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# Evaluation of Tetrahydrobiopterin Pathway in Operating Room Workers: Changes in Biopterin Status and Tryptophan Metabolism

**Mustafa Baydar · Zeynep Capan · Gozde Girgin · S. Sezin Palabiyik · Gonul Sahin · Dietmar Fuchs · Terken Baydar**

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**Abstract** The aim of the study was to evaluate the effect of anesthetics as operating room contaminants on tetrahydrobiopterin pathway in 40 operating room personnel and 30 healthy controls by measuring biopterin, dihydrobiopterin reductase, tryptophan, kynurenine and serotonin. Biopterin concentrations were 124 ± 12.3 μmol/mol creatinine in workers and 88 ± 5.7 μmol/mol creatinine in controls whereas kynurenine concentrations were 1.75 ± 0.09 μM and 1.95 ± 0.06 μM, respectively (both, $p < 0.05$). It can be claimed that enhanced biopterin and diminished kynurenine levels may play a triggering role in disruption of metabolic events in operating room personnel.

**Keywords** Tetrahydrobiopterin · Tryptophan · Kynurenine · Operating room personnel

M. Baydar
Clinic of Anesthesiology, Numune Hospital, Ankara, Turkey

Z. Capan
Clinic of Anesthesiology, Ankara Hospital, Ankara, Turkey

G. Girgin · S. S. Palabiyik · G. Sahin · T. Baydar (✉)
Department of Toxicology, Faculty of Pharmacy, Hacettepe University, 90-06100 Ankara, Turkey
e-mail: tbaydar@hacettepe.edu.tr

S. S. Palabiyik
Department of Toxicology, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey

G. Sahin
Department of Toxicology, Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, T.R. North Cyprus

D. Fuchs
Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Innsbruck, Austria

Workplaces harbor many chemical, biological and physical agents that may cause health problems. Among these agents, chemicals occupy a major place. Since long-time occupational exposures cause harmful effects even at low doses, serious measures should be taken for occupational health and safety (Phoon 1995; Thorn 2001). Operating theatres in health services have a special importance among the workplaces as a result of their ubiquitous nature. The health workers such as surgeons, anesthesiologists, technicians and nurses are exposed to a variety of noxious agents in this workplace. Trace amounts of waste anesthetic gases, cleaning substances, solvents and also various infectious agents are present in operating theatres (Thorn 2001; Gruber et al. 2002; Nilsson et al. 2005). Modern air-conditioning and -scavenging systems are established in order to reduce the risk caused by inhalational anesthetics in air. However, sometimes the residual concentrations may exceed the upper limits mentioned in national guidelines and may cause chronic exposure in operating room workers (Irwin et al. 2009; Wood et al. 1992). Nevertheless, the possible noxious agents in anesthetic work place and job stress may also affect many organs and/or functions including neurologic and immune system in these workers (Gruber et al. 2002; Byhahn et al. 2001). It was reported that, compared to non-exposed females, women workers in 8,032 hospital staff exposed to waste anesthetic gases has significantly increased frequencies of spontaneous abortion and giving birth with congenital abnormalities (Guirguis et al. 1990). Burnout, depression and reductions in cognitive, perceptual and motor skills have been observed to be common symptoms of these agents in the workers. Precise mechanisms and possible relationship between these gases and observed side effects still remain unclear (Wood et al. 1992; Sasskortsak et al. 1981). The possible waste anesthetics in operation rooms' air may also

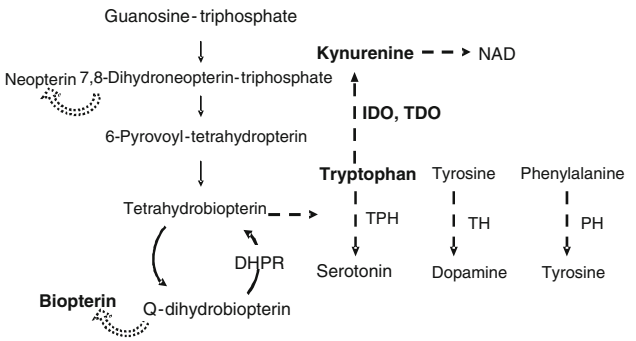


**Fig. 1** Overview of the biosynthesis of tetrahydrobiopterin as a cofactor in the production of neurotransmitters and its regeneration pathway via the enzyme dihyropteridine reductase (DHPR), and tryptophan conversion: serotonin and kynurenine as two major metabolites occur. Kynurenine pathway is the first step in the biosynthesis of cofactor nicotinic acid dinucleotides (NAD). *TPH* tryptophan hydroxylase, *TH* tyrosine hydroxylase, *PH* phenylalanine hydroxylase, *IDO* indoleamine 2,3-dioxygenase, *TDO* tryptophan 2,3-dioxygenase

produce oxidative stress, sensitization, inflammation, and modulation of the immune system (Thorn 2001; Gruber et al. 2002). Our previous study showed that operating room staff had increased urinary neopterin when compared to a non-exposed group (Baydar et al. 2011). This finding prompted a further study to obtain some idea about mechanism of their effects due to chronic low exposure. 5,6,7,8-Tetrahydrobiopterin ($BH_4$), which has a key role in metabolisms of neurotransmitters, is an endogenously synthesized co-factor in pteridine pathway. Regeneration of $BH_4$ from its oxidized forms is particularly important in biological system as shown in Fig. 1. The enzymes, pterin-4α-carbinolamine dehydratase and dihydropteridine reductase (DHPR) are responsible for the regeneration of $BH_4$ (Wei et al. 2003; Werner et al. 2011; Thony et al. 2000). The present study was undertaken to evaluate tetrahydrobiopterin pathway in operating room workers by measuring urinary biopterin concentrations, blood DHPR enzyme activity, and also to by examining tryptophan metabolism. To estimate the activities of various enzyme pathways involved, kynurenine to tryptophan, serotonin to tryptophan, serotonin to kynurenine and the biopterin to tryptophan ratios were calculated.

## Materials and Methods

The group of 40 operating room workers including surgeons, anesthesiologists, and anesthesia technicians from the surgery and anesthesiology clinics of Numune and Ankara Hospitals volunteered to participate in the study. A questionnaire was designed for each subject to yield information on sex, age, occupational history and general health status. The mean age of the operating room personnel was 34 ± 8 years (19 female and 21 male). The working durations ranged from 1 to 18 years and the 45 % of the study group had been working more than 10 years. A non-exposed control group comprised 30 healthy people (21 female and 9 male, mean age 29 ± 6 years) who did not work in the operating rooms. In both groups, all of the participants did not have any systemic diseases and were not receiving any medication during sample collection period. The principles of the Ethical Committees of the Hospitals according to the Helsinki Declaration were followed during the whole study. Blood and urine samples were collected early in the morning. Biopterin concentrations were determined in urine samples. For determination of DHPR activity, appropriate amount of blood was dropped on a filter paper. After centrifugation of blood specimens at 3,500 rpm for 15 min, serum samples were collected in order to measure tryptophan, kynurenine and serotonin concentrations. All samples were stored at −20°C until assayed and kept from direct light. Biopterin and creatinine concentrations were analyzed by high performance liquid chromatography (HPLC, HP Agilent 1100, Vienna, Austria) as described before (Girgin et al. 2010). Biopterin levels were expressed as μmol per mole creatinine. DHPR enzyme assay was performed on stored dried blood spots. Enzyme activity was measured spectrophotometrically at 550 nm wavelength (Shimadzu UV160, Japan) following the BH4-dependent reduction of ferricytochrome C in the presence of NADH (Girgin et al. 2010). The enzyme activity was expressed as nanomoles of cytochrome C reduced per min relative to the 6 mm diameter of blood spots. Serotonin levels, products of tryptophan metabolism, were measured by enzyme-linked immunosorbent assay (ELISA) kits which were obtained from DIAsource Immunoassays SA (Nivelles, Belgium) according to the kit procedure provided by manufacturer. Tryptophan and kynurenine concentrations were determined by HPLC as described before (Laich et al. 2002). The tryptophan concentrations were expressed as mM while kynurenine levels were given as μM. The kynurenine to tryptophan ratio and serotonin to tryptophan ratio were calculated to estimate the degree of tryptophan conversion. The mean values for each parameter were expressed with standard error (SEM). Differences among groups were evaluated with non-parametric Kruskal–Wallis analysis of variance while Mann–Whitney *U* test was used to compare the differences between two independent groups. Spearman rank correlation test was used to investigate the correlations of the parameters, $p < 0.05$ was considered to indicate statistical significance.

## Results and Discussion

The mean concentration of biopterin was 124 ± 12.3 μmol per mol creatinine in the exposed-workers and thus higher

than in non-exposed subjects (88 ± 5.7 μmol per mol creatinine; $p = 0.032$). The mean DHPR activity of the control and exposed groups were 1.48 ± 0.11 and 1.48 ± 0.09 nmol reduced cytochrome C $min^{-1}$ 6 $mm^{-1}$ disc, respectively ($p > 0.05$).

In our previous study in the operating room workers, urinary neopterin, as an early valuable biomarker of immune activation and inflammation has been found to be elevated compared to the controls (Baydar et al. 2011). These results prompted to design the present study as a further investigation of the pteridine pathway. Like neopterin, $BH_4$ is a vital unconjugated pteridine, which is synthesized from guanosine triphosphate by the subsequent action of various enzymes including dihydropteridine reductase (Verbeek et al. 2008). DHPR has a key role in regeneration of $BH_4$ from q-dihydrobiopterin. Biopterin is a stable, oxidized, measurable urinary marker of $BH_4$ (Fig. 1) (Netter et al. 1991). In the present study, urinary biopterin concentrations were found to be higher in operation room workers than in the non-exposed controls expectedly in parallel to urinary neopterin levels. In spite of this, DHPR activity did not differ between the study groups. Because of unchanged regenerating enzyme activity, excessive $BH_4$ regeneration cannot be considered. This situation might be the result of enhanced oxidation of $BH_4$ due to exposure to agents and occupational stress. Still, measurement of biopterin concentrations only allows a rough estimate of $BH_4$ metabolism.

The tryptophan concentrations in the controls were 66 ± 2.1 and 67 ± 1.3 μM in the workers, respectively ($p > 0.05$). Kynurenine concentrations were significantly lower in the workers (1.75 ± 0.09 μM) as compared to the controls (1.95 ± 0.06 μM, $p = 0.014$). The level of serotonin was 1.11 ± 0.07 μM in the control group and 1.32 ± 0.15 μM in the exposed group, respectively ($p > 0.05$). There were no significant correlations between the working duration and measured parameters (all, $p > 0.05$), and there was not any effect of age or gender on the tested parameters (all, $p > 0.05$). It was also tested whether there were any differences between two hospitals, no significant changes were observed (all, $p > 0.05$). The ratios of kynurenine to tryptophan, serotonin to tryptophan, serotonin to kynurenine and biopterin to tryptophan were presented in Table 1.

As shown in Table 1, the kynurenine to tryptophan ratio decreased while the rest of the calculated ratios were increased in exposed group compared to the controls. The ratio of biopterin to tryptophan showed 67 %-elevation in comparison to non-exposed group. It can be thought that this increase in the ratio may also confirm enhanced oxidation of $BH_4$. Tetrahydrobiopterin is a crucial cofactor for phenylalanine hydroxylase and tyrosine hydroxylase, as well as tryptophan hydroxylase in the biotransformation of phenylalanine to tyrosine and to dopamine, and tryptophan to serotonin, respectively (Verbeek et al. 2008). Tryptophan has two major metabolites, serotonin and kynurenine. Kynurenine pathway leads to the production of NAD which is important cofactor in many metabolic pathways, i.e., oxidative phosphorylation (Fig. 1). Tryptophan concentrations were detected and tended to increase in the exposed group, although the difference was not statistically significant. Overall, data may indicate that amine metabolism in operation room personnel is shifted away from the kynurenine pathway towards serotonin and biopterin production. In parallel to measured parameters, two products of tryptophan metabolism were determined to evaluate the direction of tryptophan pathway. In operation room personnel kynurenine levels significantly decreased while serotonin concentrations considerably increased. Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) activity are indicated as the ratio of kynurenine to tryptophan concentrations, and the calculated ratios was found to be significantly decreased in the exposed group. In addition, the ratio of serotonin to tryptophan was calculated because serotonin is a metabolic product of tryptophan. The kynurenine to tryptophan ratio exhibited 10 %-decrease while serotonin/tryptophan showed 43 %-increase. It seems that tryptophan metabolism is directed to work in favor of serotonin. Unlike serotonin depletion, synthesis of serotonin increased in

**Table 1** Ratios of the parameters related to tryptophan degradation pathway and alteration % according to the controls' values

| Ratio | Controls | Operating room personnel | Alteration(%) |
|---|---|---|---|
| Kynurenine/tryptophan (μmol/mmol) | 28.5 ± 1.0 | 25.7 ± 1.0‡ | −10 |
| Serotonin/tryptophan (μmol/mmol) | 0.016 ± 0.001 | 0.018 ± 0.002 | +21 |
| Serotonin/kynurenine (μmol/mmol) | 0.55 ± 0.04 | 0.80 ± 0.07‡ | +43 |
| (Biopterin/creatinine)/tryptophan [(μmol/mol)/mmol] | 1.2 ± 0.1 | 2.0 ± 0.2‡ | +67 |

Controls, healthy volunteers; Exposed, operating theatre personnel; Statistically significant difference: ‡ $p < 0.05$ versus controls

operation room personnel, but it was still within the normal range of serotonin (www.nlm.nih.gov/medlineplus/ency/article/003562.htm).

Inhalation anesthetics are still an important source of occupational hazard in operating theatres due to their widespread use. Possible health problems from long-exposure to inhalation anesthetic residues cannot yet be definitely excluded (Byhahn et al. 2001; Turkan et al. 2005). Routine monitoring of indoor air quality for workplaces and evaluation of health status of workers are useful and important to prevent hazards and reduce risks. New development of reliable exposure biomarkers and understanding the toxicity mechanisms are two important fields with growing interest in order to early diagnose, reduce and heal the hazards (Phoon 1995; Thorn 2001). Considering the comments that anesthesiologists have higher risk for depression, substance abuse and suicide, and anesthesia and anesthesia-related disciplines have lost too many workers through suicide, addiction, severe depression and other causes (Alexander et al. 2000; Maulen 2010), our results suggests that these effects of anesthetics as a contaminant of indoor air in operating theatres seem not to be related with pteridine pathway or serotonin production. In conclusion, enhanced biopterin and diminished kynurenine concentrations observed in this study may play a role as a triggering factor in disruption of crucial metabolic events owing to the decreased production of vital cofactors such as NAD. This situation might be one reason for the cytotoxicity related to exposure of anesthetic agents in the workplaces. It may be claimed that anesthetic gases may have untoward effects on health workers via pteridine pathway and related products. However, further studies are required in order to be clearer on precise molecular mechanisms underlying toxicity of anesthetic agents.

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