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Melatonin facts: Lack of evidence that melatonin is a radical scavenger in living systems

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Abstract : Melatonin is a small natural compound, so called a neuro-hormone that is synthesized mainly in pineal gland in animals. Its main role is to master the clock of the body, under the surveillance of light. In other words, it transfers the information concerning night and day to the peripheral organs which, without it, could not “know” which part of the circadian rhythm the body is in. Besides its main circadian and circannual rhythms mastering, melatonin is reported to be a radical scavenger and/or an antioxidant. Because radical scavengers are chemical species able to neutralize highly reactive and toxic species such as reactive oxygen species, one would like to transfer this property to living system, despite impossibilities already largely reported in the literature. In the present commentary, we refresh the memory of the readers with this notion of radical scavenger, and review the possible evidence that melatonin could be an *in vivo* radical scavenger, while we only marginally discuss here the fact that melatonin is a molecular antioxidant, a feature that merits a review on its own. We conclude four things: (i) the evidence that melatonin is a scavenger in acellular systems is overwhelming and could not be doubted; (ii) the transposition of this property in living (animal) systems is (a) theoretically impossible and (b) not proven in any system reported in the literature where most of the time, the delay of the action of melatonin is over several hours, thus signing a probable induction of cellular enzymatic antioxidant defenses; (iii) this last fact needs a confirmation through the discovery of a nuclear factor – a key relay in induction processes – that binds melatonin and is activated by it and (iv) we also gather the very important description of the radical scavenging capacity of melatonin in acellular systems that is now proven and shared by many other double bond-bearing molecules. We finally discussed briefly on the reason -scientific or else – that led this description, and the consequences of this claim, in research, in physiology, in pathology, but most disturbingly in therapeutics where a vast amount of money, hope, and patient *bien-être* are at stake.

1 Introduction

As a foreword, one should be aware that the very existence of radical scavengers usable in living system(s) has been criticized for decades by many authors, including HJ Forman¹⁻³. The curious reader will be encouraged to read those reviews up front.

The core of the present discussion lies in the claimed capacity of melatonin to (i) scavenge all sorts of radicals (see below) as it has been proven in many instances in acellular systems (see below) and (ii) that the same capacity can happen in living system, which has been wished, hypothesized but has not been proven and is denigrated by the theory, and is not issued from experimental data (see below).

Melatonin is an indole-based, tryptophan-derived neurohormone produced essentially in the pineal gland⁴. It is mainly produced during the night and barely detectable in blood during the day. Melatonin biosynthesis is well described and is mainly regulated by an enzyme, the so-called timezyme⁵. Melatonin is a relay between circadian rhythm and peripheral organs, a compound somewhat mastering the circadian rhythm process⁶. This has been proven beyond any doubts. It is also responsible for the mastering of the circannual rhythm that direct the reproduction cycles in mammals⁷⁻⁹. Melatonin exerts its main effects through the activation of two G-protein coupled receptors: MT₁ and MT₂, the pharmacology of which has been extensively studied during the last decades^{10,11}.

Beside this fundamental, extremely well documented, domain exists another avenue of research and discoveries that treats melatonin like an ultimate adjuvant or drug that has active healing properties towards almost all the main human diseases, a feature that requires far more in-depth experimentations and discoveries of the implicated mechanisms of action¹². These properties are exerted at pharmacological concentrations and not melatonin receptors mediated.

One can trace back the so-called wonderful melatonin properties to the report that initially¹³ described the radical scavenging capacity of melatonin in many acellular systems and the immediately following hypothesis emitted by the same group¹⁴ that the same thing occurs in living systems. Despite the lack of strong evidences that this observation in a test tube could be extrapolated to living system, and then to almost any disease involving oxidative stress, the same group published about 500 papers on the matter, loosely based on the hypothesis¹⁴ that melatonin is a scavenger *in vivo*. We already scrutinized these claims and criticized the lack of solid

scientific basis^{12,15}, as we also tried to elaborate on an alternative hypothesis¹⁶. One of the key points of those claims is the common belief that melatonin has no toxicity. A feature largely controverted for at least 30 years¹⁷ and for which continuous interrogations are published¹⁸⁻³¹, most of which were consistently ignored or disregarded. Even more preoccupying, sentence such as *“Therefore, in view of the lack of toxicity of melatonin, the administration of pharmacological doses of this compound may significantly improve its efficiency as an antioxidant agent.”*³² Incidentally, some have shown that such antioxidant capacity of melatonin does not correspond to the experimental evaluation, for example, of the total antioxidant activity of blood³³. These sorts of claims are often completely ignored, and unfortunately extremely poorly cited. Similarly, *“it should be noted that the results of simple in vitro model experiments cannot be directly extrapolated to in vivo systems”*³⁴, again an advice largely under regarded in the literature.

In the present commentary, we want to stress the lack of evidence that melatonin can be established as an *in vivo* radical scavenger.

1.1 What is a scavenger ?

From a chemical point of view, a scavenger refer to any chemicals that is added to a reaction mixture to remove or abolish the reactivity of a specific molecule or class of molecules. The purpose of a scavenger is numerous. It could be to allow a reaction to process without interference from a side product such as for a peptide coupling with acid chloride moiety. The first few reactions will produce chlorohydric acid that would react with the free amine moiety, thus resulting in a salt that will most likely precipitate or become unreactive. To avoid such phenomenon, hydrogen-less amine can be used in excess, like triethylamine, to scavenge the HCl molecules produced. A more modern approach can rely on the use of ionic liquid formation to scavenge the inorganic acid as a biphasic liquid³⁵.

Relying on high avidity of certain moieties to react, some scavenger strategies use an insoluble polymer (a resin) coupled with a reactive entity to scavenge specific reagent that could thus be used in large excess. For example, an aldehyde moiety can be couple onto a resin to scavenge excess of reactive amine³⁶. Metal catalysis is also a major strategy in organic and medicinal chemistry, but most of metal ions are toxics and must be removed. Scavenger coupled to resin have been developed, relying on thiol moiety or on EDTA moiety to clear out the metals from a crude reaction^{37,38}. One last example is the bio-orthogonal click chemistry, with an alkyne moiety reacting with an azide to form a triazole link between two compounds. As one of the component is often in excess, resin coupled with the other components can easily be used to scavenge it³⁹. Scavenger resins are most valuable when considering continuous-flow chemistry, when the reaction mixture can process through a column with solid-phase scavenging resin⁴⁰.

One of the main chemical applications for scavengers are heterogeneous photocatalysis, to control the nature of the generated radicals and thus control the pathway of degradation. In this case, scavengers used are hole scavenger, such as formate or oxalate, alcohols for hydroxide radical, or p-benzoquinone for superoxide ⁴¹. The issue of most of the described radical scavengers are a lack of selectivity and the generation of new radical species that can also readily react ⁴².

In these applications, melatonin would not be of use, as the goal is to photodegrade organic compounds. But from chemical acellular assays, most indolic compounds – like many other organic ones - demonstrate hydroxide radical scavenging properties while only melatonin was starting to react with superoxide, albeit poorly ⁴³.

Crossing from chemistry to biology, scavengers refer more specifically to molecules scavenging oxygen, or nitrogen or sulfur-centered radicals. Such scavengers are supposed to react readily and specifically with radicals to form stable species and thus abolish the side effects of the reactive radicals ⁴¹. Efficiency of such scavengers can be followed directly by electron paramagnetic resonance (EPR) spectroscopy, with the help of spin trapping compounds, such as DMPO or TEMP ⁴¹. Other methods rely on direct UV or colorimetric measurement of reactive species with radicals.

1.2 What it means

Because a radical scavenger is chemically modified by the radical that it traps, it is transformed in a new entity. Thus, the scavenger is consumed during the process and give rise to a new compound, distinguishable from the scavenger itself, for example by mass spectrometry.

This means that a given compound, used, presumably, as a scavenger in a system, for example, in cells, will generate the production of its “metabolite” that can be actually easily measured and quantified. Thus, whenever a compound is claimed to be a radical scavenger, one can validate this idea by experimentally proving that the compound is transformed as a function of the oxidative stress or of the generation of reactive oxygen species (ROS) or reactive nitrogen or sulfur species . Whenever possible, a simple measure of the claim can validate the scavenging capacity of the compound. But again, in cell systems, the scavenging capacity is shared by literally thousands of compounds already present in the cells, leading to a competition to the added molecule – the so-called “scavenger” – that cannot really exercise its property in this context (see below).

At least three examples do exist of such possible markers of melatonin scavenging property, Dellegar et al showed that by reacting with hypochlorous acid (ClOH), melatonin led to the formation of 2-hydroxymelatonin⁴⁴. On another study, Tan et al⁴⁵ showed that the attack of melatonin by singlet molecular oxygen led to the formation of a remarkable metabolite they called cyclic 3-hydroxymelatonin, structurally profoundly different from melatonin and that can be

“easily” measured *in vivo*, although a minor metabolite in the large number of melatonin metabolites ⁴⁶. Zhang et al suggested that in case of the peroxynitrite/melatonin reaction, the main by-product would be hydroxypyrrolo[2,3-b]indole compounds ⁴⁷.

1.3 Scavengers in living system: the limits

As clearly described and reviewed by HJ Forman ¹⁻³, scavengers do not exist in living system. Indeed, in such system, the presence of literally millions of endogenous molecules capable to trap the ROS and other radical species renders the activity of “scavengers” almost impossible to describe in those systems. ROS are reacting mostly with double bonds in any molecule, also with reducers, also with antioxidant enzymatic systems. ROS do not have an affinity for particular double bonds, thus, it is not a ratio of ‘affinity’ that can render a scavenger competitive in such complex milieu, but the amount of double bonds offered to the ROS to attract and neutralize them. This means that the “scavenger” in living systems will have to be more numerous than endogenous double bonds. Stating some simple numbers, such as the concentration of proteins in a cell (about 120 mg/mL) and remembering that each mer of a protein (that is each amide bond contains one double bond, to which the nature of the amino acid itself, add from one more to 5 more (histidine, tryptophan) to which one has to add nucleic acids (three billions of base per cell, in which the number of double bonds is at least 3 per base for DNA, plus an unknown number of mRNA molecules) and lipids, small endogenous molecules, such as ATP, NADH etc.

2 The Melatonin case

2.1 The origin

In an initial paper, Tan et al ¹³*(Note that this paper was not published in the Endocrinol J of the Japanese Society of Endocrinology, but was published in the first year of the Journal "Endocrine". This paper is not available from the publisher's site (Springer) nor it is indexed in PubMed)* measured the capacity of melatonin to scavenge ROS which was immediately followed up by another paper from the same group ¹⁴. This paper is important because it marks the start of a misunderstanding on the action of melatonin. The original paper shows very clearly that adding melatonin to a ROS-generating system, the ROS was trapped by the molecule, and thus ROS production is not impaired but their harmful actions are limited. The initial observation has been nicely complementarily documented by others, showing beyond doubts that melatonin is able to limit ROS action in acellular systems [see the following section 2.2]. But the key points of all these observations are (i) this always has been observed in an acellular systems and (ii) many molecules share this property, as long as they have double bonds, or conjugated bond systems, or reducing functions, or are electron donors, such as natural products like resveratrol ⁴⁸, flavonoids

⁴⁹ or synthetic compounds such as those bearing SH moiety ⁵⁰. Although for resveratrol at least, the picture is even more complex – if possible – due to the fact that resveratrol might have a completely different mechanism of action in this context, as it is a good inhibitor of NQO2 ⁵¹, an enzyme generating ROS as by-product of its catalytic activity ^{52,53}. This example is yet an extra source of confusion, the compound being an inhibitor of the production of ROS, its activity, including in cellular system(s) ⁵⁴, may be taken for a scavenger activity, while it is not.

The case of melatonin is even more confusing, as (i) its activity in an acellular system has been immediately suggested [as a working hypothesis ¹⁴] to be applicable *in cellulo* and *in vivo* and (ii) melatonin might have other capacities such as the inhibition of NQO2 ⁵⁵ with which it has been co-crystallized ⁵⁶, and of course (iii) melatonin is an effector of an antioxidant protein complex, as it is an inducer of antioxidant defenses although through a mechanism of action that still needs to be discovered and validated.

2.2 The working hypothesis.

As it happens in this domain, ideas are proposed through reviews and because of the way they are suggested with great details not always with experimental evidence – or only very partial ones – they are immediately adopted and become a pillar of melatonin knowledge.

2.3 Melatonin is an inhibitor of NQO2 ⁵⁵.

This has been independently confirmed in several laboratories ^{56,57}. The question is then, what is the meaning of this fact? Indeed, melatonin has an IC50 towards hNQO2 in the 200 µM range. Thus, to be able to do something at the level of this cytosolic enzyme, melatonin has to reach and penetrate the cells at an even higher concentration, a feature that might have drastic consequence in cells.

2.4 Is melatonin an inducer of antioxidant enzymatic defenses?

In order to be an inducer of antioxidant defenses which are most of the time, enzymes, melatonin as any compound need to hit and activate a so-called nuclear receptor that will in turn starts the protein neo-synthetic machinery [see Jacob & Monod ⁵⁸ for general presentation]. Alternatively, the molecule can act directly onto the gene expression regulating region of the DNA by direct binding and activation, or, another possibility, the compound can stabilize the mRNA and sustain for a longer period of time the expression of the protein. Of course, it could be a combination of these systems, and/or involving other systems that we do not know, yet. This said, the demonstration of this must be experimental. And, to the best of our knowledge such demonstrations do not exist, or were not reported in the Literature, remaining working hypotheses. A simple way to separate the inductive capacity to a scavenging one is to look at very short times

after treatment. The induction process takes several hours to be efficient, scavengers would be immediately effective (including if their source is inhibited by melatonin).

2.5 The radical scavenging properties of melatonin.

In this section, we'd like to review most of the studies that established the radical scavenging nature of melatonin.

To start, one has to know that querying PubMed (2023/08/12) by the words "melatonin" & "scavenger" retrieves 1777 publications, 425 of which are reviews. Because Science is not a democratic science – that is even if a majority of people claim something, this claim could be untrue - but an experimental science: as long as the fact has not been established at least by independent experiments, this fact is questionable.

It is almost impossible to survey 1352 experimental papers. So we chose to check the initial ones: from the Tan et al initial one¹³ until a decade later. Among them, we eliminated all the studies carried on living system(s) whether cellular or living animals, leading to about one hundred studies left, all point out at the capacity of melatonin to scavenge in acellular systems a wide variety of radicals.

The first independent experiment made after Tan report is from Pieri et al⁵⁹ and later reinforced by a second report from the same group⁶⁰. They used a fluorescent assay to measure the capacity of antioxidant molecules to scavenge peroxide radicals, generated from the 2-2'-azo-bis(2-amidinopropane) dihydrochloride (AAPH). They found melatonin to be better than any other antioxidant reference molecule to scavenge this radical, even at low concentrations (from 0.5 to 4 μM). Of note, the generating system, AAPH, was also used at a similar concentration, 1 μM .

Scaiano further dissected the reaction of indoles, including melatonin with various types of oxidants, such as *tert*-butoxyl or cumyloxyl radicals, using absorption monitoring⁶¹. These experiments permitted also to determine their respective rate constants. Other reports have been published on the search of other synthetic indole derivatives and their scavenging capacities⁶², see also review by Poeggeler et al⁶³. A common point of these studies: they were performed in acellular systems.

Other reports from acellular systems⁶⁴ determined the various constants of melatonin when it quenches radicals generated from various types of chemicals. Incidentally, in the conclusion of their work, these authors stated "*It seems unlikely, therefore, that melatonin's cytoprotective effects in vivo are mediated by direct radical scavenging activity*", despite numerous claims repeatedly done by the same group⁶⁵⁻⁶⁹.

Looking also into the lipid peroxidation systems in liposomes and the inhibiting effect of melatonin (1 μM and below), Longoni et al⁷⁰ stated that "*These results show that melatonin*

inhibits lipid damage induced by oxygen free radicals. However, melatonin is only about one one-hundredth as effective an antioxidant as vitamin E in the micelles system”, a statement that contradicts some previous reported data. Similar data were later obtained by Pahkla et al⁷¹ and Teixeira et al⁷². Indeed, lipids and membranes have been often used as models for the antioxidant activity of compounds, including melatonin^{73–77}, mostly using artificial membranes. Based on those results, extrapolations to living systems were reviewed enthusiastically⁷⁸, despite warnings that findings in artificial systems cannot be extrapolated to actual membranes from living organisms³⁴: “(..) *the antioxidant capacity of melatonin, one of the important physiological lipophilic antioxidants, in solution of pure triglycerides enriched in omega-3 polyunsaturated fatty acids is considerably different from that in subcellular membranes*”.

The potency of melatonin was also evaluated in quenching the hydroxyl radical, $\cdot\text{OH}$, generated radiolytically^{79,80} as well as radicals issued from various chemical reactions such as $\text{OH}\cdot$, $\text{tert-BuO}\cdot$, $\text{CCl}_3\text{OO}\cdot$, $\text{Br}_2\cdot$, $\text{N}_3\cdot$ ⁸¹ or phenylglyoxylic ketyl radicals⁸². Melatonin also scavenges NO^{\cdot} ⁸³ and peroxynitrite-generated radicals^{84,85}. Other compounds able to generate radicals were also studied, such as alloxan and their ROS production was also inhibited by 1 mM of melatonin⁸⁶. In their acellular systems, melatonin quenched those radical with efficient rate constants. Khaldy et al⁸⁷ added to this series of radicals those issued from dopamine auto-oxidation, a step extremely important in our understanding of neurodegenerative diseases. Similar results were already reported in an acellular system⁸⁸. A computational study of melatonin scavenging capacity, with the various steps of the chemical reactions involved in this scavenging activity has been published by Turjanski et al³².

The central question according to which melatonin is “only” a representative molecule of the indole family with scavenging properties was address by Goda et al⁸⁹. In this publication, they measured the capacity of several molecules to quench phenoxy radicals by determining their respective rate constant and concluded that melatonin was the least potent in this assay among a dozen of indole-based molecules. The same question was answered by Wolfler et al: *N*-acetylserotonin is a better antioxidant than melatonin⁹⁰, although the statement was global and did not address only their respective scavenging capacities. In another study, Daniels et al compared melatonin and serotonin and found the former a better candidate than the later⁹¹. Gulcin et al shows that in an acellular system, ~0.25 mM (20 - 60 mg/L) melatonin chelates iron ions and scavenges H_2O_2 ⁹², confirming previously obtained data⁹³. Fowler et al insisted to the fact that without metal contaminations, H_2O_2 cannot be scavenged by melatonin⁹⁴, contrarily to previous claims^{95,96}, including Gulcin et al work⁹². Data were also confirmed using xanthine oxidase-mediated production of H_2O_2 , melatonin did not inhibit the oxidation, indirectly demonstrated that even at 2 mM, melatonin is not an inhibitor of this enzyme⁹⁷, in contrast of an report where an IC_{50} of 1 mM was described⁷².

Indeed, Kladna et al, reported that there was an increase generation of $^1\text{O}_2$ from $\text{Cu}/\text{H}_2\text{O}_2$ in the presence of melatonin using a series of different measurement techniques ⁹⁸. Interestingly, they dissected the mechanisms by which melatonin leads to excited N^1 -acetyl- N^2 -formyl-5-methoxykynuramine (AFMK^{*}) that in turn is stabilized in AMFK by O_2 , leading to the emission of $^1\text{O}_2$, an electronically excited state of oxygen which spontaneously decays while emitting a photon of red light. Similar observations were later done by Zang et al ⁹⁶. This metabolic route were described by Tan et al ^{99,100}, even suggesting that this molecule was the main agent of the scavenging capacity of melatonin.

In another study, Tan et al demonstrated the capacity of melatonin and of other antioxidant molecules to scavenge ABTS^+ , issued from the reaction of ABTS with potassium persulfate. These experiments were performed in acellular system, and demonstrated the peculiar profile of melatonin, a molecule surprisingly superior to most of the reference ones, such as trolox, vitamin C and glutathione¹⁰¹. Other indole-derived compounds found in plants were also studied on the same acellular model¹⁰², although all the compounds were roughly as antioxidant than trolox, in this study, melatonin itself was surprisingly not tested by these authors.

Melatonin was also tested with regards of the protein attack by ROS. For example, using LDL as a target of the radical peroxidation, it was found that melatonin decreased the yields of lipid peroxidation products, as well as the propagation phase for conjugated dienes ¹⁰³. The effect was maximal at the highest concentration tested, 250 μM , but did not protect endogenous α -tocopherol from peroxidation, probably due to a lower scavenging capacity than this product¹⁰³. This study complemented previously published observations on LDL^{104,105}. Another example is from Salvi et al ¹⁰⁶, in which the authors, using 3 model proteins, looked at their structural modifications following the production of radicals, issued from the decomposition of 2,29-azobis(2-amidinopropane). They confirm the capacity of melatonin to impair at least partially the deformations following the oxidation process and the lack of it in the presence of melatonin, with an IC_{50} in the 150 μM range.¹⁰⁶.

2.6 From acellular to cellular living systems

In an attempt to fill the gap between acellular experiments and the reality of living systems, Tan et al ¹⁰⁷ reported the effect of melatonin, injected (0.6 mg/Kg, i.p.) to rats after a safrole treatment (100 mg/Kg, i.p.), and found that melatonin diminished significantly the DNA-adducts classically recorded in safrole-induced carcinogenesis. Although presented as evidence for the scavenger capacity of melatonin, the number of bias in such experiments is so important that the only conclusion should not have been on the mechanism of action of melatonin, but on the fact itself. Of note, in this paper, the authors found a regular amount of melatonin in control rats (not injected

of ca. 30 pg/mL) while they found the stratospheric concentration of 13 ng/mL after the i.p. injection. This might be due to the radioimmunoassay, based on anti-melatonin antibodies the use of which led to several conflicting results over the years, as review by Kennaway¹⁰⁸. Incidentally, these conflicting results led to endless conflicts over the years on actual results. The same group doubled down in a similar set of experiments but using acellular systems. In this study, they compared two things: (i) one the melatonin ability to scavenge DNA adducts with five reference molecules doing the same (xanthurenic acid, resveratrol, epigallocatechin-3-gallate, vitamin C, and lipoic acid) and (ii) the capacity of melatonin to synergize the actions of the same five molecules. Oxidation process was obtain by treating 0.5 mM Cr(III) with 0.5 mM H₂O₂, and measuring the effect on purified calf thymus DNA. Not surprisingly, they found melatonin to be superior to any reference compounds, with an IC₅₀ in the 3 µM range while 1 µM melatonin did synergize the effect of vitamin C (0.5 µM) and lipoic acid (0.5 µM), and had a more complex effect on the actions of the 3 other compounds¹⁰².

In the next paper, from the same group, another model was used. This time the BSO-induced cataract formation in lenses of new-born rats. Similarly, to the previous observation, the mechanism(s) involved in the appearance of the conditions are so numerous, that it could be hard to conclude that it was due to the merit of melatonin to be a scavenger. BSO is a compound that leads to the complete fall of GSH concentration *in vivo*, a feature largely described before and confirmed here. This depletion in glutathione was moderately (6 % *versus* 3% of the control rats) impaired by the melatonin treatment¹⁰⁹.

2.7 The perpetual quantitative question

Indeed, for several years, two schools exist on the use of melatonin *in vivo*, including in humans. Those claiming that melatonin is a safe compound, and thus can be administered to humans – including to new-born infants and children – to stratospheric dosages, without alert(s) or apparent toxicities and those thinking that melatonin safety has not been thoroughly studied, and this is not because some individuals take systematically 25 mg per day orally for years that the compound – like any other ones – could not have toxic side(s). Besides, based on the feeling that melatonin is safe at high dosages, some also claim that millimolar concentrations of melatonin can be reached locally at the level of some organs, leading to the miraculous capacity(ies) of melatonin to heal any if not all diseases (see lists in Boutin et al¹²). Nevertheless, it has been shown that melatonin could be effective as an antioxidant at concentrations 10000- to 100000-fold higher than physiologic concentrations¹⁰⁴, which questioned – as for any other compounds – the capacity of the body to handle such dosages of any given molecule.

However, since the predicted equilibrium constant for melatonin conversion to the indolyl neutral radical is very high (of the order of 10¹⁴), each molecule of melatonin could be able to scavenge

one $^{\circ}\text{OH}$ radical. Thus, it can be assumed that melatonin, even at physiological levels, would protect the cell, but as it is irreversibly oxidized, the magnitude of that protection is limited by its concentration. Therefore, in view of the lack of toxicity of melatonin, the administration of pharmacological doses of this compound may significantly improve its efficiency as an antioxidant agent.³²

Another key paper came from Giusti et al.¹¹⁰. In this experimental work, the authors clearly show that the protection exerted by melatonin onto a cellular model of excitotoxic after exposure of *primo* cultures of rat cerebellar granule neurons to glutamate. They clearly show that the effect of melatonin was not immediate – as it should be if it was a scavenger – but if melatonin was let to act for several hours. This points out, as many times afterwards^{111,112}, to an inducing effect of melatonin towards the enzymatic defenses of the cells¹¹⁰.

In a completely acellular system, Papolla et al. made a crucial observation: melatonin binds to amyloid beta protein and 5 mM of melatonin – or the *N*-t-butyl- α -phenylnitrone (PBN) another structurally unrelated scavenger - inhibits the progressive formation of beta-sheets and amyloid fibrils as evaluated by NMR¹¹³. Another previous study by the same group¹¹⁴, demonstrated that melatonin, 50 μM , was able to protect several types of neuronal cells from amyloid injury. The authors suggested that this feature was due to the scavenger capacity of melatonin, despite the fact that another well-described scavenger, PBN, was unable to produce the same effect. Since these observations, several hundred reports considered the relationship between melatonin and β -amyloid, in several more or less complex contexts. Similar observations were later made using indole-3-propionic acid¹¹⁵, a compound structurally related to melatonin, but not claimed to possess scavenging-like capacities. The molecular aspects of the melatonin/amyloid interaction has been thoroughly studied by other groups using several biophysical investigations^{116–118}.

Indeed, other groups repeated these observations, particularly Clapp-Lilly et al.¹¹⁹ whom completely characterized the way melatonin counteracts the amyloid toxicity, but also showing that at high concentration, 1 mM, melatonin induces oxidative stress markers, suggestions that the inhibitory effect can be obtained at high concentrations of melatonin, but with cautious signals appearing in the treated system¹¹⁹. What is interesting in this publication and the avalanche of subsequent reports gathering amyloid and melatonin (more than 300) is two facts: one the possible impairment by melatonin of the binding of metals in the amyloid molecule¹²⁰, that leads to the impairment of those metals to produce ROS upon amyloid binding [see recent reviews on this metal hypothesis^{121,122}]; and the second the fact that as soon as the possibility of melatonin acting on amyloid was reported, several reviews claimed that melatonin was an anti-Alzheimer drug usable in therapy^{123–126} leading to stimulating new avenues to launch new programs against neurodegenerative diseases.

3 Amplification, a reflection.

A curious phenomenon exists in Science since the early 2000s. The more one writes about something, the more that something becomes common in the readers' mind. Unfortunately, the higher the capacity of one to project a possible discovery in a bigger picture, the more frequent the abuse of repeating hypotheses becomes the equivalent for the reader to a validation of what started as an hypothesis, despite minimal or total lack of strong scientific and experimental data.

Ideas, particularly the ones out of the box, are key to the progress of science and knowledge, especially at the fringe of diseases and therapeutical applications. Those ideas should be inspirational to reach what we all want: better understanding of diseases and ways to heal them. Using the generosity of these published ideas, one could help validate the hypothesis and move forward on the basis of what then become strong evidence.

A tendency, these last years, to build strategies on the reputation of some key players has minimized all prudence in terms of translating a concept from an idea to the patient bed, instead of the usual bench to bed, leading to a waste of time, money, and probably of hopes, not only from the researchers but also from the patients, and concomitantly a decreasing trust in good science.

Ideas should be kept and cherished, but they should be opening avenues towards confirmations and validations, on which the future of human therapy should be built.

4 Conclusions

There might be a confusion between “melatonin is a radical scavenger” and “melatonin is an antioxidant”. These are two different things. A scavenger is a molecule that physically traps the ROS while a molecular antioxidant is a molecule able to reduce an oxidant like does Vitamin C.. Many compounds have the radical scavenging properties.

As very clearly documented by HJ Forman, no scavenger really exists in a living context².

In order to be an inducer of antioxidant defenses, the molecule must bind to a nuclear receptor that command the neo-synthesis of the defense enzymes – such as dismutases or peroxidases, for example. Before speculating to this property for melatonin, one should demonstrate at the bench that melatonin binds to such a receptor and show that this binding results in the neo-expression (the induction) of the defense enzymes. This, to the best of our knowledge, has never been done and remains to be demonstrated, even if a large number of indirect evidences seems to point at a central role for nrf2^{127,128}. Useless to point out, once again, that despite this lack of experimental evidence, melatonin is reported as an antioxidant inducer.

It is fair to say that reading this abundant literature is confusing, particularly those reporting data on living animals or in cells. Indeed, dozens of different protocols were reported, with major differences between them, especially in terms of incubation times (“long times” - several hours - leading to the induction of the enzymatic defenses, suggesting an antioxidant capacity, direct or

indirect, that could resemble scavenger activity, despite multiple points made by Forman's commentaries and analyses¹⁻³, as pointed earlier.

This discussion might seem to the unwarned reader a futile discussion on a side effect of melatonin. In fact, the direct consequence(s) of those claims are numerous, in particular the fact that many clinicians were attracted by the possibility that such a molecule could have a healing effect in diseases where oxidation is a key malfunctioning mechanism, supposedly. We developed elsewhere several lines of reasoning around that very concept, calling for more hard science confirmation before testing hypotheses in clinic on patients¹².

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