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Article Addendum

Phytoremediative Capacity of Plants Enriched with Melatonin

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Novel Rhythms of N1-acetyl-N2-formyl-5-methoxykynuramine and its Precursor Melatonin in Water Hyacinth: Importance For Phytoremediation

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ABSTRACT

Melatonin is an environmentally friendly-molecule with broad spectrum antioxidant capacity. Melatonin is widely present in the plant kingdom. High levels of melatonin exist in an aquatic plant, the water hyacinth, which is highly tolerant of environmental pollutants. Elevated levels of melatonin probably help plants to protect against environmental stress caused by water and soil pollutants. To investigate the potential relationships between melatonin supplementation and environmental tolerance in plants, pea plants were treated with high levels of copper in the soil. The results show that copper contamination kills pea plants; however, melatonin added to the soil significantly enhanced their tolerance to the copper contamination and, therefore, increased their survival. Based on the theory and these preliminary data, we speculate that melatonin could be used to improve the phytoremediative efficiency of plants against different pollutants. Since melatonin is safe to animals and humans as well as being inexpensive, it may be a feasible and cost-effective approach to clean environmental contaminations.

INTRODUCTION

Because melatonin was first isolated from bovine pineal glands, this molecule has been portrayed as an exclusive animal hormone for decades.[1] This concept was changed after the discovery that melatonin is a potent free radical scavenger.[2,3] Scientists realize that free radical scavengers/antioxidants are widely present in plants and, thus, this may also be the case for melatonin. In 1995, two groups independently found that melatonin indeed exists in plants including fruits, vegetables and cereals.[4,5] Thereafter, numerous publications have confirmed melatonin's presence in the plant kingdom.[6-9]

It seems that melatonin's presence in plants is a universal phenomenon. The levels of melatonin in plants are highly variable from specie to specie. The reported concentration ranges are from several pg/g tissue to multiple μg/g tissue.[10] Even in the same species, the variations in the melatonin concentration vary considerably when measured in different laboratories and using different techniques.[11] The likely reasons for these variations are the percent recovery due to the extraction procedure and the detection specificity of the assay. Use of a radioimmunoassay (RIA) could over-estimate melatonin levels in plants due to the interactions of the antibody with other indoles, which are common in plants and which are structurally related to melatonin.

It appears that melatonin concentrations in plants are substantially higher than those in the blood of animals. Considering the high levels of plant melatonin, a major question is its origin. There is little information regarding the synthetic enzymes for melatonin in plants; however, an isotope tracer method showed that a plant can convert tryptophan, the initial amino acid precursor, to the indolamine.[12] This indicates that some plants are equipped with the molecular machinery for melatonin biosynthesis. Another alternative is that plant melatonin may be partially derived from the soil or water in which it is grown.

Many microorganisms including bacteria and fungi produce melatonin.[13,14] The decomposition of microorganisms releases melatonin into the surrounding material; as a result, the rootlets of the plants may absorb this melatonin and recycle it. This speculation is also based on our recent observation that plants can rapidly absorb melatonin from the water and store it in their leaves.[15] In addition to melatonin, its major metabolite, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), has also been identified in an aquatic plant [*Eichhornia crassipes (Mart) Solms*], the water hyacinth.[15]

Melatonin and its metabolite AFMK exhibit unique rhythms in the water hyacinth with their peaks during the photophase. It appears that the higher the intensity of the light to which the plant is exposed, the greater the levels of melatonin in the water hyacinth. This is in contrast to the melatonin circadian rhythm in animals. In all vertebrates, peak melatonin levels occur in the dark phase of the light:dark cycle and acute light exposure at night immediately suppresses melatonin production.[16]

Since during photosynthesis large quantities of free radicals or reactive oxygen species are generated, elevated melatonin levels during the photophase may be necessary for plants to protect against the pending free radical damage. That melatonin prevents environmentally-induced oxidative stress in plants has been reported.[17,18]

HYPOTHESIS AND PILOT STUDY

The most serious environmental insults are agricultural and industrial contaminations. Some contaminants such as heavy metals and extremely long half-life chemicals are difficult to remove from the soil and water using conventional methods or by natural decomposition. Phytoremediation provides a promising alternative for decontamination. Theoretically, if a plant can tolerate these pollutants, the plant may be a potential candidate in phytoremediation to remove these toxic substances. The water hyacinth has been used successfully in phytoremediation due to its high tolerance to many environmental toxins.[19,20] Since the water hyacinth has high levels of melatonin and AFMK when grown under natural sun light, we hypothesize that the elevated concentration of these natural antioxidants may be associated with the high tolerance of this plant to pollutants and to its utility in phytoremediation. As highly effective antioxidants,[10, 21] melatonin and AFMK likely assist this plant in defending against the environmental stresses caused by heavy metals or toxic chemicals.

To test this hypothesis, a pilot study has been performed. In this study, pea seeds (*Pisum sativum*) were planted and grown in a green house. The temperature was controlled at 25 ± 0.5°C; humidity was roughly 70%. Ten days after sprouting, half of the pea plants received 100 ml water to the soil; the water contained 5 μM melatonin. The water/melatonin solution was provided every other day until the termination of the study. The other pea plants received water only. Six days after the onset of melatonin administration the soil of all plants was treated with 100 ml of 100 μM copper (cupric sulfate). Copper treatment retarded plant growth. Fifteen days after copper treatment, the plants that had not received melatonin died; however, the plants given melatonin-enriched water continued to survive, even though the plants were smaller than prior to copper-treatment (Fig. 1).

Copper is essential to plant growth and is referred to as a micronutrient; however, it is toxic to plants at high concentrations such as in industrially-contaminated water and soil. Exposure of the plants to copper has been associated with the production of reactive oxygen species (ROS) and oxidative damage, caused in large part by the ability of this metal to redox cycle.[22] It is obvious that the application of melatonin in water is absorbed by pea plants and high levels of the antioxidant, melatonin, are stored in plant tissue. As a result, the elevated antioxidant capacity of pea plants with melatonin treatment dramatically improves their tolerance to copper contamination and presumably retards oxidative injury.

CONCLUSION

Use of high tolerant plants to clean heavy metal and toxin-polluted water and soil is a cost-effective and environmentally friendly technology. A key step is to identify high tolerant plants. Phytoremediative capacity of plants can be substantially improved using genetic engineering technologies.[23] Our preliminary study implies that the use of a natural antioxidant, melatonin, may also achieve similar results. Melatonin is an environmentally-friendly molecule. It naturally occurs in all organisms from unicellular algae to humans. Furthermore, the melatonin concentration in plants can be enriched, as reported here and elsewhere, to increase the tolerance of plants to environmental toxins. There is no evidence of serious toxicity in any plant, animal or in humans. Its half-life in the ecosystem is unknown; however, its half-life in blood of animals and of humans is of short duration, ranging from 10 to 40 minutes. In addition, melatonin is inexpensive and immediately available in large quantities. Based on the preliminary data reported herein, use of melatonin to improve the phytoremediative capacity of different plants may be a feasible and cost-effective approach to environmental contamination.

Figure 1. Representative photos of pea plants. Upper panel, the pea plants before copper treatment; lower panel, the same plants 15 days after copper treatment. The plant on the left was watered with fountain water only; the plant on the right was given fountain water containing melatonin. The plant with water only died 15 days after copper treatment; however, the plant with melatonin continued to survive.

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