Melatonin in Patients with Cancer Receiving Chemotherapy: A Randomized, Double-blind, Placebo-controlled Trial

AUMKHAE SOOKPRASERT[1], NUTJAREE PRATHEEPAWANIT JOHNS[2,3], ANAKAPONG PHUNMANEE[1], PARICHART PONGTHAI[4], AREEWAN CHEAWCHANWATTANA[2,5], JEFF JOHNS[2,3], JULRAHT KONSIL[2,3], PREEYAPORN PLAIMEE[2,3], SUPATRA PORASUPHATANA[2,6] and SUTHIPHAN JITPIMOLMARD[1]

[1]Department of Medicine, Faculty of Medicine, [2]Melatonin Research Group, [3]Division of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, [4]Khon Kaen Hospital, [5]Division of Social and Administrative Pharmacy, Faculty of Pharmaceutical Sciences, [6]Division of Pharmacognosy and Toxicology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand

Abstract. *Background: The MIRCIT trial was a randomized, double-blind, placebo-controlled study of advanced Non-small cell lung cancer (NSCLC). Patients and Methods: Patients were randomized to receive 10 mg or 20 mg of melatonin or placebo. Assessment of health-related quality of life (HRQoL) was completed at baseline, and at 2, 3 and 7 months. Survival and adverse events were collected. DNA damage marker 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) was measured during the first three months of chemotherapy. Results: Patients in the melatonin-treated group had better adjusted HRQoL scores, with a slightly significantly better score (2.69 points, 95% confidence interval (CI)=0.01-5.38, p=0.049) being found in social well-being. Median survival was 7.3 months (95% CI=3.42-11.14) without significant difference. A great amont of DNA damage marker was observed in the placebo-treated group, and this was associated with lower survival (r^2=−0.656, p=0.02), implying the protective effect of melatonin in healthy cells. Conclusion: Melatonin in combination with chemotherapy did not affect survival and adverse events of advanced patients with NSCLC, but there was a trend for better HRQoL.*

Non-small cell lung cancer (NSCLC) accounts for about 80% of lung cancer and has a high morbidity (1, 2). It has poor prognosis, with survival of less than five months for untreated metastatic NSCLC (3). Meta-analysis has shown slightly improved survival with chemotherapy, with a median survival time of up to 12 months, depending on various prognostic factors (4). The majority of patients are diagnosed with disease at a non-operable advanced stage, and have more symptoms of distress such as fatigue, loss of appetite, shortness of breath, cough and pain (5). In addition to improved survival, symptom control of adverse events from cancer treatment and improvement of health-related quality of life (HRQoL) are, therefore, essential components of cancer care in this population.

A number of adjuvant therapies have been investigated with various proposed mechanisms (6, 7), including antioxidant actions. Few studies have shown negative effects of antioxidants, and then only for selected antioxidants *e.g.* vitamin E or *N*-acetylcysteine when used in healthy individuals for cancer prevention (8, 9). For patients with cancer, however, systematic reviews of over 2,400 patients using supplements including antioxidants during chemotherapy have shown no clinical evidence that they interfere with chemotherapy or cause any decrease in chemotherapeutic efficacy. Many of the studies show an increased response or survival time, with reduced toxicities, compared to controls (10, 11).

Melatonin is an endogenous indoleamine hormone secreted primarily from the pineal gland (12). The anticancer properties of melatonin have been reported for many types of cancer cell lines with several plausible mechanisms elucidated including: (a) modulation of cell cycle and induction of apoptosis (13); (b) reduction of migration and invasiveness (14); and (c) inhibition of tumor angiogenesis (15). Certain studies have demonstrated antiproliferative effects of melatonin through radical oxygen species (ROS)-mediated pathways (16) and immunomodulation (17). Animal models have demonstrated the antitumor effects of melatonin when used alone and enhanced effects for chemotherapy when used in combination (17, 18). Melatonin in conjunction with chemotherapy has also shown reduction of chemotherapy toxicity such as cardiotoxicity (19), renal toxicity (20) and testicular toxicity (21), among many others.

Correspondence to: Associate Professor Nutjaree Pratheepawanit Johns, Division of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002 Thailand. Tel: +66 43202378, Fax: +66 43202379, e-mail: pnutja@kku.ac.th

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Melatonin has shown positive results in a number of clinical trials on patients with cancer. Meta-analysis of 10 randomized controlled trials of patients with advanced cancer showed that melatonin reduced the risk of death at one year by 34% (22). Another meta-analysis of melatonin used as an adjuvant therapy showed improved complete response of 2.53-fold (95% confidence interval (CI)=1.36-4.71) compared to chemotherapy-alone, with significantly reduced adverse events (23). Likewise, melatonin in concurrent chemotherapy or radiotherapy for solid tumor resulted in improving the 1-year survival rate (relative risk (RR)=1.90; 95% CI=1.28-2.83, p=0.001) and significantly decreased radiochemotherapy-related side-effects (24). No severe side-effects of melatonin were reported in any of these trials.

Patients with advanced NSCLC were reported to have distorted circadian rhythms, poor sleep quality, accompanied by marked deterioration of HRQoL (25). With low toxicity, melatonin appears promising as an adjunct therapy in patients with NSCLC receiving chemotherapy; however, limitations of previous clinical studies have not as yet provided sufficiently convincing evidence for clinicians. None of the previous studies used placebo controls or were blinded, and thus there is potential risk of bias in the results. Furthermore, most of the trials were conducted by the same group of investigators, and therefore independent trials are needed to confirm the existing findings. The current randomized placebo-controlled, double-blind trial conducted outside the previous setting is, thus, warranted.

This trial of melatonin in reduction of chemotherapy-induced toxicity (MIRCIT) was conducted to evaluate the effect of melatonin compared with placebo on HRQoL, survival, biochemical analysis, and adverse events of cancer treatment of patients with advanced-stage cancer. It was originally planned to recruit patients with four types of advanced cancer, which were NSCLC, breast cancer, soft-tissue sarcoma, and head and neck cancer. Randomization was stratified by cancer type, cancer treatment and hospital settings. With the participating settings, however, mostly patients with NSCLC were recruited, which are reported in this study.

Patients and Methods

Study design. This is a randomized, double-blind, placebo-controlled trial conducted on patients with advanced NSCLC who were receiving treatment from two tertiary care hospitals. The study was approved by the Khon Kaen University Ethics Committee (HE490615) and the Khon Kaen Hospital Ethics Committee (55/02/2551) and conducted in accordance with the International Conference on Harmonization on Good Clinical Practice requirements. All patients provided their written informed consent. The study was registered at ClinicalTrials.gov Identifier: NCT01706627.

Eligibility. Patients with histologically-proven advanced NSCLC were recruited. Additional inclusion criteria included age 18 to 70 years, Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2, platelet count ≥100,000 cells/mm^3, white blood cell count ≥3,000 cell/mm^3, hemoglobin ≥10 g/dl, serum creatinine ≤1.5 mg/dl, bilirubin ≤2 mg/dl, aspartate aminotransferase (AST) ≤2.5 times the upper limit of normal (ULN) for those without metastases or ≤4 times the ULN for those with liver metastasis and New York Heart Association grade ≤2. Patients who had received prior chemotherapy or biotherapy, radiotherapy or surgery within one month preceding randomization, or had more than one type of cancer, or brain metastasis were excluded from the trial. Additional exclusion criteria included patients with moderate neuropathy with Common Terminology Criteria for Adverse Events (CTCAE) grade 2 or more, an active infection, or uncontrolled complications (*i.e.* blood glucose >200 mg/dl, uncontrolled hypertension, unstable angina, history of congestive heart failure or history of myocardial infarction within one year).

Treatment. Chemotherapy regimens given were based on the hospital protocol for cancer treatment. All patients received standard supportive care, including blood and platelet transfusions, antibiotics, and anti-emetics, as appropriate. Administration of chemoprevention (*i.e.* amifostine or dexrazoxane) to reduce toxicities associated with chemotherapy was not provided. Granulocyte colony-stimulating factor was used only in patients with history of febrile neutropenia, but not used as primary prophylaxis.

Chemotherapy treatments were monthly, with the decision on the number of months, or change to second- or third-line chemotherapy regimens being at the doctor's discretion.

Mixed-block randomization, stratified by hospital and first-line chemotherapy regimen, was used to divide eligible patients into three treatment groups: 10 mg melatonin, 20 mg melatonin, or matched placebo. Random assignment was performed on the first day of chemotherapy treatment. The patients were required to take the study drug at night (after 21.00 pm) on the first day of chemotherapy treatment and continue for six months. A diary was given to the patients for self-recording of time and date of drug administration. Pill counts and diary record of each visit were used to determine a patient's compliance.

Hard capsule formulations containing 10 mg and 20 mg melatonin and matched placebo were manufactured by General Drugs Houses, Ltd, Bangkok, Thailand. Quality control of the study drugs was cross-checked by the Faculty of Pharmaceutical Sciences, Khon Kaen University, based on weight variation, content uniformity and dissolution following US Pharmacopeia guidelines, and melatonin purity by differential scanning calorimetry and High Performance Liquid Chromatography (26).

Outcome measures. The primary study end-point was HRQoL assessed with the Functional Assessment of Cancer Therapy – Lung (FACT-L). It comprises 36 questions categorized into five domains, namely physical well-being (PWB), social well-being (SWB), emotional well-being (EWB), functional well-being (FWB) and lung cancer subscale (LCS). The questionnaire is well validated and has been used widely in lung cancer trials. The validated Thai version of FACT-L, with corresponding psychometric properties of the original, was used in this study (27).

FACT-L was completed at baseline (before treatment), and the first day of the first follow-up (month 2), second follow-up (month 3) and third follow-up (month 7) of the treatment. HRQoL scores were reported by the domain, total FACT-L (sum of all five domains), total FACT-G (sum of PWB, EWB, SWB and FWB) and TOI (sum of PWB, FWB and LCS). Higher scores represent better quality of life.

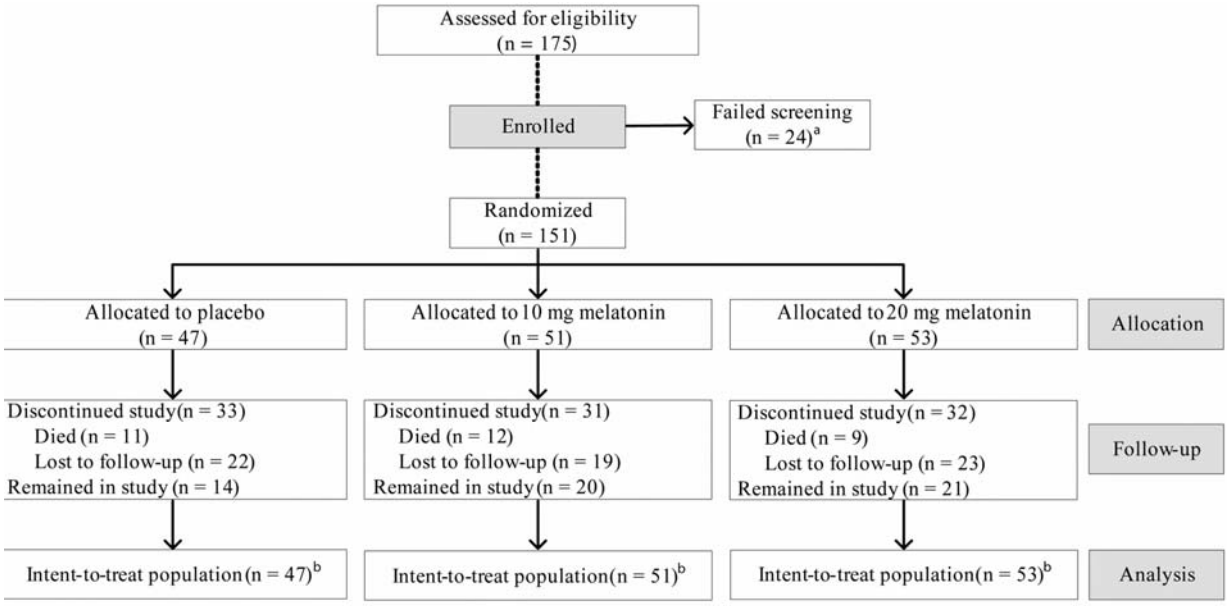


Figure 1. *Consolidated Standards of Reporting Trials diagram. [a]Among the 24 patients who failed screening, the main reason for exclusion was not NSCLC. [b]All patients who were randomly assigned to a study group were included in the intent-to-treat analysis. Died is reported as death during the trial.*

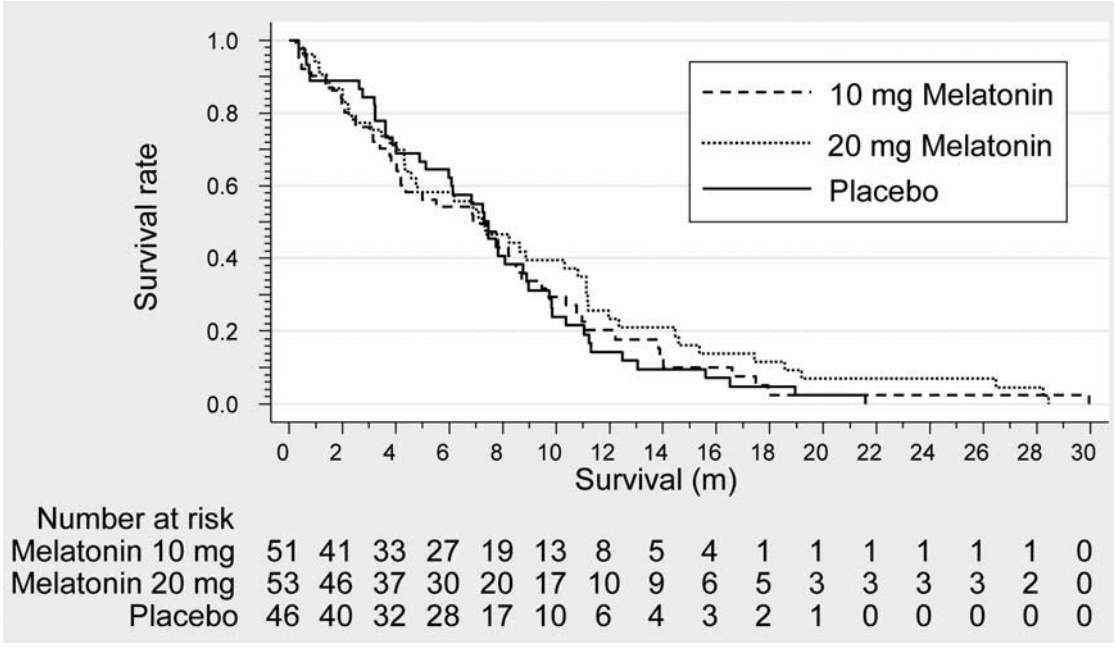


Figure 2. *Kaplan–Meier plot of overall survival for patients in the groups treated with placebo (n=46), 10 mg melatonin (n=51), and 20 mg melatonin (n=53).*

All patients were used for analysis of HRQoL. To handle missing data, for patients who were reported dead during the follow-up period, HRQoL data were reported as zero. Other outcomes evaluated every month were adverse events assessed using the CTCAE version 3, ECOG PS, standard hematology, chemistry, electrolytes, urinalysis, and physical examination. Survival data were obtained from the date of registration of the death at the National Registry.

Overnight or spot morning urine was collected every month and the marker of DNA damage and repair, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in the urine samples was measured using a DNA/RNA Oxidative Damage EIA Kit (#589320; Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions. Urine was diluted 500-fold before analysis. Levels were normalized against urinary creatinine (ng 8-oxydG/mg Cr).

Table I. *Baseline demographic characteristic of patients with non-small cell lung cancer (n=151).*

	Placebo (n=47)	10 mg Melatonin (n=51)	20 mg Melatonin (n=53)	Total (n=151)	*p*-Value
Age average (SD)	55.6 (11.5)	56.8 (9.4)	56.3 (8.8)	56.3 (9.9)	0.856‡
Male gender	33 (70.2%)	39 (76.5%)	32 (60.4%)	104 (68.9%)	0.202†
Married	38 (80.9%)	46 (90.2%)	47 (88.7%)	131 (86.8%)	0.346†
1st Chemotherapy					0.833†
Cisplatin/etoposide	23 (48/9%)	23 (45.1%)	27 (50.9%)	73 (48.3%)	
Paclitaxel/carboplatin	24 (51.1%)	28 (54.9%)	26 (49.1%)	78 (51.7%)	
Education					0.821†
≤6th grade	37 (78.7%)	38 (74.5%)	42 (79.2%)	117 (77.5%)	
>6th grade	10 (21.3%)	13 (25.5%)	11 (20.8%)	34 (22.5%)	
Hospital					0.965†
SNH	37 (78.8%)	39 (76.5%)	41 (77.4%)	117 (77.5%)	
KKH	10 (21.3%)	12 (23.5%)	12 (22.6%)	34 (22.5%)	
Insurance scheme					0.463†
Civil servant/social	16 (34.0%)	22 (43.1%)	17 (32.1%)	55 (36.4%)	
UC	31 (66.0%)	29 (56.9%)	36 (67.9%)	96 (63.6%)	
Quality of life scores, average (SD)					
TOTAL	88.7 (16.7)	87.6 (16.7)	88.8 (15.6)	88.4 (16.2)	0.922‡
TOI	52.3 (12.2)	52.0 (11.6)	53.5 (12.3)	52.6 (12.0)	0.798‡
FACTG	71.3 (12.9)	71.1 (14.0)	71.4 (12.8)	71.2 (13.2)	0.993‡
LCS	17.4 (5.3)	16.6 (4.6)	17.4 (4.6)	17.2 (4.8)	0.570‡
PWB	21.0 (4.2)	20.8 (4.6)	22.2 (4.3)	21.4 (4.4)	0.251‡
SWB	18.7 (4.7)	17.7 (5.2)	16.9 (4.3)	17.8 (4.8)	0.176‡
EWB	17.7 (5.1)	17.9 (5.0)	18.4 (4.1)	18.0 (4.7)	0.751‡
FWB	13.8 (5.9)	14.6 (5.9)	13.9 (5.9)	14.1 (5.9)	0.778‡
ECOG score	(n=41)	(n=49)	(n=49)	(n=139)	
0 or 1	36 (87.8%)	45 (91.8%)	39 (79.6%)	120 (86.3%)	0.200†
≥2	5 (12.2%)	4 (8.2%)	10 (20.4%)	19 (13.7%)	
Weight loss	(n=41)	(n=42)	(n=40)	(n=123)	
None or <5%	27 (65.9%)	23 (54.8%)	28 (70.0%)	78 (63.4%)	0.332†
≥5%	14 (34.1%)	19 (45.2%)	12 (30.0%)	45 (36.6%)	
Adverse events					
Anemia	(n=44)	(n=49)	(n=52)	(n=145)	
9 (20.5%)	12 (24.5%)	6 (11.5%)	27 (18.6%)	0.231†	
Liver dysfunction	(n=43)	(n=46)	(n=50)	(n=139)	
6 (14.0%)	6 (13.0%)	5 (10.0%)	17 (12.2%)	0.827†	
Neuropathy	(n=45)	(n=50)	(n=49)	(n=144)	
2 (4.4%)	2 (4.0%)	2 (4.1%)	6 (4.2%)	1.000*	
Fatigue	(n=44)	(n=50)	(n=50)	(n=144)	
20 (45.5%)	21 (42.0%)	17 (34.0%)	58 (40.3%)	0.504†	
Anorexia	(n=44)	(n=50)	(n=50)	(n=144)	
12 (27.3%)	19 (38.0%)	21 (42.0%)	52 (36.1%)	0.314†	
Mucositis	(n=44)	(n=50)	(n=50)	(n=144)	
0	3 (6.0%)	0	3 (2.1%)	0.108*	
Nausea	(n=44)	(n=50)	(n=51)	(n=145)	
1 (2.3%)	3 (6.0%)	2 (3.9%)	6 (4.1%)	0.776*	

†Pearson Chi square, ‡one-way ANOVA, *Fisher's exact test. SD=standard deviation, SNH=Srinagarind Hospital, KKH=Khon Kaen Hospital, UC=universal coverage, Total=functional assessment of cancer therapy - lung, TOI=trial outcome index, FACT-G=functional assessment of cancer therapy – general, LCS=lung cancer subscale, PWB=physical well-being, SWB=social well-being, EWB=emotional well-being, FWB=functional well-being.

Statistical analysis. Descriptive statistics were tabulated and summarized. Analyses were performed by using intent-to-treat principle with percentage of main outcome (survival, mean HRQoL and adverse events). Kaplan–Meier analysis was performed to assess overall survival. The relative hazard for each patient was calculated from co-efficients determined from Cox regression. HRQoL scores for each follow-up were calculated by using paired *t*-test. The mean difference of estimated HRQoL at first (month 2), second (month 3) and third (month 7) follow-up compared to baseline was analyzed. All *p*-values and reported CIs are two-sided at 95%. Data analysis was conducted using STATA version 10 (StataCorp LP, College Station, TX, USA).

Table II. *Mean HRQOL scores reported before treatment (baseline) and after treatment at the first (2 month), second (3 month) and third (7 month) follow-up (FU), categorized by treatment group.*

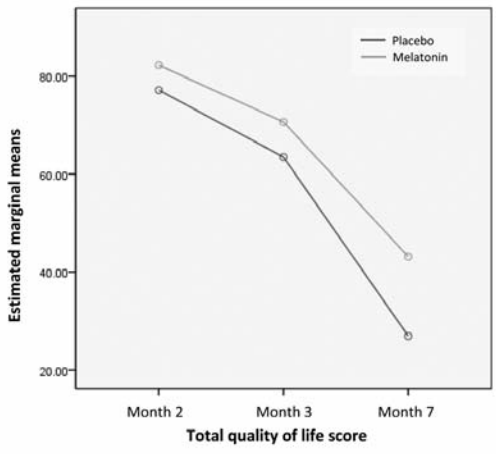
	Mean (SD) HRQoL score		Estimated mean (95% CI)		Mean difference (95% CI)	*p*-Value
	Placebo	Melatonin	Placebo	Melatonin		
TOTAL	N=38	N=88	55.85 (45.47-66.24)	65.35 (58.56-72.14)	9.50 (–2.97-21.97)	0.134
Baseline	89.99 (17.80)	87.89 (16.25)				
1st FU	79.75 (38.26)	81.15 (34.48)				
2nd FU	66.68 (45.70)	69.28 (43.42)				
3rd FU	28.25 (45.72)	42.54 (48.62)				
TOI	N=38	N=88	33.84 (27.62-40.06)	38.59 (34.52-42.65)	4.74 (–2.72-12.21)	0.211
Baseline	52.89 (13.17)	52.55 (12.33)				
1st FU	47.09 (23.29)	47.79 (21.21)				
2nd FU	40.26 (27.96)	41.17 (26.28)				
3rd FU	16.55 (26.88)	25.76 (29.74)				
FACTG	N=38	N=88	44.81 (36.51-53.11)	52.36 (46.93-57.79)	7.55 (–2.42-17.52)	0.136
Baseline	72.35 (13.75)	70.98 (13.23)				
1st FU	63.54 (30.63)	64.84 (27.69)				
2nd FU	52.34 (36.36)	55.77 (34.05)				
3rd FU	23.32 (37.79)	34.41 (39.30)				
LCS	N=38	N=89	11.30 (9.05-13.55)	13.15 (11.69-14.61)	1.85 (–0.85-4.55)	0.177
Baseline	17.63 (5.38)	16.88 (4.68)				
1st FU	16.21 (8.33)	16.32 (7.41)				
2nd FU	14.34 (10.02)	14.31 (9.49)				
3rd FU	4.92 (8.17)	8.14 (9.58)				
PWB	N=38	N=89	14.00 (11.45-16.56)	15.24 (13.58-16.90)	1.23 (–1.83-4.29)	0.427
Baseline	20.87 (4.37)	21.51 (4.55)				
1st FU	19.00 (9.29)	19.13 (8.43)				
2nd FU	16.26 (11.15)	16.34 (10.06)				
3rd FU	6.63 (10.73)	10.29 (11.67)				
SWB	N=38	N=89	10.67 (8.44-12.90)	13.36 (11.92-14.80)	2.69 (0.01-5.38)	0.049
Baseline	19.18 (4.40)	17.38 (4.67)				
1st FU	15.61 (8.23)	16.70 (7.53)				
2nd FU	12.97 (9.74)	14.08 (8.91)				
3rd FU	5.64 (9.42)	8.36 (9.59)				
EWB	N=38	N=89	12.00 (9.70-14.29)	13.36 (11.86-14.85)	1.36 (–1.39-4.12)	0.330
Baseline	17.91 (5.31)	17.97 (4.48)				
1st FU	17.05 (8.22)	16.75 (7.45)				
2nd FU	13.45 (9.53)	14.56 (8.97)				
3rd FU	6.05 (9.77)	8.52 (9.85)				
FWB	N=38	N=88	8.66 (6.89-10.44)	10.31 (9.15-11.47)	1.64 (–0.49-3.78)	0.130
Baseline	14.39 (6.17)	14.15 (5.90)				
1st FU	11.88 (6.97)	12.40 (6.77)				
2nd FU	9.66 (8.12)	10.94 (7.82)				
3rd FU	5.00 (8.58)	7.35 (8.95)				

Note: Covariates were gender, age, medical benefit scheme, Eastern Cooperative Oncology Group score and fatigue at baseline. FU=follow-up, SD=standard deviation, CI=confidence interval, HRQoL=health-related quality of life, Total=functional assessment of cancer therapy - lung, TOI=trial outcome index, FACT-G=functional assessment of cancer therapy – general, LCS=lung cancer subscale, PWB=physical well-being, SWB=social well-being, EWB=emotional well-being, FWB= functional well-being.

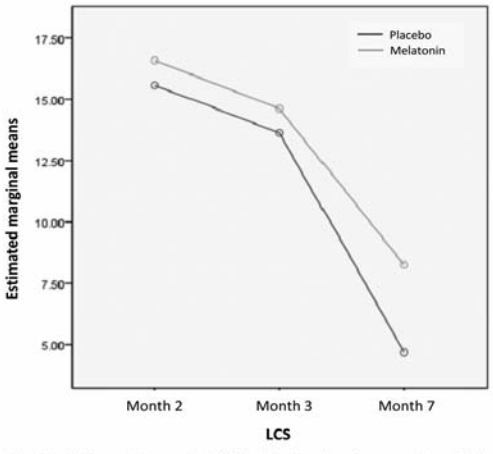
Results

Baseline characteristics. A total of 151 patients with NSCLC were recruited to this study and allocated as to treatment follows: placebo, n=47; 10 mg melatonin, n=51; and 20 mg melatonin, n=53. The proportion of patients who remained in the study through out the 6-month period was 39% in both melatonin-treated groups (10 mg melatonin, n=20; 20 mg melatonin, n=21) and 30% in the placebo-treated group (n=14) (Figure 1). Overall, the main reasons for discontinuing the study were loss to follow-up (42%), or death (21%).

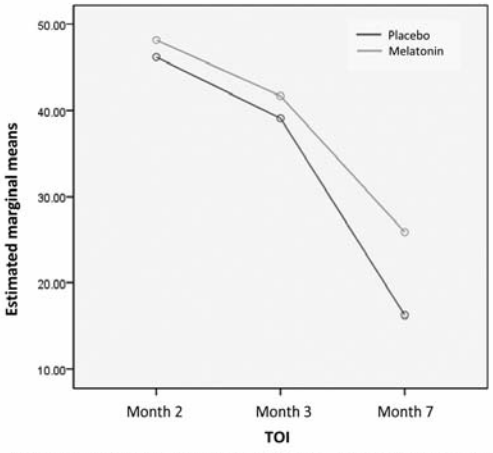
Demographic characteristics and quality of life scores at baseline of the participating patients were comparable for all groups (Table I). The average age of the patients was 56 years



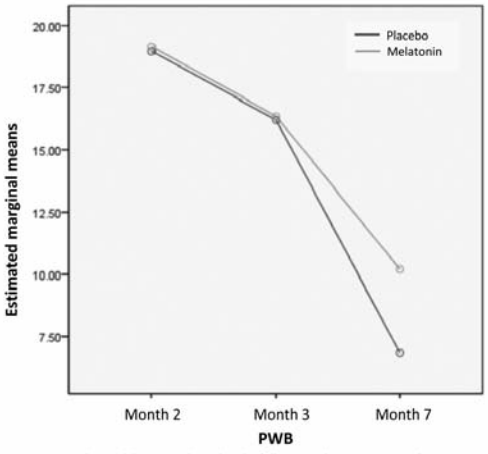
Covariates in the model were evaluated at the following values: insurance scheme=2.27, sex=1.33, age=55.9696, ECOG score at baseline=1.07, Total QOL score at baseline=88.5220, fatigue at baseline=1.57



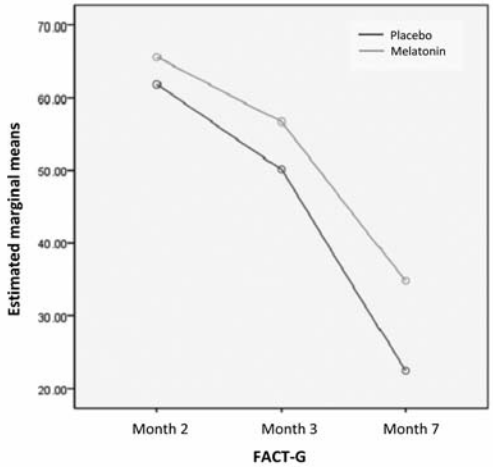
Covariates in the model were evaluated at the following values: insurance scheme=2.26, sex=1.33, age=55.9987, ECOG score at baseline=1.07, LCS score at baseline=17.1076, fatigue at baseline=1.57



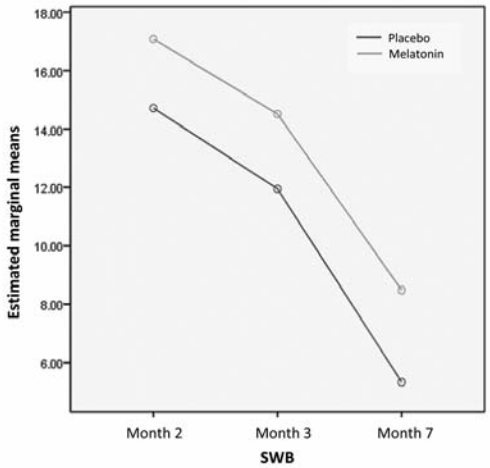
Covariates in the model were evaluated at the following values: insurance scheme=2.27, sex=1.33, age=55.9696, ECOG score at baseline=1.07, TOI score at baseline=52.6548, fatigue at baseline=1.57



Covariates in the model were evaluated at the following values: insurance scheme=2.26, sex=1.33, age=55.9987, ECOG score at baseline=1.07, PWB score at baseline=21.3150, fatigue at baseline=1.57



Covariates in the model were evaluated at the following values: insurance scheme=2.27, sex=1.33, age=55.9696, ECOG score at baseline=1.07, FACT-G score at baseline=71.3976, fatigue at baseline=1.57



Covariates in the model were evaluated at the following values: insurance scheme=2.26, sex=1.33, age=55.9987, ECOG score at baseline=1.07, SWB score at baseline=17.9186, fatigue at baseline=1.57

Figure 3. *continued*

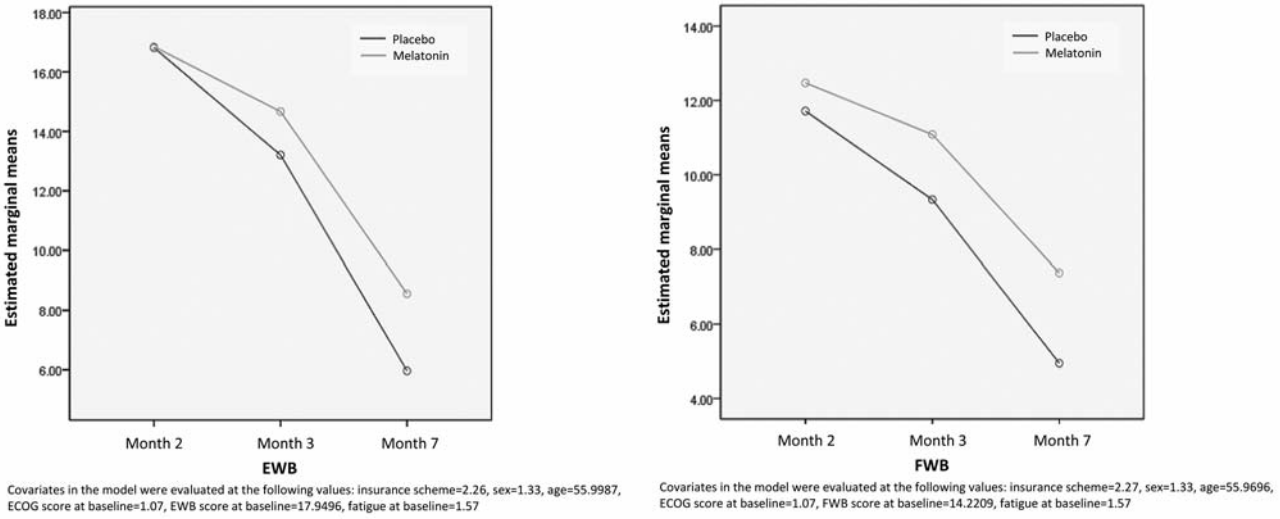


Figure 3. *Health-related quality of life scores for each domain reported at the first (month 2), second (month 3) and third (month 7) follow-up. Total quality of life score=functional assessment of cancer therapy - lung, TOI=trial outcome index, FACT-G=functional assessment of cancer therapy – general, LCS=lung cancer subscale, PWB=physical well-being, SWB=social well-being, EWB=emotional well-being, FWB= functional well-being.*

and most patients were male (69%), with primary school education (78%). Around one-third of the population reported adverse events at baseline, such as fatigue (40%), weight loss greater than 5% (37%) and anorexia (36%). It should be noted that about 14% had poor PS, reported as ECOG scores of 2 or more (Table I). Overall compliance rates for the FACT-L questionnaire were 97.8% (98.5% in the melatonin-treated group and 96.1% in the placebo-treated group).

Overall survival. From death registration data, out of 151 patients recruited, 67 (44%) died within six months of the study period, and one patient who was recruited less than six months from the end of the trial was not included for analysis. The overall median survival of the patients was 7.3 (95% CI=3.42-11.14) months [7.46 (95% CI=3.61-9.86) months for those treated with placebo, 7.23 (95% CI=3.15-10.78) months for those treated with 10 mg melatonin and 6.9 (95% CI=3.45-11.96) months for those treated with 20 mg melatonin). No significant difference in survival was reported among the groups (Figure 2). Of significant note was that at 22 months after the start of the study, only patients in the melatonin-treated groups had survived, with higher numbers of patients surviving in the 20-mg than 10-mg melatonin group.

Health-related quality of life. There were 130 patients who completed HRQoL assessment at the first follow-up (month 2), 114 at the second follow-up (month 3) and 55 at the final follow-up (month 7). With intention-to-treat analysis, all patients recruited are included for HRQoL analysis, with scores for missing data due to death being replaced as '0'. Data from 10-mg and 20-mg melatonin group were combined for HRQoL analysis. The results showed that HRQoL of the studied patients decreased over time. Adjusting for gender, age, medical benefit scheme, ECOG score and fatigue at baseline, patients in the melatonin-treated group had better HRQoL, *i.e.* higher estimated mean HRQoL scores, in all domains (Table II) (Figure 3). The total FACT-L score was 65.35 (95% CI=58.56-72.14) in melatonin-treated groups and 55.85 (95% CI=45.47-66.24) in the placebo-treated group, with the average score being 9.5 points higher in the melatonin-treated groups (95% CI=−2.97-21.97, p=0.134). For social well-being, the melatonin-treated groups reported a slightly significantly better score than those treated with placebo (2.69 points, 95% CI=0.01-5.38 points, p=0.049).

Adverse events. Adverse events at any cycle were counted and reported during treatment. With high numbers of missing data due to loss to follow-up and death, the comparison of frequency and intensity of adverse events reported during each month of treatment could not be calculated. The incidence of adverse events reported within six months of treatment is shown in Table III. There was no difference in adverse events reported by melatonin-or placebo-treated groups. All reported adverse events were expected and relevant to patients with cancer, receiving chemotherapy. Main adverse events during six months of treatment were fatigue (88%), anorexia (78%), neuropathy (64%) and anemia (50%). No serious adverse event relevant specifically to melatonin was reported.

Table III. *Adverse events reported during six months of chemotherapy treatment.*

Adverse event	Number of events during treatment month 1-7		
	Placebo	Melatonin	Total
Fatigue	39	89	128
	86.7%	89.0%	88.3%
	(n=45)	(n=100)	(n=145)
Anorexia	31	82	113
	68.9%	82.0%	77.9%
	(n=45)	(n=100)	(n=145)
Neuropathy	31	62	93
	67.4%	62.6%	64.1%
	(n=46)	(n=99)	(n=145)
Anemia	26	48	74
	56.5%	47.1%	50.0%
	(n=46)	(n=102)	(n=148)
Nausea	18	41	59
	40.0%	40.6%	40.4%
	(n=45)	(n=101)	(n=146)
Liver dysfunction	13	21	34
	28.3%	21.0%	23.3%
	(n=46)	(n=100)	(n=146)
Mucositis	10	24	34
	22.2%	24.0%	23.4%
	(n=45)	(n=100)	(n=145)
Low glomerular filtration rate	7	12	19
	15.2%	11.8%	12.8%
	(n=46)	(n=102)	(n=148)
Febrile neutropenia	3	7	10
	7.1%	7.1%	7.1%
	(n=42)	(n=98)	(n=140)
Thrombocytopenia	2	4	6
	4.3%	4.0%	4.1%
	(n=46)	(n=100)	(n=146)

DNA damage marker. Patients who provided urine samples during all the first three months of treatment were included for 8-oxydG analysis. There were 12 such patients in the placebo-treated group, 9 in the group treated with 10 mg melatonin, and 10 in that treated with 20-mg melatonin. Slightly higher levels were found at baseline in the group treated with 10 mg melatonin (median=14.3 ng 8-oxydG/mg Cr) than the others (20 mg melatonin, median=5.7 ng 8-oxydG/mg Cr; placebo, median=8.7 ng 8-oxydG/mg Cr), but not significant. Figure 4 shows that the levels of 8-oxydG between pre-treatment (month 1) and post-treatment (month 2 and 3) remained rather stable in the melatonin-treated groups, compared to greater fluctuation in the placebo-treated group. Only in the placebo group were higher levels of 8-oxydG during the 3-month chemotherapy treatment correlated with lower survival (r^2=–0.656, p=0.02).

Discussion

Rapid deterioration of disease places greater emphasis on the importance of the quality of the remaining life of such patients. Despite patients having advanced cancer stages, melatonin seemed to affect patients' HRQoL showing slight improvement in the melatonin-treated group compared to those treated with placebo. The sample size, however, precludes detection of statistical significance. Nevertheless, a slightly better score (2.69 points, 95% CI=0.01-5.38, p=0.049) was found in social well-being. Future studies with a larger sample size are required to confirm the findings. Likewise, initiation of melatonin in earlier stages of cancer might have a more marked effect on a patient's HRQoL.

The current findings show that using melatonin in combination with chemotherapy did not affect adverse events and survival of patients with advanced NSCLC. The results are different from previous studies where reduced adverse events and improved survival were reported. It is believed that poorer prognosis and less responsiveness to chemotherapy treatment in this study population was the underlying cause for the limited effect of melatonin observed in this study. In Lissoni *et al.*'s study of NSCLC, patients were more responsive to treatment, with a median survival of 12 months (28). In our study, the median survival was only 7.3 months. This poorer response could be due to less aggressive chemotherapy treatment, as 30% of our patients did not receive second-line chemotherapy. It is also possible that our population had a more aggressive cancer subtype as there is growing evidence that this Thai cohort may represent a genetically predisposed cisplatin-resistant group (29).

To be representative of the true population of patients with cancer, patients with poorer prognosis with ECOG scores greater than 1 were recruited into the study, and unfortunately, a higher ratio into the melatonin group, which greatly affected the survival results. Most previous melatonin studies have recruited patients with ECOG score less than 2, and one recruited patients with ECOG scores of less than 1 (28, 30). In the present study, however, 19 patients (14%) had ECOG scores of 2 or more, of whom 14 were treated with melatonin and five with placebo. The negative effect of poorer ECOG scores was confirmed in analysis of the 1-year survival rate, adjusted for age, gender, treatment, weight loss, baseline HRQoL, and ECOG score. It showed that each point of increase in ECOG score increased the risk of death by two-fold (hazard ratio=2.05; 95% CI=1.25-3.38), p=0.005).

Additionally, in previous studies, patients had taken melatonin until disease progression (28, 30), some of whom had survived up to five years. In our study, patients had a much lower survival rate, with 44% (67 out of 151) of patients dying within six months of the treatment. It should be noted that for patients who survived and completed the six months of study medication, those in the melatonin-

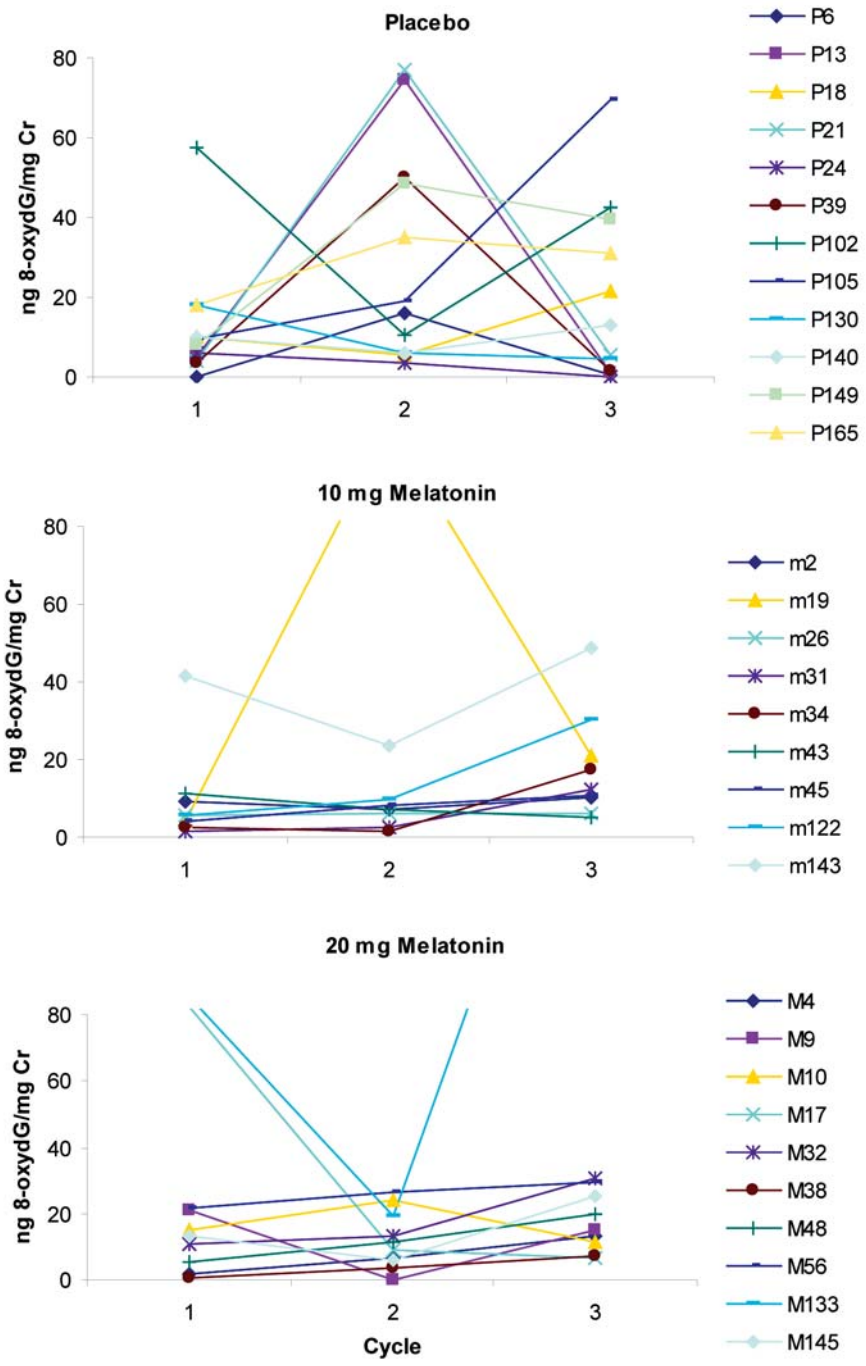


Figure 4. *8-oxydG levels relative to urinary creative (Cr) at each month of chemotherapy (cycle) for each treatment group. Each line represents an individual patient.*

treated group survived up to 28 months, but patients only survived 20 months when taking placebo. Therefore, it is believed that the initiation of melatonin treatment in earlier stages of disease and for – duration longer than six months should be more beneficial for patient survival.

This study did not find a significant reduction of adverse events in the melatonin-treated groups. This could be due to small sample size and limited evaluable data as many reports were missing due to death and loss to follow-up. Unlike the quality of life assessment where there are methodologies for

handling such missing data, this study cannot report the true effects of melatonin on adverse events.

Cisplatin and its related drugs are the first-line chemotherapeutic agents for NSCLC. The anticancer activity of cisplatin is the result of formation of inter- and intrastrand DNA crosslinks or monoadducts that inhibit DNA replication and transcription, preventing tumor growth (31, 32). Oxidative stress is a secondary effect, pushing cancer cells towards apoptosis, but also reducing systemic serum antioxidant concentrations as they are consumed by ROS (33). Toxicity as a direct result of high levels of ROS (34, 35) causes oxidative damage, not just to cancer cells, but also to healthy cells systemically, giving rise to undesirable adverse events. Therefore, protecting healthy cells from the undesirable side-effects of oxidative stress (ROS) during chemotherapy by using antioxidants seems highly desirable. Anticancer activity, however, should not be affected, as cancer cells undergo death *via* a different mechanism (cisplatin-adducts preventing DNA replication). A recent cell line study of melatonin in combination with cisplatin showed synergistic effect for pathways leading to cancer cell apoptosis (36).

We have previously shown in this same study group that chemotherapy causes markedly increased oxidative stress (measured by 8-isoprostane marker) due to cellular and subcellular membrane damage (37). Measuring 8-oxydG, a marker of DNA damage/repair, indicates the level of damage from ROS to both healthy and cancer have cells as a result of chemotherapy. Previous studies shown that after chemotherapy, 8-oxydG levels remain high and do not return to baseline (38). In this study, the baseline 8-oxydG levels were comparable with those reported for healthy people (1-28 ng 8-oxydG/mg Cr) (39), with a few patients having levels higher than 40 ng 8-oxydG/mg Cr. After chemotherapy, 8-oxydG levels in the placebo-treated group increased markedly for almost all patients (compared to the melatonin-treated groups). This might indicate that melatonin reduces the amount of DNA damage and repair occurring by protecting healthy cells against oxidative damage. Interestingly, higher levels of 8-oxydG in the placebo-treated group were also associated with worse survival. Reduced systemic damage to healthy cells might contribute to improved HRQoL as seen in this study despite deterioration of the disease. The decrease in adverse events reported by Lissoni *et al*. are also likely due to these same mechanisms (28, 30).

Conclusion

When used in combination with chemotherapy, melatonin did not significantly affect survival and adverse events of patients with advanced NSCLC, but there was a trend for better HRQoL in the melatonin-treated groups, with a significantly better score in social well-being. Higher levels of 8-oxydG, reflecting DNA damage/repair was observed in the placebo-treated than melatonin-treated groups and was associated with lower survival (r2=–0.656, p=0.02), implying the protective effect of melatonin on healthy cells. A limited sample size and a study population with potentially poor prognosis and less responsive to chemotherapy treatment might have undermined the full benefit of melatonin. Future studies with a larger sample size are warranted and initiation of melatonin in earlier stages of cancer and for a longer duration should be considered clinically important.

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