The Impact of Different Anesthesia Methods on Stress Reaction and Immune Function of the Patients with Gastric Cancer during Peri-Operative Period

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Objective: *To explore a better anesthesia method for the patients with gastric cancer who will undergo operation by investigating the impact of different anesthesia methods on stress reaction and immune function.*
Material and Method: *The present prospective study was carried out in Xiangyang Central Hospital, China, between January 2008 and August 2014. Sixty-four cases of antral carcinoma, subjected to radical resection of gastric cancer, were randomly divided into general anesthesia group (GA group) and general anesthesia combined with continuous epidural anesthesia group (GCEA group). The peripheral venous bloods were sampled to determine the content of TNF-α, IL-6, IL-8, T-lymphocyte subsets, and natural killer (NK) cells. The data was analyzed by SPSS 13.0.*
Results: *After operation, the content of TNF-α, IL-6, and IL-8 in GCEA group were significant lower than that in GA group ($p<0.05$). At the end of the operation, the content of T-lymphocyte subsets and NK cells in GA and GCEA groups descended significantly, but GA group was larger ($p<0.01$ vs. $p<0.05$). Seventy-two hours after the operation, the content of T-lymphocyte subsets and NK cells in GCEA group were back to normal, but it was significantly lower than before anesthesia ($p<0.05$) in the GA group.*
Conclusion: *Compared to GA, GCEA is a better anesthesia method for gastric cancer patient who will undergo operation because it has lower stress reaction and less impact on immune function.*

Keywords: *Anesthesia, Stress reaction, Immune function, Gastric cancer, Operation*

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Gastric cancer is one of the most common malignant tumors in the world. Based on recent clinical epidemiology study, gastric cancer is the second-highest incidence rate in all malignant tumors[1]. Surgical treatment has remained the most important method for it. During the operation, a uniform standard for anesthesia in stomach cancer surgery has not yet been established, and anesthetists tend to opt for continuous epidural anesthesia (CEA), general anesthesia (GA), or general anesthesia combined with continuous epidural anesthesia (GCEA) depending on their personal preference or the medical ability of their hospital. In general, as long as the effect of anesthesia can be achieved, different anesthesia methods do not affect the surgical operation in a notable way. However, the surgical operation is a strong stressor for the patient that may cause internal environment disorder and metabolic imbalance[2]. In addition, immune system disorder is common in malignant tumor patients, and the stress reactions caused by surgery may weaken the body's immune function, which would potentially increase the risk of postoperative complications, and increase the proliferation and metastasis of tumor cells[3]. For this reason, it is necessary to find an anesthesia method that has the least impact on human body, can reduce the stress reaction during operation, and can improve immune status of the body to achieve better outcomes post-operation of the patients with gastric cancer.

Inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-8, play very important roles in the procedures of intra-operative and postoperative stress reactions[4]. At the same time, cellular immunity is a significant part of immune function. The detection of T-lymphocyte subsets in peripheral blood is an important method for observing the level of cell-mediated immunity[5]. On the other hand, natural killer (NK) cells are a major

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lymphocyte for the regulation of the immune system. It can prevent the formation, growth, spread, and metastasis of tumor cells. It also can defend viral infections[6]. It was reported that the reduction of NK cells might result in survival of tiny tumor thrombus caused by the operation[7].

Based on the theories listed above, between January 2008 and August 2014, the Department of Anesthesiology, Xiangyang Central Hospital of Hubei Province, China, took GA or CGEA as the anesthesia methods for 64 cases of gastric cancer surgery. The variations of T-lymphocyte subsets, NK cells, TNF-α, IL-6, and IL-8 during the peri-operative period were measured in both GA and GCEA groups, for the purpose of investigating the different anesthesia methods impact on stress reaction and immune functions during peri-operative period.

Material and Method

This is a prospective study designed to explore a better anesthesia method for the patients with gastric cancer who will undergo radical resection of cancer. The study took place at Department of Anesthesiology, Xiangyang Central Hospital of Hubei Province, China, between January 2008 and August 2014. The experiment was performed according to Principles of Helsinki Declaration and under ethical approval by the Ethical Committee of Xiangyang Central Hospital. All the participating patients had joined with informed consent.

These were our inclusion criteria for the present study. 1) The antral cancer was diagnosed by gastroscopy and biopsy in our hospital, or pathological sections of other hospitals were confirmed by our hospital. 2) Patients underwent radical resection of antral cancer in our hospital. 3) No distant metastasis was found pre-operation. 4) Patients were free from severe heart, lung, liver, kidney, or endocrine diseases, with the score of anesthesia classification less than or equal to three. 5) Patients had not undergone radiation, chemical, hormone, or immunological adjuvant therapy pre-operation. These were our exclusion criteria for the present study. 1) Patients that underwent emergency operation for gastric perforation due to perforation of gastric cancer. 2) Patient older than 70-years-old. 3) Blood transfusion was performed intra-operatively. 4) Tumor was found to be unremovable, or total gastrectomy was found to be required during operation. The criterion of anesthesia classification was adopted from American Society of Anesthesiologists (ASA)[8].

Sixty-four cases of antral cancer were randomly divided into the GA and GCEA group, with 31 cases in the GA group, and 33 cases in the GCEA group. No significant differences were present regarding to the ages, sexes, weights, scores of ASA classification, surgical approaches, operation duration, and the amount of intra-operative bleeding in both groups (p>0.05), making the two groups comparable.

Protocols of anesthesia methods

Before operation, 0.1 g of phenobarbital sodium and 0.5 mg of atropine were administered through muscular injection in both GA and GCEA group. After entrance into the operating room, a monitor was connected to the patients to continuously observe the patient's bispectral index (BIS), cardiogram, blood pressure, and partial pressure of oxygen. A venous channel was created. For the GA group, 3 μg/kg of fentanyl, 2 mg/kg of propofol, and 1 μg/(kg•min) of vecuronium bromide was administrated for anesthesia induction through intravenous injection. Twenty to 30 mL/L of isoflurane was inhaled, 0.02 μg/(kg•min) of fentanyl, and 1 μg/(kg•min) of vecuronium bromide was injected by venous pump to maintain anesthesia. After tracheal intubation, fentanyl and atracurium was administered by intermittent intravenous drip, combined with isoflurane inhalation throughout the process of anesthesia. For the GCEA group, an external catheter was placed at thoracic vertebrae (T7-8) for epidural anesthesia, which was inducted by 13 g/L of lidocaine. When the level of anesthesia reached T4-10, the GA induction was performed using the same agents as the GA group. Lidocaine at 20 g/L was pushed periodically through the epidural catheter for the GCEA group until the end of operation, accompanied by inhalation of 10 to 20mL/L isofulrane and intravenous infusion of 1 μg/(kg•min) vecuronium bromide, in order to maintain anesthesia. Mechanical ventilation was used after tracheal intubation, with O_2:N_2O = 2:3, and tidal volume at 8 to 12 mL/L. Breathing was controlled throughout the operation, with end-tidal carbon dioxide partial pressure ($PETCO_2$) maintained at 30 to 35 mmHg (1 mmHg = 0.133 kPa). The inhalation of isofulrane and injection of propofol and vecuronium bromide was stopped after abdominal cavity had been closed. The inhalation of N_2O was stopped after the skin had been sutured.

Monitoring of indicators

The content of TNF-α, IL-6 and IL-8 in serum were detected by using enzyme-linked immunosorbent

assay (ELISA) Kits (purchased from Terli Biological Technology Co., Ltd., Guangzhou, China), the procedure was strictly in accordance with the instruction manual of ELISA Kits. The sample was obtained with the following steps. For both groups, 10 mL of venous blood was sampled 30 minutes before and 30 minutes after anesthesia, and two hours post-operation respectively. Samples were placed at room temperature for two hours, and then were centrifuged at 1,000 g for 20 minutes. After that, the supernatants were aspirated and stored in a -70°C refrigerator, without repeated freezing and melting. The content of T-lymphocyte subsets (CD3+, CD4+, CD4+/CD8+) were measured by using combined alkaline phosphatase anti-alkaline phosphatase (APAAP) method (APAAP Kits donated by Institute of Basic Medical Sciences, Military Medical Science Academy of the PLA, Beijing, China). The sample was obtained with the following steps. For both groups, 5 mL of peripheral venous blood was sampled pre-anesthesia, at the end of operation, and 72 hours post-operation respectively. The samples were centrifuged at 3,000 r/minute for two minutes, and then 2 mL of supernatants were aspirated for separating mononuclear cells smears. After that, the smears were dehydrated and stored in a -20°C refrigerator. The NK cells were detected by using flow cytometer (Kits purchased from EPICS-Elite Beckman-Coulter Company, USA). The sample was obtained with the following steps. For both groups, 2 mL of peripheral venous blood was sampled pre-anesthesia, at the end of operation, and 72 hours post-operation respectively. The blood was mixed in specially made anti-coagulation tubes, and measured by a flow cytometer.

Statistics

Statistical analysis was performed by the SPSS System (version 22.0, SPSS Inc., Chicago, IL, USA). All the values were expressed as mean ± standard deviation (SD). Means were compared by paired Student's t-test for intro-group comparisons, and for inter-group comparisons, single factor analysis of variance (one-way ANOVA) was adopted. The limit of statistical significance was set at p less than 0.05.

Results

Comparisons of inflammatory cytokines

In both groups, compared to pre-anesthesia, the content of TNF-α, IL-6, and IL-8 in serum at 30 minutes post-anesthesia and two hours post-operation were significantly increased ($p<0.05$). Comparisons between the two groups showed that the contents of TNF-α, IL-6, and IL-8 in GCEA group at 30 minutes post-anesthesia and two hours post-operation were all significantly lower than that in GA group ($p<0.05$, Table 2).

Comparisons of T-lymphocyte subsets and NK cells

At the time of operation ending, the contents of CD3+, CD4+, CD4+/CD8+, and NK cells in GCEA group were all significantly lower than that at pre-operation ($p<0.05$), while the same changes can be found in GA group but more obviously ($p<0.01$). In GCEA group, 72 hours after operation, the contents of CD3+, CD4+, CD4+/CD8+, and NK cells were close to pre-anesthesia with no statistical difference from intra-group comparison, while in GA group, all the parameters were still significantly lower than that at pre-anesthesia ($p<0.05$, Table 3).

Discussion

The radical gastrectomy for gastric antrum cancer involves not only the removal of distal stomach and greater omentum, but also dissection of station 1 and station 2 lymph nodes. This means, it is difficult to avoid stimulating the dense plexi surrounding the celiac trunk, which would cause the secretion of hormones and catecholamine controlled by the

Table 1. Comparative of clinical data

Parameters	GA group	GA combined CEA group	t-value	*p*-value
Number of case	31	33		
Male/female	14/17	15/18		
Age (years), mean ± SD	52.8±3.4	53.4±2.9	0.000	0.992
Weight (kg) , mean ± SD	61.7±3.1	63.1±2.8	0.049	0.826
Operation duration (hour)	3.5±0.2	3.4±0.3	0.595	0.444
Amount of bleeding (mL)	210±32	190±26	1.735	0.193

GA = general anesthesia; CEA = continuous epidural anesthesia

Table 2. Comparative for the content of inflammatory cytokines

Parameter	Group	Before GA	30 minutes after GA	2 hours post-operation	F-value	*p*-value
TNF-α (pg/mL)	A	21.53±4.97	65.05±10.16	100.01±6.92	28.10*	0.000*
	B	20.19±4.47	53.16±7.66	85.81±6.44	53.76$^{\Delta}$	0.000$^{\Delta}$
IL-6 (pg/mL)	A	28.84±6.80	61.62±10.42	77.12±8.19	14.04*	0.000*
	B	27.04±6.61	52.91±8.09	72.36±6.27	6.87$^{\Delta}$	0.011$^{\Delta}$
IL-8 (pg/mL)	A	32.69±7.17	75.85±6.73	89.22±8.48	54.02*	0.00*
	B	35.37±7.18	63.61±6.49	78.83±8.33	24.42$^{\Delta}$	0.00$^{\Delta}$

TNF = tumor necrosis factor; IL = interleukin

* Indicated the comparative for the content of inflammatory cytokines 30 minutes after GA between group A and B

$^{\Delta}$ Indicated the comparative for the content of inflammatory cytokines 2 hours after GA between group A and B

Table 3. Comparative for the content of T lymphocyte subsets and NK cells

Parameter	Group	Before GA	30 minutes after GA	72 hours post-operation	F-value	*p*-value
CD3+ (/μL)	A	1,882	1,213	1,502	10.81	0.002
	B	1,967	1,410	1,893		
CD4+ (/μL)	A	976	552	721	9.74	0.003
	B	952	671	939		
CD4+/CD8+ (%)	A	1.87	1.01	1.34	8.99	0.004
	B	1.77	1.24	1.73		
NK cells (%)	A	14.89	9.06	11.93	10.50	0.002
	B	14.99	10.49	14.69		

NK = natural killer

F-value and *p*-value was the comparative for the date 72 hours post-operation between Group A and B.

hypothalamic-pituitary-adrenal axis, leading to strong stress reactions[9]. In recent years, many studies have proven that cytokines and their receptors are suitable parameters for representing and predicting incidence rate and death rate of stress diseases caused by abdominal surgery, with TNF-α, IL-6, and IL-8 as the most crucial cytokines[10]. It has been reported that anti-TNF-α treatment may reduce the incidence rates of acute respiratory distress syndrome (ARDS) and multi-organ dysfunction syndrome (MODS) after hemorrhage[11]. The abnormal increase of IL-6 is a dangerous signal for a patient after operation, while IL-8 is a risk factor for the possibility of patient complicated by MODS[12].

On the other hand, the changes in cell-mediated immunity (CMI) after surgical stress have also been noticed by researchers. Current studies have proven that surgical stress may cause an increase in immune-inhibiting hormones, and a notable decrease in immune-enhancing hormones and the amount of lymphocytes[13]. The T-lymphocytes act as immune effecter and regulation cells in cell-mediated immunity. Among the T cell subset parameters, the value of CD3+ represents the overall level of cell-mediated immunity[14], and the molecule of CD3+ expresses on all the surface of T cells, which can assist the antigen receptors on T cells to recognize the antigen epitopes of major histocompatibility complex provided by antigen presenting cells[14]. Reduction of CD3+ cells will reduce this recognition ability, and weaken the immune function of body[15]. The CD4+ cell is T helper cells that assists the differentiation of B cell to generate antibodies, and secretes lymphatic factor that activate other cells to cause inflammatory reaction, at the same time, the CD4+ can mediate inflammatory reaction[16]. The CD8+ cell is an immune-inhibiting cell that assists other immune cells by suppressing the synthesis and secretion of antibodies, also suppressing proliferation of T cells[17]. Variations of CD4+/CD8+ ratio indicate immune system dysfunction, and a significant decrease of the ratio often means severe disease and unfavorable prognosis[18]. The NK cells is an important cell in immune regulation that regulates T cells, B cells, bone marrow stem cells, etc., and controls the body's immune functions by releasing lymphatic factors[19]. NK cells play a major role in the prevention of tumor formation, growth, spread and metastasis, can defend against viral infections. Decrease of NK cells may cause survival of tiny tumor thrombus drifted during the surgical treatment[7].

In the present study, compared to GA group, the content of TNF-α, IL-6, and IL-8 in GCEA group at 30 minutes post-anesthesia and two hours post-operation were significantly lower ($p<0.05$). Based on the theories listed above, the decrease of TNF-α, IL-6 and IL-8 may reduce stress reactions throughout the body, which is beneficial for the patient's postoperative recovery, and reduces the risks of sepsis, ARDS and MODS. Meanwhile, the intro-group comparison showed that in both groups, compared to pre-anesthesia, the contents of CD3+, CD4+, CD4+/CD8+, and NK cells at the end of operation were significantly lower, but it was more obviously in GA group than that in GCEA group ($p<0.01$ vs. $p<0.05$). Seventy-two hours post-operation, the contents of CD3+, CD4+, CD4+/CD8+, and NK cells in GCEA group was close to pre-anesthesia levels, while in GA group, the parameters were still significantly lower than pre-anesthesia ($p<0.05$). Such results indicates that for the patients with antral cancer who will undergo radical resection, compared to general anesthesia, the method of general anesthesia combined with continuous epidural anesthesia can decrease the stress reactions in the peri-operative period. It can decrease the suppression of T cell subsets and NK cells caused by narcotic. It is beneficial to the recovery of the patient's immune functions after surgical treatment.

At present, most anesthetists are unaware that anesthesia and surgical treatment themselves are special injuries for the body and can activate stress reactions. It is a major reason of immuno-suppression during peri-operative period. Therefore, most anesthetists apt to choice general anesthesia or continuous epidural anesthesia for the patients with gastric cancer who will undergo radical resection. It has been proven by animal experiments that removal of nerves can prevent the changes of stress hormone caused by trauma[20]. In addition, the stress reactions of the patients with spinal cord injury decrease. Both indicate that the stimulations from injured regions are the main causes of stress reactions during peri-operative period[21]. The epidural anesthesia may obstruct the transmission of traumatic stimulation from the operative region. However, its anti-stress effect is incomplete due to the patient's psychological stress and vagus reflex during operation. Compared to epidural anesthesia, general anesthesia can only suppress the limbic system of cerebral cortex, or the projection system of hypothalamus on cortical. It is ineffective to block the transmission of traumatic stimulations from operative region to the central nervous system. Additionally, most anesthetics used in general anesthesia, as well as the inhalation of high concentration oxygen, would weaken the body's immune functions[22]. The results of the present study had shown that general anesthesia combined with continuous epidural anesthesia might block the traumatic stimulations transmitted through sympathetic nervous system at a nerve root. Furthermore, the stimulation transmitted from secondary afferent pathway such as phrenic and vagus nerve was suppressed by general anesthesia from central nervous system. This would reduce the impact on stress and damage of immune functions from radical surgery of gastric antral cancer. In the present study, we also discovered that in GCEA group, it was not only showing weaker stress reactions, but also using a smaller amount of anesthetics than that in GA group by about 30%, smaller amount of anesthetics would both accelerate recovery from anesthesia and reduce the effects on immune system from the anesthetics itself.

Given the results, we believe that general anesthesia combined with continuous epidural anesthesia is a better choice for patients with gastric antral cancer who will undergo radical resection. Of course, the conclusion needs to be confirmed by more clinical tests from multi centers.

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Author contribution

Jun Zhao performed the research and wrote the paper. Huiqin Mo designed the research and analyzed the data.

Potential conflicts of interest

None.

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