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Melatonin, Metallothionein and Naringin Reduce Nodularin-Induced Hepatic 8- Hydroxydeoxyguanosine in Mice

[1]S. Al-Jassabi, [2,3]R. Saif-Ali and [4]Ali Saad

[1]Institute of Biological Sciences, University of Malaya, Malaysia
[2]Faculty of Medicine, University of Malaya
[3]Faculty of Medicine, Sana'a University, Yemen
[4]Faculty of Pharmacy, Jordanian University of Science and Technology, Jordan

Abstract: Nodulain initiates the formation of reactive oxygen species (ROS) followed by damage to DNA and other cellular components. We investigated the ability of melatonin, metallothionein and naringin to reduce nodularin-induced hepatic 8-hydroxydeoxyguanosine in mice. Melatonin, metallothionein and naringin markedly inhibited the formation of hepatic 8-hydroxydeoxyguanosine. The concentration that reduced DNA damage by 50% (IC_{50}) was 0.59, 37.8 and 41.7 μM for melatonin, metallothionein and naringin, respectively. Results showed that melatonin is 40 and 50- fold more effective than metallothionein and naringin, respectively, in reducing oxidative DNA damage. These findings are consistent with the conclusion that melatonin is highly protective against nodularin toxicity and the protective action relates, at least in part, to its direct free radical scavenging ability.

Key words: Nodularin · DNA damage · Melatonin · Metallothionein · Naringin

INTRODUCTION

Cyanobacteria (blue-green algae) blooms are commonly observed in eutrophic bodies of water [1], posing serious water quality problems because of the potent toxins that they can produce [2]. The most commonly reported algal toxins are hepatotoxins and neurotoxins [3, 4] which are produced by a wide range of unicellular and filamentous genera (e.g., *Microcystis, Anabaena, Oscillatoria, Planktothrix, Chroococcus and Nostoc)*. The King Talal Reservoir is the largest over-ground water basin in Jordan (located about 30 km north of Amman with geographical co-ordinates of 32° 11 N and 35° 48 E. The summer bloom of the cynobacteria *N. spumigena* is an annual occurrence [5]. The presence of *N. spumigena* in the King Talal Reservoir and possibly in recreational water is of particular concern as it produces nodulain, a cyclic pentapeptide with hepatotoxic activity.

Nodulain is chemically stable [6-8] and, if not diluted, can persist in water for several days or weeks [9]. Nodularin is taken up into hepatocytes by multi-specific bile acid transporters [10-12] whereas it induces the production of potentially harmful free radicals and their reactive oxygen species [13-14]. These reactants interact with DNA strand breaks, DNA-protein cross links and oxidative DNA base modification such as the formation of 8- hydroxudeoxyguanosi ne (8-OH-dG) [15-16].

Melatonin, an indoleamine product of the pineal gland [17-18], is an endogenous. OH scavenger and is a highly effective antioxidant [19-20]. Melatonin, as well as its metabolites, is more effective than other antioxidants in reducing. OH toxicity [19]. Melatonin is highly lipophilic as well as being somewhat hydrophilic. Therefore, it easily passes all known morphophysiological barriers and enters all subcellular compartments [21]. Also, melatonin has a high affinity for the nucleus of mammalian cells, whereas its concentration can be 5 times higher than levels found in blood [22]. Melatonin has been shown to scavenge radicals in the nucleus of cells [23]. Melatonin, as well as other dietary antioxidants, including metallothionein and naringin have been identified as factors in reducing the risk of cancer [24]. In the present study, we compared the ability of these antioxidants to reduce nodularin- induced oxidative DNA damage in Balb/c mice.

Corresponding Author: S. Al-Jassabi, Institute of Biological Sciences, University of Malaya, Malaysia.
Tel: +603-17-306-7907, E-mail: saaam84@live.com.

MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma St. Louis (USA) and were of analytical grade.

N. spumigena samples were collected from selected sites at the KTR and isolated from other types using a light microscope; they were then grown on Z8 medium without nitrogen and with 8.75 g/ litre NaCl and 3.75 g/litre $MgSO_4 \bullet 7H_2O$ as described in the literatures [25]. *N. spum igena* cells were harvested at the end of the exponential growth phase and lyophilized.

Toxin was extracted from freeze-dried cells using 70% aqueous methanol (80 µ1/mg dry cell mass). The suspension was sonicated overnight (16h) in a sonicating water bath and centrifuged to remove cell debris; then the supernatant fluid was assayed to determine the toxin concentration using the protein phosphatase according to [26]. The nodularin were analyzed and isolated by running the toxin extract in HPLC (Knauer, Germany) on an RP C- 18 column. The samples were run in parallel with known nodularin (NOD) standard under the same conditions.

Sixty-four male Balb/c mice (6-7 weeks old and about 30g body weight) were divided into 8 groups (of 8 mice each) as follows: the control group (CON), mice received neither nodularin nor any supplement (only 0.5 ml normal saline daily). The melatonin control group [MELC] received melatonin supplementation orally (0.55 µM per mouse daily for two weeks) but no nodularin. The metallothionein control group (METC) received metallothionein supplementation orally (8mg/kg bwt. per mouse daily for two weeks, but no nodularin). The naringin control group (NARC) received naringin supplementation orally (300mg/kg bwt. per mouse daily for two weeks) without nodularin. The toxin group (TOXC) received nodularin in normal saline at the LD_{50} dose (28 µg toxins/kg bwt) as intraperitoneal injection (i.p.), without any supplementation. The toxin and melatonin group (TOXMEL) received melatonin (0.55 µM per mouse) daily for two weeks; they were then treated with nodularin. The toxin with metallothionein group (TOXMET) received metallothionein (8mg/kg bwt. per mouse daily for two weeks) and they were then treated with nodularin. The toxin and naringin group (TOXNAR) received naringin (300mg/kg bwt. per mouse daily for two weeks) and then they were injected with nodularin. Experimental mice were killed 24h after injection of nodularin.

RESULTS AND DISCUSSION

The *Nodularia* blooms were identified and characterized based on the method described before [27]. Using light microscopic examination, toxic *N. spumigena* were recognized by their gas-filled vesicles and on the basis of cell size, length and width of vegetative cells which varied from 3.1-4.7 micrometres and from 4.6-8.3 micrometres, respectively. Nodularin was identified by its retention time and characteristic absorption spectrum with a maximum 238 nm which is due to the conjugated diene in the structure of the unusual amino acid, Adda [28, 29]. The Balb/c mouse LD_{50} intraperitoneal (i.p) dose for nodularin, as determined in our laboratory conditions (28 µg/kg bwt) was found to be well within the range of that reported in the literature [30]. The nodularin treated mice showed a 4-fold inhibition of protein phosphatase 1(PP1) activity of liver homogenates compared to control mice (Table 1). A consequence of PP1 inhibition might lead to excessive phosphorylation of structural filaments, subsequent cytoskeletal degradation and the breakdown of the hepatic ultra structure [31-33]. This ultimately results in local tissue damage and liver cell destruction [34]. This destruction was clear in nodularin treated mice as the serum values of alanine transaminase (ALT) was 10-fold as compared to those in control mice (Table 1).

Table 1: Summary of the results of the effects of melatonin, metallothionein and naringin on the toxicity of nodularin from the King Talal Reservoir/Jordan

	Control	Melatonin	Metallothionein	Naringin	Nodularin	Nodularin + melatonin	Nodularin + metallothionein	Nodularin + naringin
PP1 (U/mg)	0.574±0.002	0.512±0.002	0.553±0.001	0.562±0.003	0.165±0.001	0.313±0.001	0.301±0.004	0.188±0.002
ALT(U/L)	61.62±3.103	52.14±1.546	57.66±1.593	55.76±1.404	648.33±1.961	127.69±1.658	138.77±1.358	146.31±0.881
MDA (µM)	0.066±0.001	0.037±0.001	0.041±0.001	0.044±0.001	3.266±0.001	0.805±0.002	0.608±0.001	0.866±0.002
8-OH-dG/1^{05} 2dG	4±0.894	2±0.894	3±1.095	3±0.894	68±1.789	18±1.264	24±2.000	27±2.615

ALT, alanine transaminase: MDA, Malondialdehyde; PP 1, protein phosphatase 1; 8-OH-dG, 8-hydroxudeoxyguanosine; 2dG, 2-deoxyguanosine

However, intraperitoneal injection of nodularin LD_{50} into mice pretreated with melatonin, metallothionein or naringin significantly decreased the inhibition effect of nodularin on PP1 as shown in Table (1). This protection effect was confirmed by significantly decreased serum levels of ALT.

Results in Table (1) showed that the liver homogenate of mice exposed to nodularin contained high levels of MDA (about a 50-fold increase) when compared with control values. Nodularin increased lipid peroxidation producing high levels of MDA as a main product of lipid breakdown. The present studies showed that melatonin, metallothionein and naringin reduced the formation of MDA, most likely via their ability to scavenge free radicals [35, 36]. There was a 17-fold increase in the level of 8-OH-dG in nodularin treated mice. The generation of 8-OH-dG resulted from oxidative modification of DNA [37]. A strong correlation between higher amounts of 8-OH-dG and a greater degree of oxidative stress, DNA strand breaks, or DNA, damage has been reported [37-38]. As expected, the pretreatment of mice with melatonin, metallothionein or naringin inhibited nodularin induced the formation of 8-OH-dG. Melatonins, metallothionein and naringin function as free radical scavengers and markedly inhibit the formation of 8-OH-dG in a concentration-dependent manner but clearly with different efficacies [39].

A comparison of the efficacy of melatonin, metallothionein and naringin reveals that melatonin was the strongest inhibitor for nodularin oxidative stress since it markedly decreased the level of ALT in the serum and lipid peroxidation in the liver homogenate. IC_{50} is the concentration of antioxidant that reduces nodularin induced 8-OH-dG in DNA by 50%. The IC_{50} for melatonin was 0.59 μM, much less than that of metallothionein (37. 8μM) or naringin (41.7μM). In the present study, melatonin provided highly effective protection against the nodularin- induced formation of 8-OH-dG in DNA. Firstly, melatonin is a direct free radical scavenger and is a particularly efficient scavenger of the highly toxic $^{\cdot}OH$ [39]. Secondly, melatonin not only detoxifies the highly toxic $^{\cdot}OH$, but also scavenges its precursor, H_2O_2. It was found that the melatonin is highly lipophilic as well as being somewhat hydrophilic [40] and, therefore, it easily enters cells and subcellular compartments [41-42].

In conclusion, nodularin inhibited hepatocyte PP1 resulted in hepatocyte damage and increased serum levels of ALT. In addition, it induced lipid peroxidation and DNA damage. Pretreatment of mice with melatonin, metallothionein or naringin inhibited the nodularin-induced hepatotoxicity and oxidative stress, while melatonin was the strongest nodurlarin inhibitor.

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