Intraoperative Flurbiprofen Treatment Alters Immune Checkpoint Expression in Patients Undergoing Elective Thoracoscopic Resection of Lung Cancer

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Highlights of the Study

• CD4(+) and CD8(+) T cells in the peripheral blood of lung cancer patients express higher levels of PD-1, which indicates a poorer clinical outcome.
• PD-1 expression levels were markedly induced in the peripheral blood of lung cancer patients following surgery.
• Perioperative administration of flurbiprofen attenuated the postoperative increase in PD-1 levels on circulating CD8(+) T cells for up to 72 h.

Keywords
Immune cells · Lung cancer · Flurbiprofen · Programmed death 1

Abstract

Objectives: This study aimed to determine the effect of intraoperative administration of flurbiprofen on postoperative levels of programmed death 1 (PD-1) in patients undergoing thoracoscopic surgery. Materials and Methods: In this prospective double-blind trial, patients were randomized to receive intralipid (control group, n = 34, 0.1 mL/kg, i.v.) or flurbiprofen axetil (flurbiprofen group, n = 34, 50 mg, i.v.) before induction of anesthesia. PD-1 levels on T cell subsets, inflammation, and immune markers in peripheral blood were examined before the induction of anesthesia (T0) and 24 h (T1), 72 h (T2), and 1 week (T3) after surgery. A linear mixed model was used to determine whether the changes from baseline values (T0) between groups were significantly different. Results: The increases in the percentage of PD-1(+)CD8(+) T cells observed at T1 and T2 in the control group were higher than those in the flurbiprofen group (T1: 12.91 ± 1.65 vs. 7.86 ± 5.71%, p = 0.031; T2: 11.54 ± 1.54 vs. 8.75 ± 1.73%, p = 0.004), whereas no differences were observed in the changes in the percentage of PD-1(+)CD4(+) T cells at T1 and T2 between the groups. Moreover, extensive changes in the percentage of lymphocyte subsets and inflammatory marker concentrations were observed at T1 and T2 after surgery and flurbiprofen attenuated most of these changes. Conclusions: Perioperative administration of flurbiprofen attenuated the postoperative increase in PD-1 levels on CD8(+) T cells up to 72 h after surgery, but not after this duration. The clinical relevance of changes in PD-1 levels to long-term surgical outcome remains unknown.

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Introduction

Lung cancer is a major cause of cancer-related deaths globally and approximately 80% of lung cancers occur as non-small cell lung cancer (NSCLC) [1–3]. Some well-established immune-checkpoint molecules expressed on activated T cells, such as programmed death 1 (PD-1) or CD279 and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4 or CD152), functionally suppress T-cell-mediated immunity against tumors and are considered a hallmark of exhausted T cells following persistent stimulation with tumor antigens [4–8]. Increasing evidence indicates that higher levels of immune-checkpoint molecules predict poorer outcomes in cancer patients [9, 10]. Furthermore, immune-checkpoint blockade (e.g., anti-PD-1 therapy) elicits persistent and significant therapeutic responses in multiple tumor types, including lung cancer [1, 11–16]. However, it remains unclear whether perioperative use of analgesic drugs can directly influence these immune-checkpoint molecules in cancer patients undergoing surgery.

Interestingly, PD-1 and its ligands are markedly inducible by cyclooxygenase (COX) enzymes and downstream prostaglandins (PGs), which are prominent tumor-sustaining inflammatory mediators [17, 18]. Additionally, nonsteroidal anti-inflammatory drugs (NSAIDs), which have anti-inflammatory and analgesic activities, including inhibiting COX enzymes, pharmacologically cooperate with anti-PD-1 therapy to increase its efficacy in a preclinical cancer model [19]. In clinical settings, the perioperative administration of flurbiprofen, which is regularly prescribed as a perioperative analgesic, efficiently elicits a short-term increase in the number of innate and adaptive immune cells in postoperative peripheral blood [20]. Since less is known about how NSAIDs synergize with anti-PD-1 therapy in clinical settings, we aimed to determine whether flurbiprofen had a direct effect on postoperative PD-1 levels in circulating T cell subsets, in patients undergoing elective thoracoscopic resection of NSCLC.

Materials and Methods

Study Sample

This study was approved by the Biomedical Research Ethics Committee of Anhui Medical University and was registered with the Chinese Clinical Trial Registry (No. ChiCTR-IPR-15006482). All participating patients provided written informed consent. This study was a prospective, double-blind, randomized, controlled clinical trial. Patients were screened at the outpatient department or inpatient wards and underwent randomization between October 15, 2016, and May 10, 2017, at Anhui Provincial Hospital, Hefei, China. Patients were eligible for participation in this study if they met the following criteria: (1) adults undergoing elective thoracoscopic resection of lung cancer, (2) American Society of Anesthesiologists (ASA) status of I–II, (3) aged 40–65 years, and (4) weighing 45–80 kg for both genders. Patients were excluded if they met any of the following criteria: (1) allergy or contraindication to NSAIDs; (2) history of peptic ulceration; (3) blood coagulation disorder; (4) severe cardiac, hepatic, or renal dysfunction; (5) perioperative blood transfusion; (6) bronchial asthma; (7) current or recent use of radiotherapy, chemotherapy, immune depressant, or glucocorticoid; (8) autoimmune disease or acute inflammation; (9) severe hypertension or diabetes mellitus; (10) pregnancy; (11) use of enoxacin, lomefloxacin, or norfloxacin; and (12) duration of operation < 120 min.

Anesthesia and Analgesia

General anesthesia was induced with 0.05 mg/kg midazolam, 2 mg/kg propofol, and 0.4 µg/kg sufentanil, and double-lumen endobronchial tube insertion was facilitated with 1 mg/kg rocuronium. The site of the tube was confirmed by fiberoptic bronchoscopy after intubation and by changing the position of the patient in order to achieve an optimal view. Double-lumen ventilation was performed using a Narcotrend monitor (MonitorTechnik, Bad Bramstedt, Germany). Postoperatively, all patients received the same regimen of patient-controlled intravenous analgesia with sufentanil (100 µg, diluted to a total volume of 100 mL with 0.9% sodium chloride). Patient-controlled intravenous analgesia was performed with a loading dose of 2 mL, a background infusion of 2 mL/h, a bolus of 2 mL, and a lockout time of 15 min.

Intervention and Randomization

Using a computer-generated random number sequence, patients were allocated in a 1:1 ratio to receive treatment with either flurbiprofen (flurbiprofen axetil, 50 mg, i.v.) or placebo (intralipid, 0.1 mL/kg, i.v.) before the induction of anesthesia. Data collection was performed by an independent researcher who was not involved in the trial. In addition, another researcher was in charge of the preparing the study drugs. The drugs were placed into unmarked syringes, and treatment assignments were concealed in sealed, opaque envelopes. All of which was blinded to patients, anesthetists and other investigators involved in the study. The statistician was unaware of the assignments until all data analyses were completed. In addition, tramadol was administered postoperatively as a rescue analgesic for unbearable pain, when the Visual Analogue Scale score was ≥5.

Outcomes

Venous blood (2 mL) was collected from the noninfused peripheral vein before the induction of anesthesia (T₀) and 24 h (T₁), 72 h (T₂), and 1 week (T₃) after surgery. Blood samples were preserved in an EDTA anticoagulation tube at 4 °C for subsequent testing within 24 h. The primary outcomes were counts of circulating PD-1⁺ CD8⁺ and PD-1⁺ CD4⁺ T cells at each perioperative time point. The secondary outcomes were the percentages of lymphocyte subsets in peripheral blood mononuclear cells (PBMCs).
and the concentrations of inflammatory markers, including tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-6 (IL-6), and C-reactive protein (CRP), in peripheral blood. Moreover, data from full blood count tests, including platelet count, total white blood cell count, hemoglobin concentration, neutrophil count, lymphocyte count, monocyte count, eosinophil count, and basophil count, were tracked simultaneously.

**Immune-Checkpoint and Lymphocyte Subset Analyses**

Circulating PD-1$^{(+)}$CD8$^{(+)}$ and PD-1$^{(+)}$CD4$^{(+)}$ T cell counts were determined as the percentage of total T lymphocytes. The percentages of total CD3$^{(+)}$ T cells, CD4$^{(+)}$ helper T cells, CD8$^{(+)}$ cytotoxic T cells, and CD3$^{(+)}$CD16$^{(+)}$CD56$^{(+)}$ NK-cells in PBMCs were measured by flow cytometry (CytoFLEX; Beckman, Brea, CA, USA). PBMCs were separated from peripheral blood by Ficoll-Hypaque density-separated method.

**Fig. 1.** Flow cytometric analysis results. **a** Flow cytometric gating strategy and analysis of PD-1 expression on CD45$^{(+)}$CD3$^{(+)}$CD4$^{(+)}$ and CD45$^{(+)}$CD3$^{(+)}$CD8$^{(+)}$ T-cell populations. **b** Representative plots of PD-1 expression on the CD8$^{(+)}$ T-cell population at different time points. PD-1, programmed death 1.
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Results

Study Population

The CONSORT diagram is shown in Figure 2. A total of 70 patients were screened for our study, with 68 patients ultimately participating. Of the 2 patients who were not included, one refused to participate and one did not satisfy the inclusion criteria. Four patients (2 in the flurbiprofen group, 2 in the control group) were withdrawn from the statistical analysis, despite being randomized into study groups. In the flurbiprofen group, one of these patients refused phlebotomy for laboratory testing after the operation, and one patient was withdrawn because of blood loss >1.5 L. Both patients in the control group withdrew due to tumor metastasis into the pleura, not having undergone a lung parenchyma resection procedure. Baseline and intraoperative characteristics of patients in both groups are shown in online supplementary Table 1 (see www.karger.com/doi/10.1159/000503166). No significant differences in age, body mass index, gender, or ASA status were observed between groups. There were also no significant between-group differences in surgery type, duration of surgery, the use of antibiotics, or intraoperative propofol consumption. Remifentanil consumption was higher in the control group than in the flurbiprofen group. Outcome values are shown in Table 1. There were no significant differences in the percentages of PD-1(+)CD8(+) T cells, PD-1(+)CD4(+) T cells, or T lymphocyte subsets or inflammatory marker concentrations between groups at baseline and 1 week after surgery (data not shown).

Outcomes

As shown in Table 2, the increases in the percentage of PD-1(+)CD8(+) T cells observed 24 h (T1) and 72 h postoperatively (T2) in the control group were higher than those in the flurbiprofen group (T1: 12.91 ± 1.65 vs. 7.86 ± 5.71%, p = 0.031; T2: 11.54 ± 1.54 vs. 8.75 ± 1.73%, p = 0.004). Original images of PD-1(+)CD8(+) T cells at different time points are shown in Figure 1b. No differences were observed in the change in the percentage of PD-1(+)CD4(+) T cells at T1 and T2 between the groups. However, changes in the percentages of PD-1(+)CD8(+) T cells and PD-1(+)CD4(+) T cells from baseline (T0) were similar between groups 1 week after surgery (T3; data not shown). Moreover, the decreases observed for all lymphocyte subsets at T1 and T2 were markedly greater in the control group than in the flurbiprofen group, with the exception of CD8(+) T cells, which showed a significant change only at T1. In addition, the significant increases...
observed in the concentrations of TNF-α, IFN-γ, IL-6, and CRP at T1 and T2 were greater in the control group than in the flurbiprofen group. At a longer postoperative follow-up period (T3), no significant differences were observed in the abovementioned data from baseline (data not shown). Samples were also collected 3 weeks (T4) after surgery; however, at this time point, there were no significant differences in the concentration of these inflammatory markers between groups (data not shown). Figure 1b shows the expression of PD-1 (+) on CD8 (+) T cells in 2 groups.

There were no significant differences between groups with respect to the proportion of patients who were unexpectedly subjected to respiratory depression after surgery. Respiratory depression was defined as a respiratory rate <8 breaths per minute and oxygen saturation either below 92% or showing a decrease of >5% from baseline in patients with a baseline SPO₂ <90% [21]. There were also no significant between-group differences in the incidence of nausea or skin pruritus. Eight patients in the control group experienced vomiting and retching, compared with 1 patient in the flurbiprofen group (p = 0.026). Furthermore, the use of antiemetics in the control group was higher than that in the flurbiprofen group (p = 0.011). A significant reduction in score for cough was observed in the flurbiprofen group 24 h postoperatively, but no difference was observed between groups 72 h postoperatively. Moreover, 10 patients in the control group required postoperative rescue analgesia (tramadol) for unbearable pain, compared to three patients in the flurbiprofen group (p = 0.030). In addition, no differences were observed between the groups in terms of postoperative wound infection or the length of hospital stay.

Assessed for eligibility (n = 70)

- Excluded (n = 2)
  - Did not satisfy inclusion criteria (n = 1)
  - Declined to participate (n = 1)

Randomized (n = 68)

- Allocated to flurbiprofen group (n = 34)
  - Received allocated intervention (n = 34)
  - Did not receive allocated intervention (n = 0)

- Lost to follow-up (n = 1)
  - Refused phlebotomy for laboratory testing after operation (n = 1)
  - Discontinued intervention (n = 0)

- Analyzed (n = 32)
  - Excluded from analysis (n = 1)
  - Blood loss more than 1.5 L (n = 1)

- Allocated to control group (n = 34)
  - Received allocated intervention (n = 34)
  - Did not receive allocated intervention (n = 0)

- Lost to follow-up (n = 0)
  - Discontinued intervention (n = 0)

- Analyzed (n = 32)
  - Excluded from analysis (n = 2)
  - Tumor metastasis and pleural invasion (n = 2)

**Fig. 2.** CONSORT diagram of the trial process.
Table 1. Differences in perioperative PD-1 expression on CD4<sup>(+)</sup> and CD8<sup>(+)</sup> T-cells and other inflammation and immune markers between the flurbiprofen and control groups

| Variable | Control group | Flurbiprofen group | Difference (95% CI) | p value |
|----------|---------------|--------------------|---------------------|---------|
| **24 h after surgery** | | | | |
| PD-1<sup>(+)</sup> on CD4<sup>(+)</sup> T-cells | 15.74 (3.41) | 18.06 (4.56) | −2.33 (−6.64 to 1.99) | 0.268 |
| PD-1<sup>(+)</sup> on CD8<sup>(+)</sup> T-cells | 22.68 (1.67) | 16.85 (6.83) | 5.83 (0.50 to 11.16) | 0.034 |
| CD3<sup>(+)</sup> T-cells | 33.50 (7.27) | 45.38 (5.48) | −11.88 (−18.78 to −4.97) | 0.002 |
| CD4<sup>(+)</sup> T-cells | 27.25 (3.73) | 32.00 (4.78) | −4.75 (−9.35 to −0.15) | 0.044 |
| CD8<sup>(+)</sup> T-cells | 14.13 (1.25) | 17.13 (3.00) | −3.00 (−5.58 to −0.42) | 0.027 |
| CD4:CD8 ratio | 0.74 (0.19) | 1.16 (0.34) | −0.43 (−0.72 to −0.13) | 0.008 |
| NK cells | 7.58 (0.78) | 10.04 (0.93) | −2.46 (−3.39 to −1.54) | <0.001 |
| Platelet count, 10<sup>9</sup>/L | 228.75 (60.07) | 215.25 (56.74) | 13.50 (−49.16 to 76.16) | 0.651 |
| Total WBC count, 10<sup>9</sup>/L | 12.85 (2.32) | 12.44 (1.40) | 0.41 (−1.65 to 2.47) | 0.674 |
| Hemoglobin, g/L | 120.25 (8.23) | 121.63 (6.63) | −1.38 (−9.39 to 6.64) | 0.718 |
| Neutrophil count, 10<sup>9</sup>/L | 12.01 (3.38) | 13.20 (3.32) | −1.19 (−4.78 to 2.41) | 0.490 |
| Lymphocyte count, 10<sup>9</sup>/L | 0.84 (0.32) | 0.95 (0.27) | −0.11 (−0.43 to 0.20) | 0.453 |
| Monocyte count, 10<sup>9</sup>/L | 0.66 (0.10) | 0.62 (0.17) | 0.04 (−0.11 to 0.18) | 0.622 |
| Eosinophil count, 10<sup>9</sup>/L | 0.183 (0.157) | 0.114 (0.099) | 0.069 (−0.074 to 0.212) | 0.315 |
| Basophil count, 10<sup>9</sup>/L | 0.010 (0.011) | 0.011 (0.008) | −0.001 (−0.012 to 0.009) | 0.798 |
| TNF-α, pg/mL | 1,680.13 (273.48) | 1,350.38 (283.36) | 329.75 (31.13 to 628.37) | 0.033 |
| IFN-γ, pg/mL | 44.16 (3.48) | 36.71 (4.99) | 7.45 (2.84 to 12.06) | 0.004 |
| IL-6, pg/mL | 178.38 (13.17) | 145.50 (20.33) | 32.88 (14.51 to 51.24) | 0.002 |
| CRP, mg/L | 44.11 (13.46) | 16.74 (3.00) | 27.38 (16.05 to 38.70) | 0.001 |
| Serum glucose, mmol/L | 7.78 (1.22) | 7.25 (1.12) | 0.53 (−0.73 to 1.78) | 0.384 |
| **72 h after surgery** | | | | |
| PD-1<sup>(+)</sup> on CD4<sup>(+)</sup> T-cells | 15.13 (2.87) | 18.39 (4.64) | −3.26 (−7.40 to 0.87) | 0.113 |
| PD-1<sup>(+)</sup> on CD8<sup>(+)</sup> T-cells | 21.31 (1.45) | 17.74 (3.69) | 3.57 (0.57 to 6.58) | 0.023 |
| CD3<sup>(+)</sup> T-cells | 33.88 (3.87) | 43.50 (7.71) | −9.63 (−16.17 to −3.08) | 0.007 |
| CD4<sup>(+)</sup> T-cells | 20.88 (2.80) | 28.38 (4.81) | −7.50 (−11.72 to −3.28) | 0.002 |
| CD8<sup>(+)</sup> T-cells | 15.88 (1.81) | 17.0 (2.0) | −1.13 (−3.17 to 0.92) | 0.258 |
| CD4:CD8 ratio | 0.86 (0.36) | 1.39 (0.37) | −0.53 (−0.92 to −0.13) | 0.012 |
| NK cells | 7.29 (0.80) | 9.12 (1.26) | −1.84 (−2.97 to −0.71) | 0.004 |
| Platelet count, 10<sup>9</sup>/L | 232.38 (75.64) | 209.50 (71.30) | 22.88 (−55.95 to 101.70) | 0.544 |
| Total WBC count, 10<sup>9</sup>/L | 9.60 (2.59) | 8.74 (1.06) | 0.86 (−1.36 to 3.09) | 0.405 |
| Hemoglobin, g/L | 125.25 (5.73) | 122.25 (4.27) | 3.0 (−2.42 to 8.42) | 0.255 |
| Neutrophil count, 10<sup>9</sup>/L | 6.20 (1.13) | 6.28 (2.43) | −0.08 (−2.11 to 1.96) | 0.938 |
| Lymphocyte count, 10<sup>9</sup>/L | 0.95 (0.26) | 1.13 (0.27) | −0.18 (−0.46 to 0.11) | 0.206 |
| Monocyte count, 10<sup>9</sup>/L | 0.55 (0.09) | 0.47 (0.16) | 0.09 (−0.05 to 0.22) | 0.210 |
| Eosinophil count, 10<sup>9</sup>/L | 0.115 (0.068) | 0.086 (0.022) | 0.029 (−0.029 to 0.086) | 0.284 |
| Basophil count, 10<sup>9</sup>/L | 0.010 (0.001) | 0.021 (0.016) | −0.004 (−0.018 to 0.011) | 0.593 |
| TNF-α, pg/mL | 1,611.00 (298.82) | 1,221.13 (187.55) | 389.88 (122.35 to 657.41) | 0.007 |
| IFN-γ, pg/mL | 41.43 (2.50) | 35.25 (3.90) | 6.18 (2.66 to 9.69) | 0.002 |
| IL-6, pg/mL | 160.38 (8.18) | 137.00 (14.84) | 23.38 (10.53 to 36.22) | 0.002 |
| CRP, mg/L | 48.68 (10.67) | 24.55 (9.52) | 24.13 (13.28 to 34.97) | <0.001 |
| Serum glucose, mmol/L | 6.36 (1.14) | 5.58 (0.47) | 0.79 (−0.15 to 1.72) | 0.092 |

Data are presented as mean (SD) or median (IQR) and compared using an independent Student t test or Mann-Whitney U test, respectively.

PD-1, programmed death 1; NK, natural killer; WBC, white blood cells; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; IL-6, interleukin-6; CRP, C-reactive protein; NA, not applicable; IQR, interquartile range.
Table 2. Changes in perioperative PD-1\(^{(+)}\) expression on CD4\(^{(+)}\) and CD8\(^{(+)}\) T-cells and other inflammation and immune markers, after receiving flurbiprofen or placebo, at 24 and 72 h from the baseline values before induction of anesthesia

| Variable | Control group \((n = 32)\) | Flurbiprofen group \((n = 32)\) | Difference (95% CI) | \(p\) value |
|----------|-----------------|-----------------|-------------------|----------|
| **24-h postoperative data compared with baseline** | | | | |
| PD-1\(^{(+)}\) on CD4\(^{(+)}\) T cells | 1.76 (0.58) | 1.46 (1.11) | 0.30 (–0.65 to 1.25) | 0.509 |
| PD-1\(^{(+)}\) on CD8\(^{(+)}\) T cells | 12.91 (1.65) | 7.86 (5.71) | 5.05 (0.55 to 9.55) | 0.031 |
| CD3\(^{(+)}\) T-cells | –21.25 (6.71) | –9.75 (3.37) | –11.50 (–17.20 to –5.80) | 0.001 |
| CD4\(^{(+)}\) T-cells | –7.75 (2.44) | –4.25 (1.49) | –3.50 (–5.66 to –1.34) | 0.004 |
| CD8\(^{(+)}\) T-cells | –3.63 (2.00) | –1.25 (1.83) | –2.38 (–4.43 to –0.32) | 0.026 |
| CD4:CD8 ratio | –0.70 (0.31) | –0.35 (0.12) | –0.35 (–0.62 to –0.08) | 0.016 |
| NK cells | –3.54 (1.31) | –1.49 (0.66) | –2.05 (–3.17 to –0.93) | 0.001 |
| Platelet count, \(\times 10^9/L\) | 16.00 (–22.50 to 20.25) | 2.00 (–24.50 to 24.75) | NA | 0.645 |
| Total WBC count, \(\times 10^9/L\) | 6.80 (4.58 to 9.08) | 5.90 (4.43 to 7.58) | NA | 0.161 |
| Hemoglobin, g/L | 5.85 (5.55 to 11.23) | 10.00 (7.10 to 12.0) | NA | 0.105 |
| Neutrophil count, \(\times 10^9/L\) | –0.80 (0.17) | –0.85 (0.16) | 0.05 (–0.13 to 0.23) | 0.554 |
| Monocyte count, \(\times 10^9/L\) | 0.32 (0.09) | 0.31 (0.14) | 0.01 (–0.12 to 0.13) | 0.933 |
| Eosinophil count, \(\times 10^9/L\) | 0.084 (0.045) | 0.021 (0.075) | 0.063 (–0.065 to 0.190) | 0.302 |
| Basophil count, \(\times 10^9/L\) | –0.016 (0.009) | –0.015 (0.009) | –0.001 (–0.011 to 0.009) | 0.790 |
| TNF-α, pg/mL | 817.13 (131.75) | 552.13 (146.24) | 265.0 (115.74 to 414.26) | 0.002 |
| IFN-γ, pg/mL | 25.83 (3.30) | 18.96 (4.87) | 6.86 (2.40 to 11.33) | 0.005 |
| IL-6, pg/mL | 40.34 (11.59) | 13.65 (6.27) | 26.69 (16.97 to 36.41) | <0.001 |
| Serum glucose, mmol/L | 3.29 (1.05) | 2.26 (1.40) | 1.03 (–0.30 to 2.35) | 0.119 |
| **72-h postoperative data compared with baseline** | | | | |
| PD-1\(^{(+)}\) on CD4\(^{(+)}\) T cells | 1.15 (1.42) | 1.79 (1.96) | –0.64 (–2.47 to 1.20) | 0.469 |
| PD-1\(^{(+)}\) on CD8\(^{(+)}\) T cells | 11.54 (1.54) | 8.75 (1.73) | 2.79 (1.04 to 4.55) | 0.004 |
| CD3\(^{(+)}\) T-cells | –20.88 (6.45) | –11.63 (4.03) | –9.25 (–15.02 to –3.48) | 0.004 |
| CD4\(^{(+)}\) T-cells | –14.13 (5.22) | –7.88 (1.55) | –6.25 (–10.67 to –1.83) | 0.011 |
| CD8\(^{(+)}\) T-cells | –2.00 (1.69) | –1.38 (1.30) | –0.63 (–2.24 to 0.99) | 0.421 |
| CD4:CD8 ratio | –0.55 (–1.00 to –0.01) | –0.10 (–0.18 to –0.03) | NA | 0.005 |
| NK cells | –3.83 (1.23) | –2.40 (0.67) | –1.43 (–2.49 to –0.36) | 0.012 |
| Platelet count, \(\times 10^9/L\) | 18.50 (–19.00 to 23.25) | –5.00 (–33.50 to 23.25) | NA | 0.442 |
| Total WBC count, \(\times 10^9/L\) | 3.75 (2.45 to 4.58) | 2.25 (0.83 to 3.95) | NA | 0.105 |
| Hemoglobin, g/L | –6.13 (3.04) | –6.63 (7.35) | 0.50 (–5.53 to 6.53) | 0.863 |
| Neutrophil count, \(\times 10^9/L\) | 2.05 (0.85 to 3.18) | 2.65 (0.73 to 4.98) | NA | 0.878 |
| Lymphocyte count, \(\times 10^9/L\) | –0.69 (0.22) | –0.68 (0.26) | –0.01 (–0.27 to 0.25) | 0.919 |
| Monocyte count, \(\times 10^9/L\) | 0.22 (0.08) | 0.16 (0.13) | 0.06 (–0.06 to 0.17) | 0.338 |
| Eosinophil count, \(\times 10^9/L\) | 0.016 (0.034) | –0.006 (0.012) | 0.023 (–0.006 to 0.051) | 0.110 |
| Basophil count, \(\times 10^9/L\) | –0.009 (0.015) | –0.005 (0.017) | –0.004 (–0.021 to 0.013) | 0.642 |
| TNF-α, pg/mL | 748.00 (133.71) | 422.88 (95.49) | 325.13 (200.53 to 449.72) | 0.000 |
| IFN-γ, pg/mL | 44.09 (9.94) | 21.46 (7.26) | 23.44 (14.10 to 32.77) | <0.001 |
| Serum glucose, mmol/L | 23.09 (3.08) | 17.50 (3.46) | 5.59 (2.07 to 9.10) | 0.004 |
| IFN-γ, pg/mL | 87.25 (11.99) | 59.50 (9.68) | 27.75 (16.07 to 39.43) | <0.001 |
| IL-6, pg/mL | 44.09 (9.94) | 21.46 (7.26) | 23.44 (14.10 to 32.77) | <0.001 |
| Serum glucose, mmol/L | 1.88 (1.26) | 0.59 (1.29) | 1.29 (–0.08 to 2.65) | 0.062 |

Data are presented as mean (SD) or median (IQR) and compared using an independent Student’s \(t\) test or Mann-Whitney \(U\) test, respectively.

PD-1, programmed death 1; NK, natural killer; WBC, white blood cells; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; IL-6, interleukin-6; CRP, C-reactive protein; NA, not applicable; IQR, interquartile range.
Flurbiprofen Treatment Alters Immune Checkpoint Expression

Sensitivity Analysis

Linear mixed models confirmed the significant differences in the percentage of CD8(+) T cells, CD4(+) T cells expressing PD-1(+) and CTLA-4(+) T cells in peripheral blood of NSCLC patients up to 72 h postoperatively, without any change in the percentage of circulating PD-1(+) CD8(+) T cells. COX activity and COX-dependent inflammatory mediators, such as PGs, facilitate the increase in the inhibitory immune-checkpoints. PD-1/CTLA-4 and low levels of PD-1/CTLA-4 have been demonstrated in COX-2 MEC knock-out mice bearing tumors, suggesting that COX activity and downstream PGs are potentially linked with PD-1. Although NSAIDs may cooperate with anti-PD-1 blockade in inducing the eradication of tumors in preclinical studies, our study primarily demonstrated that flurbiprofen altered PD-1 levels on the circulating CD8(+) T cell population in NSCLC patients up to 72 h postoperatively, without any change in the percentage of circulating CD8(+) T cells observed after that time point. Flurbiprofen administration during the perioperative period had little influence on the postoperative percentage of PD-1(+) CD8(+) T cells. The percentage of lymphocytes after surgery in the control and flurbiprofen groups was, in general, lower compared with hospital reference values. It is interesting to note that there were extensive changes in lymphocyte subsets and inflammatory markers following administration of flurbiprofen in the short-term postoperative period up to 72 h.

Discussion

PD-1, expressed in tumor-infiltrating T cells and circulating T cells, has been shown to predict prognosis and serve as a candidate therapeutic target in several malignant tumors, including NSCLC [8, 12, 13, 16]. Recent evidence indicates that a higher level of PD-1 on circulating CD8(+) T cells in peripheral blood is correlated with poorer clinical outcome and shorter overall survival time [10]. In addition, recent clinical studies have shown that treatment with the PD-1 monoclonal antibody, Nivolumab, resulted in improved overall survival among NSCLC patients and cancer patients who improved significantly after receiving immune-checkpoint blockade therapy [22]. Despite the preclinical and clinical progress in immune-checkpoint blockade therapy for cancer, it remains unclear whether the perioperative use of analgesics has a direct impact on these key inhibitory molecules during surgery. Interestingly, it is has been reported that COX inhibitors act synergistically with immune-checkpoint blockade therapy, implying that NSAIDs commonly used as perioperative analgesics may be a useful adjuvant for anti-PD-1/anti-CTLA-4 therapies in cancer patients [19]. To the best of our knowledge, this is the first clinical study providing direct evidence that NSAIDs alter the postoperative levels of PD-1, thus inhibiting the increase in PD-1 expression on CD8(+) T cells in the peripheral blood of lung cancer patients undergoing resection surgery.

Numerous studies have demonstrated that PD-1 and CTLA-4 have distinct cellular mechanisms for attenuating T cell activation [14, 23–25]. Anti-PD-1 predominantly induces expansion of tumor-infiltrating exhausted-like CD8(+) T cells, whereas anti-CTLA-4 engages both subsets of the ICOS(+)Th1-like CD4(+) effector population and exhausted-like CD8(+) T cells in the tumor microenvironment [26]. Furthermore, PD-1 and CTLA-4 are detected in peripheral blood samples under different conditions. Although both markers are constitutively expressed on lymphocytes, only PD-1 is usually detectable without any T cell stimulation in the majority of patients. By contrast, CTLA-4 is rarely expressed on peripheral blood T cells, except under certain conditions of stimulation [27]. While tumor-infiltrating lymphocytes collected by tumor biopsy offer intuitive and fundamental perspectives on the immune response for tumor site analysis, analyses of circulating lymphocytes are easier to perform, can be repeated at several time points, and may provide a more systemic view of the immune response, especially in patients with visceral tumors. Peripheral blood analysis has recently provided insights into the changes in PD-1 levels in circulating T cells after immunotherapy. Therefore, we collected peripheral blood and examined the changes in postoperative PD-1 levels in circulating T cells.

Based on the results of our pilot study, CD4(+) and CD8(+) T cells in the peripheral blood of NSCLC patients generally have an exhausted phenotype and express higher levels of PD-1 than those of healthy subjects (NSCLC patients versus healthy volunteers; PD-1(+) CD8(+) T cell: 9.76 ± 0.67 vs. 8.61 ± 0.98%, p = 0.016; PD-1(+) CD4(+) T cell: 16.60 ± 5.20 vs. 10.40 ± 1.37%, p = 0.012). COX activity and COX-dependent inflammatory mediators, such as PGs, facilitate the increase in the inhibitory immune-checkpoints. PD-1/CTLA-4 and low levels of PD-1/CTLA-4 have been demonstrated in COX-2 MEC knock-out mice bearing tumors, suggesting that COX activity and downstream PGs are potentially linked with PD-1 [18]. Although NSAIDs may cooperate with anti-PD-1 blockade in inducing the eradication of tumors in preclinical studies [19], our study primarily demonstrated that flurbiprofen altered PD-1 levels on the circulating CD8(+) T cell population in NSCLC patients up to 72 h postoperatively, without any change in the percentage of circulating CD8(+) T cells observed after that time point. Flurbiprofen administration during the perioperative period had little influence on the postoperative percentage of PD-1(+) CD8(+) T cells. The percentage of lymphocytes after surgery in the control and flurbiprofen groups was, in general, lower compared with hospital reference values. It is interesting to note that there were extensive changes in lymphocyte subsets and inflammatory markers following administration of flurbiprofen in the short-term postoperative period up to 72 h.
by a proangiogenic factor in the tumor microenvironment [28]. Moreover, COX-derived PGE2 promotes tumor progression by sustaining angiogenesis through the induction of VEGF. This is largely required for a stable blood supply to facilitate tumor growth [29, 30]. We speculate that the inhibition of COX and PGE2 by flurbiprofen attenuated the increase in PD-1 levels, partly by abrogating the induction of VEGF.

There are several limitations of this study. First, further studies are needed to determine the exact mechanism by which NSAIDs downregulates antitumor immunity and immune escape. Second, although the randomization of participants in our study was strict, some baseline and perioperative factors were not equal between the 2 groups. Third, we did not subdivide CD4(+) T cells into conventional CD4(+) T cell and regulatory CD4(+) T cell categories. Fourth, our study is a single-center investigation. A large multicenter study would be ideal to confirm our findings. Last, we have not investigated whether perioperative flurbiprofen administration affected the clinical outcome and overall survival of patients after leaving the hospital.

Conclusions

Perioperative administration of flurbiprofen attenuates the increase in levels of PD-1 on CD8(+) T cells up to 72 h postoperatively, with no effect identified after this time.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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