study comprising asthmatic children, a positive association between urinary MDA and 8-OHdG and urinary aMT6 concentrations was reported, possibly due to a physiological response which led to mitochondrial melatonin production and the alleviation of oxidative stress. Despite evidence that short-term air pollutants exposure did not lead to an increase in urinary aMT6 levels, possibly due to the annulation of endogenous systemic melatonin by generated ROS, accompanied by an increase in melatonin release, there was also a positive association between ozone and airborne fine particles and urinary aMT6 levels after 2 to 5 days of exposure. If the same argument is considered, melatonin production triggered by exposure to air pollutants may take longer to surpass the levels of neutralized endogenous melatonin. The same study also reported greater airway inflammation and lung elasticity with increased endogenous melatonin production [29].

The NLRP3 inflammasome, as well as IL-1β, are related to the development of airway inflammation that characterizes chronic obstructive pulmonary disease (COPD). While melatonin can upregulate SIRT1 expression, which in turn suppresses the NLRP3 inflammasome and IL-1β activity, the activity of SIRT1 is dampened in the context of COPD, and melatonin levels are lower in patients experiencing acute exacerbation of the pathology. Aside from the antioxidant enzyme activity and GSH/GSSG ratio reduction, the ROS derived from cigarette smoke can mediate NLRP3 inflammasome activation, due to an interaction with thioredoxin-interacting protein (TXNIP) in the context of ER stress. ER stress can lead to both ROS build-up and protein folding. A disruption in calcium ions (Ca^{2+}) homeostasis provoked my mPTP dysfunction associated with ER stress can lead to an accumulation of mitochondrial ROS and aggravated NLRP3 inflammasome production. Treatments based on exogenous melatonin play a protective role in rats with COPD, as airway inflammation was alleviated, with a push for total cell count normalization, such as neutrophils and macrophages, as well as a decrease in IL-1β levels in bronchoalveolar lavage fluid. Melatonin activity led to an inhibition of the SIRT1-dependent NLRP3/IL-1β pathway, with an increase in SIRT1 expression in lung tissue. In addition, mice who underwent exogenous melatonin administration saw a Nrf2-mediated upregulation of antioxidant enzymes SOD2 and HO-1, as well as an increase in thioredoxin-1 (TRX-1) levels, which antagonize TXNIP activity and NLRP3 inflammasome activation. Melatonin administration promoted phosphatase and tensin homolog-induced putative kinase 1 (PINK1)/Parkin-mediated mitophagy, aiding in the destruction of damaged mitochondria that resulted from ER stress, which would otherwise lead to NLRP3 inflammasome activation as well [30 – 32].

2.2.5. Roles in female and male reproduction

TXNIP/NLRP3 inflammasome activation under ER stress circumstances, as well as IL-1β production, can apply to endometritis too. Not surprisingly, melatonin was able to inhibit this phenomenon. In the case of LPS-derived endometritis in mice, due to an increase in 5' adenosine monophosphate-activated protein kinase (AMPK) phosphorylation, NF-κB induction was also suppressed and ER stress alleviated in melatonin-treated mice. In the same situation, melatonin also inhibited the phosphorylation-stimulated activation of protein kinase R-like ER kinase (PERK) and inositol-requiring enzyme 1α (IRE-1α). Both these enzymes intervene in the signalling pathway that works towards NLRP3 inflammasome activation under ER stress [32].

Exogenous melatonin has also the capacity to inhibit inflammatory processes associated with benzo(a)pyrene (BaP)-induced ovarian corpus luteum dysfunction, with damage to metabolic enzymes associated with estrogen and progesterone production, oocyte nuclear maturation and promotion of cell apoptosis, partly caused by mitochondrial membrane potential decrease, as Ca^{2+} is consumed by CAT, SOD and GSH-Px. The levels of these antioxidant enzymes are decreased thanks to BaP activity, as melatonin synthesis is inhibited, as well as the expression of MT1 and MT2; and MDA concentration is increased. Inhibition of the PI3K/Akt/GSK3β pathway increased CASP activity and led to a dysregulation of ovarian cell apoptosis, as overexpressed GSK3β leads to an increase in Bax and Bcl-2 expression. Under normal physiological circumstances, the corpus luteum produces estrogen and progesterone in the post-ovulation phase of the menstrual cycle, in a process called luteinization. BaP metabolization by cytochrome P4501A1 and epoxide hydrolase can lead to benzo(a)pyrene-7, 8-dihydrodiol- 9, 10-epoxide (BPDE) synthesis, a carcinogenic metabolite whose detoxification leads to an excess of ROS levels and ovarian granulosa cell apoptosis, compromising ovulation and possibly leading to luteolysis. Moreover, BPDE has been shown to increase Bax/Bcl-2 levels, which led to mitochondrial secretion of Cyt-C in luteinized cells. In the context of luteinization, ovary-produced melatonin is dominant, the suppression of its production could be one of the major causes of corpus luteum dysfunction. Exogenous melatonin administration could be useful in protecting ovaries against corpus against BaP-associated dysfunction, given that it annulled the inhibitory effect of BaP and BDPE in estrogen and progesterone production and in enzyme expression. Under this treatment in mice, oxidative stress and ovarian granule cell apoptosis in vivo and in vitro was dampened, and pathway signalling balance was restored [33].

In roosters, melatonin has successfully decreased hydrogen peroxide-related oxidative stress and apoptosis of Leydig cells, which are the principal synthesizer and secretor of androgens involved in spermatogenesis and reproductive health, capable of producing melatonin. Given that oxidative stress is the main factor for Leydig cell apoptosis, melatonin could be useful through its induction of MT1 and MT2-dependent Akt/Nrf2 signalling. The amelioration of oxidative stress was evidenced by the decrease in ROS, 8-OHdG and MDA levels, and an increase in GSH-Px and SOD activity. In addition, an increase in mitochondrial membrane potential and a decrease in intracellular Ca^{2+} and Cyt-C levels were reported, which leads to the conclusion that suppression of oxidative stress in rooster Leydig cells was possible in a mitochondria-dependent approach [34].

2.3. Role in neurodegenerative diseases

2.3.1. Roles in AD pathophysiological hallmarks

As aforementioned, Aβ accumulation opens a precedent in favour of the development of AD. The accumulation of abnormal Aβ in the brain occurs due to insufficient Aβ degradation (mostly by insulin-degrading enzyme and neprilysin (NEP)) and an imbalance between Aβ synthesis and clearance through the brain-blood barrier, granted by receptor advanced glycation end productions (RAGE) proteins (responsible for Aβ influx to the brain) and low-density lipoprotein receptor-related protein-1 (LRP1) (responsible for Aβ efflux to systemic circulation) under normal physiological conditions. Given that aluminium (Al) accumulation in the hippocampus and cerebral cortex can lead to memory and cognitive dysfunction in mice, and to the development of AD (and PD), it is relevant to consider that when Al chloride-treated mice are subjected to exogenous melatonin administration in an AD model, Aβ homeostasis was promoted with an increase in LRP1 and NEP expression, and a decrease in RAGE expression and Aβ and MDA levels [35].

Aβ build-up can lead to an interruption in mitochondrial genesis of neurons located in the hippocampus, as its accumulation in mitochondria leads to the suppression of mitochondrial DNA replication, oxidative stress and neurodegeneration. SIRT1 increases mitochondrial transcription factor A (TFAM) levels, which are representative of the quality of mitochondrial DNA replication, and both proteins have increased expressions with melatonin supplementation in rats, considering that dorsal hippocampal SIRT1 levels (and, consequently, TFAM levels) were decreased in rats injected with Aβ. Activation of the SIRT1/PGC 1α pathway led to TFAM production and the reestablishment of normal mitochondrial biogenesis, and can prevent mitochondrial fission in the central nervous system. Moreover, Aβ

accumulation-derived ER and oxidative stress can be alleviated by melatonin. In another study, transgenic mice who underwent an exogenous, long-term melatonin treatment saw a diminishment in mitophagic activity in neurons of the hippocampus, evidenced by the decrease in mitophagy-related markers PINK1, Parkin, LC3-I and LC3-II, and an improvement in mitochondrial structure. A decrease in β-secretase 1 (BACE1) and C-terminal fragment of APP (CTFβ) levels was also noted [36, 37].

To address the issue of tau protein aggregation, a study was conducted to assess the potential for melatonin to separate tau protein molecules located in the same aggregate, by disrupting salt bridges between tau protein molecules and by weakening hydrophobic interactions in pre-formed tau protein aggregates, compromising the structure of paired helical filaments. It did, however, require high concentrations of melatonin for disaggregation efforts to be effective, given the weak interaction between melatonin and tau protein molecules. The same study recognized the potential for a combination of melatonin and another molecule for optimal results in this issue. Another *in vitro* study proved inefficacy of melatonin in disassembling full-length tau proteins after exposure to Neuro2A cells, while disaggregating intermediate, toxic, higher order oligomers [38, 39].

Cellular exosomes may also contribute to AD pathophysiology, as they may carry tau proteins, whether they are hyperphosphorylated or in a normal state. Even though melatonin can diminish hyperphosphorylated tau proteins *in vitro* and *in vivo*, a study based on an *in vitro* Aβ toxicity model revealed absence of hyperphosphorylated tau protein in exosomes. While $A\beta_{1-42}$ administration triggered the release of exosomes in a human SH-SYS5 cell line, melatonin pre-treatment (before $A\beta_{1-42}$ administration) reduced the extension of exosome release (without reduction of total tau protein), but not with melatonin post-treatment or when combined with $A\beta_{1-42}$. Nevertheless, melatonin post-treatment was successful in reducing the amount of total tau protein, proving melatonin can reduce both exosome release and total tau protein under specific settings [40].

In the context of an AD model, rats who underwent melatonin pre-treated, brain mesenchymal stem cell (BMSC) transplantation and were subject to Aβ administration thereafter saw improvements in cognitive and memorial performance and in spatial learning. Melatonin pre-treatment in these conditions caused an increase in MT1 and MT2 expression as well. Through MT1/MT2 signalling, Bcl-2, CAT and SOD1 expression was increased, and Bax expression has diminished, proving melatonin plays a role against Aβ accumulation-related apoptotic efforts in *in vitro* BMSCs [41].

Exogenous melatonin administration in the hippocampi of rats can also inhibit methamphetamine-induced changes to the PI3K/Akt/GSK3β pathway in an AD model. GSK3β

activation (caused by a decrease in its phosphorylation) is associated with Aβ synthesis and tau protein hyperphosphorylation. PI3K/Akt activation and, consequently, inhibition of GSK3β activity, was possible thanks to melatonin pre-treatment, which led to a decrease in tau protein hyperphosphorylation. While methamphetamine can increase the expression of $A\beta_{42}$ and AD biomarkers such as BACE1 or presenilin-1 (PS1), the suppression of $A\beta_{42}$-sourced GSK3β by melatonin inhibited their expression. Melatonin administration also prevented abnormalities in hippocampal dopamine transporter protein, *N*-methyl-D-aspartate (NMDA) receptor and Ca2+/calmodulin-dependent protein kinase II (CaMKII) expressions [42].

When combined with melatonin, the neuroprotective effects of resveratrol are improved. The roles of melatonin and resveratrol against AD-like neurodegeneration (caused by Al chloride and D-galactose) in the prefrontal cortex and hippocampus of mice were assessed. Melatonin has improved recognition memory in AD mice, when either administered alone or as a resveratrol adjuvant. Melatonin, resveratrol and melatonin + resveratrol-treated mice saw an improvement in AchE levels, which had been diminished in the present AD model. BDNF expression in the prefrontal cortex was increased with either melatonin or resveratrol administration, and melatonin administration alone increased cAMP-response element binding protein 1 (CREB1) expression. As both BDNF and CREB1 expression was decreased in the prefrontal cortex of AD mice, an increase in BDNF transcription by CREB1 may restore normal memory production and retention [43].

2.3.2. Roles in PD pathophysiological hallmarks

Melatonin can suppress α-synuclein aggregation and promote its disaggregation in neurons. Ubiquitin-mediated proteasomal degradation of phosphorylated α-synuclein at serine 129 in PD cell models is possible with melatonin exposure. The expression of epigenetic polycomb repressor complex-1 subunit BMI-1 in neurons may be regulated by melatonin and can contribute to phosphorylated α-synuclein at serine 129 clearance by activating a non-canonical E3 ubiquitin-ligase function [44].

PD-related neurotoxicity can be caused by homocysteine, which is a compound capable of inducing oxidative stress in the brain and contribute to the development of PD. With previous knowledge that melatonin can suppress plasmatic homocysteine levels, a study in a PD model successfully assessed the role of melatonin post-treatment in reverting homocysteine-induced motor disturbances, dopamine rarefaction (with the decrease in dopamine turnover) and loss of tyrosine hydroxylase-positive dopaminergic neurons (in high melatonin doses) in the striatum, inhibition of respiratory complex-I and oxidative stress in

the substantia nigra of rats. Oxidative stress was ameliorated by melatonin at high doses through CAT and SOD upregulation, increase in GSH levels and ROS scavenging [45].

When it comes to melatonin supplementation in patients with PD, a study encompassing 60 eligible patients suggests an indirect role of exogenous melatonin intake in ameliorating sleep disturbances, which are a symptom related to PD, although a need for further research was recognized. Melatonin supplementation also aided in the elevation of biochemical markers such as GSH, and in TNF-α downregulation, without affecting IL-8 and TGF-β expression [46].

An assessment of the expression of BMAL1 and PER1 clock genes in 28 PD patients confirmed PD-related changes in BMAL1 and PER1 expression. This study recognizes a decrease in PER1 expression with age. While BMAL1 and PER1 suffer transcription in negative feedback-mediated cycles, melatonin supplementation can influence those cycles. For example, patients with melatonin supplementation for 3 months saw a bigger increase in BMAL1 expression in the morning than the placebo group. While the same study presented mixed results in sleep quality, an overall improvement of sleep comfort was reported [47].

3. Synthetic analogs of melatonin

3.1. Agomelatine

Agomelatine (Figure 3) is an MT1/MT2 agonist and a serotonin receptor 5-HT_{2C} antagonist. This synergy may justify its antidepressant activity. Efficacy against bipolar depression and anxiety disorder has been observed in agomelatine. It is also known for circadian rhythm synchronization through its activity in melatonin receptors of SCN. Agomelatine can reduce levels of IL-1β and IL-6 in the brain, as well as the expression of metabotropic glutamatergic receptor genes [51].

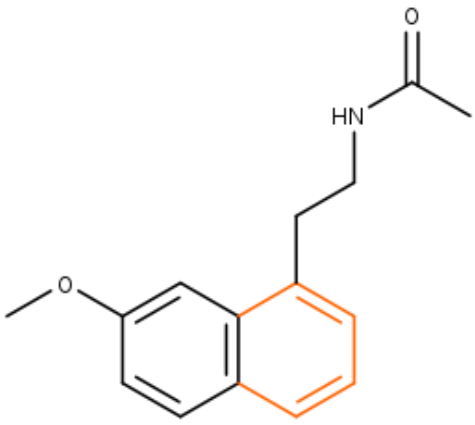

Figure 3 – Chemical structure of agomelatine. The colored aromatic ring of the formula highlights the structural difference when compared with melatonin, which presents an indole group instead.

An increase in Nrf2/HO-1 signalling in an MT1/MT2-independent manner in rats is indicative of neuroprotective efforts of agomelatine under the context of cerebral I/R injury. Consequently, Nrf2 and antioxidant enzymes HO-1 and NAD(P)H: quinone oxidoreductase 1 (NQO1) upregulation occurred, SOD activity was amplified and MDA levels were diminished, reducing the risk of neuronal apoptosis [48].

Furthermore, agomelatine administration prevented LPS-triggered neuronal damage and apoptosis in rats by NF-κB suppression and increase of SIRT1 levels. Oxidative stress and inflammation were attenuated with SIRT1 upregulation, as well as of peroxisome proliferator-activated receptor gamma (PPARγ), as a consequence of the inhibition of TLR-4/MAPK/NF-κB signalling by agomelatine, in the context of gentamicin-induced nephrotoxicity in rats as well [49, 50].

A study comprising chronic unpredictable mild stress-induced rats revealed that an agomelatine-based treatment can increase hippocampal BDNF levels, preserve hippocampal morphology and ameliorate model-related, inflicted behavioral changes [51].

Under the context of evidenced depressive-like behavior in AD patients, a study in an AD rat model was conducted to investigate a possible connection. Indeed, the administration of $A\beta_{1\text{-}42}$ led to the development of depressive and anxiety-like behavior, and agomelatine successfully ameliorated induced $A\beta_{1\text{-}42}$ build-up in the frontal cortex and hippocampus of rats, exerting specific neuroprotection primarily through an inhibition of γ-secretase levels, in both early and late stages of AD [52].

Lastly, agomelatine aids in post-fracture bone healing in rats at 21 days, with increase in gene expression of bone creation markers such as bone alkaline phosphatase and osteocalcin, despite the assessment of osteopontin expression being inconclusive. [53].

3.2. Ramelteon

Ramelteon is a selective MT1/MT2 agonist that acts on the SCN with higher affinity for these receptors (six-fold and three-fold, respectively) and increased lipophilicity compared to melatonin. It is known for improving sleep quality and circadian rhythmicity in patients with insomnia [54 – 56].

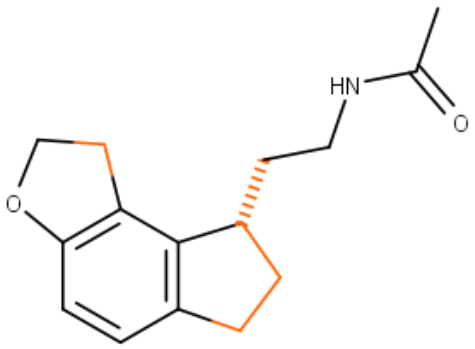

Figure 4 – Chemical structure of ramelteon. Highlighted are changes in structure compared to melatonin.

Similarly to agomelatine, in the same study, ramelteon achieved identical results in post-fracture bone healing, with increase in bone alkaline phosphatase and osteocalcin in rats [53].

Prophylactic ramelteon administration has been recently associated with a reduction in frequency and duration of delirium manifestations in intensive care units, in a triple-blinded, randomized placebo-controlled trial. A possible explanation for these results may reside in a decrease in nightly awakenings. The addition of suvorexant (a selective dual orexin receptor antagonist) to ramelteon in treatment of post-acute stroke patients has also shown efficacy in improving sleep, with lower delirium incidence [54, 55].

Ramelteon administration as an adjuvant for antipsychotics could improve sleep quality and the symptoms of patients with schizophrenia, with the increase of serum and melatonin levels, which would otherwise be dampened at night in these patients. An increase in SNAT levels was also reported [56].

3.3. Other synthetic melatonin analogs

In order to explore the potential for melatonin analogs to exert antidepressant and antioxidant activity with modifications on the *N*-acetyl-2-aminoethyl chain in C3, the activity of three C3-modified analogs with hydrazine features (Figure 5), which are known for their antidepressant activity (among others), as well as of melatonin, was assessed [57].

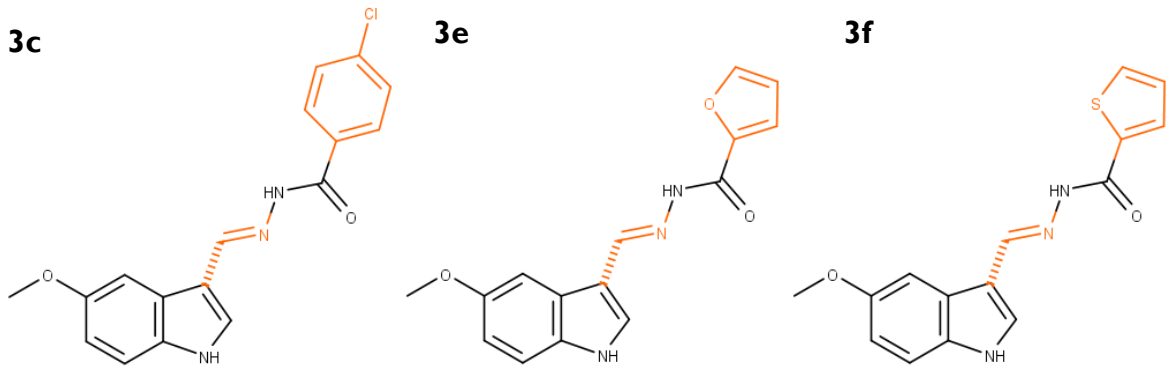

Figure 5 – Structural formulae of synthetic melatonin analogs 3c, 3e and 3f. Aside from hydrazine derivation, each analog presents 2-chlorophenyl, 2-furyl and 2-thienyl groups in the C3-modified chain, respectively [57].

None of the analogs or melatonin presented anxiolytic activity, but 3c, 3e and 3f presented antidepressant-like effects, possibly through monoamine oxidase (MAO) inhibition- 3c and 3f also presented analgesic activity. While melatonin increased SOD and GPx activity in both the frontal cortex and the hippocampus of mice, the three analogs were incapable of doing so in the hippocampus, and only 3c and 3f increased SOD activity. However, 3f also increased MDA levels in the frontal cortex, contributing to pro-oxidant effects [57].

In another study with colon and gastric cancer cell models, the compound P-3 (Figure 6) presented greater anti-proliferative efforts *in vitro* compared to 14 other studied compounds [58].

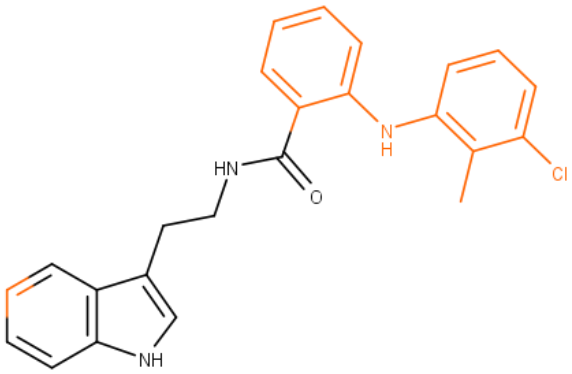

Figure 6 – Structural formula of *N*-salicyloyl derivative P-3. Highlighted are the structural features that differ from melatonin.

P-3 also inhibited signal transducers and activators of transcription 3 (STAT3) expression and activation and the release of IL-6 and TNF-α *in vitro*, which makes it a candidate for further research on its anti-inflammatory and anti-carcinogenic potential [58].

Research that suggests MT1 and MT2 are G protein-coupled receptors able to present homodimers, oligomers and heterodimers (and the possibility of them having two selective binding sites) motivated the study of bivalent ligands comprised of *O*-linked dimers which would ideally target these receptors, and how those receptors would function under dimerization. Compounds 3c and 7 (Figure 7) had the most promising results *in vitro* [59].

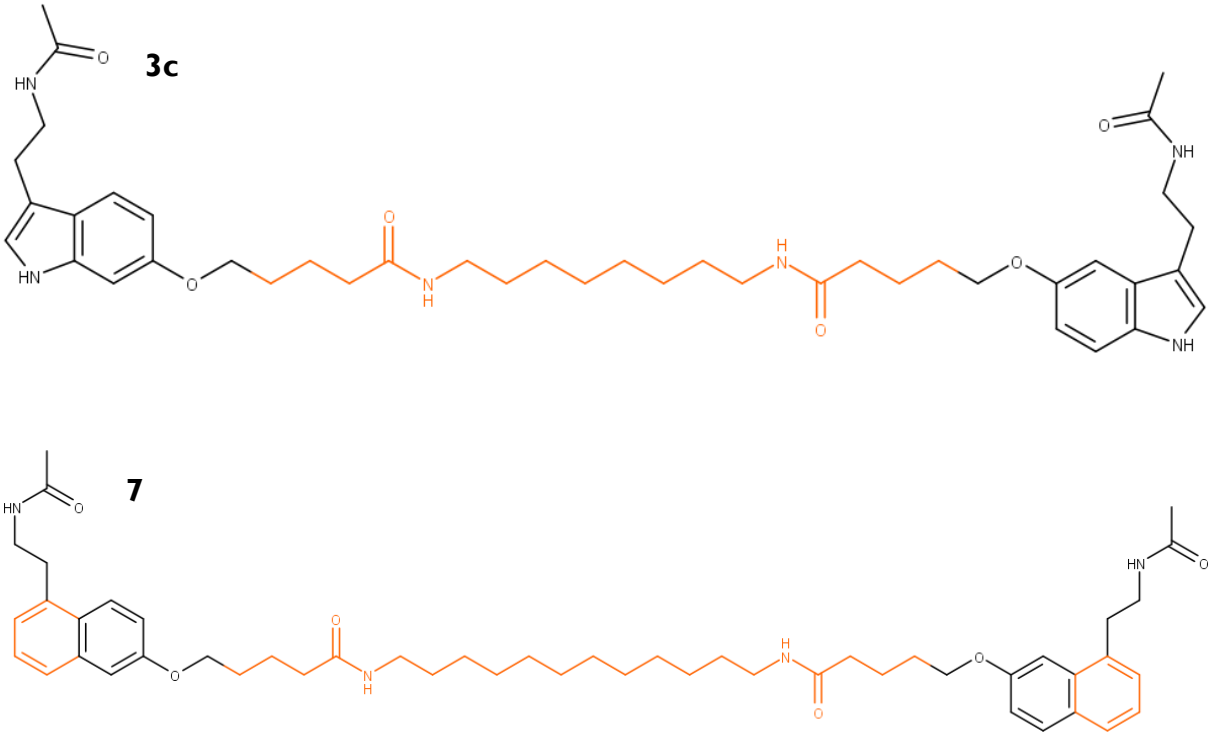

Figure 7 – Structural formulae of compounds 3c and 7. Highlighted are the structural differences compared to melatonin.

However, the same study recognized that the dual binding of both pharmacophores and increased binding activity are not mutually exclusive. The alkyl chain between two pharmacophores may also exert additional effects other than setting the distance between them, such as conformational alterations between two protomers [59].

Bioluminescence resonance energy transfer (BRET) is associated with receptor conformation, and its signal varies with the location of both donating and accepting entities. 3c, with its two protomers, caused the greatest BRET alterations regarding MT1 homodimers, and the compound was also capable of occupying both binding sites of MT2 homodimers as well. BRET changes were also associated with the transition of 3c from MT1 agonist to MT2 inverse agonist. Compound 7 also worked as a promoter of cAMP synthesis [59].

4. Final remarks

Melatonin is a pleotropic hormone that can act in several pathophysiological hallmarks in the human body. Currently, its purpose for approved clinical use resides mostly in its hypnotic properties, as it is commonly known as the "sleep hormone", but current research is more directed to its role against inflammation hallmarks, as they are linked to most human pathologies.

The potential for melatonin in a clinical context for treatment of inflammation-related pathologies and NDDs is yet to be fully understood, as the shift of research efforts to those directions is still premature. Nevertheless, the present review revealed that melatonin, whether in an endogenous capacity or as a supplement, may exert activity through the same signalling pathways, but those may apply to different pathologies.

It is also established in the present review that melatonin can present efficacy in both melatonin receptor-independent and dependent fashion.

The current research in melatonin synthetic analogs may also reside in attributing different clinical uses for current analogs (in the case of agomelatine) and in the validation of new ligands for fairly understood pharmacological targets.

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