

Does Post-Surgery Placement of Rectus Sheath Block Analgesia Alter the Oxidative Stress Biomarker 8-OHdG Concentrations: A Randomised Trial of Patients with Cancer and Benign Disease

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Abstract. *Background:* The aim of the study was to evaluate whether the post-surgery placement of the rectus sheath block (RSB) analgesia could alter the oxidative stress response. The main hypothesis of our study was to find some correlation between patients' pain experience, numeric rating scale (NRS) and the concentration of oxidative stress marker, 8-OHdG (8-hydroxy-2'-deoxyguanosine) in patients with benign disease and cancer. *Materials and Methods:* Initially, 46 patients were randomized to the placebo group (n=11) and to one of the three active groups; single-dose (n=12), repeated-dose (n=12) and continuous infusion (n=11) RSB analgesia group. The plasma concentrations of the hs-C-reactive protein (CRP) and 8-OHdG were measured at three time points: just before, immediately after and 24 h after operation. The primary end-point was to compare plasma concentrations of the hs-CRP and 8-OHdG in the placebo group and in the three different RSB analgesia groups in patients with benign disease and cancer. *Results:* The placebo group and three active groups were similar in terms of demographic variables and the perioperative data. The patients in the continuous infusion group had a trend for lower median 8-OHdG values post-operatively than the three other study groups (p=0.147; in all patients with benign

disease and cancer). The patients in the cancer group showed a trend for higher median 8-OHdG values in the repeated-dose group than the patients in the benign group (p=0.241). There was a significant inverse correlation between the individual values of the plasma hs-CRP and 8-OHdG in patients with benign disease and cancer (r=-0.40, p=0.02). However, there was no significant correlation between the individual values of the NRS score and 8-OHdG post-surgery in patients with benign disease and cancer. *Conclusion:* The results suggest that the placement of RSB analgesia does not significantly alter the oxidative stress marker 8-OHdG concentrations in patients with benign disease or cancer patients. A new finding with possible clinical relevance is a significant inverse correlation between the individual plasma values of the hs-CRP and 8-OHdG in patients with benign disease and cancer.

Surgical trauma is associated with the generation of mutagenic reactive oxygen species (ROS). 8-hydroxy-2'-deoxyguanosine (8-OHdG) is frequently used as a biomarker of oxidative stress. When exposed to oxidative radicals, endogenous oxidative damage to DNA leads to formation of 8-OHdG. It can serve as a sensitive indicator of physiological damage to DNA (1-3). ROS are known to be linked to cell damage, leading to carcinogenesis, and augmented levels of 8-OHdG are found in malignancies such as colorectal, ovarian and bladder cancer (1-5). Abdominal surgery is associated with generation of oxidative stress. Elevated levels of ROS are found to associate with the degree of trauma in surgery and oxidative stress can lead to chronic inflammation by activating several transcription

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factors (6) Arsalani-Zadeh *et al.* (7) reviewed the literature for development of oxidative stress in laparoscopic (LAP) and open surgery (OS) procedures. After review of the abstracts, 17 papers met the inclusion criteria, but it was not possible to perform a meta-analysis due to heterogeneity of the patient data, patient selection criteria and diversity of the markers used. However, they concluded that the oxidative stress response to surgery depends on the degree of trauma, and the reduction of surgical trauma by LAP seems to diminish the stress response compared to OS. Pappas-Gogos *et al.* (8) studied the effect of surgical techniques in response to oxidative stress markers in 60 consecutive patients with colorectal cancer who received either LAP (n=30) or OS (n=30). They found, that the postoperative 8-OHdG values were significantly lower in the LAP group compared to the OS group ($p < 0.05$) and they concluded that the LAP surgery is associated with lower oxidative stress compared to OS. To our knowledge, the assessment of oxidative stress response to surgical trauma in laparotomy with rectus sheath block (RSB) analgesia has not been studied in a placebo controlled, randomised trial. Therefore, the plasma concentrations of the hs-CRP and 8-OHdG were measured at three time points; before (PRE), immediately after (POP1) and 24 h after operation (POP2). The primary end-point of the study was to compare plasma concentrations of the hs-CRP and 8-OHdG in patients with midline laparotomy randomized to the placebo group and to the three active groups; single-dose, repeated-dose and continuous infusion RSB analgesia groups. The main hypothesis of our study was, whether the post-surgery placement of the RSB could reduce the oxidative stress following surgery in patients with benign disease and cancer.

Materials and Methods

The present study was approved by the Ethics Committee of Kuopio University Hospital District, Finland (DNRO 120/2011, November 11, 2011), it was registered in the EudraCT database (EudraCT number 2011-005136-25, Consort diagram, Figure 1), and it was conducted in accordance with the Declaration of Helsinki. Participants gave written consent after receiving verbal and written information. Operations were carried out in Kuopio University Hospital, Kuopio between 2012 and 2015. The flowchart of the study is presented in Figure 1. The study design was a prospective, randomised, clinical trial with four parallel groups. The patients with midline laparotomy were randomized to the placebo group and to one of the three active groups; single-dose, repeated-dose and continuous infusion RSB analgesia groups. The patients had intravenous oxycodone pumps (PCA, the patient controlled analgesia). The randomisation list was generated by computer (www.randomization.com) and a sealed enveloped method was used for blinding.

All RSB procedures were performed by an experienced surgeon in the operating room before the wound closure. Bilateral RSB catheters were placed with completely aseptic technique to the 'railway-like line' in the rectus sheath between the rectus muscle and the posterior rectus sheath in the lateral third of the sheath. The correct position of

catheters was confirmed and 20 ml injection of levobupivacaine (Chirocaine, AbbVie, Espoo, Finland) was performed to separate the planes and achieve hydrodissection for placement of the catheters. In the blocks, including the single-dose block, bilateral rectus sheath catheters were used (InfiltraLong Pajunk, Geisingen, Germany). In the single-dose group, the block was performed at the end of operation by injecting 20 ml levobupivacaine 1.25 mg/ml for both catheters, a total dose of levobupivacaine of 50 mg. In the repeated-dose group, a similar starting injection was performed, and after that a 10 ml of 1.25 mg/ml for both catheters, a total dose of two injections 25 mg, was repeated at every 4 hours for the first 48 h. In the continuous infusion group, the 20+20 ml block was performed as described above and continued with bilateral rectus sheath catheters, infusion of 5 ml/h of levobupivacaine 1.25 mg/ml for each (a total dose of 12.5 mg/h) with ambulatory infusion pumps (Autofuser pumps, Acemedical, Seoul, Korea). The patients in the placebo group had no RSB catheters inserted. However, the patients in the placebo group were blinded using the similar wound dressing as the patients in the active groups.

The exclusion criteria included high body mass index (BMI) ≥ 35 kg/m², age under 18 or ≥ 80 years, pregnancy, re-operations in the same hospital visit or another hospital, history of drug or narcotics abuse and earlier allergic reactions to local anaesthetics and contraindications to oxycodone. All study patients were informed by the investigator about different post-operative analgesia methods before they gave a written consent.

For laboratory measurements EDTA-plasma samples were taken at the pre-specified time-points and centrifuged at 1,000 \times g for 15 min. Plasma was separated and stored frozen at -70°C until analyzed. The plasma 8-OHdG assays were performed using the HT 8-oxo-dG ELISA Kit II (Trevigen, Gaithersburg, MD, USA). Plasma Hs-CRP was analyzed with a Cobas 6000-analyzer (Hitachi, Tokyo, Japan).

The primary outcome measures were the plasma levels of Hs-CRP and 8-OHdG measured at three time points with high-sensitivity assays.

The data were entered and analyzed with a statistical software program SPSS (IBM SPSS Statistics 22.0, IBM, Somers, IL, USA). Baseline characteristics between groups were tested by Fisher exact test and if variable were continuous then analysis were performed by analysis of variance (ANOVA). Group differences in three time points were tested by Mann-Whitney *U*-test and Kruskal-Wallis-test. The results of the marker values are presented as median with interquartile range because distributions were right skewed. A two-sided *p*-value of less than 0.05 was considered statistically significant. The overall satisfaction and an opinion on the success of the analgesia procedure were surveyed and filed on an 11-point numeric rating scale (NRS; 0=fully unsatisfied; 10=fully satisfied). The results of the individual NRS values *versus* the 8-OHdG, *versus* the Hs-CRP marker correlations, are shown as jitter plots with Spearman correlation coefficients in Figures 2 and 3.

Results

The demographics and perioperative data were similar in the four study groups, but BMI was higher in patients in the placebo group and in the single-dose group compared to the two other study groups (Table I).

At baseline, the Hs-CRP median values were similar between the placebo group and the three active groups and

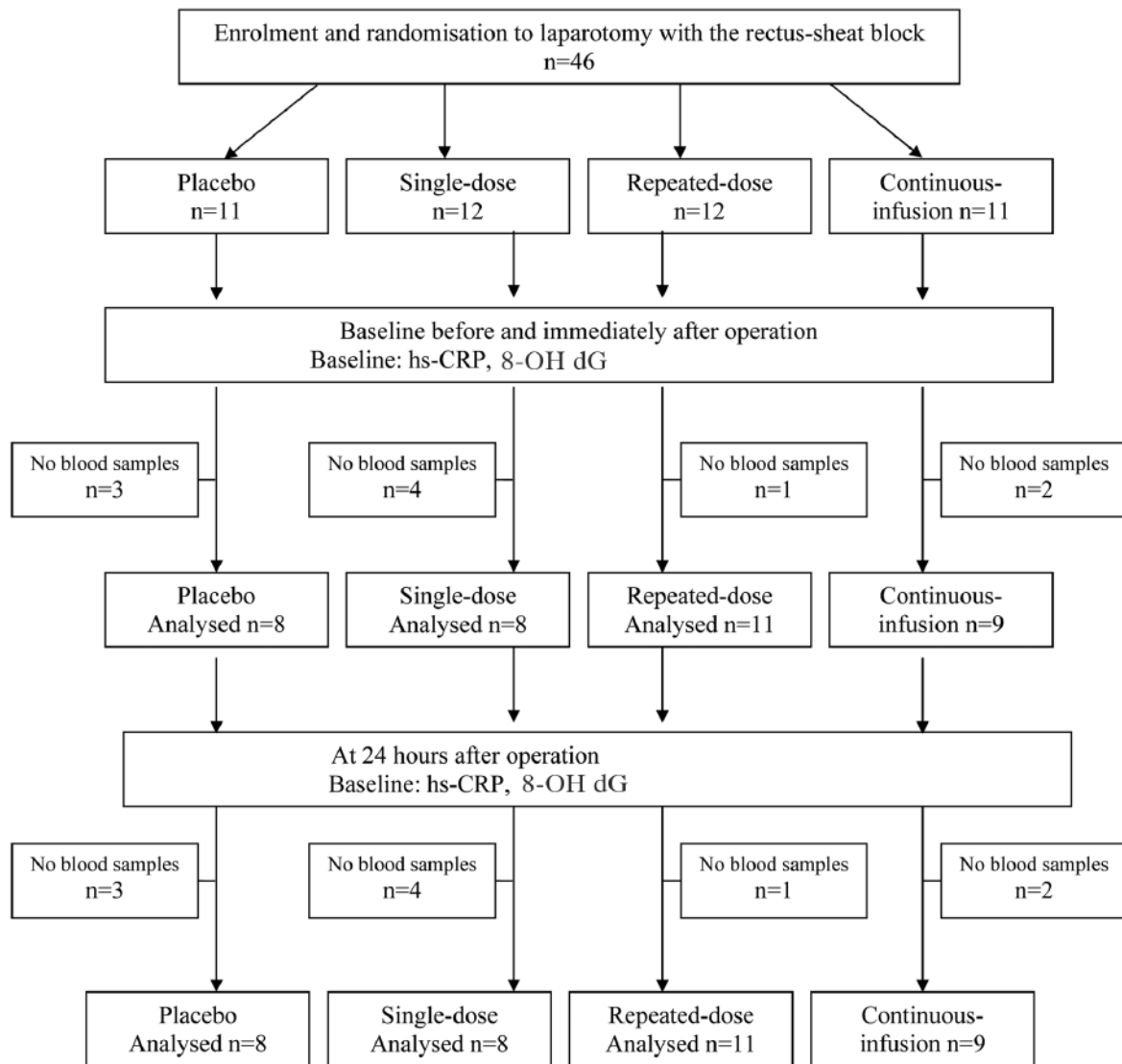


Figure 1. Flowchart of the study design.

the median values increased in all four study groups post-operatively (Table II). However, the elevation of the Hs-CRP median values 24 h postoperatively was less in the patients in three active groups combined (RSB combined, Table III) than the Hs-CRP in the placebo group, respectively (Table II).

Plasma levels of 8-OHdG marker were decreased post-operatively in all four groups (POP1 and POP2). No differences were detected in the 8-OHdG values between the placebo and the three active groups preoperatively and immediately after operation. However, the patients in the single-dose group had a slightly higher decrease of the 8-OHdG level immediately after operation (POP1) than the two other active groups separately (Table II).

There was no statistical significant difference in the Hs-CRP median values between benign and cancer patients pre- and post-operatively. No statistically significant differences were detected in the 8-OHdG median values between the benign and cancer patients pre- and postoperatively in the placebo group and in the single dose and in the continuous dose group.

There was a statistically significant inverse correlation between the individual values of the plasma Hs-CRP and 8-OHdG in patients with benign disease and cancer ($r=-0.40$, $p=0.02$) (Figure 2). However, there is no significant correlation between the individual values of the NRS and 8-OHdG postoperatively in patients with benign disease and cancer (Figure 3).

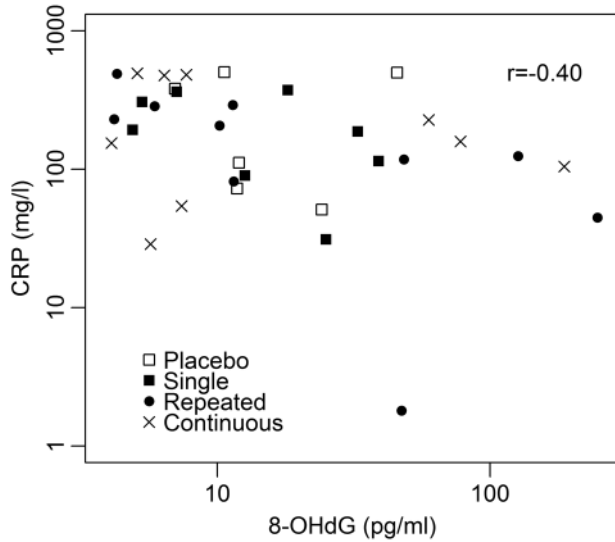


Figure 2. The jitter plots of the individual hs-CRP (C-reactive protein) values versus 8-OHdG (8-hydroxy-2'-deoxyguanosine) values post-surgery (POP1) for the placebo group and three study groups ($r=-0.4$, $p=0.02$).

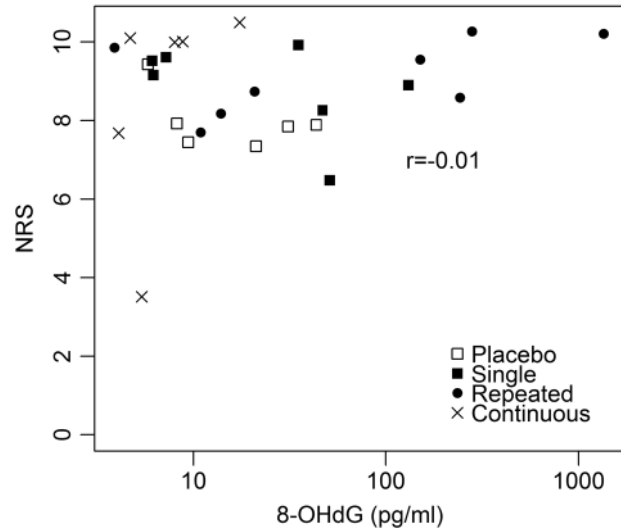


Figure 3. The jitter plots of the individual postoperative NRS (11-point numeric rating scale) values versus 8-OHdG (8-hydroxy-2'-deoxyguanosine) values post-surgery (POP1) for the placebo group and three study groups ($r=-0.01$, $p=ns$).

Table I. Baseline demographic characteristics and surgical data for the placebo group and the three active groups; single-dose, repeated-dose and continuous infusion RSB analgesia groups.

Variable	Placebo n=11	Single n=12	Repeated n=12	Continuous n=11	p-Value
Age (years)	62.6 (14.3)	60.8 (12.6)	63.3 (10.8)	58.0 (10.1)	0.74
Sex (male/female)*	1/10	3/9	3/9	4/7	0.22
Height (cm)	166.6 (8.6)	168.4 (7.9)	165.7 (7.2)	164.3 (6.6)	0.62
Weight (kg)	78.6 (11.8)	83.7 (12.8)	67.8 (13.7)	68.8 (10.6)	0.007
BMI (kg/m ²)	28.3 (3.8)	29.6 (4.4)	24.6 (4.3)	25.7 (4.9)	0.03
Time in the operative room (min)	229.4 (113.4)	274.9 (148.4)	235.7 (112.0)	279.7 (178.5)	0.85
Operative time(min)	209.6 (141.2)	221.8 (156.4)	154.4 (95.0)	253.3 (168.9)	0.55
Perioperative-bleed (ml)	696 (741)	822(906)	697 (967)	1340(928)	0.31
ASA 1/2/3/4*	1/8/2/0	0/7/4/1	1/5/6/0	2/6/3/0	0.43
Length of the scin incision(s) (mm)	27.2 (6.6)	24.4 (7.8)	24.2 (7.9)	29.7 (7.3)	0.31
Type of disease* (Benign/GI-cancer/Gyn-cancer/other)#	1/3/6/1	3/3/4/2	5/2/4/1	3/2/6/0	0.32

#Benign disease, n=12/Gastrointestinal tract cancer, n=10/Gynecological cancer, n=20/Other malignancy, n=4. BMI: Body mass index; ASA: American Society of Anesthesiologists physical status score. Data are mean (standard deviation) or *number of cases.

Discussion

High concentrations of ROS and RNS, can damage both nuclear and mitochondrial DNA, RNA, lipids, and proteins by nitration, oxidation and halogenation reactions leading to mutations and genomic instability (2, 9). ROS and RNS products can also modulate signaling molecules and alter functions of enzymes and proteins involved in carcinogenesis (2, 9).

Our study group (10) has previously reported that the inflammatory response in patients with minilaparotomy (MC) versus laparoscopic cholecystectomy (LC) was quite similar based on the IL-8, IL-10, and IL-1 β values post-surgery (4), although the LC patients reported significantly lower pain score 24 h post-operatively and a shorter convalescence than the MC patients in a randomised trial (11). Okholm *et al.* (12) reviewed studies published between

Table II. The plasma 8-OHdG (8-hydroxy-2'-deoxyguanosine) concentrations for the placebo group and the three active groups; single-dose, repeated-dose and continuous infusion RSB analgesia groups.

Marker	Placebo	Single	Repeated	Continuous	p-Value
Hs-CRP (mg/l)					
PRE	3.48 (1.21-13.05)	4.65 (0.89-48.23)	1.20 (0.59-4.00)	9.60 (1.46-73.96)	0.455
POP1	6.60 (1.01-13.24)	9.18 (2.90-83.25)	2.30 (0.59-5.13)	25.87 (1.04-231.81)	0.289
POP2	246.11 (82.25-469.10)	189.55 (96.15-348.97)	206.50 (81.50-284.42)	159.00 (79.33-478.93)	0.875
8-OHdG (pg/ml)					
PRE	17.10 (5.33-59.93)	47.30 (6.90-297.70)	24.25 (8.02-211.00)	10.40 (6.20-19.25)	0.449
POP1	15.25 (7.60-34.05)	29.20 (6.45-50.20)	20.80 (7.65-261-85)	8.00 (4.70-8.80)	0.147
POP2	11.90 (9.70-29.58)	15.35 (5.75-30.88)	11.45 (5.50-67.98)	7.40 (5.40-68.80)	0.925

Values are median (interquartile range). Plasma levels of Hs-CRP and 8-OHdG were measured at three time points; before operation (PRE), immediately after operation (POP1) and 24 h after operation (POP2).

Table III. The plasma 8-OHdG marker concentrations for the placebo group and for the RSB group; three active groups, single-dose, repeated-dose and continuous infusion groups combined.

Marker	Placebo	RSB	p-Value
Hs-CRP (mg/l)			
PRE	3.48 (1.21-13.05)	3.30 (0.82-48.23)	0.723
POP1	6.60 (1.01-13.24)	4.35 (0.82-59.86)	0.889
POP2	246.11 (82.25-469.10)	189.55 (93.60-302.50)	0.466
8-OHdG (pg/ml)			
PRE	17.10 (5.33-59.93)	18.35 (6.88-107.33)	0.524
POP1	15.25 (7.60-34.05)	12.40 (6.13-50.20)	0.980
POP2	11.90 (9.70-29.58)	11.40 (5.70-47.50)	0.733

Values are median (interquartile range). Plasma levels of Hs-CRP and 8-OHdG were measured at three time points; before operation (PRE), immediately after operation (POP1) and 24 h after operation (POP2).

1999 and 2013 including three randomized trials and seven retrospective studies, and concluded that the stress response to surgery depends on the degree of trauma, and the reduction of surgical trauma by laparoscopy-assisted techniques seems to diminish the immune response compared to open surgery. Since recent evidence suggests that oxidative stress, inflammation and cancer are related (3, 6), we compared the plasma concentrations of the hs-CRP and the 8-OHdG in patients with cancer and benign disease with RSB analgesia randomized to the placebo group and to the three active groups. The oxidative stress is defined as an imbalance between production of so-called oxidants or ROS and their elimination by protective antioxidants (3). Oxidative stress can activate a variety of transcription factors including p53, beta-catenin, AP-1, PPAR-gamma, and Nrf2. Activation of these factors can lead to the expression of over 500 genes of chemokines, inflammatory cytokines, growth factors, and anti-inflammatory molecules. It is not yet fully understood how oxidative stress and inflammation lead to

transformation of a normal cell to tumor cell, but recent observations suggest that chronic inflammation and oxidative stress are closely related (3, 6).

The present study showed that the inflammatory response in benign group *versus* cancer group was similar based on the plasma concentrations of Hs-CRP, one of the most studied acute phase protein after surgery. CRP activates the complement pathway and stimulates phagocytosis by macrophages and neutrophils (12). A new finding is a statistically significant inverse correlation between the individual plasma values of the hs-CRP and 8-OHdG in patients with benign disease and cancer ($r=-0.4$, $p=0.02$). Interestingly, although the plasma levels of 8-OHdG decreased after surgery, the plasma levels of hs-CRP, the inflammatory response marker, did increase post-operatively.

From a methodological point of view, the limitation of our study is its small sample size of 46 analyzed patients. However, this research question is very specific and therefore we did not expect a high number of patients. This could be

taken in account when planning new scientific studies of the RSB analgesia in cancer patients in the future. In all active groups, the RSB catheters were inserted at the end of operation, while the patients in the placebo group had no RSB catheters at all. However, the patients in the placebo group were blinded using the similar wound dressing as the patients in the active groups. Therefore, it is unlikely that there is a study bias from the placement of the RSB catheters between the placebo group and the three study groups.

In conclusion, the results suggest that post-surgery placement of RSB analgesia does not significantly alter the oxidative stress marker 8-OHdG concentrations following surgery in patients with benign disease or cancer. Unfortunately, we don't know the explanation for the inverse correlation between the postoperative ns-CRP and 8-OHdG plasma values at the moment.

Disclosure

No conflicts of interest exists. The authors alone are responsible for the content and writing of this original article.

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