Effects of Different Anesthetic Methods on Cellular Immune and Neuroendocrine Functions in Patients With Hepatocellular Carcinoma Before and After Surgery

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Background: Many anesthesia methods have been studied in hepatocellular carcinoma (HCC). We aimed to explore the effects of combined intravenous and inhalation anesthesia and combined general and epidural anesthesia on cellular immune function and neuroendocrine function in patients with HCC before and after surgery. **Methods:** Between September 2012 and April 2014, 72 patients who underwent a hepatectomy in our hospital were enrolled. **Results:** Compared with the combined intravenous and inhalation anesthesia group, the combined general and epidural anesthesia group demonstrated increased CD4⁺/CD8⁺ T cells 0 hr after surgery, increased CD3⁺, CD4⁺, CD4⁺/CD8⁺ cells, and IFN- γ levels 12 hr after surgery, and increased CD3⁺, CD4⁺, and CD4⁺/CD8⁺ cells 24 hr after surgery (all $P < 0.05$). At 72 hr after surgery, the levels of ACTH and Cor in the combined general and epidural anesthesia group, and the levels of CD3⁺, CD4⁺, CD4⁺/CD8⁺

cells, and IFN- γ in both the combined intravenous and inhalation anesthesia and the combined general and epidural anesthesia groups decreased to pre-surgery levels. Significant differences were observed in the comparisons of CD3⁺, IL-6, and IL-10 between the combined intravenous and inhalation anesthesia and the combined general and epidural anesthesia groups 72 hr after surgery (all $P < 0.05$). **Conclusion:** Our results revealed that combined general and epidural anesthesia plays a crucial role in hepatectomy via the mitigation of the inhibition of immunologic function in HCC patients during the peri-operative period. Combined general and epidural anesthesia also hastens the recovery of immunologic suppression after surgery, which can provide a certain reference for the selection of clinical anesthesia in the treatment of HCC. *J. Clin. Lab. Anal.* **30**:1175–1182, 2016. © 2016 Wiley Periodicals, Inc.

Key words: anesthesia; cellular immune function; hepatocellular carcinoma; IFN- γ ; IL-10; IL-6; Neuroendocrine function

INTRODUCTION

Hepatocellular carcinoma (HCC), which is the most common primary hepatic malignancy, is derived from epithelial and mesenchymal tissue of the liver and is the third leading cause of cancer-related deaths worldwide (1, 2). Observational data indicated approximately more than 500,000 new patients diagnosed with HCC, and approximately 600,000 die every year worldwide (3). A related study revealed that such high mortality is mainly caused by late diagnosis and high tumor recurrence after surgery (4). Except for the higher mortality rate, HCC also demonstrates regional differences, with higher incidences in populations of

Eastern Asia, sub-Saharan Africa and Southern Europe (5). With regard to the risk factors, obesity was deemed one of the most important risk factors for HCC. Other factors that can also influence the risk of HCC include alcoholism, hepatitis B, hepatitis C, exposure to aflatoxin, and cirrhosis of the liver (6–8). The treatment of HCC involves multiple

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disciplines, including hepatology, surgery, diagnostic and interventional radiology, oncology, and pathology (9). Statistics have shown that the 5-year survival rate of HCC patients was approximately 30–40% after curative resection (10). Despite promising therapeutic strategies for HCC, surgical resection or liver transplant remain the best options that are currently available (11).

Hepatectomy is considered a major surgery for HCC and is conducted under general anesthesia alone or in combination with epidural analgesia (12). General anesthesia results in a medically induced coma and loss of protective reflexes, which occur after the administration of one or more general anesthetic agents (13). It has been demonstrated that general anesthesia may offer improved hemodynamic stability relative to regional anesthesia (14). Epidural anesthesia is known to be a routine component of abdominal surgery due to the high quality of analgesia and the early mobilization of patients, including patients at high risk for perioperative complications (15). Previous evidence revealed that combined general and epidural anesthesia provided better analgesia and a better reduction in blood loss and postoperative complications than general anesthesia (16). While, study also confirmed that the low concentration of general anesthesia used in surgery cannot only reduce the interference of normal physiologic processes, but also can help in the removal of drugs from the patient's system and prompt patients return to a physiological state (17). Due to the controversy surrounding the different methods of anesthesia, our study was conducted to explore the effects of two anesthetic methods (general anesthesia and combined general and epidural anesthesia) on cellular immune function and neuroendocrine function in HCC patients before and after surgery.

MATERIALS AND METHODS

Ethical statement

This study was approved by the Ethical Committee of Guangrao People's Hospital. Written informed consent was obtained from all study subjects. This study complied with the guidelines and principles of the Declaration of Helsinki (18).

Clinical data

Between September 2012 and April 2014, 72 patients with HCC (males: $n = 51$; females: $n = 21$) underwent hepatectomy at Guangrao People's Hospital were enrolled in our study. These patients were considered level I–II according to the American Society of

Anesthesiologists (ASA) classification system and received a Child Pugh score of A. The diagnostic criteria were based on the criteria of the American Association for the Study of Liver Disease (AASLD), which were published in the Clinical Practice Guidelines for hepatocellular carcinoma (HCC) (updated in 2010) (19). All patients had pathologically confirmed HCC after surgery. The age of enrolled patients ranged from 26 to 73 years old (average: 43.35 ± 13.70). All patients were divided into two groups as follows: group A (general anesthesia group, combined intravenous and inhalation anesthesia) and group B (combined general and epidural anesthesia). The inclusion criteria were as follows: (a) all patients were pathologically diagnosed with primary HCC; (b) HCC patients did not show distant recurrence by iconography who were eligible for radical resection, and they did not display any abnormal cell counts of white blood cells or leukomonocytes; (c) this was the patient's initial surgery for HCC; (d) patients were able to provide informed consent and complete the tables. The exclusion criteria were as follows: (a) chemotherapeutics were given before surgery; (b) immunopotentiator was given after surgery; (c) a history of sedative dependence; (d) patients did not want to participate in the study or they withdrew; (e) after surgery, patients were pathologically diagnosed with a disease other than HCC; (f) surgery was performed again within 1 month after the initial surgery; (g) patients failed to return to the hospital within 14 days and/or 1 month after surgery.

Anesthetic methods

None of the patients received drug treatment before surgery, and all patients fasted for more than 8 hr. Sodium salt (0.1 g, purchased from Tian jin kingyork anjisan CO., Ltd., Tianjin, China) and atropine (0.5 mg, purchased from Beijing Double-Crane Pharmaceutical CO., Ltd., Beijing, China) were given as intramuscular injections 30 min before surgery. Vital signs were monitored by conventional means after the patients entered the operating room; the bispectral index (BIS) was also used to monitor the anesthesia. After the vein was opened, venous blood (5 ml) was extracted and injected into an anticoagulant tube and was then stored at 4°C until further use. For patients in group B, a catheter was inserted at T8-9, and 1% lidocaine (5 ml, purchased from Astra Company, London, UK) was used for the epidural block. Midazolam (0.04 mg/kg, Yichang Humanwell Pharmaceutical CO., Ltd.), propofol (2.0 mg/kg, Astra Company), fentanyl (4 µg/kg, Yichang Humanwell Pharmaceutical CO., Ltd., Yichang, China), and vecuronium bromide (0.1 mg/kg, Organon Pharmaceutical Co., Ltd., Holland, the Netherlands) were

successively injected intravenously in patients in both groups A and B. Breathing was controlled in all patients by the induction of tracheal intubation. For patients in group B 1% lidocaine (5 ml) was added to the epidural every 60 min, and the dosages of propofol (8–10 mg/(kg·h)) plus remifentanyl (1 mg/h, Yichang Humanwell Pharmaceutical CO., Ltd.) and traciun (30 mg/h, Shanghai Hengrui Pharmaceutical CO., Ltd., Nanjing, China) were adjusted to maintain the depth of anesthesia. In contrast, for patients in group A, propofol was continuously added and intermittently injected intravenously along with sufentanil (Yichang Humanwell Pharmaceutical CO., Ltd.) to maintain the depth of anesthesia. The two groups had the same BIS and train of four stimulation (TOF) values. The drugs were discontinued 10–15 min before the end of surgery. Venous blood (5 ml) was extracted after surgical suture and was stored at 4°C for future experiments. During surgery, traciun was intermittently added according to the flabby condition, and the transfusion consisted primarily of hetastarch and lactate ringer's solution (all purchased from Nanjing Chia Tai Tianqing Pharmaceutical CO., Ltd., Nanjing, China). Packed red blood cells were added when the hematocrit (HCT) was $\leq 25\%$, and plasma was injected if necessary. The catheter was removed when patients could open their eyes when asked to do so, and when they demonstrated better autonomous respiration, active cough, and deglutition reflex. The patients were then sent to the ward for further monitoring and therapy.

Outcome measures

The following data were monitored and recorded: the time the patients entered the room (T0), the blocking of the fossa transversalis hepatis (T1), the recovery of blood flow to the liver (T2), 1 hr after recovery of blood flow to the liver (T3), 0 hr after surgery (T4), heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP). Venous blood in the median cubital vein was collected before surgery and at 0, 12, 24, and 72 hr after surgery in both A and B groups. Adrenocorticotrophic hormone (ACTH) and cortisol (Cor) were detected by radioimmunoassay with the use of a tissue homogenate machine (CNNC Beijing Nuclear Instrument Factory), a Varifuge 3.0 RS version centrifugal machine and an FT-613 Automatic Radioimmunoassay Assembly (China). Flow cytometry (FACSCalibur flow cytometry; Becton Dickinson Company, Franklin Lakes, NJ) was applied to test T lymphocyte subsets ($CD3^+$, $CD4^+$, and $CD4^+/CD8^+$), while a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (R and D Systems, Minneapolis, MN) was used to detect interleukin-6 (IL-6), IL-10, and IFN- γ levels. The

experiment was performed strictly according to the instructions of the kit.

Statistical analysis

SPSS 20.0 (SPSS, Chicago, IL) software was used for statistical analysis. Categorical data were measured by χ^2 test and were presented as a ratio or a percentage. Continuous data were presented as the mean $\pm$ the standard deviation (SD) and tested by Student's *t*-test. Two-way analysis of variance (Two-way ANOVA) was used to compare the expressions of different time points in each group before and after surgery and to compare the expressions of the same time point between two groups. Moreover, factor analysis of variance was also applied for the comparison of different time points between groups. *P* values < 0.05 were considered statistically significant.

RESULTS

Baseline characteristics between groups A and B

Comparison of the baseline characteristics of age, gender, ASA classification, height, weight, and surgery time between groups A and B showed no significant difference (all *P* > 0.05) (Table 1).

Hemodynamics in the perioperative period between groups A and B

No significant difference was observed in the comparisons of HR, SBP, and DBP between groups A and B at T0, and the comparisons of the HR between groups A and B at T1, T2, T3, and T4 presented no significant differences (all *P* > 0.05). Higher SBP and DBP were found in group B at T1 and T2 compared with group A, and a significant difference was observed in SBP and DBP at T0 in group A (all *P* < 0.05). However, in group B, no obvious change was seen in SBP and DBP at any of the time points, and a comparison of SBP and DBP

TABLE 1. Comparison of Baseline Characteristics Between Group A and B ($\bar{x} \pm s$)

Group	A (<i>n</i> = 36)	B (<i>n</i> = 36)	<i>t</i> / χ^2	<i>P</i>
Age	44.42 $\pm$ 13.37	42.28 $\pm$ 14.14	38.333	0.409
Male:Female	27: 9	24:12	0.605	0.605
ASA physical status (I/II)	19/17	13/23	2.025	0.236
Body weight (kg)	65.27 $\pm$ 5.00	66.30 $\pm$ 5.45	63.333	0.500
Operation time (min)	154.86 $\pm$ 13.63	152.81 $\pm$ 13.37	31.333	0.835

ASA, American Society of Anesthesiologists.

Fig. 1. Changes in the HR, SBP, and DBP in the two groups during the perioperative period ($\bar{x} \pm s$) (HR: heart rate; SBP: systolic pressure; DBP: diastolic pressure; T0: before surgery; T1: 0 hr after surgery; T2: 12 hr after surgery; T3: 24 hr after surgery; T4: 72 hr after surgery); (a) changes in the HR in the two groups during the perioperative period; (b) changes in SBP in the two groups during the perioperative period; (c) changes in DBP in the two groups during the perioperative period; *: compared with T0, $P < 0.05$; #, compared with the same time point in the combined general and epidural anesthesia group, $P < 0.05$).

values within the group showed no statistical significance (all $P > 0.05$) (Fig. 1).

Changes in ACTH and Cortisol (Cor) in groups A and B

A comparison of ACTH and Cor levels between groups A and B showed no significant difference before surgery, while the levels of ACTH and Cor were

obviously higher 0, 12, and 24 hr after surgery compared with the levels before surgery. Moreover, a comparison of ACTH and Cor within each group showed significant differences. Lower levels of ACTH and Cor were observed in group B at different time points compared with the levels in group A, and a comparison between the groups showed a statistical significance (all $P < 0.05$). At 72 hr after surgery, ACTH and Cor levels in group B had decreased to pre-surgery levels. However, at 12 hr after surgery, ACTH and Cor levels in group A began to decrease but were still higher than those before surgery and were clearly higher than those of group B 72 hr after surgery. Furthermore, comparisons of the levels of ACTH and Cor at different time points within the groups showed significant differences (all $P < 0.05$) (Fig. 2).

T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD4^+/CD8^+$) in the peripheral blood of patients in groups A and B

Compared with the levels before surgery, the levels of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ T cells were

Fig. 2. Changes in ACTH and Cor at different time points in the two groups (ACTH: adrenocorticotrophic hormone; Cor: cortisol; T0: before surgery; T1: 0 hr after surgery; T2: 12 hr after surgery; T3: 24 hr after surgery; T4: 72 hr after surgery); (a) changes in ACTH levels at different time points in the two groups; (b) changes in Cor at different time points in the two groups; *: compared with T0, $P < 0.05$; #, compared with the same time point in the combined general and epidural anesthesia group, $P < 0.05$).

obviously decreased in both groups A and B at 0, 12, and 24 hr after surgery, and differences within groups were statistically significant (all $P < 0.05$). The levels of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ T cells were found to be clearly decreased 0 hr after surgery, and no significant difference was observed between the levels of $CD3^+$ and $CD4^+$ T cells within each group. The levels of $CD4^+/CD8^+$ T cells in group B were higher than those in group A ($P < 0.05$). An upward trend of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ T cells was found 12 hr after surgery in both groups A and B, but patients in group B had significantly higher numbers of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ T cells than patients in group A (all $P < 0.05$). Seventy-two hours after surgery, the numbers of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ T cells had increased to pre-surgery levels in both groups A and B, but the number of $CD3^+$ T cells in group B was higher than that in group A ($P < 0.05$). However, a comparison of $CD4^+$ and $CD4^+/CD8^+$ T cells at different time points within each group revealed no significant differences (all $P > 0.05$) (Fig. 3).

Concentration of inflammatory cytokines (IL-6, IL-10, and IFN- γ) in the peripheral blood of patients in groups A and B

Figure 4 shows the change in the concentration of IL-6, IL-10, and IFN- γ in groups A and B before and after anesthesia. Before surgery, no significant difference was found in a comparison of the concentration of IL-6, IL-10, and IFN- γ in groups A and B (all $P > 0.05$). The levels of IL-6 and IL-10 were significantly increased in both groups A and B, and patients in group A had significantly higher levels than patients in group B 0 hr after surgery (both $P < 0.05$). Additionally, the level of IFN- γ was decreased in both groups, which showed statistically significant differences before and after surgery (both $P < 0.05$), but no significant difference was observed between groups A and B ($P > 0.05$). Moreover, 12 hr after surgery, the IL-6 level in group B presented a downward trend, but the IL-6 level was still clearly higher than that before surgery; moreover, group A demonstrated a higher IL-6 level compared with group B (all $P < 0.05$). A significant difference was also found in the comparison of IL-6 and IL-10 levels 24 hr after surgery between the two groups, and IL-6 and IL-10 levels in group B were obviously lower than those in group A (both $P < 0.05$). The level of IFN- γ presented an uptrend trend, which suggested a significant difference compared with the pre-surgery level (both $P < 0.05$), while no such difference was observed in a comparison of the level of IFN- γ among groups A and B ($P > 0.05$). Furthermore, the levels of IL-6, IL-10 and IFN- γ in both groups A and B recovered to the pre-

Fig. 3. Changes in T lymphocyte ($CD3^+$, $CD4^+$, and $CD4^+/CD8^+$) subsets at different time points in the two groups (T0: before surgery; T1: 0 hr after surgery; T2: 12 hr after surgery; T3: 24 hr after surgery; T4: 72 hr after surgery); (a) changes in the number of $CD3^+$ T cells at different time points in the two groups; (b) changes in the number of $CD4^+$ T cells at different time points in the two groups; (c) changes in the number of $CD4^+/CD8^+$ T cells at different time points in the two groups; *: compared with before surgery, $P < 0.05$; #, compared with the same time point in the combined intravenous and inhalation anesthesia group, $P < 0.05$).

surgery levels 72 hr after surgery, and the levels of IL-6 and IL-10 in group A were slightly higher than those before surgery (all $P < 0.05$). In contrast, no such difference was found in a comparison of the level of IFN- γ between the two groups (the level of IFN- γ in group B was slightly higher than that in group A) (all $P > 0.05$).

DISCUSSION

Through an investigation of the influence of two different anesthetic methods on cellular immune function

Fig. 4. Changes in inflammatory cytokines (IL-6, IL-10 and IFN- γ) at different time points in the two groups (IL-6: interleukin-6; IL-10: interleukin-10; IFN- γ : interferon- γ ; T0: before surgery; T1: 0 hr after surgery; T2: 12 hr after surgery; T3: 24 hr after surgery; T4: 72 hr after surgery); (a) changes in IL-6 levels at different time points in the two groups; (b) changes in IL-10 levels at different time points in the two groups; (c) changes in IFN- γ levels at different time points in the two groups; *: compared with before surgery, $P < 0.05$; #, compared with the same time point in the combined intravenous and inhalation anesthesia group, $P < 0.05$).

and neuroendocrine function after a hepatectomy in patients with HCC, our study found that combined general and epidural anesthesia plays a crucial role in hepatectomy. It does so via the mitigation of the inhibition of immunologic function in HCC patients during the perioperative period and by hastening the recovery of immunologic suppression after surgery, which can provide a certain reference for the selection of clinical anesthesia in HCC treatment.

Previous evidence indicates that surgery and related inflammation have been shown to suppress immune competence, which causes surgical stress that can activate the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). These mechanisms evoke a neuroendocrine response and increase the release of hormones, such as Cor, catecholamines, and adrenocorticotrophic hormone, which inhibit pro-inflammatory T cell responses (20–22). Ample evidence has demonstrated that hypovolemia, hypothermia, fever and pain can all induce a robust activation of the HPA axis, which leads to increased Cor levels, and both very high and low plasma concentrations of Cor have been correlated with adverse outcomes in cases of critical illness and surgery (23, 24). T cells were deemed to play a crucial role in anti-tumor immunity, and T lymphocyte subsets can be divided into two types based on their different effects on tumor immunity: $CD4^+$ and $CD8^+$ T cells (25). $CD4^+$ T cells can be further divided into diverse subsets, such as T helper (Th) 1, Th2, Th17 cells, and regulatory T cells (Tregs), which play opposite roles in the regulation of immune responses to tumor cells (26). Moreover, these immunocompetent cells secrete different types of effector molecules and cytokines, including interferon (IFN)- γ , transforming growth factor (TGF)- β 1, interleukin (IL)-4, as well as IL-17A, which also mediate anti-tumor immunity (27). Furthermore, myeloid-derived suppressor cells (MDSCs), which comprise a population of heterogeneous cells, are derived from immature myeloid cells, such as cells that are $CD11b^+$, $CD14^-$, $CD15^+$, and/or $CD33^+$, as well as cells that are HLA-DR $^-$ (28). It was also demonstrated that MDSCs can negatively regulate anti-tumor immunity in cancer patients, that these cells can inhibit the T cell activity of natural killer (NK) cells and macrophages and that they enhance angiogenesis, which promotes the growth and metastasis of cancer cells (29–32). Our results also revealed that compared with pre-surgery levels, the levels of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ T cells were obviously decreased in both groups A and B 0 hr, 12 hr after surgery and 24 hr after surgery. In addition, the levels in group B were significantly higher than those in group A, which suggests that anesthesia and surgical stress may have a certain degree of influence on the immune function of HCC patients. In summary, the anesthesia method used in group B can better reduce the stress reaction caused by surgery compared with the method used in group A. Finally, the method used in group B may also result in a lower inhibitory effect of T lymphocyte subsets.

Surgery is the first-line treatment for liver cancer, and the different anesthetic methods that are used in

the treatment of HCC may exert different effects in terms of the hormone and enzyme levels in patients, these hormones and enzymes play an important role in the differences in systemic stress responses that are controlled by inflammatory cytokines (33, 34). Inflammatory cytokines can regulate a variety of physiologic functions within an organism, and they may exert some effects on reactions to stress, including trauma, pain and infection (35). IL-6 is an important mediator in the process of stress pathophysiology, and studies have demonstrated that surgery can directly or indirectly promote the synthesis and secretion of IL-6; in addition, an increased level of IL-6 has been shown to be positively correlated with the incidence of postoperative complications and the degree of damage caused by surgery (36). Moreover, IFN- γ is of great importance for the induction of a cytotoxic T cell response (37). Furthermore, IL-10 is recognized because of its ability to inhibit the production of Th1 cytokines, especially IFN, during an inflammatory response (38). Our study demonstrated that patients who received combined general and epidural anesthesia had lower levels of IL-6 and IL-10 at the five mentioned time points compared with patients who received combined intravenous and inhalation anesthesia. The study conducted by Snyder et al. also confirmed that compared with general anesthesia alone, combined epidural anesthesia and analgesia might be more beneficial because epidural anesthesia might hinder the neuroendocrine stress response to surgery by blocking afferent neural transmission. Epidural anesthesia may also restrain the activation of the HPA, which results from noxious stimuli and functions to decrease cortisol secretion, and may provide better postoperative pain relief (39).

In summary, our study provided evidence that combined general and epidural anesthesia plays a crucial role in hepatectomy via the mitigation of the inhibition of immunologic function in HCC patients during the perioperative period and by hastening the recovery of immunologic suppression after surgery. This can provide a certain reference for the selection of clinical anesthesia in the treatment of HCC. However, this study has limitations because we did not analyze or compare the type of anesthetic, the depth of anesthesia or the dosage of anesthesia. Therefore, further studies with a detailed comparative analysis of the above items should be conducted to further confirm our conclusion.

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