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ORIGINAL ARTICLE

Influence of radiotherapy on 6-sulphatoxymelatonin levels in the urine of brain cancer patients

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Abstract

OBJECTIVES: The synthesis of melatonin, an endogenous compound synthesized by the pineal gland in the brain, is reported to be depressed in patients with primary cancers of the breast, prostate, stomach and rectum. It is not known whether patients with brain cancer exhibit altered melatonin synthesis. Also unknown is whether radiotherapy given to the region of the brain where the pineal gland is located affects the synthesis of melatonin. This information could be relevant to the clinician for the successful treatment of brain cancer patients since melatonin has been reported to be a potent oncostatic agent.

METHODS: Urinary levels of 6-sulphatoxymelatonin, the chief metabolite of melatonin, are routinely used as an index of pineal melatonin production and secretion. In this study, the concentrations of 6-sulphatoxymelatonin (aMT6S) excreted in the urine before and during radiotherapy of patients with primary or metastatic brain cancer were determined and compared with the values obtained in breast or lung cancer patients who also received radiotherapy (excluding exposure of the brain where the pineal gland is located).

RESULTS: The results showed a wide variation in the mean concentration of aMT6S excreted in the urine.

CONCLUSION: The data from this preliminary study suggested that radiotherapy given to the region of human brain, where the pineal gland is located, does not significantly affect the excretion of aMT6S, the chief metabolite of melatonin.

Abbreviations used:
aMT6S - 6-sulphatoxymelatonin

Introduction

Melatonin (5-methoxy-N-acetyltryptamine) is a highly conserved molecule, existing in organisms as diverse as algae and humans [1 4]. In mammals including man, it is synthesized and secreted by the pineal gland in the brain. It exhibits a distinct circadian rhythm with maximum production occurring during the night [2, 3, 5, 6]. Melatonin has been reported to participate in the regulation of a number of important physiological processes in mammals. It functions as a soporific [7] and as a timing signal [8]. Recent investigations have reported melatonin to be an antioxidant [9 12], capable of scavenging reactive species such as hydroxyl and peroxyl radicals and the peroxynitrite anion [13 18]. The radical scavenging activity of melatonin is hypothesized to be involved in its ability to reduce ionizing radiation-induced genetic damage in peripheral blood lymphocytes collected from healthy human volunteers [19 24]. The radioprotective ability of melatonin was also demonstrated in whole-body irradiated mice, where pre-treatment with melatonin increased survival after exposure to an $LD_{50/30}$ radiation dose. Melatonin pre-treatment also decreased the extent of radiation-induced genetic damage in both peripheral blood and bone marrow cells of whole-body irradiated mice [25, 26].

The synthesis of melatonin decreases with increasing age of the individual [27, 28] and after exposure to bright light [29]. It has also been reported in some cases to decrease as a result of exposure to extremely low frequency electromagnetic fields [30, 31], intake of alcohol [32, 33], caffeine [34] and certain drugs, such as dexamethasone [35], β-blockers [36] and calcium antagonists [37]. Melatonin production has also been reported to be altered in individuals who experience headaches [38, 39] and in those who suffer from seasonal affective disorder [40]. It is not known if radiotherapy given to the region of the brain where the pineal gland is located affects the synthesis and/or degradation of melatonin. This information could be relevant to the clinician for the successful treatment of primary or metastatic brain cancer patients; melatonin has been shown to be a potent oncostatic agent and to inhibit the growth of experimental tumors *in vivo* [41], including those of the breast [42], liver [43] and prostate [44].

Urinary levels of 6-sulphatoxymelatonin are routinely used as an index of pineal melatonin production and secretion [8]. The current study was designed to investigate the concentrations of 6-sulphatoxymelatonin (aMT6S), the chief enzymatic metabolite of melatonin, excreted in urine before and during radiotherapy of patients with primary or metastatic brain cancer. The data are compared with the values obtained in breast or lung cancer patients who also received radiotherapy (excluding exposure of the brain where the pineal gland is located).

Materials and methods

A protocol approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio was followed. Informed consent was obtained from patients with primary or metastatic brain cancer who received radiotherapy to the region of pineal gland at the Cancer Therapy and Research Center. Informed consent was also obtained from patients receiving radiotherapy for breast and lung cancers: these patients served as controls. Patients who were taking β- and calcium channel blockers or those who were unable to collect or unreliable to collect 24-hour urine samples were excluded from the study. The radiotherapy was given in fractionated doses, daily Monday through Friday. Each participating patient was asked to collect the first 24-hour urine sample before the radiation exposures began and a second sample one week after the start of radiotherapy, and weekly thereafter until the completion of the course of radiotherapy. A 24-hour urine sample was also collected from some patients at one month after completion of the therapy regimen.

All urine samples were stored at $20\pm1^{o}C$ and coded before determining the concentration of 6-sulphatoxymelatonin (aMT6S) using a competitive binding radioimmunoassay [45, 46]. Briefly, an aliquot of each urine sample (containing aMT6S) was diluted, mixed with a specific antiserum to aMT6S raised in a sheep, and incubated for 30 minutes at room temperature. A trace amount of ^{125}I-aMT6S (Stockgrand Ltd., Guilford, UK) was then added to this mixture, which was incubated overnight at $4\pm1^{o}C$. The free and antibody-bound fractions of aMT6S were separated: the free aMT6S fraction was precipitated with dextran-coated charcoal suspension by incubation for 15 minutes at $4\pm1^{o}C$, and the radioactivity counted in a gamma counter. A standard curve using known amounts of aMT6S was constructed at the same time. The concentrations of aMT6S in the urine samples were calculated from this standard curve. The aMT6S binding was found to be 76% with a specificity of 2 pg/ml and a curve correlation of 0.9978. The values were expressed as ng of aMT6S per mg of creatinine.

Statistical Analysis: The data were collected from 9 brain, 7 breast and 2 lung cancer patients and compared between the three types of cancers investigated. The data were also analyzed for the regression of aMT6S excreted in the urine with the radiation dose received by individual cancer patients over their treatment period. These measures were also correlated adjusting for group and patient variability. In addition, based on all available data, the regression estimates of the amount of aMT6S excreted in the urine for each group of patients at 3000, 4000 and 5000 cGy radiation dose were computed.

Results and discussion

The results are presented in Table 1. Before, during and after radiotherapy, the mean concentration of aMT6S excreted in the urine was not significantly different between the three cancer patient groups (p=0.72) and showed wide variation. The regression slopes for aMT6S excreted in the urine with increasing radiation dose received by the three cancer patient groups were not significantly different (p=0.68). However, brain cancer patient #3 and #6 indicated a linear prediction of aMT6S excretion with

Table 1. Concentration of 6-Sulphatoxymelatonin (ng of aMT6S/mg of creatinine) in urine samples of brain cancer patients receiving radiotherapy (cumulative radiation dose in cGy) in the region of pineal gland.

Brain Cancer Patients											(*)	p value
1	cGy	0	900	1800	2700	3600	4500	5040	5260		0.0	0.8
	aMT6S	15.3	5.5	2.8	14.4	10.6	16.3	9.0	2.4			
2	cGy	180	900	1800	2700	3600	4320	5040	6300		0.1	0.5
	aMT6S	7.4	8.6	7.6	11.2	5.3	7.7	9.0	10.7			
3	cGy	0	1260	1980	3060	3420	4380				0.8	0.0
	aMT6S	20.3	25.9	58.1	28.9	28.2	37.8					
4	cGy	0	800	2600	3400	4000					0.2	0.6
	aMT6S	10.0	10.2	6.5	10.7	6.8						
5	cGy	0	1000	2000	2800	4000					0.1	0.7
	aMT6S	2.2	11.0	2.6	3.5	8.7						
6	cGy	400	1200	2200	3200						1.0	0.0
	aMT6S	10.2	15.3	17.2	21.2							
7	cGy	0	1000	2600	3600						0.1	0.7
	aMT6S	9.5	13.8	21.2	10.3							
8	cGy	0	2200	3400	4000						0.2	0.5
	aMT6S	3.5	8.7	2.0	13.2							
9	cGy	0	1000	2800	3000						0.0	0.8
	aMT6s	10.7	7.6	12.6	5.7							
Breast Cancer Patients												
1	cGy	0	720	1620	2520	3240	4140	5100	5900	**	0.3	0.2
	aMT6S	13.7	19.0	16.5	12.0	20.7	16.4	24.4	19.3	29.6		
2	cGy	0	720	1800	2880	3600	4500	5400	6120	**	0.0	0.9
	aMT6S	7.1	8.5	10.1	11.4	9.8	8.5	7.3	8.1	9.2		
3	cGy	0	720	1620	2520	3420	4320	5220	6120		0.2	0.2
	aMT6S	3.3	5.4	4.8	4.0	5.4	5.4	7.2	4.4			
4	cGy	0	1000	2000	3000	4000	5000	6000		**	0.0	1.0
	aMT6S	9.2	8.6	10.1	10.3	11.4	10.5	7.5		9.7		
5	cGy	0	1200	1600	2800	4000	5600			**	0.6	0.1
	aMT6S	1.4	7.8	1.6	5.4	15.8	10.9			11.0		
6	cGy	0	1000	2000	2800	3800	4800	6000			0.5	0.1
	aMT6S	2.9	6.9	3.0	5.0	10.3	6.1	9.6				
7	cGy	0	800	1800	2600	3600					0.2	0.5
	aMT6S	12.3	13.2	11.8	9.9	12.1						
Lung Cancer Patients												
1	cGy	0	1000	2000	3000	3800	4800			**	0.2	0.2
	aMT6S	11.9	20.1	13.4	19.9	19.1	15.3			23.3		
2	cGy	600	1000	2800							0.1	0.8
	aMT6S	2.2	9.4	2.4								

(*): Correlation Coefficient Squared between aMT6S and radaiton dose. ** : follow-up value

the cumulative radiation dose received, with p-values of 0.03 and 0.02, respectively. When computed for the best fit for exposure to similar radiation doses of 3000, 4000 and 5000 cGy, the data indicated that the concentration of aMT6S excreted in the urine of brain cancer patients (12.7, 13.7 and 14.7, respectively) were higher than those observed in breast (9.6, 10.1 and 10.7, respectively) and lung cancer patients (10.4, 10.4 and 10.4, respectively). When the data were all pooled and analyzed by adjusting the two sources of variability (cumulative radiation dose and the type of cancer), the partial correlation of aMT6S and radiation dose was found to be 0.32 ($p=0.003$). Within groups, this partial correlation was 0.25 ($p=0.12$), 0.41 ($p=0.007$) and 0.37 ($p=0.32$) for brain, breast and lung cancer patients, respectively. Perhaps, the larger correlation observed in breast cancer patients ($p=0.007$) could be due to receiving higher doses of radiation. For some patients, a curvilinear fit was better compared to the linear fit, but several patients had only 4 urine collection points and a full analysis of this more complicated model was not possible.

A careful examination of the records of patients who participated in this study indicated intake of a variety of drugs (other than those which are shown to have an influence on melatonin synthesis, as mentioned in the introduction). These included ambien, azmacort, dilantin, diazepam, tagamet, tylenol and zocor. Four brain cancer patients (#1, 2, 8 and 9) and one lung cancer patient (#1) were taking dilantin in various dose regimens. The possible effect of this drug on the mean aMT6S level excreted in the urine was evaluated using the analysis of variance test. The radiation exposure was used as a covariate and the drug was tested as a factor and for interaction with the type of cancer. The drug was found to have no significant effect on the mean aMT6S level excreted in the urine.

The data from this preliminary study suggests that radiotherapy given to the region of the human brain where the pineal gland is located does not significantly affect the excretion of the chief melatonin metabolite during treatment. The evidence for a linear relationship between aMT6S excretion and cumulative radiation dose in two brain cancer patients (#3 and 6) could be due to an enhanced synthesis of melatonin or due to an increase in the metabolic degradation of melatonin (resulting in greater amounts of aMT6S excretion in the urine). The urinary excretion level of aMT6S was the highest recorded for patient #3 (58.1 ng/mg creatinine) and the patient was observed to have difficulty to stay awake during weekly checkups: based on this one patient, it is not possible to conclude the increased excretion of aMT6S is due to increased synthesis of melatonin, which in turn is the result of the radiotherapy given to the region of the brain where the pineal gland is located. Investigations with larger numbers of cancer patients and long-term follow-ups are necessary to determine possible differences in melatonin synthesis and degradation.

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