Original Article

YKL-40 Gene Expression and Plasma Levels of CD30 are not Affected by Isoflurane or Propofol: Pilot Study

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Abstract

Background: It has been hypothesized that the body's response to anesthesia techniques can increase risk of cancer recurrence and metastatic disease after surgery and also can modulate immune responses. Some acute inflammatory markers have been measured to survey the immunomodulatory effect of anesthesia, but in this research, we studied the plasma level of CD30 and YKL-40 gene expression which can present major changes of the immune system.

Materials and Methods: Our study was a controlled before and after study**.** 34 women with biopsy-proven breast cancer were randomized to receive either propofol general anesthesia (n=17) or standard isoflurane general anesthesia (n=17). There were no significant differences between the two patient groups in age, body weight, and height, length of general anesthesia, operative time and group of surgery. The blood samples were collected in two different sets, before anesthesia and 72-h postoperatively. Soluble CD30 (sCD30) plasma level was measured by ELISA and YKL-40/CHI3L1 gene expression was evaluated by real-time-PCR.

Results: The results showed that the anesthetics, propofol and isoflurane, have no effect on the expression of YKL-40. Despite increased in the expression of YKL-40 that was observed in patients receiving isoflurane, this increase was not statistically significant. There was no significant increase or decrease in plasma concentrations of sCD30.

Conclusion: YKL-40 and sCD30 are not affected by isoflurane or propofol. So, in immunological perspective, there is no preference in use of isoflurane or propofol in breast cancer patients.

Keywords: YKL-40, CD30, Propofol, Isoflurane

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Introduction

Breast cancer is one of the main causes of cancer-related death in women and associated morbidity and mortality relates mainly to metastatic disease and not the primary tumor[1]. A number of preoperative factors

during primary breast cancer surgery, including anesthetic technique, might influence whether minimal residual micro-metastases elimination or full-blown metastatic disease. Major surgery is associated with immunosuppression, which can facilitate angiogenesis, tumor metastasis, and tumor invasion[1-3]. There is increasing evidence from experimental studies and a limited number of clinical studies that some anesthetic drugs may be contributing factors to the development of metastases after cancer surgery[3-10].

YKL-40 is a glycoprotein involved in cellular growth, migration, and the inflammatory process. Overexpression of YKL-40 has been associated with worse prognosis in various localized and metastatic malignancies, including breast cancer[11,12]. CD30 and CD30L are members of the tumor necrosis factor receptor and TNF super family. It is an important costimulatory molecule and a marker for the physiological balance between TH1/TH2/TH17/Treg immune responses[13]. Ligation of CD30 on normal effector T cells induces interleukin 13 (IL13) productions. IL-13 can promote cancer progression. YKL-40 is a ligand for the IL13 receptor which acts as a decoy receptor[14,15].

We sought to determine the postoperatively immunomodulatory effects of propofol general anesthesia in comparison with standard isoflurane general anesthesia among breast cancer patients. In this research, we studied the impact of anesthetic regimes by assessment of two biomarkers (YKL-40 and CD30) which regulate and modify immune responses to tumor.

Methods

Study subjects: This study was conducted after approval by the local ethics committee. Thirty-four women undergoing surgery for biopsy-proven primary breast cancer were randomized to receive either propofol general anesthesia or standard isoflurane general anesthesia. Patients had been randomly assigned for the study by using prepared opaque envelopes. Patients with a history of smoking, anemia, endocrine disorders, immune system disorders, malignant disease, chronic inflammatory disease, marked obesity, kidney or liver disorders, or steroid therapy were not studied. The inclusion criteria were women aged 18–60 years, undergoing mastectomy or wide local tumor excision with or without axillary node sampling or excision. There were no significant differences between the two patient groups in age, body weight, and height (Table 1), and also operative time and group of surgery.

Patients were consented to contribute a sample of venous blood before operation and 3 days after operation. Based on previous studies[16] a peak in immunosuppression is said to occur at day three. Hence, it can be suggested that the early postoperative period represents an immunological window of opportunity during which the extracellular milieu may be increasingly permissive to minimal residual disease growth and spread. Blood samples (10 ml each) were drawn immediately before induction of anesthesia and 72h postoperatively. Separated plasma samples and buffy coat were stored at -80°C until assayed.

Anesthesia: All patients were premeditated with diazepam 5 mg orally 2 h pre-operatively. The patients were randomly allocated to receive either propofol (n=17) or isoflurane (n=17). For the propofol group, anesthesia was induced with 1–2 $mg.kg^{-1}$ propofol and 0.5-l $g.kg^{-1}$ Atracurium and anesthesia was maintained with 10 $mg.kg^{-1}.h^{-1}$ propofol for the first 10min, 8 $mg.kg^{-1}.h^{-1}$ for the second 10min, 6 $mg.kg^{-1}.h^{-1}$ for the third 10 min and titrated doses of 3– 6 $mg.kg^{-1}.h^{-1}$ thereafter. For the isoflurane group, anesthesia was induced with 3–4 $mg.kg^{-1}$ thiopental and 0.5 $mg.kg^{-1}$ atracurium. Anesthesia was maintained with isoflurane 0.5–1%. In both groups fentanyl was given at 2-4 $mg.kg^{-1}$, neuromuscular blockade was maintained with intermittent doses of atracurium and ventilation was controlled to obtain an end-tidal carbon dioxide tension of 30-35 mmHg. Nitrous oxide was not used in either group. Anesthetists unaware of the purpose of the study performed all the anesthetic procedures described above.

CD30 Plasma Levels: To measure the plasma levels of soluble CD30 (sCD30), a sandwich enzyme-linked immunosorbent assay was used (human sCD30, Invitrogen, USA). Briefly, subjects' plasma was added to peroxidase-conjugated mouse anti-CD30 monoclonal antibodies. After 3 h incubation and washing, to remove unbound materials, a chromogenic substrate was added to the wells; then, the enzyme reaction was stopped and the absorbance on a spectro-

photometer using 450nm.

YKL-40 Gene Expression: Blood cells were used for real time PCR analysis. RNA was purified from cell with RNX-Plus kit (SinaClon, IRAN) following the manufacturer's instructions. One µg of total RNA was used for synthesizing cDNA by using a commercial kit (Intron, Korea). cDNA was used for the real time PCR reaction in the mixture of primers and the 2X Maxima SYBR Green q-PCR Master Mix (Thermo Fisher Scientific, Waltham, U.S.).

The specific primers were designed to amplify a 141bp fragment of the human CHI3L1 (GenBankTM code NM_001276) gene, using Beacon Designer software. To normalize differences in efficacy of sample extraction, we used beta-actin as a housekeeping gene. The sequences of primers represented in (Table 2). A non-template control (NTC) consisting of water in place of template DNA was used in each run. The analysis of melting curve was performed in the temperature range of 65 to 95°C at the end of each run and specificity of all individual amplification reactions was confirmed by melting curve analysis and also gel electrophoresis (Fig 1).

Statistical Analysis: Statistical analyses were performed using the SPSS program (v.17; SPSS, Chicago, IL). The student's t-test was performed to compare the groups with normal distribution. The Mann–Whitney U test was used in the case of non-normal distribution. P=0.05 was considered as statistically significant. The relative levels of mRNA were analyzed by the REST 2009 Software (QIAGEN).

Results

Soluble CD30 (sCD30): Pre-incisional values of sCD30 did not differ significantly between the two groups (p=0.77). Before surgery, all patients, had sCD30 levels more than 17.5 pg/ml (17.5-86.5 pg/ml). The plasma concentration sCD30 among patients after surgery ranged from 13-70 pg/ml (Fig. 2).

In group of isofluran, the sCD30 levels did not show any significant change (P=0.84) in their concentration throughout the study period. In this group, pre-incisional value of sCD30 ranged from 18-64.6 pg/ml and postoperative value of sCD30 ranged from 13-70 pg/ml (Fig. 2). In group of propofol, the sCD30 levels did not show any significant change in their concentration throughout the study period (P=0.83), pre-incisional value of sCD30 ranged from 17.5-86.5 pg/ml and postoperative value of sCD30 ranged from 19-61.5 pg/ml in this group (Fig. 2). Postoperative sCD30 plasma levels of isofluran group in relation to the preoperative period revealed a wide rang. Suggesting the changes is more pronounced during isofluran than propofol anesthesia. However; mean comparison of sCD30 levels between groups did not show a statistically significant difference after surgery (P=0.81).

There was not any significant correlation between plasma levels of sCD30 and absolute WBC counts and age among our study subject (data not shown).

YKL-40 gene expression rate in blood cells: At baseline (before surgery), the YKL-40 gene expression rate did not show a significant difference (P=0.065) between groups (Table 3). Postoperatively YKL-40 gene expression rate did not show a significant difference (P=0.068), as well. Therefore the YKL-40 gene expression is not affected by anesthetic drugs.

Despite of propofol group that postoperative expression in relation preoperative expression was 1.092 fold change (P=0.65), the YKL-40 gene expression rate showed slightly increased after surgery among isoflurane group. Pre-incisional value of YKL-40 fold change was 0.88 less than postoperative but this difference did not show statistically significant (P=0.86). Overlay our data according to the YKL-40 gene expression, suggesting that propofol may not be preferred over isofluran.

Discussion

In tumor patients postoperative immunosuppression could cause spreading minimal residual diseases, so surgery is a double-edged sword which in one hand is a tumor treatment and on the other hand may be ending with metastatic disease. Preoperative factors influencing immune components and might be increased the risk of recurrence or metastasis. There is a question: can anesthetic technique for surgery affect immune responses? To answer this question we compered the effects of isofluran and propofol anesthesia on sCD30 plasma level and YKL-40 gene

Table 1: Demographic data in both groups. Values are Mean (SD) or number (Proportion).

	Propofol (n=17)	**Isoflurane (n=17)**	
Demographic Detail			**P-value**
Gender	Female	Female	-
Age Average(year)	44.94±2.1	44.64±2.3	0.772
Weight Average(Kg)	70.29±1.8	70.11±2.3	0.179
Height Average(Cm)	160.70±1.6	160.76±1.1	0.357
(Kg/m^2)BMI	29.1±1.6	28.7±1.2	0.624
Operation Length (min)	120	120	-
Clinical History			
Asthma	-	-	
Inflammation	-	-	
Operation	-	-	
Blood transfer	-	-	
Smoking	-	-	
Hormone Therapy	-	-	
First Blood Sample (Before)			
W.B.C (3^/mm)	7.30±0.29	6.74±0.33	0.660
R.B.C (6^/mm)	4.36±0.12	4.87±0.17	0.721
PLT (g/dl)	245±2.5	224±3.1	0.582
Neut(%)	75±2.8	66.12±2.5	0.300
Lymph(%)	31.29±1.3	32.35±1.9	0.608
HGB (g/dl)	12.96±0.16	12.02±0.42	0.074
Second Blood Sample (After)			
W.B.C (3^/mm)	7.28±0.32	7.76±0.40	0.398
R.B.C (6^/mm)	4.64±0.29	4.92±0.51	0.754
PLT (g/dl)	250	258	0.234
Neut(%)	75.11±2.2	73.05±3.6	0.164
Lymph(%)	26.70±1.60	27.64±2.7	0. 342
HGB (g/dl)	12.15±0.25	11.89±0.44	0.137

expression rate in blood cell during mastectomy operation. The results showed that the anesthetics, propofol and isoflurane, have no effect on the gene expression of YKL-40 in blood cells and also plasma

Table 2: The primers sequences of genes of interest and housekeeping genes.

F: GTGAAGGCGTCTCAAACAGG
R: GAAGCGGTCAAGGGCATCT
F: CCTGGGCATGGAGTCCTGT
R: ATCTCCTTCTGCATCCTGTCG

Table 3: YKL-40 mRNA expression among isoflurane group in relation to propofol group before surgery (A) and after surgery (B).

Gene	Type	Reaction Efficiency	Expression	Std. Error	95% C.I.	P(H1)	Result
A							
B-Actin	REF	0.8038	1.000				
YKL-40	TRG	0.8482	1.401	0.055 - 35.562	0.010 - 377.068	0.652	
B							
B-Actin	REF	0.8140	1.000				
YKL-40	TRG	0.8597	1.060	0.689 - 1.382	0.446 - 4.166	0.678	

concentrations of sCD30.

Recently, Baki et al. assess the effect of propofol versus desflurane anesthesia on systemic immune modulation and central nervous system on patients undergoing coronary artery bypass grafting and found that in terms of S100β plasma levels propofol is more preferable for anesthetic management[17]. However, this study were assessed, the effects of isoflurane anesthesia based on the acute inflammatory markers. A comparable study in Finland supports this idea that there isn't notably adverse immune response after surgery and anaesthesia[18], researchers compared the effects of propofol-based (n=15) and isoflurane-based anaesthesia (n=15) on mucous host defenses by measuring the salivary flow and the concentrations/activities of salivary total protein and amylase, and of salivary immunological (IgA, IgG and IgM) and non-immunoglobulin defense factors (lysozyme, myeloperoxidase, total salivary peroxidase and thiocyanate) in patients undergoing elective abdominal hysterectomy. The changes were similar in both groups. In contrast to our findings, Inada et al.[19] found that "The Th1⁄Th2 ratio decreased significantly after isoflurane anaesthesia, while it did not change after propofol anaesthesia. They concluded that propofol anaesthesia attenuated the surgical stress-induced adverse immune response.However; an obvious limitation of this study was the size of the population. A small sample size (n=9 in each group) greatly underpowered any potential associations. In another study, researcher considered 15 patients in two groups of propofol and isofluran and evaluate whether propofol stimulates the activation and differentiation of T helper cells in patients undergoing pulmonary lobectomy. They

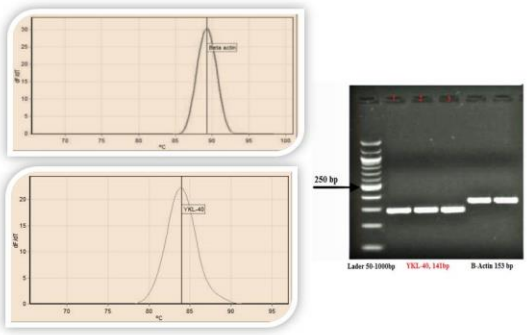

Figure 1. The specificity of PCR products.
To verify the specificity, the dissociation curves of PCR products were generated. The *y*-axis was expressed as first derivative of the fluorescence as a function of temperature. The PCR products were analyzed agarose gel electrophoresis, followed by ethidium bromide staining.

found that interferon-γ: interleukin-4 plasma levels increased with propofol but showed no change with isoflurane. However, they considered brief periods after surgery (24h post-operation) for their evaluation[20].

Our study suggests that isofluran and propofol could not lead to a major change in immune system homeostasis. However; our study could not reject different biological impact of these drugs. Recently, there is a theory that using isoflurane could increase alzheimer risk and cause hippocampi cells apoptosis[21]. So immunological approach is not enough to choose one of them and all the other aspects should be surveyed too.

Conclusion

Based on our data there is no advantage in using

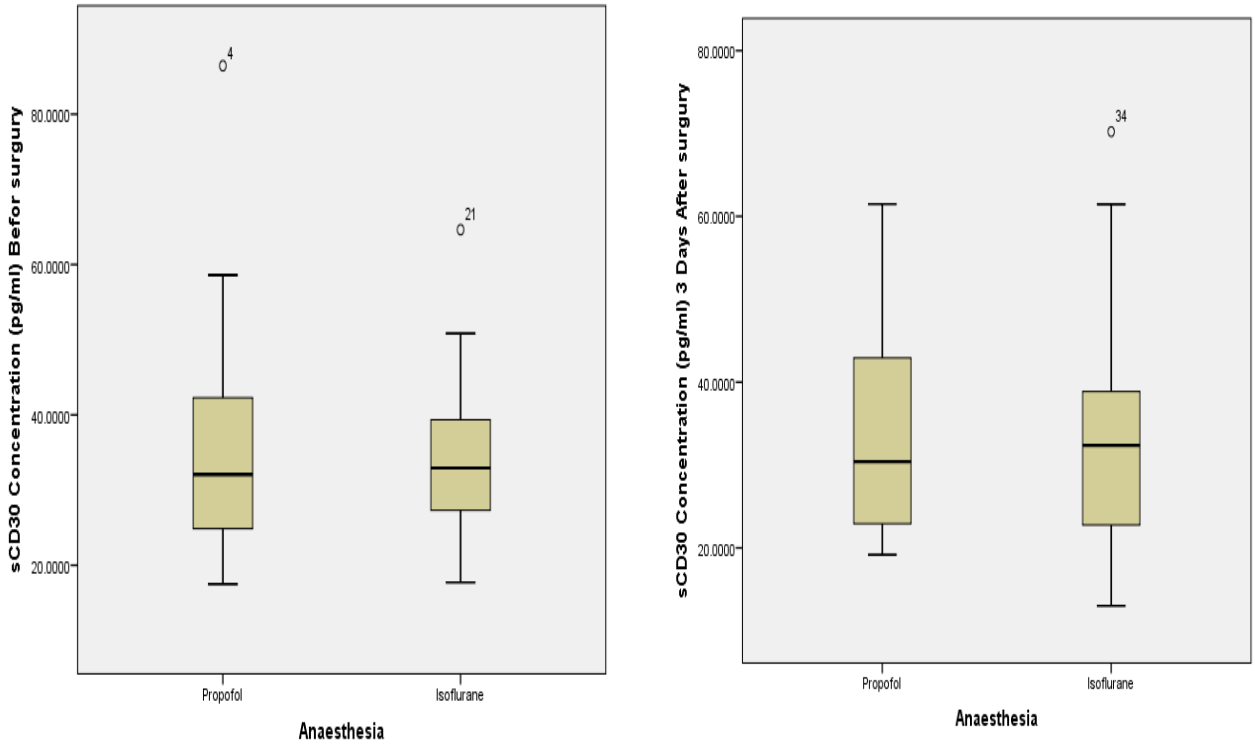

Figure 2. Plasma levels of sCD30 in breast cancer patients.
Comparison of Plasma levels of sCD30 among propofol and isoflurane groups. Boxes represent values between the 25th and 75th percentiles. The horizontal lines correspond to the median, minimum, and maximum. The highest level of sCD30 (86.5pg/ml) belonged to the patient among propofol group, who was 63 years old that had $7*10^3$ WBC/µl before surgery but had 36.62 pg/ml sCD30 concentration and $7.1*10^3$ WBC/µl counts after surgery. The highest level of sCD30 (70.2pg/ml) among isofluran group belonged to the patient with $10.7*10^3$ WBC/µl after surgery , who was 40 years old with $6.9*10^3$ WBC/µl and 42.92 pg/ml sCD30 before surgery.

isoflurane or propofol in mastectomy. YKL-40 and sCD30 are not affected by isoflurane or propofol. So that, from an immunological perspective, there is no preference in use of isoflurane or propofol in breast cancer patients. Actually, based on the patient's condition, diagnose of anesthesiologists, and other factors such as the price and the availability of drugs either propofol or isoflurane could be used in mastectomy candidate patients.

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Conflict of Interest

The authors declare no conflicts of interest.

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