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SCIENTIFIC ARTICLE

The effects of remifentanil used during cesarean section on oxidative stress markers in correlation with maternal hemodynamics and neonatal outcome: a randomized controlled trial☆

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Advanced oxidation protein products

Abstract
Background and objective: Remifentanil is used to attenuate maternal hemodynamic response to intubation and surgical stress during Induction–Delivery period of cesarean section. The goal was to compare the effects of two remifentanil dosing regimens on oxidative stress level, in correlation with its hemodynamic and neonatal effects.
Methods: Fifty-one patients, 17 per group, enrolled for elective cesarean section were randomly divided by computer-generated codes into three parallel groups: (A) patients received a 1 $\mu g.kg^{-1}$ remifentanil bolus immediately before induction, followed by 0.15 $\mu g.kg^{-1}.min^{-1}$ infusion, that was stopped after skin incision; (B) patients received a 1 $\mu g.kg^{-1}$ remifentanil bolus immediately before induction; (C) (control), patients did not receive remifentanil until delivery. Maternal venous blood samples were taken at basal time, at extraction and 30 minutes after the end of operation for spectrophotometrical determination of malondialdehyde and advanced oxidation protein products concentration. The same was conducted for umbilical venous sample.
Results: Systolic blood pressure and heart rate remained significantly lower in group A compared to B and C during entire Induction–Delivery period ($p < 0.001$, $p = 0.02$ after intubation; $p = 0.006$, $p = 0.03$ after skin incision; $p = 0.029$, $p = 0.04$ after extraction; respectively). Malondialdehyde concentration was lower at time of extraction in maternal blood in group A compared to B and C ($p = 0.026$). All neonatal Apgar scores were ≥ 8 and umbilical acid–base values within normal range.

☆ The study was carried out at Clinic of Obstetrics and Gynaecology Department of Anaesthesiology, University Clinical Centre Niš, Serbia.

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Conclusions: The remifentanil dosing regimen applied in group A significantly attenuated lipid peroxidation and maternal hemodynamic response during entire I–D period, without compromising neonatal outcome.

PALAVRAS-CHAVE
Anestesia;
Obstetrícia;
Remifentanil;
Malondialdeído;
Produtos proteicos de oxidação avançada

Os efeitos do remifentanil sobre os marcadores do estresse oxidativo durante a cesariana, em correlação com a hemodinâmica materna e o desfecho neonatal: um estudo randômico controlado

Resumo
Justificativa e objetivo: O remifentanil é usado para atenuar a resposta hemodinâmica materna à intubação e ao estresse cirúrgico durante o intervalo indução-parto cesariana. O objetivo foi comparar os efeitos de dois regimes posológicos de remifentanil sobre o nível de estresse oxidativo, em correlação com seus efeitos na hemodinâmica materna e no neonato.
Métodos: Mediante códigos gerados por computador, 51 pacientes (17 por grupo) programadas para cesariana eletiva foram randomicamente divididas em três grupos paralelos (A, B e C). No Grupo A, as pacientes receberam remifentanil em *bolus* de 1 $\mu g.kg^{-1}$ imediatamente antes da indução, seguido por infusão de 0,15 $\mu g.kg^{-1}.min^{-1}$ que foi interrompida após a incisão da pele; no Grupo B, as pacientes receberam remifentanil em *bolus* de 1 $\mu g.kg^{-1}$ imediatamente antes da indução; no Grupo C (controle), as pacientes não receberam remifentanil até o parto. Amostras de sangue venoso materno foram colhidas no momento basal, na extração do feto e 30 minutos após o término da operação para determinar espectrofotometricamente as concentrações do malondialdeído e dos produtos proteicos de oxidação avançada. O mesmo foi feito para a coleta das amostras de sangue venoso umbilical.
Resultados: A pressão arterial sistólica e a frequência cardíaca permaneceram significativamente menores no Grupo A, comparado aos grupos B e C durante todo o intervalo indução-parto ($p < 0,001$, $p = 0,02$ após a intubação; $p = 0,006$, $p = 0,03$ após a incisão da pele; $p = 0,029$, $p = 0,04$ após a extração do feto, respectivamente). No momento da extração do feto, a concentração do malondialdeído foi menor no sangue materno do Grupo A, comparado aos grupos B e C ($p = 0,026$). Todos os escores de Apgar neonatais foram ≥ 8 e os valores da avaliação ácido-base do cordão umbilical estavam dentro da faixa normal.
Conclusões: O regime posológico de remifentanil aplicado ao Grupo A atenuou de modo significativo a peroxidação lipídica e a resposta hemodinâmica materna durante todo o intervalo indução-parto, sem comprometer o desfecho neonatal.

Introduction

The time from induction of general anesthesia for cesarean section to the delivery of the baby (I–D interval) is very vulnerable period for both mother and fetus/neonate and is particularly challenging for the anesthesiologist. Since the anesthetics that the mother receives cross the placental barrier, there is a great probability that they will affect the fetus and cause neonatal respiratory depression. On the other hand, traditionally performed anesthesia, with reduced doses of anesthetics until extraction of the baby, could increase the risk of intraoperative maternal awareness and provoke an excessive neuroendocrine stress response to endotracheal intubation and surgical incision, leading to severe psychological and cardio/cerebrovascular complications.[1,2]

Remifentanil, an ultra-short acting synthetic opioid, has been used, among various medications, for the attenuation of the maternal pressor response during the I–D period. With its rapid onset of action (1–1.5 minutes), rapid redistribution and metabolism dependent on nonspecific tissue and plasma esterases, and context sensitive half time of 3 minutes, remifentanil seems to be an adequate choice when there is a need for prompt and intense but brief analgesia, without residual effects.[1,2] The main challenge for the anesthesiologist is to create a remifentanil dosing regimen that provides hemodynamic stability during the I–D period without adversely affecting the neonate.

In this study, we compared the effects of two remifentanil dosing regimens and traditionally performed anesthesia on the maternal hemodynamic response to operative stress and on neonatal outcome. However, our main goal was

to explore whether the expected positive hemodynamic effects of remifentanil would lead to protective effects at the cellular level and to reduction of oxidative stress, so we measured maternal and umbilical levels of oxidative stress markers, malondialdehyde (MDA – a secondary product of lipid peroxidation), and advanced oxidation protein products (AOPP). In addition to being reliable markers of a prooxidative state, they also cause further propagation of oxidative stress (with damaging effects on cell membranes, proteins and DNA), systemic inflammation and apoptosis.[3–11]

With its hyperdynamic circulation, high metabolic and oxygen demand and with the placenta as a major source of reactive species, pregnancy itself represents a prooxidative state, that increases with gestational age.[6–8,12] During operative delivery, excessive free radical formation could additionally be triggered by various mechanisms: hypoxia (i.e. hypotensive episodes, respiratory depression), elevation of proinflammatory cytokines (TNFα, IL6), the arachidonic acid cascade, pain, a neuroendocrine stress response with subsequent hypertension, vasoconstriction and reduced tissue perfusion, tissue trauma with neutrophil activation, or hyperoxia (mechanical ventilation or oxygen supplementation).[8,12] Our hypothesis was that if hemodynamic stability induced by remifentanil was achieved, this could also have beneficial effects at the cellular level, by reducing the generation of deleterious Reactive Oxygen Species (ROS).

Methods

This prospective, randomized controlled trial was institutionally approved, received local Research Ethics Committee approval no. 12-2466-1, and was performed in conformance with the Declaration of Helsinki ethical guidelines at the Clinic of Gynecology and Obstetrics from April 2015 until July 2017. Sixty women with ASA physical status II and singleton term pregnancies scheduled for elective cesarean section under general anesthesia were enrolled in this study after having given written informed consent. General anesthesia was administered in patients who refused or had some absolute or relative contraindication to regional anesthesia, such as thrombocytopenia, coagulopathy or lumbar scoliosis. The exclusion criteria were maternal morbidity or signs of fetal compromise. Fifty-one patients (17 in each group) fully completed the study (Fig. 1).

In the operating room, patients were placed supine with left uterine displacement. Standard monitoring including noninvasive blood pressure measurement, electrocardiography, pulse oxymetry, capnography (using a bedside monitor, model BSM-2301k, Nihon Kohden Corporation, Tokyo, Japan) and Bispectral Index (BIS) electroencephalogram (BIS-Vista monitoring system Norwood, Massachusetts, USA) was initiated, and two intravenous lines were established, one for remifentanil infusion (using Perfusor fm B/Brown, Melsungen AG, Germany), and the other for the administration of other medications and fluids.

Patients were randomly allocated, using computer-generated codes in closed envelopes that were opened just before the operation, to one of three parallel groups:

A – Patients received a 1 μg.kg^{-1} remifentanil bolus over 30 s, immediately before the induction, followed by 0.15 μg.kg^{-1}.min^{-1} infusion that was stopped after skin incision.
B – Patients received a 1 μg.kg^{-1} remifentanil bolus over 30 s immediately before induction.
C (Control) – Patients did not receive remifentanil until delivery.

After the induction with thiopentone (3–5 mg.kg^{-1}) and succinylcholine (1.5 mg.kg^{-1}), direct laryngoscopy and endotracheal intubation were performed by an anesthesiologist blinded to group assignment, who graded intubating conditions as excellent, good or poor.[13] Anesthesia was maintained with 1%–1.5% end-tidal sevoflurane and 50% nitrous oxide in oxygen. Further muscle relaxation has been provided with rocuronium 0.6 mg.kg^{-1}. The lungs were mechanically ventilated to maintain end-tidal PCO_2 of 28–32 mmHg, with fresh gas flow of 6 L.min^{-1}.

Blood pressure (systolic – SAP, diastolic, main arterial pressure) and heart rate (HR) were measured at 2-min intervals until 30 min after the end of the operation and specifically recorded at basal time (T0), 30 s after induction to anesthesia (T1), endotracheal intubation (T2), skin incision (T3), extraction of neonate (T4) and 30 min after the end of surgery (T5).

After delivery, neonatologists blinded to group assignment assessed neonates and recorded the Apgar score at the 1st and 5th minute and, if required, performed resuscitative measures. Arterial and venous blood samples were taken in heparinized syringes from a double-clamped umbilical cord for blood gas analysis (using a Gem Premier 3000 Blood Gas/Electrolyte Analyzer, Model 5700, Instrumentation Laboratory Company, Bedford, MA, USA).

In the later part of the operation, sevoflurane and remifentanil were titrated according to BIS values and the presence/absence of signs of intraoperative surgical stress (autonomic, somatic and hemodynamic). Thirty minutes before the anticipated end of surgery, patients were given a 0.1 mg.kg^{-1} IV morphine bolus, to achieve peak analgesic effect at the time of discontinuation of remifentanil infusion. At the moment of skin closure, anesthetics were discontinued, and residual neuromuscular block antagonized using neostigmine and atropine. The trachea was extubated when spontaneous respiratory rate reached >10 breaths/min, end-tidal PCO_2 <45 mmHg, and the patient became responsive to verbal commands. Within the following 5–10 minutes patients were transferred to the PACU for routine 6 hours surveillance. Pain intensity (Verbal Rating Score 0–3: no/mild/moderate/severe pain), BP, HR, sedation level, the occurrence of shivering and PONV were recorded and graded at 15, 30, 60 and 120 minutes postoperatively by an anesthesiologist blinded to group assignment. Moderate pain was treated with NSAIDs, shivering with magnesium sulfate and PONV with metoclopramide.

Biochemical analysis

Biochemical analyses were performed by G.K., who was completely blinded to group assignment, at the Biochemical Institute of the Medical Faculty. Blood samples were

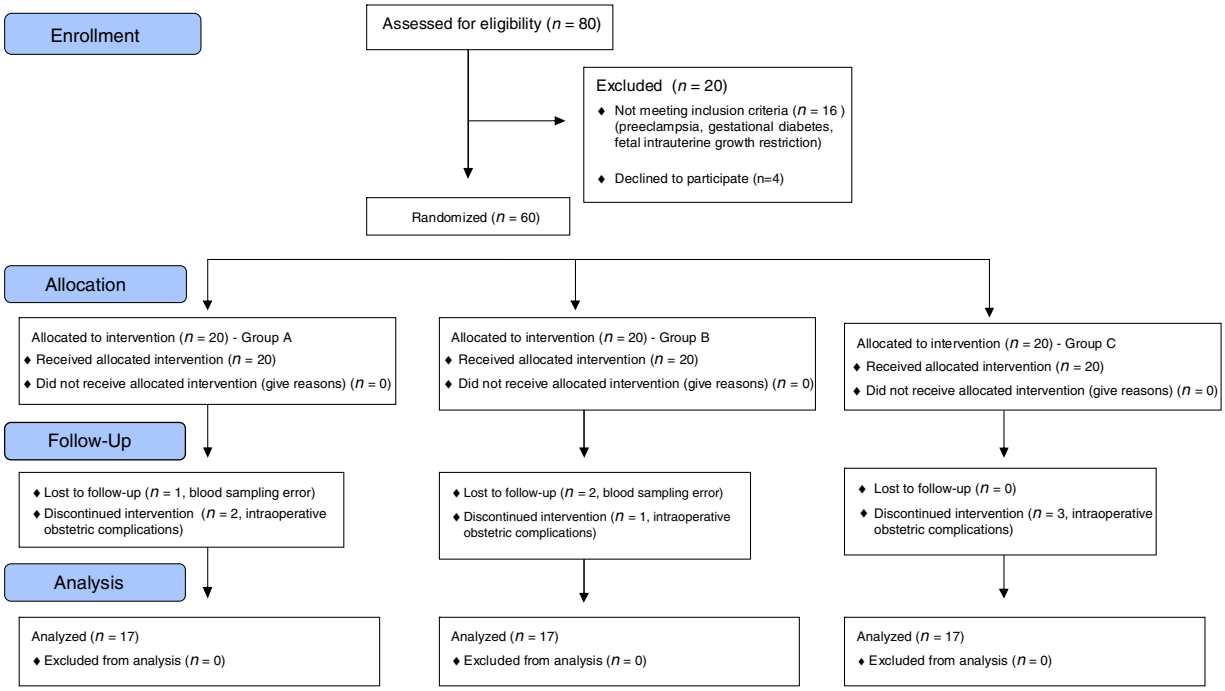

Figure 1 CONSORT flow diagram.

obtained by venous puncture from the maternal antecubital vein at three time points (Sample 1 – basal time, Sample 2 – time of extraction, Sample 3 – 30 minutes after the end of operation) and from the vein of the double-clamped umbilical cord immediately after delivery (Sample 4). Venous blood (3 mL) was collected in standard sterile vacuum tubes containing 5 mM EDTA and immediately centrifuged at 3500 rpm for 10 minutes. Separated plasma was divided into aliquots, frozen and stored at −17 °C until final analysis (within nine months of sampling).

MDA plasma concentration (μmoL.L^{-1}) was determined spectrophotometrically by the method of Janero et al.[3] based on MDA reaction with Thiobarbituric Acid (TBA). In acidic medium and at high temperature (100 °C), the pink color chromogen MDA-TBA2 is generated, and its absorbance was measured at λ = 553 nm by fluorescence detector.

AOPP plasma concentration in chloramine units/L or μmoL.L^{-1} was determined by spectrophotometric method of Witko-Sarsat et al.[4] based on measurement of the absorbance of released chloramines, which in the presence of potassium iodide absorb at 340 nm), using chloramine-T as a standard.

Statistical analyses

The primary outcome was defined as the difference in MDA and AOPP blood concentrations between groups; the secondary outcome was defined as the difference in SAP in response to intubation and surgical incision. In our preliminary study (unpublished data), a significant difference ($p < 0.05$) in MDA concentrations between term pregnant patients and nonpregnant control (1.64 ± 0.73 vs. 0.83 ± 0.19 μmoL.L^{-1}) was found. We calculated that 16 patients per group would have 80% power with $p < 0.05$ to detect 0.8 μmoL.L^{-1} difference in MDA concentrations (with SD = 0.50) among groups. An estimation of the sample size necessary to observe a significant difference in AOPP concentration was based on data derived from a study by Kalousová et al.[11] We calculated that 16 patients per group would have 80% power with $p < 0.05$ to detect 28.56 μmoL.L^{-1} difference among groups (with SD = 17.5). The calculation of a sufficient sample size to observe a difference in SAP showed that 15 patients per group would have 80% power with $p < 0.05$ to detect a difference in SAP of 15 mmHg (difference value chosen according to Maguire et al.[14]). Anticipating possible subject dropout during collection, 60 patients were enrolled (20 per group).

Statistical analysis was performed using the SPSS statistical package, version 13. Normality of the data was evaluated with the Kolmogorov–Smirnov test. Analysis of variance (ANOVA) was used for parameter comparison between the three groups, with subsequent post hoc analysis. In cases of non normal data distribution, the Kruskal–Wallis test was utilized, with subsequent post hoc analysis with the Mann–Whitney *U* test. The Chi-square test was used to verify the relation between categorical variables. Pearson's correlation coefficient (r) was used to test the linear correlation between two variables. The statistical hypothesis was tested on the significance level for risk of $\alpha = 0.05$; the difference between samples was considered significant if p was <0.05.

Results

Patient's characteristics and operation details are presented in Table 1. There were no differences between groups regarding patient age, gestation age, body weight, I–D and U–D (uterine incision–delivery) time.

Table 1 Parturient characteristics and surgical details.

	n	Group A Mean ± SD	*n*	Group B Mean ± SD	*n*	Group C Mean ± SD	*p*[a]
Age (years)	17	32.18 ± 4.86	17	30.76 ± 5.54	17	31.18 ± 3.32	0.571
Gestation (weeks)	17	38.94 ± 0.83	17	39.0 ± 1.12	17	39.47 ± 0.94	0.131
Weight (kg)	17	78.59 ± 11.67	17	77.76 ± 9.67	17	73.53 ± 11.3	0.242
I–D interval (min)	17	11.23 ± 1.52	17	10.18 ± 1.67	17	10.35 ± 1.8	0.353
U–D interval (s)	17	58.0 ± 17.28	17	59.47 ± 15.2	17	58.0 ± 21.1	0.871

[a] ANOVA.
I–D, Induction–Delivery Interval; U–D, Uterine Incision–Delivery.

Table 2 Serial systolic arterial pressure (mmHg) measurements.

	Group A		Group B		Group C		*p*[a]	Post hoc
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD		
SAP T0	17	131.71 ± 15.25	17	129.59 ± 10.33	17	135.53 ± 9.49	0.781	
SAP T1	17	109.76 ± 13.67	17	108.29 ± 13.98	17	116.87 ± 9.61	0.221	
SAP T2	17	119.06 ± 16.31	17	122.59 ± 15.94	17	149.13 ± 15.85	< 0.001	[c]$p < 0.001$ [d]$p < 0.001$
SAP T3	17	118.88 ± 15.98	17	124.88 ± 13.68	17	131.33 ± 7.91	0.006	[c]$p = 0.006$
SAP T4	17	117.0 ± 14.15	17	126.41 ± 9.43	17	125.60 ± 5.11	0.02	[b]$p = 0.029$
SAP T5	17	128.47 ± 8.35	17	123.47 ± 8.47	17	126.82 ± 6.85	0.355	[c]$p = 0.016$

SD, standard deviation; SAP, systolic arterial pressure in mmHg; T0, basal time; T1, after induction to anesthesia; T2, after endotracheal intubation; T3, after skin incision; T4, after extraction of fetus; T5,30 minutes after surgery.
[a] ANOVA.
[b] A vs. B.
[c] A vs. C.
[d] B vs. C.

Table 3 Serial hearth rate (beats per minute) measurements.

	Group A		Group B		Group C		*p*[a]	Post hoc
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD		
HR T0	17	101.12 ± 15.0	17	100.06 ± 13.29	17	98.18 ± 14.9	0.699	
HR T1	17	96.47 ± 10.19	17	96.29 ± 9.82	17	102.87 ± 11.05	0.367	
HR T2	17	101.53 ± 8.49	17	100.88 ± 8.51	17	108.20 ± 9.70	0.02	[c]$p = 0.027$ [d]$p = 0.017$
HR T3	17	99.06 ± 15.19	17	103.82 ± 12.53	17	109.33 ± 12.79	0.03	[c]$p = 0.031$
HR T4	17	92.24 ± 13.85	17	100.32 ± 16.34	17	101.67 ± 12.21	0.04	[b]$p = 0.040$
HR T5	17	81.06 ± 11.7	17	76.82 ± 9.22	17	80.88 ± 8.92	0.455	[c]$p = 0.032$

HR, hearth rate (beats per minute); T0, basal time; T1, after induction to anesthesia; T2, after endotracheal intubation; T3, after skin incision; T4, after extraction of fetus; T5, 30 minutes after surgery.
[a] ANOVA.
[b] A vs. B.
[c] A vs. C.
[d] B vs. C.

Both remifentanil regimens significantly attenuated blood pressure and HR response to intubation compared to controls, but beneficial effects persisted until extraction only in group A, with significant differences between variables in comparison with Groups B and C. Tables 2 and 3 present serial changes in SAP and HR from initial values to time of extraction. After delivery until 120 minutes postoperatively, all patients were normotensive, with no difference between groups. Although there were no serious problems with airway management, the estimated intubation conditions were significantly better in the remifentanil groups, especially in Group A ($p = 0.01$).

Table 4 illustrates neonatal outcome. There were no differences between the remifentanil groups and the control in Apgar scores (all ≥ 8) or umbilical acid–base values (all within normal range).

Table 4 Newborns characteristics.

	Group A		Group B		Group C		*p*
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	
Ap^1	17	8.71 ± 0.47	17	8.76 ± 0.41	17	8.59 ± 0.51	0.493[b]
Ap^5	17	8.94 ± 0.24	17	8.88 ± 0.33	17	8.88 ± 0.33	0.706[b]
Venous pH	17	7.30 ± 0.02	17	7.32 ± 0.03	17	7.32 ± 0.03	0.144[b]
Venous PCO_2 (mmHg)	17	39.46 ± 3.49	17	38.29 ± 5.21	17	37.0 ± 4.03	0.068[b]
Venous PO_2 (mmHg)	17	35.01 ± 7.43	17	36.12 ± 9.59	17	31.49 ± 5.99	0.317[a]
Venous BD ($mmoL.L^{-1}$)	17	4.49 ± 1.25	17	4.49 ± 1.65	17	4.88 ± 1.33	0.616[a]
Venous lactate ($mmoL.L^{-1}$)	17	1.33 ± 0.18	17	1.35 ± 0.25	17	1.22 ± 0.18	0.338[a]
Arterial pH	17	7.27 ± 0.02	17	7.28 ± 0.03	17	7.28 ± 0.03	0.312[b]
Arterial PCO_2 (mmHg)	17	48.58 ± 5.16	17	50.61 ± 6.08	17	48.26 ± 4.31	0.455[a]
Arterial PO_2 (mmHg)	17	19.04 ± 2.86	17	19.12 ± 2.64	17	19.38 ± 1.59	0.658[a]
Arterial BD ($mmoL.L^{-1}$)	17	4.29 ± 1.55	17	4.22 ± 1.70	17	4.07 ± 0.94	0.762[a]
Arterial lactate ($mmoL.L^{-1}$)	17	1.35 ± 0.35	17	1.35 ± 0.34	17	1.39 ± 0.24	0.788[a]

[a] ANOVA.
[b] χ^2_{KW}, Kruskal–Wallis test.
Ap^1, Apgar score in 1st minute; Ap^5, Apgar score in 5th minute; PCO_2, CO_2 partial pressure; PO_2, O_2 partial pressure; BD, base deficit.

Table 5 Serial malondialdechide plasma concentration measurements.

Sample	Malondialdechide ($\mu mol.L^{-1}$)						
	n	Group A Mean ± SD	*n*	Group B Mean ± SD	*n*	Group C Mean ± SD	*p*[a]
First	17	1.65 ± 0.86	17	1.91 ± 0.83	17	1.64 ± 0.73	0.762
Second	17	1.30 ± 0.57	17	2.11 ± 0.81	17	2.07 ± 0.85	0.026
Third	17	1.63 ± 0.78	17	1.98 ± 0.96	17	1.59 ± 0.91	0.671
Fourth	17	1.46 ± 0.77	17	1.80 ± 0.53	17	2.58 ± 1.44	0.054

First, sample at basal time; Second, sample at the extraction of baby; Third, sample 30 min after the surgery; Fourth, umbilical venous sample.
[a] ANOVA.

The recorded postoperative VRS for pain was significantly lower in group A than in Groups B and C ($p < 0.001$). The greatest pain intensity (VRS 2 – moderate pain) was recorded in all groups at 15 and 30 minutes, when NSAIDs had to be administered. During the patients' further stay in the PACU, no additional analgesics were requested. Mild PONV and shivering occurred in 20% of the patients, with no difference between groups.

Measured MDA plasma concentrations are presented in Table 5. There was significantly lower MDA concentration at the time of extraction in Group A (Sample 2) than in Groups B and C (A vs. B: $p = 0.026$; A vs. C: $p = 0.040$). Umbilical venous MDA concentration was also lower in group A than in other groups, but the difference did not reach significance ($p = 0.054$). A significant positive correlation between MDA levels in Samples 4 and 2 ($r = 0.352$, $p = 0.015$), as well as between MDA levels in sample 4 and thiopentone consumption ($r = 0.308$, $p = 0.035$) was found. There was no significant difference between groups in pre- and postoperative MDA levels (Samples 1 and 3).

The AOPP concentration decreased in the remifentanil groups at the time of extraction (Sample 2) compared to the preoperative level (Sample 1), while it increased in the control group. Nevertheless, the difference did not reach significance. Postoperative (Sample 3), as well as umbilical venous (Sample 4) AOPP concentrations, were also lower in the remifentanil groups than in the control group, but not significantly (Table 6).

A significant positive correlation was found between MDA level in Sample 2 and sevoflurane consumption during the I–D period ($r = 0.343$, $p = 0.015$), as well as between AOPP level in Sample 2 and thiopentone and sevoflurane consumption ($r = 0.301$, $p = 0.036$ and $r = 0.283$, $p = 0.049$, respectively).

Discussion

Remifentanil effects on maternal hemodynamics and neonatal outcome

Studies reporting the use of remifentanil during the I–D period of cesarean section vary regarding hemodynamic effects and neonatal outcome. Attenuation of the pressor response to surgical stress has been observed either not to last through the whole I–D period, or to often be achieved at the expense of neonatal respiratory depression.[1,2,15–17] By measuring umbilical arterial and venous remifentanil

Table 6 Serial advanced oxidation protein products plasma concentration measurements.

Sample	Advanced oxidation protein products (μmoL.L^{-1})						p^a
	n	Group A Mean ± SD	*n*	Group B Mean ± SD	*n*	Group C Mean ± SD	
First	17	58.64 ± 26.22	17	59.34 ± 41.3	17	67.57 ± 49.83	0.318
Second	17	47.02 ± 27.69	17	47.98 ± 26.42	17	71.94 ± 55.53	0.103
Third	17	46.50 ± 30.06	17	40.54 ± 32.13	17	54.13 ± 26.88	0.296
Fourth	17	44.94 ± 28.84	17	51.19 ± 46.19	17	60.20 ± 47.16	0.564

First, sample at basal time; Second, sample at the extraction of baby; Third, sample 30 min after the surgery; Fourth, umbilical venous sample.

[a] χ^2_{KW}, Kruskal–Wallis test.

concentrations at delivery, Hu et al. showed that remifentanil is metabolized rapidly in the fetal circulation, but emphasized that it can be affected by the applied dosing regimens.[18] Based of existing data we created a remifentanil dosing regimen of an initial bolus plus infusion that was interrupted after skin incision. In that sense two stressful events (intubation and skin incision) were covered, and since the I–D interval usually lasts for 10–11 min, there was still enough time for the remifentanil to be metabolized in fetal circulation, leaving less possibility for the development of neonatal respiratory depression. We compared the effects of the above dosing regimen (Group A) with a sole remifentanil bolus regimen (Group B) and with controls (Group C), who received traditionally performed anesthesia (thiopentone at the induction, sevoflurane for maintenance of anesthesia, and omission of opioids until delivery). Both remifentanil regimens successfully attenuated the maternal hemodynamic response to endotracheal intubation, which is in accordance with previous reports.[15–17] Subsequently, from intubation until extraction of the neonate, measured hemodynamic variables remained significantly lower in group A compared to both B and C groups. We focused on changes in SAP because systolic hypertension is considered to be the most important predictor of cerebrovascular complications[19] and on changes in HR, which, in combination with SAP, reflects myocardial oxygen demand.[20] It seems that only the remifentanil bolus plus infusion regimen attenuated the maternal hemodynamic stress response throughout the whole I–D period. The important fact is that it did not adversely affect neonatal outcome. There was no difference in Apgar score, as all the scores were $\geq$8 or umbilical acid–base status between the remifentanil groups and the control group with traditionally performed anesthesia. All values were within physiological limits.

Postoperative pain treatment and the possibility of Acute Opioid Tolerance (AOT) and Opioid-Induced Hyperalgesia (OIH) expression represent a particular challenge after the use of remifentanil.[21] To prevent the development of severe postoperative pain, we administered a 0.1 mg.kg^{-1} IV morphine bolus 30 minutes before the anticipated end of the surgery, aiming to obtain its peak analgesic effect at the time of discontinuation of remifentanil infusion. Nevertheless, our patients reported pain of moderate intensity (VRS 2) 15–30 minutes after surgery. This was most likely the consequence of visceral pain caused by coadministration of continuous uterotonic infusion that our patients received intra- and postoperatively. Indeed, our patients responded to NSAID medications that are more efficient in visceral pain treatment. Signs of AOT/OIH were not present, possibly because of the low applied remifentanil doses: 0.14 μg.kg^{-1}.min^{-1} intraoperative remifentanil consumption in Group A and 0.17 μg.kg^{-1}.min^{-1} in Groups B and C, as well as the short duration of exposure to the drug (less than 60 minutes).

Remifentanil effect on oxidative stress markers

We were interested in examining whether beneficial remifentanil effects on maternal hemodynamics could have any impact on cellular metabolism and the level of oxidative stress in maternal venous and umbilical venous blood. Since studies reporting the effect of remifentanil on oxidative stress during cesarean section were lacking, our expectations were based on data from nonobstetric populations and experimental studies.

The mechanisms of remifentanil-induced tissue protection include the following:

Activation of opioid receptors (members of the G-protein coupled receptor family), signaling kinases (MAPK, ERK ½, p38), protein kinase C and mitochondrial ATP-dependent potassium channels, which prevents cellular calcium overload and subsequent activation of degrading enzymes, such as nucleases, proteases and phosphatases;
Increased expression of antiapoptotic proteins (Bcl-2), suppression of proapoptotic proteins (Bax) and of caspases activation;
Reduced expression of proinflammatory cytokines (TNFα, IL6, IL8), decreased neutrophil adhesion and transmigration.[22,23]

Remifentanil has shown cardio/hepato/neuro/utero/small intestine protection against ischemia/reperfusion injury.[22,23] Compared to other opioids, remifentanil is the only drug that has been shown to attenuate the human inflammatory response, possibly due to more successful attenuation of the neuroendocrine stress response.[24]

The results of MDA concentration measurements performed at the time of delivery (Sample 2), illustrate remifentanil effects at the cellular level. The MDA concentration in Group A (remifentanil bolus plus infusion)

decreased, while the values in Groups B and C increased compared to preoperative values. The MDA level was significantly lower in group A than in the other groups. Since the remifentanil dosing regimen represented the only difference between groups as there was no difference with respect to other anesthetics, inspiratory oxygen concentration, intensity of surgical stress, maternal characteristics, or duration of I–D and U–D interval, our result proves that remifentanil, given at a sufficient dose, provides protection from the deleterious effect of lipid peroxidation on cell membranes. The lower MDA concentration in group A umbilical venous blood compared to that in Groups B and C suggests the same conclusion.

Maternal and umbilical venous MDA concentrations (Samples 2 and 4) were similar. The tendency of leveling maternal and placental ROS secretion has been demonstrated in several studies.[12] We found a significantly positive correlation between maternal and umbilical samples ($p = 0.015$). On the other hand, a correlation between maternal (Sample 2) and umbilical (Sample 4) MDA concentration with neonatal Apgar scores and umbilical acid–base status was not found. It has been reported that a negative Apgar score/MDA concentration correlation existed only with an Apgar score $\leq$7.7. In our investigation, Apgar scores were higher and blood gas values were within the normal range. Although umbilical acid–base status represents the gold standard for the estimation of intrapartal events, both pH and BD reflect only the fetal metabolic state. It has been suggested that the umbilical lipid peroxide level would be a more accurate measure of fetal intrapartal hypoxic insult, as it reflects the extent of ROS-mediated cell membrane damage.[5]

At the time of delivery (Sample 2), we noted a rise in AOPP level in group C and a fall in remifentanil groups compared to preoperative values. Nevertheless, the difference between groups in Sample 2 did not reach statistical significance. The umbilical venous AOPP concentration (Sample 4) in the remifentanil groups was also slightly lower than in Group C. The umbilical venous AOPP levels corresponded to maternal values. The correlation of maternal and umbilical AOPP concentrations with Apgar scores and umbilical acid–base status was not found, presumably because all values were within the normal range.

The postoperative MDA concentrations (Sample 3) did not differ between groups, which could have been predicted, since the remifentanil dosing regimen after delivery was the same in all groups. This was also noted for AOPP concentrations. There was no difference between pre- and postoperative MDA and AOPP values, as if the operation had no impact on oxidative stress. This might be the result of general anesthetic-induced protection. Karabayırlı et al. reported a significantly lower oxidative stress index in the general anesthesia group (propofol/remifentanil/sevoflurane) compared to spinal and epidural anesthesia for elective cesarean sections.[25] Since we found a positive correlation between anesthetic (thiopentone and sevoflurane) consumption and MDA/AOPP concentrations, we believe that remifentanil was the drug that prevented the rise of prooxidants.

The main limitation of our study is that it was not completely blinded. Although anesthesiologist who performed the intubations and estimated intubation conditions and postoperative VRS scores as well as the neonatologists who examined the babies and the biochemist were blinded to the study design, the anesthesiologist who performed the anesthesia procedure was not. Since this was the first time at our clinic that remifentanil was used for general anesthesia during cesarean section, we thought that it would be prudent for the anesthesiologist to be aware of the procedure. In any case, all decisions during the procedure and all our analyses and conclusions were based on objective measurements derived from monitors or laboratory results.

Conclusion

The remifentanil dosing regimen (bolus plus infusion) that we proposed for use during the I–D period of cesarean section attenuated the maternal hemodynamic response to surgical stress until extraction of the neonate and provided tissue protection from lipid peroxidation without compromising neonatal outcome. Therefore, remifentanil can be considered very useful in clinical practice of obstetric anesthesia.

Conflicts of interest

The authors declare no conflicts of interest.

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