Effect of preoperative fever-range whole-body hyperthermia on immunological markers in patients undergoing colorectal cancer surgery†

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Editor’s key points
- Therapeutic hyperthermia might have beneficial immunological effects as an adjunct to cancer surgery.
- The effects of preoperative hyperthermia on a panel of immune system indicators were studied in patients undergoing cancer surgery.
- Two hours of hyperthermia before surgery had potentially beneficial effects on the immune responses to the stress of cancer surgery in this small pilot study.

Background. Previous studies have demonstrated beneficial immunological effects of fever-range whole-body hyperthermia (FR-WBH) as an adjunct to non-surgical cancer therapy. We conducted a study of preoperative FR-WBH in patients undergoing colorectal cancer surgery to evaluate perioperative, hyperthermia-induced immunomodulation.

Methods. The trial was conducted as a subject-blinded, controlled, randomized study. Subjects in the FR-WBH group (n=9) were treated with FR-WBH before operation under propofol sedation; the target core temperature was 39 (0.5)°C with 1 h warming and 2 h plateau phase. Subjects in the control group (n=9) were treated with propofol sedation only. Blood samples were acquired before and after treatment, after operation, and 24, 48 h, and 5 days after the end of surgery. The following parameters were measured: lipopolysaccharide (LPS)-induced tumour necrosis factor (TNF-α), procalcitonin (PCT), interleukin (IL)-6/10, heat shock proteins (HSPs) 60, 70, and 90, human leucocyte antigen-DR (HLA-DR), and LPS-binding protein (LBP).

Results. HSPs were increased in the FR-WBH group after treatment [HSP60, 48 h postop: 143 (41)% vs 89 (42)%, P=0.04; HSP90, postop: 111 (33)% vs 64 (31)%, P=0.04; HSP70: P=0.40; FR-WBH vs control, P-values for area under the level/time curve]. TNF-α levels were elevated after surgery in the control group and remained near baseline in the FR-WBH group [24 h postop: 73 (68)% vs 151 (72)%, P=0.04]. PCT increased in both groups 24 h after surgery; in the control group, this increase was significantly higher (P=0.02). There were no significant differences for IL, HLA-DR, or LBP.

Conclusions. The immune system to react to surgical stress, as measured by a panel of laboratory indicators, might be improved by preoperative FR-WBH.

Keywords: hyperthermia, induced; immunomodulation; immunosuppression; perioperative period

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After surgical cancer therapy, immunological host defence mechanisms of patients are often significantly impaired. This immunological impairment can increase the likelihood of postoperative infections, delay postoperative recovery, and promote metastatic growth.1–2 Perioperatively, immunomodulation is caused to some degree by a stress response to surgical trauma. However, animal and human experiments have demonstrated additional contributing immunosuppressive effects of general anaesthesia. Several inhalation and i.v. drugs have immunomodulatory effects,3,4 and opioids are currently seen as the primary culprits.5,6 Regional anaesthesia, alone or as an adjunct to general anaesthesia, would thus be an obvious choice to avoid or reduce opioid-induced immunosuppression.7 Minimization of surgical trauma, preservation of normothermia, and avoidance of allogeneic blood transfusions are other evidence-based approaches.8 However, the general problem of perioperative immunosuppression remains mostly unresolved.
Induction of fever-range core temperatures in patients has been used as supportive oncological therapy in combination with radiotherapy and/or chemotherapy. The American Cancer Society mentions fever-range whole-body hyperthermia (FR-WBH) as a mostly experimental technique intended to boost the activity of the immune system. Several studies have demonstrated beneficial immunological effects of FR-WBH, such as increased survival in experimental sepsis, induction of immunostimulatory heat shock proteins (HSPs), monocyte stimulation, and improved immunocompetence during radiotherapy/chemotherapy.

We performed the present study to investigate if preoperative FR-WBH can enhance perioperative immunocompetence in a subject-blinded, controlled, randomized study.

Methods

The study was approved by the research ethics committee of the Medical University of Vienna as medical device study (Ref: 16/2009) and registered at Clinicaltrials.gov (Ref: NCT00876954). After obtaining written informed consent, 18 subjects were included in the study. Inclusion criteria were age of 18–75 yr and undergoing curative, colorectal cancer surgery. Exclusive criteria included pregnancy, ongoing immunosuppressive treatment, immunosuppressive treatment in the last 3 months, ongoing chemotherapy, acute infections, or palliative surgical treatment. The trial was conducted as a subject-blinded, controlled, randomized study.

Whole-body hyperthermia system

Over the last decade, patient warming with infrared radiation has been established as a standard procedure for FR-WBH treatment. FR-WBH systems differ with regard to the spectrum of infrared radiation used and the area of application (front or back of the patient). The HECKEL HT-3000 (Heckel medizintechnik GmbH, Esslingen, Germany), used in the present study (Fig. 1), uses water-filtered infrared radiation (wIRA) delivered by four wIRA emitters to the chest, and two heating elements for warming the air under the tent-like canopy.

Treatment protocol

Subjects were transferred to the preoperative holding area ~3.5 h before the scheduled start of surgery. In the preoperative holding area, which doubles as the postoperative recovery unit, subjects were first moved to a bed with the warming device attached, and heart rate, electrocardiography, oxygen saturation, mean arterial pressure, and core temperature via a rectal probe were monitored. After the start of sedation with propofol (3–5 mg kg\(^{-1}\) h\(^{-1}\)), an opaque envelope containing a computer-generated randomization list was opened and treatment allocation was revealed. In the control group, only the heating elements for warming the air under the canopy were activated to avoid accidental hypothermia during sedation. In the FR-WBH group, all four wIRA emitters and the air heaters were activated. The core temperature of the hyperthermia group was raised to 39.0 °C for a duration of 2 h. Once 39.0 °C was reached, the wIRA emitters were turned off, and were turned on again if temperature decreased below 39.0 °C.

Oxygen saturation, mean arterial pressure, and heart rate were registered at 5 min intervals. Isotonic crystalloid solution (Elo-mel isoton, Fresenius Kabi, Graz, Austria) was administered by continuous infusion during control and

Fig 1. Heckel HT-3000 whole-body hyperthermia device. The four water-filtered infrared emitters and the tent-like structure enclosing a regular patient bed are displayed.
hyperthermia treatment (5–10 ml kg\(^{-1}\) h\(^{-1}\)). In both groups, after treatment and during surgery, normothermia was maintained with forced air (BairHugger, Arizant, Eden Prairie, MN, USA) until arrival in the postoperative recovery unit. Anaesthesia was induced with fentanyl (1–3 \(\mu g\) kg\(^{-1}\)), propofol (2–3 mg kg\(^{-1}\)), and rocuronium (0.6 mg kg\(^{-1}\)) and maintained with sevoflurane (1.5–2.0%) and fentanyl to keep mean arterial pressure within ±20% of the preinduction value according to a standardized protocol.

Blinding of researchers during treatment was not possible due to safety concerns as close control of core temperature and other vital parameters was paramount. However, subjects, surgeons, attending anaesthesiologists, and laboratory personnel were blinded to group assignment.

To evaluate immunomodulation, the following parameters were assessed: lipopolysaccharide (LPS)-induced tumour necrosis factor (TNF-\(\alpha\)), procalcitonin (PCT), interleukin (IL)-6, IL-10, HSPs 60, 70, and 90, and human leucocyte antigen (HLA)-DR. Blood samples were obtained: (i) before and (ii) after study treatment; (iii) after surgery; (iv) 24, (v) 48 h, (vi) 5 days after surgery.

**Measurements**

**Quality of Recovery questionnaire**

Twenty-four hours after surgery, the Quality of Recovery (QoR)-40 questionnaire for the quality of recovery after anaesthesia was assessed. The score ranges from 40 to 200, with higher values representing better quality of recovery. Five clinically relevant dimensions are derived, which encompass most aspects of postoperative recovery. The dimensions are emotional state, physical comfort psychological support, physical independence, and pain.\(^{13}\)

**Blood sampling**

Each blood sample consisted of 14 ml collected into a heparinized blood collection tube. After removing 100 \(\mu l\) for TNF-\(\alpha\) and HLA-DR measurements, the blood was centrifuged (2500g, 10 min), the supernatant was removed, and serum samples were stored at −80°C for later analysis of PCT, HSPs, and other cytokines.

**Heat shock proteins 60, 70, and 90**

Stressgen\(^{®}\) and Assay Designs\(^{®}\) ELISA kits (Enzo Life Sciences, Farmingdale, NY, USA), and a Wallac Victor 3 V microplate reader (PerkinElmer Life Sciences, Boston, MA, USA) were used for HSP measurements.

**LPS-induced TNF-\(\alpha\)**

Immediately after blood sampling, 50 \(\mu l\) of whole blood was mixed with 20 \(\mu l\) of FITC Mouse Anti-Human HLA-DR (BD Pharmingen\(^{®}\)) and 20 \(\mu l\) of fresh whole blood was mixed with 20 \(\mu l\) of LPS solution (0.5 ng ml\(^{-1}\)) at a clean bench. The mixture was then incubated in a water bath at 37°C for 4 h. After centrifugation, the supernatant was frozen at −80°C and measured with a standardized kit (Milenia\(^{®}\) QuickLine TNF-\(\alpha\) ex vivo, Milenia Biotec GmbH, Gießen, Germany) at a later date.

**Human leucocyte antigen-DR**

Immediately after blood sampling, 50 \(\mu l\) of whole blood was mixed with 20 \(\mu l\) of FITC Mouse Anti-Human HLA-DR (BD Pharmingen\(^{®}\)) and incubated for 30 min at room temperature, 450 \(\mu l\) of BD FACs Lysing solution (BD Pharmingen\(^{®}\)) was added to lyse red blood cells, and the sample was incubated for 30 min at room temperature. HLA-DR expression was measured by flow cytometric analysis on a FACs Cytomics FC500 (Becton Dickinson, San Jose, CA, USA).

**Cytokine analysis**

Cytokine analysis [IL-6, IL-10, TNF-\(\alpha\), LPS-binding protein (LBP)] was performed using enzyme-linked immunoassay kits (DPC/Siemens Healthcare Diagnostic, Eschborn, Germany). PCT levels were measured by a fully automatic, multi-channel analyser using electrochemiluminescence technology (Cobas e 411, Roche Diagnostics Limited, Burgess Hill, UK). TNF-\(\alpha\) was measured additionally as a validation with Siemens DPC; values in the paper are from the Milenia\(^{®}\) QuickLine TNF-\(\alpha\) ex vivo measurements.

**Statistical analysis**

As data of the immunomodulatory effect of preoperative hyperthermia were not available at the time of protocol design, sample size estimation relied on data of perioperative, LPS-induced TNF-\(\alpha\) levels in another context.\(^{14}\) Assuming a slightly larger standard deviation for both groups of 0.3, a sample size of eight subjects per group was calculated for a difference of 30% with an \(\alpha\)-error of 0.05 and a power (1−\(\beta\) error) of 90%. To account for a 10% dropout of subjects, who might not be able to tolerate sedation, hyperthermia treatment, or both, nine subjects per group were included in the study.

As primary analysis, the area under the variable–time curve (AUC) for each variable was calculated and compared using Student’s t-test. Differences between treatment groups for variables over time were assessed by analysis of variance (ANOVA) for repeated measurements with group as the between-subject factor and time as the within-subject factor. If a significant difference between the two groups was detected, Fisher’s least significant differences contrast was performed to assess differences at individual time points. The preoperative baselines of all parameters subsequently compared as percentage of baseline were initially compared using Student’s t-test between the groups to exclude any \(a\) priori imbalances as sensitivity analysis. All immunological parameters except IL-10 were standardized to the preoperative baseline (i.e. before treatment=100%); IL-10 was below the limit of detection before operation. All data are presented as mean (so), unless otherwise specified. PASW 18.0.3 (IBM, Armonk, NY, USA) was used for all statistical analysis; \(P<0.05\) was considered statistically significant.
Results

All enrolled subjects (n=18) completed the study. Patient characteristic, morphometric, anaesthesiological, and surgical data are displayed in Table 1. The core temperature of 38.5°C was reached in 71.2 (18.4) min; the mean peak temperature was 39.4 (0.4)°C; the mean duration of hyperthermia treatment was 132 (16.2) min.

There were no significant differences for preoperative baselines between the groups in any immune parameter (results not displayed). The following HSPs were significantly increased in the FR-WBH group compared with the control group: HSP60 (P=0.04) and HSP90 (P=0.04). A trend towards increased plasma levels was measured for HSP70 only after FR-WBH treatment; however, no overall difference was detected between the groups (P=0.40, Figs 2–4).

The results of LPS-induced TNF-α levels are displayed in Figure 5. TNF-α levels remained near baseline at time points 4–6 in the FR-WBH group and were significantly elevated in the control group (P=0.04).

PCT increased in both groups 24 h after surgery (time points 4–6); in the control group, this increase was significantly higher than in the FR-WBH group (P=0.02; Fig. 6).

There were no differences for IL-6, IL-10, HLA-DR, and LBP between the groups (Table 2). Results are summarized in Table 3.

Similarly, there was no significant difference for the quality of postoperative recovery as assessed by the QoR-40 questionnaire between the groups, with a global QoR-40 score of 167 (15) in the FR-WBH group and 159 (16) in the control group and similarly no significant differences for the individual dimensions of the questionnaire (P=0.81).

Sixty per cent of all subjects in the hyperthermia group showed transient erythema on the chest. One major hyperthermia-related side-effect was observed. Two round, thermal lesions appeared in one subject after FR-WBH treatment (Combustion grade II, 1.5 cm in diameter on both lower breasts). There were no sedation-related side-effects in either group, and no subject had any memory of hyperthermia-related stress.

Discussion

After surgical interventions for cancer surgery, postoperative immunosuppression can occur. We tested if preoperative FR-WBH was able to modulate this immunopathology. Based on various correlative markers, we found markedly different immunological reactions between the control and the FR-WBH group, indicating that the ability of the immune system to react to surgical stress might be improved.

Over the last years, many studies have been published addressing the problem of postoperative immunosuppression. Postoperative immunosuppression is particularly undesirable in cancer surgery, when the immune system’s role in the fight against metastatic growth is essential. In non-cancer surgery, postoperative immunosuppression promotes infection, sepsis, and increases duration of recovery. But few evidence-based options to improve postoperative immunocompetence are available. A surgical option is minimization of the surgical trauma itself since there is clear evidence that the extent of trauma correlates with the extent of immunosuppression.

Anaesthetic drugs also contribute to deterioration of postoperative immunocompetence. Most drugs used during general anaesthesia, and in particular opioids, have immunosuppressive effects—reducing cell-mediated immunity and promoting metastasis. In contrast, propofol is immunologically relatively inert, and features even anti-tumour properties; thus, it was used in the present study. Regional anaesthesia alone or as an adjunct to general anaesthesia would be an option to improve postoperative immunocompetence, as regional anaesthesia has an opioid-sparing effect.
There is evidence that the optimal management of peri-
operative core temperature has a major impact on post-
operative immunocompetence. Accidental hypothermia
enhances immunosuppressive effects in animal models and
humans leading to increased infection rates\(^2\) and promoting
inhibition of cell-mediated immune defence.\(^2\)

The use of therapeutic whole-body, fever-range hyper-
thermia as a booster of immunological function has a long
history. The American surgical oncologist Dr William Coley
(1862–1936) popularized a drug for cancer treatment that
consisted of a fever-inducing infusion containing dead
bacteria. With the advent of modern radiation therapy and
chemotherapy, this treatment mostly disappeared. Despite
the disappearance of Coley’s toxin, both regional and whole-
body hyperthermia induced not by pharmacological but by
physical methods has remained an increasingly important
therapy in oncology. The positive effects of FR-WBH are
partly due to a direct physical anti-tumour action on a cellu-
rar level, and due to enhanced mobilization and migration of
immune cells, improved anti-tumour immune-response
through activation of NK-cells with increased cytotoxic
action, and up-regulation of effector molecule expression
among others. A concise overview of the known immunological effects of FR-WBH has recently been published.22 There is also increasing evidence of long-term survival benefits in cancer patients treated with hyperthermia in combination with radiotherapy or chemotherapy.22 However, specific immunological findings after hyperthermia treatment are often conflicting, since immunological effects seem to be highly dependent on actual height of core temperature, on duration, and on timing of treatment.22

In the present study, we chose a regimen of preoperative moderate hyperthermia, with a 2 h plateau phase at 39.0 (0.5) °C. The levels and time courses of several HSPs (60, 70, 90) were assessed. HSPs are a class of highly conserved proteins whose expression is increased during exposure of cells to elevated temperatures or to other stress (oxidative, bacterial, chemical, etc.). The HSPs serve as molecular chaperones during all aspects of protein synthesis, assembly, and transport, and they also are highly active immunological cytokines.23 Therapeutically, HSPs have been proposed as adjuvants for cancer vaccines to boost vaccine response.24 Current evidence suggest that HSPs play a central role in the induction of peptide and tumour-specific immunity, while possessing important anti-inflammatory properties.23

The increase in HSPs 60, 70, and 90 in the FR-WBH group can thus be interpreted as part of an improved immunoregulatory response controlling an otherwise overshooting inflammatory response. For HSPs 70 and 90, anti-tumour-specific properties have been reported, and the increased levels in the FR-WBH group can therefore be hypothesized as beneficial.25

The study also shows that LPS-induced TNF-α levels were significantly elevated 24 h after surgery until the 5th day in the control group and remained near baseline for the FR-WBH group. TNF-α is a pleiotropic inflammatory cytokine

| Table 2 | IL-6, IL-10, HLA-DR, and LBP in control and WBH subjects. Values as mean (SD), statistical analysis: P for Student’s t-test—comparison of AUC between the groups. *Preoperative baseline normalized to 100%; #preoperative baseline at 0.0 pg ml⁻¹ |
|----------|-----------------|-----------------|-----------------|-----------------|-----------------|
|          | Pre-treatment   | Post-treatment  | Postoperative   | 24 h            | 48 h            | 5 days          |
| IL-6 (%)*| 100             | 117 (43)        | 3020 (3380)     | 2470 (3120)     | 999 (1300)      | 927 (1820)      |
| Placebo  | FR-WBH          | P=0.93          |                 |                 |                 |                 |
| IL-10 (pg ml⁻¹)#| 0             | 0.6 (1.8)       | 28.3 (25.6)     | 0 (0)           | 0 (0)           | 0 (0)           |
| Placebo  | FR-WBH          | P=0.36          |                 |                 |                 |                 |
| HLA-DR (%)*| 100             | 103 (17)        | 65 (12)         | 61 (12)         | 59 (13)         | 62 (17)         |
| Placebo  | FR-WBH          | P=0.68          |                 |                 |                 |                 |
| LBP (%)* | 100             | 141 (127)       | 114 (65)        | 392 (362)       | 468 (242)       | 313 (226)       |
| Placebo  | FR-WBH          | P=0.79          |                 |                 |                 |                 |

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Summary of results and implications. *P&lt;0.05; ↔ remaining at baseline; ↑↑ major increase, moderate increase; ↓↓ major decrease, moderate decrease</th>
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<tbody>
<tr>
<td>Parameter</td>
<td>Placebo FR-WBH Implications</td>
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<tr>
<td>HSP60*</td>
<td>↔ ↑ HSPs play a central role in the induction of tumour-specific immunity and possess anti-inflammatory properties</td>
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<tr>
<td>HSP70</td>
<td>↔ ↔</td>
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<tr>
<td>HSP90*</td>
<td>↔ ↑</td>
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<tr>
<td>TNF-α*</td>
<td>↑ ↔ Pro-inflammatory; can promote growth of malignancies</td>
</tr>
<tr>
<td>PCT*</td>
<td>↑ ↔ Marker of infection and inflammation</td>
</tr>
<tr>
<td>IL-6</td>
<td>↑ ↑ Both anti- and pro-inflammatory actions</td>
</tr>
<tr>
<td>IL-10</td>
<td>↑ ↑ Anti-inflammatory cytokine</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>↑ ↔ Antigen presentation</td>
</tr>
<tr>
<td>LBP</td>
<td>↑ ↔ Acute phase protein binding to bacterial LPS to promote immune response</td>
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that serves a wide variety of functions, has growth stimulating and inhibitory properties, and exhibits pro-inflammatory modulation of the immune response to bacterial, fungal, viral, and parasitic invasions. Interestingly, TNF-α also promotes growth of malignancies.26 27 TNF-α-blocking drugs play a major role in the treatment of rheumatic diseases and TNF-α levels are being investigated as prognostic markers to predict occurrence, severity, and outcome of sepsis.27 In the present study, TNF-α levels remained at baseline in the FR-WBH group. This is in line with previous observations that moderate (39°C), longer duration hyperthermia represses an overshoot of the production of pro-inflammatory cytokines after stimulation with LPS.28 The persisting increase in LPS-induced TNF-α secretion in the control group might indicate a detrimental immunological status, considering the evidence that TNF-α has tumour-promoting properties and that TNF-α blockers have been proposed as cancer therapies.

Similarly, PCT concentrations increased significantly in the control group compared with the FR-WBH group, where it remained at baseline. PCT levels in the circulation are very low (<0.05 ng ml⁻¹), infection and inflammation induce release of PCT from various cell types with significant elevations of PCT levels (up to 1000 ng ml⁻¹), for example, with severe bacterial infection or sepsis.29 Lower PCT values, as in the study’s FR-WBH group, were generally associated with a better prognosis for perioperative complications after cancer surgery.

No differences between the groups were found for LBP, IL-6, IL-10, HLA-DR, and LBP. IL-6 possesses both anti- and pro-inflammatory actions and increased in both groups after surgery, as has been reported in a previous study.30 Immediately after FR-WBH therapy, IL-6 is increased in the hyperthermia group, albeit not significantly. The anti-inflammatory cytokine IL-10 peaked after operation in both groups, and decreased 24 h after surgery to baseline levels as expected. HLA-DR and LBP did not differ between the groups, HLA-DR decreased in both groups below baseline until the end of the study, and LBP increased in both groups after surgery comparably.

This study has several limitations. The immunological markers studied are only able to identify certain aspects of perioperative immune status and are unable to give a complete picture of overall perioperative immunocompetence. Furthermore, the parameters provide results that are not easy to interpret and incongruous. This is in line with previous publications;22 a reason for this is likely the vast heterogeneity of hyperthermia studies with regard to the target temperature, timing, type, and duration of hyperthermia application. We cannot exclude that the hyperthermia treatment was not adequate or even excessive. Furthermore, it was not possible to blind research personnel during hyperthermia therapy for safety reasons. Another limitation is that core temperature was measured rectally to ensure patient comfort, and it has been reported that rectal temperature lags behind core temperature.31 However, the protocol took this lag into account, and rectal core temperatures remained within the desired temperature boundaries throughout hyperthermia treatment. Finally, results might have been different if FR-WBH had been performed without sedation, although propofol seems to have less effect on the immune system than other anaesthetic drugs.3 However, hyperthermia treatment would not have been tolerated by the majority of patients without sedation.

In summary, immunological reactions to surgery were different between the control group and the FR-WBH group. The combination of elevated HSP levels, lower TNF-α, and lower PCT in the FR-WBH group indicates that the ability of the immune system to react to surgical stress might be improved by preoperative FR-WBH.

**Declaration of interest**

None declared.

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