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General anesthesia-associated DNA damage in peripheral blood mononuclear cells of surgical patients

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ABSTRACT

Objective: To evaluate retrospectively the effect of general anesthesia on DNA damage in the blood mononuclear cells (PBMCs) of surgical patients in order to provide evidence for a better nursing care during the procedure. **Methods:** Clinical charts of 76 patients who underwent operation under general anesthesia and 76 healthy control subjects with documented results of DNA damage extent in PBMCs from the single-cell gel electrophoresis (SCGE) or comet assay and serum contents of superoxide dismutase (SOD) and malondialdehyde (MDA) from biochemical analyses were reviewed. The percentage of comet PBMCs and tail DNA and serum contents of SOD and MAD were analyzed by student *t*-test. **Results:** Compared with healthy control subjects, generally anesthetized surgical patients had significantly higher % comet PBMCs and % tail DNA ($P<0.05$) and significantly lower serum concentrations of SOD ($P<0.05$) and significantly higher serum concentrations of MAD ($P<0.05$). Compared with levels before general anesthesia in surgical patients, % comet PBMCs, % tail DNA, and serum levels of MAD were significantly higher ($P<0.05$ or 0.01), and serum levels of SOD were significantly lower ($P<0.05$), after general anesthesia. **Conclusions:** General anesthesia during surgery causes a certain degree of hypoxia and PBMC damage. Particular attention should be paid to monitoring and maintenance of blood oxygen saturation in patients undergoing surgery under general anesthesia.

1. Introduction

Oxidative stress response and subsequent oxidative injury and inflammation in patients undergoing surgery and anesthesia may cause a certain degree of damage to the human body. During surgery, anesthesia can reduce pain to some extent and traction-related physical discomfort. However, anesthetics can not completely block autonomic nervous system responses to traumatic stimuli. The autonomic nerve system-dependent responses to traumatic stress remains progressive, resulting in increased oxygen consumption and oxygen free radical production. Moreover, blood flow blockage and physical stretch constantly results in local tissue hypoxia and ischemia during surgery; after recovery of perfusion, atypical ischemia-reperfusion injury occurs, thereby worsening the body damage due to an increased production of oxygen free radicals and a decreased activity of antioxidant[1]. Nevertheless, the detrimental effect of general anesthesia on peripheral blood mononuclear cells (PBMCs) has not been evaluated clinically. This retrospective study was therefore conducted to determine DNA damage extent in PBMCs and blood levels of SOD and MDA in normal healthy subjects as well as in patients undergoing surgery before and after general anesthesia.

2. Materials and methods

2.1. Ethical considerations

The study was reviewed and approved by the Institutional Ethical Review Boards of both Hainan Medical College and

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2.2. Data access

Access was attained to clinical records of the patients who were operated in the Department of Surgery in the Affiliated Hospital of Hainan Medical College between September 2008 and December 2010 and the healthy control subjects who underwent regular annual physical examination in the Outpatient Department of the Affiliated Hospital of Hainan Medical College and at Haikou Physical Examination Center during the same time period.

2.3. Case inclusion criteria

From the access-granted clinical records, surgical cases were retrieved for analyses based on the following criteria: 1) surgical operation under general anesthesia; 2) documented single-cell gel electrophoresis (SCGE) assay of DNA damage in peripheral mononuclear phagocytes and biological measurement of superoxide dismutase (SOD) and malondialdehyde (MAD) contents in serum; 3) free of respiratory disease, cerebrovascular disease, hypertension, hyperlipidemia, kidney disease, diabetes or autoimmune diseases. The inclusion criteria for control subjects were the same as for surgical cases except that no surgery and anesthesia were performed.

2.4. Review and statistical analysis of retrieved cases

The information on major demographic parameters was reviewed for both surgical patients and healthy control subjects. Data on mononuclear cell DNA damage and serum concentrations of SOD and MDA were expressed as mean ±SD and analyzed by student *t*-test. Statistical analysis software SPSS11.5 was used. Difference was considered significant when $P<0.05$.

3. Results

3.1. Demographic characteristics

Seventy-six surgical patients and 76 healthy subjects met the corresponding inclusion criteria were included in the retrospective analyses. The surgical patients aged from 5 to 65 years and the normal control subjects from 3 to 65 years. In the surgical group, 43 patients were male and 33 were female. In the control group, 40 were male and 36 were female. Of the 76 surgical patients, 26 had gastric cancer, 15 had rectal cancer, 27 had lung cancer, and 3 had liver rupture.

3.2. Extent of DNA damage in blood monocytes

Presented in Table 1 are the SCGE assay results. As compared with norm healthy control subjects, the generally anesthetized surgical patients had significantly higher percentage of comet cells and percentage of tail DNA in peripheral blood monocytes both before ($P<0.05$) and after ($P<0.01$) the anesthesia. In surgical patients, the values for both parameters were significantly higher after general anesthesia ($P<0.05$).

3.3. Serum concentration of SOD and MAD

As shown in Table 2, SOD and MAD displayed different patterns of changes between surgical patients and healthy control subjects as well as before and after general anesthesia in surgical patients. More specifically, compared with control subjects, surgical patients had a significantly lower level of serum SOD both before ($P<0.05$) and after ($P<0.01$) general anesthesia. Within surgical patients, serum level of SOD was significantly reduced after general anesthesia ($P<0.05$). Opposite to SOD, MAD concentration in the serum was significantly higher in surgical patients before ($P<0.05$) and after ($P<0.01$) general anesthesia than

Table 1
Percentage of comet cells and percentage of tail DNA in generally anesthetized (GA) surgical patients and healthy control subjects.

Subjects	% comet cells		% tail DNA	
	Prior to GA	1 h Post GA	Prior to GA	1 h post GA
GA	28.95±2.13*	64.47±6.75**	13.16±3.43*	39.47±3.52**
Healthy control	10.53±4.45		7.33±2.53	

Values marked with * and ** are significantly different at $P<0.05$ and $P<0.01$ respectively as compared with the control value within the test parameter.

Table 2
Serum concentration of SOD and MAD in generally anesthetized (GA) surgical patients and healthy control subjects.

Subjects	SOD (U/L)		MAD (μ mol/L)	
	Prior to GA	1 h Post GA	Prior to GA	1 h post GA
GA	105.0±11.2*	45.0±9.3**	15.23±5.70*	141.00±28.70**
Healthy control	224.0±8.4		7.54±3.60	

Values marked with * and ** are significantly different at $P<0.05$ and $P<0.01$ respectively as compared with the control value within the test parameter.

that in normal control subjects; general anesthesia robustly increased the serum level of MAD in surgical patients (P<0.01).

4. Discussion

General anesthesia may result in hypoxia due to malfunction of the respiratory and circulatory systems. It is known that hypoxia injury leads to production of large amount of oxygen free radicals, which are strong DNA breakage agents and can directly damage DNA molecules in a dose- and time-dependent manner[3].

SCGE, which is also known as comet assay, is a sensitive and simple technique for the detection of DNA damage at the level of the individual eukaryotic cell. It involves the embedding of cells in agarose suspension, lysis of the cells in neutral or alkaline (pH>13) conditions, and electrophoresis of the suspended lysed cells which results in an overall structure resembling a comet (a circular head and a tail corresponding to intact and damaged DNA respectively) when observed under a fluorescence microscopy. Given that the fluorescence intensity and the migration length of the comet tail show a good linearity with the extent of DNA damage, % tail DNA is the most meaningful parameter in the SCGE assay[3-5]. In this retrospective study, analyses of the SCGE data showed significant differences in % comet cells and % tail DNA not only between normal healthy subjects and surgical patients but also before and after general anesthesia in the surgical patients. These results indicate that malignant tumors as well as general anesthesia during surgical procedures are associated with increased PBMC DNA damage.

Hypoxia may be one of the mechanisms underlying the general anesthesia-induced DNA damage in PBMCs, as suggested by Jugdutt[5,6]. It is known that general anesthesia results in hypoxia due to malfunction of the respiratory and circulatory systems. Hypoxia injury leads to production of large amount of oxygen free radicals, which are strong DNA breakage agents and can directly damage DNA molecules in a dose- and time-dependent manner[3,6]. In PBMCs, hypoxia-induced release of oxygen free radicals may lead to down-regulation of nitric oxide synthase (eNOS) expression and nitric oxide production, eventually resulting in a decreased antioxidant capacity and oxidative DNA damage and fracture.

SOD can scavenge oxygen anion free radicals, thus protecting cells against oxidative damage. As a result, measurement of SOD contents in serum may indirectly reflect the body's ability to scavenge oxygen free radicals. In contrast, MDA as a degraded metabolite of membrane polyunsaturated fatty acids is associated with the degree of tissue oxidative damage. In this study, our analyses showed a significantly higher serum content of SOD in healthy subjects as compared with the surgical patients before general anesthesia[(224.0±8.4) U/L versus (105.0±11.2) U/L] as well as a significantly decreased serum level of SOD after general anesthesia in surgical patients[(45.0±9.3) U/L versus (105.0±11.2) U/L]. The change in the serum content of MAD showed an opposite pattern: a significant increase in surgical patients both before[(15.23±5.70) μmol/L and after[(141.00±28.70) μmol/L] general anesthesia as compared with that in the normal control subjects[(7.54 ±3.60) μmol/L). Taken together, these changes in serum contents of SOD and MAD indicate damage of PBMCs under general anesthesia.

In summary, this retrospective study has demonstrated that general anesthesia in patients undergoing surgery for various tumors and internal organ lesions like liver rupture, in addition to the surgical procedure itself, may result in a certain degree of hypoxia, which subsequently leads to damage of PBMCs. Our analysis results suggest during the surgical procedure under anesthesia. Therefore, blood oxygen saturation must be tightly monitored in patients undergoing surgery under general anesthesia, which may have profound implications in improving the quality of nursing care of surgical patients.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Pintaudi AM, Tesoriere L, D'Arpa N, D'Amelio L, D'Arpa D, Bongiorno A, et al. Oxidative stress after moderate to extensive burning in humans. *Free Radic Res* 2000; **33**:139-146.

[2] Liu Yumei, Zhang Runling, Dan Zhenghong. Observation of DNA damage in patients with cerebral infarction. *J Fourth Mil Med Univ* 2004; **25** (6) :535-537.

[3] Hei TK, Liu SX, Waldren C. Mutagenicity of arsenic inmammalian cells:role of reactive oxygen species. *Proc Natl Acad Sci USA* 1998, **95**(14): 8103-8107.

[4] Migliore L, Petrozzi L, Lucetti C, Gambaccini G, Bernardini S, Scarpato R, et al. Oxidative damage and cytogenetic analysis in leukocytes of Parkinsons disease patients. *Neurology* 2002; **58**(12): 1809-1815.

[5] Terris DJ, Ho EY, Ibrahim HZ, Dorie MJ, Kovacs MS, Le QT. Estimating DNA repair by sequential evaluation of head and neck tumor radiation sensitivity using the comet assay. *Arch Otolaryngol Head Neck Surg* 2002; **128**(6): 698-702.

[6] Jugdutt BI. Nitric oxide and cardioprotection during ischemiar reperfusion. *Heart Fail Rev* 2002; **7**(4) :391-405.