Original Article

The effect of sleep deprivation and disruption on DNA damage and health of doctors

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Summary

Observational studies have highlighted the detrimental health effects of shift work. The mechanisms through which acute sleep deprivation may lead to chronic disease have not been elucidated, but it is thought that increased DNA damage or decreased repair can lead to disease. The objective of this study was to examine the effects of acute sleep deprivation on DNA damage. This was a cross-sectional observational study on 49 healthy, full-time doctors. Baseline blood was sampled from each participant after three consecutive days of adequate sleep. Participants (n = 24) who were required to work overnight on-site had additional blood sampled on a morning after acute sleep deprivation. DNA damage and expression of DNA repair genes were quantified. Information on health, working patterns and sleep diaries were collected. Independent t-tests were used to compare differences between groups and standardised mean differences expressed as Cohen's *d*. Overnight on-site call participants had lower baseline DNA repair gene expression and more DNA breaks than participants who did not work overnight (d = 1.47, p = 0.0001; and 1.48, p = 0.0001, respectively). In overnight on-site call participants, after acute sleep deprivation, DNA repair gene expression was decreased (d = 0.90, p = 0.0001) and DNA breaks were increased (d = 0.87, p = 0.0018). Sleep deprivation in shift workers is associated with adverse health consequences. Increased DNA damage has been linked to the development of chronic disease. This study demonstrates that disrupted sleep is associated with DNA damage. Furthermore, larger prospective studies looking at relationships between DNA damage and chronic disease development are warranted, and methods to relieve, or repair, DNA damage linked to sleep deprivation should be investigated.

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Accepted: 12 November 2018
Keywords: antioxidants; clinician on call; DNA damage; sleep deprivation
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This article is accompanied by an editorial by Fuller and Eikermann, *Anaesthesia* 2018; doi:10.1111/anae.14479.

Introduction

DNA damage and repair are dynamic processes that may be affected by various conditions and cellular events. Animal studies have shown that sleep loss can induce genetic damage in different organs [1–4]. Sleep deprivation is known to induce oxidative stress, and there is increasing interest in genomic changes related to sleep deprivation [5, 6]. In a study involving participants aged over 60 years, one night of partial sleep deprivation increased gene expression indicative of DNA damage and gene expression consistent with biological ageing [7]. Studies in older adults may not be applicable to a younger, working population,

but sleep deprivation is not uncommon in this group, and studies of sleep deprivation and its effect on DNA damage in younger people are lacking. Chronic sleep deprivation can lead to respiratory disease, and experimental studies using urinary 8-hydroxydeoxyguanosine as an indirect marker of DNA damage suggest night-shift workers might display impaired functioning of DNA repair [8]. However, urinary 8-hydroxydeoxyguanosine concentrations are dependent on levels of DNA damage and DNA repair activity, so interpretation can be complicated.

Direct quantification of DNA strand breaks or oxidised bases would be helpful in understanding the effect of sleep deprivation on DNA damage and repair. Both the alkaline comet assay and formamidopyrimidine DNA glycosylase (FPG)-assisted comet assay are widely applied, highly sensitive tests for the most common DNA lesions, including single- or double-strand breaks and alkali-labile sites (e.g. base and phosphate alkylation), and oxidised purines (specifically 8-oxoguanine) and alkylation damage, respectively [9]. By employing both versions of the comet assay, we aimed to detect the presence of DNA damage as a result of different damage mechanisms in an occupationally sleep-deprived population.

Methods

We obtained ethical approval from the Hong Kong East and Hong Kong West Cluster Research Ethics Committees, and recruited 51 subjects from two hospitals. Written informed consent was obtained from all participants. Participants were assigned into two groups according to their occupational requirement to work on call overnight on-site.

The on-site call group were full-time clinicians required to work regular overnight on-site calls no less than three times per month. The control group were full-time clinicians who were not required to work overnight on-site calls. Exclusion criteria were as follows: all chronic and acute illnesses; the consumption of any medications or dietary supplements, including vitamins and fish liver oil; all smokers; those consuming alcohol outside of UK Chief Medical Officer guidelines; habitual over-consumption of alcohol; those with a BMI of greater than 27.5; self-reported insomnia; and those who had undergone general anaesthesia in the past month.

We conducted the study over a four-month period. Baseline blood samples were taken on a morning after three consecutive days of self-reported adequate, undisturbed sleep from both groups of participants. Additional blood was sampled from the on-site call group participants on the morning after acute sleep deprivation, which was defined as after an overnight call with participants sleeping less than two sleep cycles (3 h) during their call. All blood samples were taken in the morning, kept at 4°C and used within 3 h of sampling. Data on health status, lifestyle, working patterns and a sleep diary were collected from all participants.

The outcomes of interest were oxidation-induced lesions in DNA and 8-oxoguanine DNA glycosylase (OGG1) activity, measured using two versions of the comet assay performed on lymphocytes harvested from venous blood, and the expression in lymphocytes of several DNA repair genes. Lymphocytes were harvested from venous blood. DNA damage and OGG1 activity were measured using the FPG-assisted comet assay. The comet assay measures strand breaks, and FPG (a microbial analogue of OGG1) creates breaks at sites of oxidation-induced lesions in DNA. The extent of damage was measured as a percentage of DNA in the comet tail of examined cells. Cells treated with a buffer in place of FPG revealed the amount of pre-existing DNA damage (as strand breaks) in lymphocytes, and cells treated with buffered FPG solution revealed the pre-existing strand breaks plus those created by the action of the enzyme on oxidation-induced lesions.

For the detailed protocol, please refer to [9]. For each sample, 50 nucleoids at random were scored in each of two gels treated with FPG, and in each of two gels treated, in parallel, with buffer. The difference between the average DNA score (as a percentage of DNA in comet tail) of (1) the 100 buffer-treated nucleoids (a measure of pre-existing single strand breaks) and (2) the 100 FPG -treated nucleoids (a measure of pre-existing plus single strand breaks created by the action of FPG at oxidation-induced lesions) of each sample was taken as the measure of oxidation-induced DNA damage.

We extracted total RNA within 2 h of collection from blood samples using commercial equipment (Roche, Basel, Switzerland). The mRNA extracted was converted immediately after extraction to cDNA using commercial equipment (RevertAid First Strand cDNA Synthesis Kit, Thermo Scientific). We aliquoted and stored cDNA at −80°C. One aliquot was used for the simultaneous testing in triplicate of the expression of the three genes of interest, namely *XRCC1*, *OGG1* and *ERCC1*, and the housekeeping gene, *HPRT1*, by real-time polymerase chain reaction (PCR). Details of the primers used are available upon request.

Plasma total antioxidant capacity was determined using the ferric reducing ability of plasma (FRAP) assay [10].

Based on data from a pilot study (conducted by the same investigators at Queen Mary Hospital), 23 participants were required in each group to detect a true difference in oxidation-induced DNA lesions (using the FPG-assisted

Table 1 Characteristics of on-site call participants and control participants. Values are number (proportion) and mean (SD).

	On-site call n = 24	Control n = 25
Men	9 (38%)	15 (60%)
Women	15 (63%)	10 (40%)
Age; yrs	28.04 (2.42)	33.88 (7.78)
Hours of sleep before baseline blood taken	7.02 (0.74)	6.94 (0.68)
Hours of sleep 2 days before baseline blood taken	7.06 (1.24)	7.48 (1.24)
Hours of sleep 3 days before baseline blood taken	7.60 (2.44)	6.90 (1.14)
Specialties		
Anaesthesia	10 (42%)	4 (16%)
Ear, nose and throat		3 (12%)
Intensive Care Unit	5 (21%)	
Internal medicine	8 (33%)	1 (4%)
Neurosurgery		2 (8%)
Nuclear medicine		1 (4%)
Ophthalmology		2 (8%)
Orthopaedics		1 (4%)
Pathology		2 (8%)
Paediatrics	1 (4%)	
Radiology		8 (32%)
Urology		1 (4%)

comet assay) of at least ± 1.60% at 80% power and $p < 0.01$ with a mean (SD) of 4.5 (1.58)%. We used the paired t-test to compare differences between baseline and postacute sleep deprivation results of on-site call group participants. We used the unpaired t-test to compare results between the on-site call group and no on-site call group. Results were analysed cautiously, using a smaller p value ($p < 0.005$) as a threshold for statistical significance [11]. Effect-size differences between groups and between baseline and postcall values are given as Cohen's *d*.

Results

We analysed data from 24 on-site call group participants and 25 control group participants (Table 1). The on-site call participants had been working these overnight shifts for between one and ten years. Most on-site call group participants were required to work five to six overnight on-site calls per month and achieved between 2 h and 4 h of sleep during calls. Only three out of 24 on-site call group participants habitually napped for between 1-2 h before starting their overnight call shift. Control group participants included those clinicians who were not required to work overnight on-site calls, and also those with no call duties. A majority of control group participants had been exempt from on-site call duties for between 3–5 years, and the time ranged from < 1 to > 10 years. Most control group participants reported having to travel back to hospital for work after midnight once to twice per month during off-site calls. The mean number of hours slept during the three consecutive days before baseline blood sampling was 7.60 h and 6.90 h for on-site call group and control group participants, respectively.

Gene expression data are given as the ratio to the housekeeping gene *HPRT1*. The on-site call group participants demonstrated statistically significantly lower *OGG1* and *ERCC1* gene expression at baseline compared with the control group (Table 2). The on-site call group exhibited lower baseline *XRCC1* expression, although this did not reach statistical significance. On-site call group participants demonstrated more DNA breaks and alkali-labile sites at baseline. There were no differences in baseline oxidised purines and antioxidant capacity between groups.

After acute sleep deprivation in on-site call group participants, DNA repair gene expression (Fig. 1) and plasma antioxidant capacity were decreased. DNA breaks/alkaline-labile sites and oxidised purines were increased after sleep deprivation (Fig. 2 and Table 2).

Two on-site call group participants were subsequently found to be regular consumers of dietary supplement pills. The results from these two participants have been removed from the data presented in this manuscript, but it is to be noted that the overall results with these participants included, and subsequently with them excluded, did not differ.

Sub-group analysis of on-site call group participants (n = 24) comparing anaesthetists (n = 10) with doctors working outside operating theatres (n = 14) showed no difference in DNA damage and antioxidant capacity at baseline and after acute sleep deprivation.

Discussion

This study is the first to quantify DNA damage directly in young adults who are required to work overnight shifts. The results demonstrate that acute sleep deprivation and a frequently disrupted sleep cycle are associated with DNA damage, which is concordant with findings of genotoxicity

Table 2 Gene expression, DNA damage and repair and plasma antioxidant capacity of on-site call and control group participants. Values are mean (SD).

	On-site call n = 24 baseline	Control n = 25 baseline	p value (on-site call vs. Control)	Cohen's *d* (on-site call vs. control)	on-site call n = 24 post-call	p value (baseline vs. post-call)	Cohen's *d* (baseline vs. post-call)
XRCC1	0.68 (0.015)	0.75 (0.014)	0.0025*	0.83	0.65 (0.054)	0.0061*	0.49
OGG1	0.84 (0.011)	0.93 (0.011)	0.0001*	1.47	0.79 (0.056)	0.0001*	0.90
ERCC1	0.88 (0.011)	0.94 (0.005)	0.0001*	1.27	0.83 (0.013)	0.0001*	0.87
DNA breaks;	1.79 (0.37)	1.37 (0.15)	0.0001*	1.48	2.23 (0.63)	0.0012*	0.87
Oxidised purines;	8.17 (1.31)	9.06 (1.54)	0.0303	0.64	11.14 (1.39)	0.0001*	2.25
Antioxidant capacity; μM	945.3 (232.5)	936.9 (204.4)	0.8912	0.04	830.2 (171.4)	0.0015*	0.50

*Statistically significant.

in sleep-deprived animals and elderly adults [4]. This present study shows that DNA repair gene expression is lower at baseline among night workers and further decreases after acute sleep deprivation, which supports the postulation that night workers demonstrate impaired DNA repair [12, 13].

DNA damage is a change in the basic structure of DNA that is not repaired when the DNA is replicated. Double-strand breaks are particularly hazardous, as repair failure causes genomic instability and cell death, whereas disrepair can lead to inappropriate end-joining events that commonly underlie oncogenic transformation. This study has shown that doctors who are required to work overnight on-site calls demonstrate 30% higher DNA breaks and alkali-labile sites as compared with those doctors not required to work overnight, and this DNA damage is further increased by over 25% after a night of acute sleep deprivation.

The DNA repair genes, *ERCC1*, *OGG1* and *XRCC1*, examined in this study are involved in nucleotide excision repair, base excision repair and recombinational repair. Decreased or absent expression of these genes is associated with accumulation of DNA damage, increased rates of mutation and tumorigenesis [14, 15]. Significantly lower baseline *OGG1* and *ERCC1* expression, and decreases in expression of all three genes investigated after acute sleep deprivation, suggest impairment of DNA repair activity in sleep-deprived people. It is important to note that overnight on-site call participants in this study were young and healthy, and people from this age group are generally known to demonstrate better tolerance of sleep disturbance [7, 16]. It should be noted that participants in the on-site call group appeared to be younger than those in control group; this is because younger residents are required to work overnight on-site calls, and this may be a limitation of the current study.

Given the paucity of relevant human literature, we should question how these findings should be interpreted and applied clinically. However, sleep deprivation and shift work have been implicated as risk factors for the development and progression of various chronic diseases. A meta-analysis of sixteen prospective cohort studies involving more than 2,000,000 participants suggested a dose–effect relationship between night-shift work and the incidence of breast tumours (risk ratio 1.057) [12]. The findings with respect to cancer at other sites are inconsistent [17]. Two studies suggested that shift workers are at increased risk of prostate cancer, contrary to findings from a large prospective cohort [17, 18]. With regard to colorectal cancer, nurses on rotating night shifts for 15 years or more were found to be at increased risk, whereas a prospective

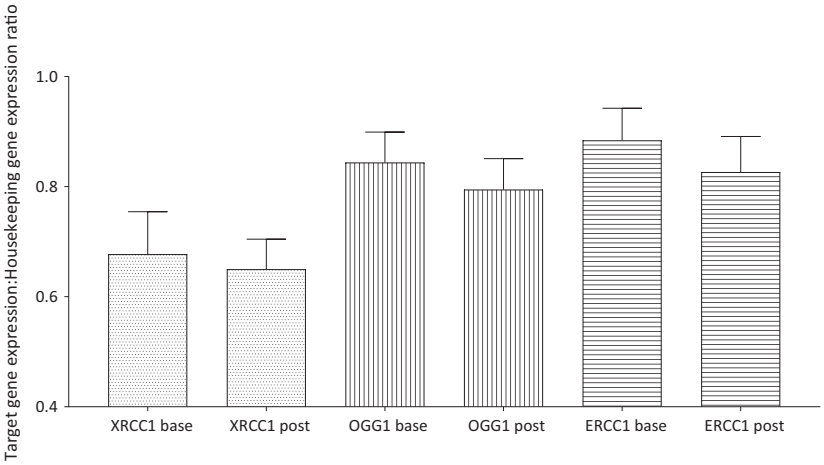

Figure 1 Baseline (base) vs. post-call (post) gene expression in 24 subjects. Values are mean (SD). The paired t-test was used to detect differences between the two time-points. Data are expressed as the ratio of gene expression between the gene of interest compared with expression of housekeeping gene.

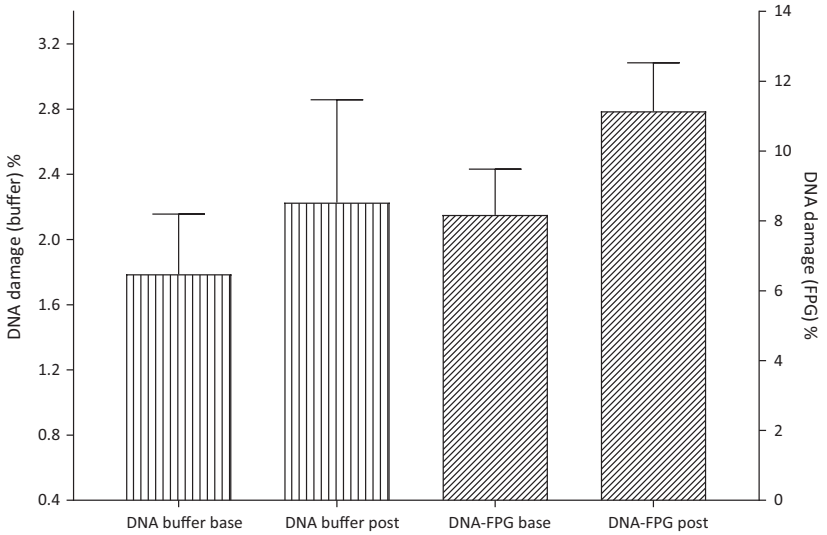

Figure 2 Baseline vs. post-call DNA damage in cells treated with buffer (buffer) and cells treated with FPG. The paired t-test was used to detect differences between the two time-points. Data are expressed as proportion of DNA damage. FPG, formamidopyrimidine DNA glycosylase.

study on the general population and telegraph operators showed no significant association between night shifts and colorectal cancer [19, 20].

Short sleep duration (< 6.5 h) is associated with mortality in advanced cancer patients in a curvilinear manner [21]. Meta-analyses have shown that shift work is associated with increased risk of myocardial infarction, coronary events and ischaemic stroke [22]. Short sleep duration (< 5 h or 6 h) is associated with a significant increase in the risk of the metabolic syndrome [23]. Meta-analysis of 12 studies involving > 226,000 participants showed that shift work may increase the risk of diabetes [24]. Sleep disruption may be implicated in neurodegeneration. In a prospective actigraphy study of > 1200 healthy elderly women, decreased circadian rhythm amplitude and robustness was associated with an increased risk for mild cognitive impairment or dementia over the next 5 years [25]. In a mouse model, chronic mild sleep restriction has been associated with impairments in contextual and cued memory, and an increase in amyloid-β and insoluble tau, hallmarks of the development of Alzheimer's disease [26]. Whether sleep disturbance is an early symptom or a contributor to the pathophysiology of neurodegenerative disease is still unknown.

Multiple mechanisms to explain the predisposition of shift workers to chronic diseases have been postulated. Disrupted circadian rhythm and suppression of nocturnal melatonin causes altered endogenous sex hormone balance, desynchrony of clock genes and the expression of genes implicated in cancer development. Heightened neuroendocrine stress responses, higher glucocorticoid and catecholamine levels, disrupted appetite control, pro-inflammatory responses, immunosuppression and changes in lifestyle, including diet and physical activity, are possible contributing factors to increased chronic disease susceptibility during periods of disrupted sleep [27–29].

Our study presents evidence of genetic damage and impairment of DNA repair associated with occupational sleep deprivation in young people. This biomolecular evidence may serve as another basis for the increased risk for malignancies and cardiovascular, metabolic and neurodegenerative diseases. However, further mechanistic studies evaluating the relationship between DNA damage and development of these chronic diseases is required.

We undertook sub-group analysis of on-site call group participants dividing this group into anaesthetists and clinicians who worked outside the operating theatre. No significant differences in DNA damage and antioxidant capacity at baseline and after acute sleep deprivation were found (data not shown), suggesting that chronic occupational exposure to volatile gases used in operating theatres is not associated with genotoxicity [30].

This study has a number of strengths. Participants were all healthy and did not suffer from any chronic disease. Secondly, we used the alkaline comet assay, the current gold standard for evaluation of DNA damage, rather than using surrogate biomarkers of DNA damage. This study is the first to employ the comet assay to investigate the effect of sleep deprivation and sleep cycle disturbance on DNA damage in humans. The successful application of these techniques represents a novel approach to evaluating sleep deprivation and disruption on human genotoxicity. Thirdly, we used a stringent p value for statistical significance, increasing the reproducibility of the results.

DNA damage results obtained using the comet assay have not been investigated in prospective cohort studies, and therefore its predictive value for chronic disease development and the clinical significance of the laboratory findings are unclear. In addition, higher baseline DNA damage in on-site call clinicians could possibly be linked with a frequently disrupted sleep cycle, but it could also be confounded by other factors, including greater work stress in specialties with on-site calls, different occupational risk exposure (e.g. to radiation) between specialties, and other personal factors which are difficult to quantify. We attempted to recruit clinicians from as many different specialties as possible in each group to balance out the confounding effect of the different nature of various specialties.

Although data on the number of sleeping hours on the preceding three consecutive days before baseline blood sampling was collected, sleep adequacy was self-defined. Similarly, acute sleep deprivation was self-reported, and it was not possible to monitor this. Nevertheless, the data as presented reflect the real-life situation of clinicians who are required to perform shift duties. Sleep demand and habits can vary greatly between individuals, and in addition to the number of hours slept, sleep quality is also an important factor determining sleep adequacy. It was beyond the scope of this study to perform psychosocial and behavioural tests after sleep deprivation, and therefore it was not possible to ascertain the extent that one night of sleep deprivation would have on the mental performance of clinicians.

Finally, participants were mostly young Chinese doctors, and therefore the results of this study can only be applied to this population. The genomic consequence of sleep deprivation and disruption may vary as a function of ethnicity, locality and age groups. Another limitation to this study was that sleep scale questionnaires were not collected from the participants. This is because the sleep scales, although validated, are nevertheless subjective and sleep diaries were considered adequate for our current study.

In conclusion, this current study has clearly demonstrated that oxidatively damaged DNA is increased after only one night of acute sleep deprivation. The significance of this DNA damage requires verification in larger, prospective studies. The theory that oxidative stress leading to impaired DNA repair is the 'common soil' of pathogenic mechanisms underlying chronic disease development was first proposed over two decades ago [31]. Future studies should be conducted to evaluate if this DNA damage can be attenuated by the use of antioxidants or agents to promote recovery sleep after acute sleep deprivation.

Acknowledgements

Special thanks to Dr. D. LAM Ming-hon, Department of Anaesthesiology, Queen Mary Hospital for his assistance in data collection and blood-taking. SWC is statistical advisor for *Anaesthesia*, and therefore the manuscript has undergone external independent review. The other authors

have no competing interests to declare. This study was funded by the Department of Anaesthesiology, University of Hong Kong.

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