Modification of surgical stress response by perioperative melatonin administration

Bülent Kücükakin

This review has been accepted as a thesis together with four original papers by University of Copenhagen 31th of July 2009 and defended on 8th of January 2010.

Tutors: Jacob Rosenberg, Ismail Gögenur and Jens Lykkesfeldt.

Official opponents: Else K. Tønnesen,
Ana Beatriz Rodriguez Moratinos and Morten Møller.

Correspondence: Department of Surgical Gastroenterology, Herlev Hospital, Herlev Ring vej 75, 2730 Herlev, Denmark.

E-mail: bulentkucukakin@hotmail.com

Dan Med Bull 2010;57(5):B4144

THE 4 ORIGINAL PAPERS ARE

1. Kücükakin B, Lykkesfeldt J, Nielsen HJ, Reiter RJ, Rosenberg J, Gögenur I. Utility of melatonin to treat surgical stress after major vascular surgery--a safety study. J Pineal Res 2008;44:426-31.
2. Kücükakin B, Wilhelmsen M, Lykkesfeldt J, Reiter RJ, Rosenberg J, Gögenur I. No effect of melatonin to modify surgical stress response after major vascular surgery: a randomized placebo-controlled trial. Submitted.
3. Kücükakin B, Klein M, Lykkesfeldt J, Reiter RJ, Rosenberg J, Gögenur I. No effect of melatonin to modify surgical stress response after laparoscopic cholecystectomy: a randomized placebo-controlled trial. Submitted.
4. Kücükakin B, Kocak V, Lykkesfeldt J, Nielsen HJ, Magnussen K, Rosenberg J, Gögenur I. Storage-induced increase in biomarkers of oxidative stress and inflammation in red blood cell components. Submitted.

INTRODUCTION

The "classical" surgical stress response

Surgical injury elicits a well-known stress response involving activation of inflammatory, endocrine, metabolic and immunologic mediators [1]. The surgical stress response is believed to be a necessary and beneficial response. However, exaggerated activation of various components of the surgical stress response can result in hemodynamic instability, and metabolic derangement leading to multiple organ failure and mortality [2, 3].

The immunologic and inflammatory responses are largely orchestrated by endogenous mediators referred to as cytokines produced by activated leucocytes, fibroblasts and endothelial cells. Cytokines influence immune cell activity, differentiation, proliferation and survival[2]. They regulate the activity of other cytokines, which may either augment (proinflammatory) or attenuate (anti-inflammatory) the inflammatory response [2]. The main cytokines released during surgery are interleukin-1 (IL-1), IL-6 and tumour necrosis factor-α (TNF-α). IL-6 is the main cytokine responsible for production of acute phase proteins in the liver including C-reactive protein (CRP) [4, 5] and may activate the hypothalamic-pituitary-adrenal axis [6].

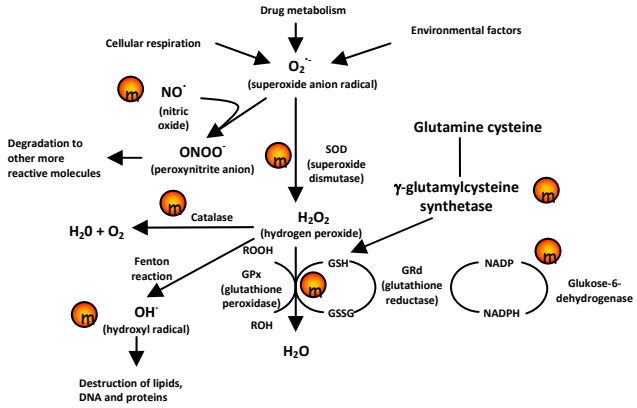
The endocrine and metabolic responses are characterized by an increased secretion of pituitary hormones and activation of the sympathetic nervous system [7]. The hypothalamic-pituitary-adrenal axis results in secretion of growth hormone (GH) and adrenocorticotrophic hormone (ACTH) [5, 8]. The latter stimulates the adrenal cortical secretion of glucocorticoids. During surgery, increased plasma concentrations of both hormones can be measured within minutes of the start of surgery [5]. After production, GH stimulates protein synthesis, lipolysis and glycogenolysis, and inhibits protein breakdown. GH and cortisol have an anti-insulin effect by inhibiting glucose uptake and use by cells [5]. Cortisol displays anti-inflammatory activity by inhibiting the accumulation of macrophages and neutrophils into areas of inflammation. During surgery, cytokines augment pituitary ACTH secretion and subsequently increase the release of cortisol.

In several studies it has been suggested that increased storage time of blood products may have a negative influence on the surgical stress response resulting in both postoperative morbidity and mortality. The concentration of oxidative stress markers in stored red blood cell products have not been investigated previously. Thus, it is also not known if the transfusion of stored blood may have an impact on the measurement of oxidative and inflammatory stress parameters.

Oxidative stress response and antioxidants

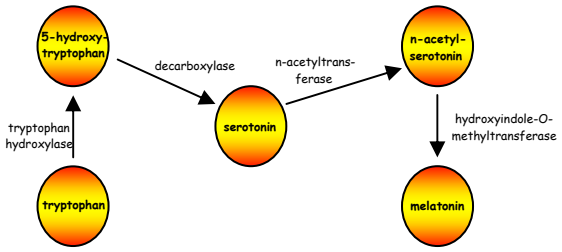
Oxidative stress describing a biological condition with generation of oxygen/nitrogen molecules higher than the capacity of the system to detoxify them, is believed to be an integrated part of the surgical stress response [9-11]. Reactive oxygen (ROS) and nitrogen (RNS) molecules include both free radicals and molecules that are not radicals but that exhibit strong oxidizing properties. Free radicals have an unpaired electron in their valence orbital making them instable and highly reactive. ROS include toxic products derived from oxygen including singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$). RNS include nitric oxide ($NO^{\bullet}$) and peroxynitrite anion ($ONOO^-$) (Figure 1). Both ROS and RNS are produced in mitochondria. ROS is produced continuously through reduction of molecular oxygen by the electron transport chain. Activation of immune and endothelial cells results in the production of reactive oxygen/nitrogen molecules [12, 13]. Activated phagocytes as neutrophils, monocytes, macrophages and eosinophils generate $O_2^{\bullet -}$ as part of the mechanism by which foreign

Figure 1:



*Oxidative stress and sites of action of melatonin (**m**). Melatonin reduces oxidative damage by multiple means. H_2O, aqua. ROH, lipid alkoxid. ROOH, organic hydroperoxide. GSH, reduced glutathione. GSSG, glutathione disulfide. NADP, nicotinamide adenine dinucleotide phosphate. NADPH, the reduced form om NADP.*

Figure 2:



Melatonin synthesis from tryptophan.

organisms are killed [13]. NO• is made by vascular endothelium and by phagocytes [13]. Neither $O_2^{\bullet-}$ nor NO• are highly reactive chemically, but formation of these to highly reactive OH• and ONOO⁻ results in damage of subcellular structures and molecules.

Through the antioxidant defence mechanisms the production of free radicals are counteracted including enzymatic as well as non-enzymatic mechanisms. The antioxidative enzymes are the superoxide dismutase (SOD), catalase and glutathione peroxidise (GPx), whereas non-enzymatic antioxidants are vitamin C and E, glutathione, β-carotene, allopurinol, N-acetylcysteine and melatonin [13].

Basic physiology of melatonin

In 1958 Lerner et al isolated the molecule N-acetyl-5-methoxy-tryptamine from the bovine pineal gland. The synthesis of melatonin is from tryptophan, which first is hydroxylated and then decarboxylated to serotonin. The latter is N-acetylated to N-acetylserotonin, and finally hydroxyindole-O-methyltransferase converts this molecule to melatonin (Figure 2). It is mainly produced in the pineal gland but other cells in the retina, gut, skin, platelets and bone marrow [14, 15] also produce melatonin.

Melatonin is secreted in a rhythmic pattern that is generated by an endogenous clock located in the suprachiasmatic nuclei of the hypothalamus. The neural pathway from the suprachiasmatic nuclei to the pineal gland is believed to pass the paraventricular nuclei, the intermediolateral column of the thoracic cord grey matter and the superior cervical ganglion. The main zeitgeber of the regulating system of melatonin secretion is the light/dark cycle; stimulation of the special light-sensitive photoreceptors in retina is transmited through the retinohypothalamic tract and inhibits the melatonin production whereas the dark period has the opposite effect [14]. Melatonin is therefore a nocturnal hormone, whereas the daytime secretion is low or undetectable. Once melatonin is produced, it is released to the circulation. In the blood melatonin is mainly bound to albumin (70%) and to orosomucoid [14]. The primary site for metabolism for endogenous and exogenous melatonin is the liver [16]. Here it is first metabolised to 6-hydroxymelatonin and then conjugated with either sulphuric acid to 6-sulfatoxymelatonin or with glucuronic acid. The conjugated metabolites and 6-sulfatoxymelatonin (aMT6s) are excreted in the urine [14,16]. In healthy subjects urinary excretion of aMT6s parallels melatonin secretion from the pineal gland [17].

Melatonin is believed to synchronize circadian rhythms [14]. Through peripheral vasodilatation melatonin reduces the core temperature and this coincides with increased sleepiness14, whereas light exposure at night has been shown to suppress melatonin synthesis and enhance alertness [18]. Melatonin is a hypnotic [19] and improves the quality and duration of sleep in elderly people with insomnia [20]. Melatonin also alleviates jet lag [21] and inhibits ACTH-stimulated cortisol production [14]. Melatonin is also thought to function in the regulation of mood, reproduction, tumor growth, immune response and aging [22].

Basic pharmacology of melatonin

In humans orally administrated melatonin higher than 0.3 mg produced supraphysiological plasma levels [23]. Thus, melatonin administration at pharmacological doses increased plasma levels of melatonin from 350 to 10.000 times compared with physiological levels [24]. The bioavailability of melatonin is approximately 15% [25]. Oral administration of melatonin has an absorption half-life of 0.4 hr, and an elimination half-life of 0.8 hr [24], but the bioavailability after oral administration varies substantially [22]. The variability seems to be due to inter-individual differences in expression and activity of hepatic cytochrome CYP1A2 [16]. The function of melatonin in humans seems to be mediated through activation of two high-affinity G protein-coupled receptors, MT1 and MT2 [26]. MT1 is present in kidney and intestine, whereas MT2 is present in the retina and melanoma cells [22]. Activation of MT1 modulates neuronal firing, arterial vasoconstriction, cell proliferation in cancer cells, and reproductive and metabolic functions [27]. Activation of MT2 inhibits dopamine release in the retina, induces vasodilatation, inhibits leukocyte rolling, enhances immune response and phase shifts circadian rhythms of neuronal firing in the suprachiasmatic nucleus [27].

Antioxidant effects of melatonin

Through the last couple of decades melatonin has been shown to possess direct and indirect functions as a free radical scavenger (Figure 1). The direct effect of it may be receptor independent [28, 29], whereas the indirect effect involves receptors30. Melatonin directly scavenges NO• and suppresses the activity of the rate limiting enzyme nitric oxide synthase thereby inhibiting the formation of ONOO⁻. Furthermore, melatonin stimulates gene expression and activities of the superoxide dismutase thereby providing rapid conversion of $O_2^{\bullet-}$ to the less toxic H_2O_2 [31]. This leads to lower formation of the highly reactive and damaging ONOO⁻. Catalase and GPx enzymes that metabolize H_2O_2 to H_2O have also been shown to be stimulated by melatonin. These processes prevent the production of OH•, an agent which is considered to be the most damaging of all endogenously generated

reactive agents. When $OH^{\bullet}$ is produced, melatonin directly detoxifies it. Melatonin stimulates the rate-limiting enzyme, gammaglutamylcysteine synthase thereby increasing the level of glutathione (GSH), which acts as an important antioxidant and which is used by GPx as a substrate to metabolize H_2O_2. The use of GSH leads to conversion of oxidized glutathione (GSSG). To maintain high levels of GSH, melatonin promotes the activity of glutathione reductase (GRd) thereby reformatting GSH [31]. The metabolites produced during the scavenging actions of melatonin (i.e. cyclic 3-hydroxymelatonin, aMT6s and N-acetyl-5-methoxykynuramine) also seem to be efficient scavengers [31, 32]. Contrary to other antioxidants melatonin is an amphiphilic molecule. This character of melatonin allows it to cross all physiological barriers and to interact with toxic molecules throughout the cell, thereby reducing oxidative damage to molecules in both lipid and aqueous environments of cells [33].

Safety of melatonin

Melatonin is believed to have very low toxicity. Maximum doses of melatonin that have been given in vivo are 200 mg/kg in pregnant rats throughout pregnancy or 800 mg/kg body weight in mice without any toxicity or death [34, 35]. In rats and mice, LD_{50} experiments have indicated a very low acute toxicity for melatonin [30, 36]. Thus, the LD_{50} oral dose in Sprague-Dawley rats was over 3.2 g/kg body weight. The LD_{50} oral dose for mice was 1.3 g/kg body weight. Finally, treatment with 1 g melatonin daily for a month given to humans orally was without any side effects [37]. In a systematic review by Buscemi et al [38], where they assessed the effects of melatonin on primary and secondary insomnia, they reported that the most commonly observed adverse events were headaches, dizziness, nausea, and drowsiness. Most of these side effects have been reported in patients with pre-existing psychiatric diseases. Anton-Tay et al [39] tested the efficiency of intravenously administered melatonin in healthy volunteers as well as in patients with Parkinson's disease and temporal lobe epilepsy. They used 0.25 mg/kg and 1.25 mg/kg melatonin, respectively, and did not observe any undesirable effects. In a study in newborns treated with melatonin (up to 10 doses of 10 mg/kg) orally [40] as well as intravenously [11, 41, 42], no side effects were reported [11, 40-42]. In a clinical safety study, chronic high-dose of (300 mg/day) rectal melatonin was well tolerated in patients with amyotrophic lateral sclerosis during an observation period of up to 2 years [43]. Finally, intravenously administration of melatonin up to 60 mg during abdominal aortic aneurism surgery was safe and without any unexpected events [44].

Aims

In study I [44], we wanted to examine if various doses of melatonin (10-60 mg) as an intraoperative intravenous infusion was safe in patients undergoing elective abdominal aortic surgery. In study II and III we wanted to examine if perioperative intravenous administration of melatonin affected markers of oxidative and inflammatory stress, after minor and major elective abdominal surgery. In study IV we wanted to examine the concentration of oxidative and inflammatory markers in stored blood (SAG-M) during a storage period of 35 days

MATERIAL AND METHODS

Study design

Study I [44] was an un-blinded, observational study on the clinical safety of three different doses of melatonin. Studies II and III were randomized, placebo-controlled, double blinded studies of the clinical effect of melatonin.

The patients in all studies were included after written informed consent and approval by the Ethics Committee, the Danish Data Protection Agency and The Danish Medicines Agency. These studies were also monitored by the Good Clinical Practice unit (GCP-unit) at the Copenhagen University Hospital.

Study IV was designed to evaluate the quality of red blood cells at the local department of clinical immunology. The study did not need approval by the local Ethics Committee.

Study setting and population

Patients in study I [44] and II were recruited from the department of vascular surgery at Copenhagen University Hospital, Gentofte. Included patients were between 18 and 80 years of age and were planned to undergo elective abdominal aortic aneurysm repair or aorto-bifemoral bypass surgery. Patients in study III were recruited from the department of surgical gastroenterology at Gentofte Hospital in Copenhagen. Included patients were female between 18 and 70 years of age undergoing elective laparoscopic cholecystectomy.

Exclusion criteria for all studies were: current anticoagulation therapy with warfarin or phenprocoumon, renal and/or hepatic insufficiency, continuous medication for psychiatric disease, regular medication with opioids and benzodiazepines, alcohol abuse, pregnancy or breast feeding, insufficient compliance and lack of written informed consent. Furthermore, patients in Study I and II with American Society of Anaesthesiologist (ASA) physical status classification higher than 3 were excluded. Patients in study III were not included if any of the following exclusion criteria were present preoperatively: men, acute cholecystitis, known ischemic heart disease or arrhythmia, current therapy with digoxin, Ca-antagonists, amiodaron or beta-blockers, and known endocrine disease (necessitating medical treatment).

10 units of non-filtered SAG-M blood were used in study IV. The blood was donated the day before the first sample collection, stored under standard conditions and collected aseptically on storage days 1, 3, 7, 14, 21, 28 and 35, respectively. The aim was to examine a representative sample of ten units, and therefore no inclusion or exclusion criteria's were applied for the donors.

Surgery, anaesthesia and postoperative care

Studies I [44] and II included patients undergoing either abdominal aortic aneurysm reconstruction or aorto-bifemoral bypass surgery. The general anaesthesia was according to the standards of the anaesthesiology department. Few hours before the planned surgical intervention, all patients received 1200 mg of gabapentin. The anaesthetic care comprised a preoperative epidural catheter for pre-, intra- and postoperative epidural blockade for 3 days after surgery. At induction a bolus infusion containing 3 ml of 2 % lidocaine with adrenalin and 5-8 ml of 0.5 % bupivacaine was used. The latter was maintained during the operation with an infusion rate of 4 ml/hour. Muscle relaxation was carried out with 0.6 mg/kg of rocuronium. For induction, all patients received 3-5 mg/kg of thiopental, 0.2-0.4 mg of fentanyl, 1.5-2.5 % sevoflurane, 15 g of mannitol and 1.5 g of cefuroxime, and anaesthesia was maintained with fentanyl, sevoflurane, bupivacaine and rocuronium. At the end of surgery, patients with continued muscle relaxation were given 0.5 mg of glycopyrron and 2.5 mg of neostigmine. The patients were intubated until admission to the intensive care unit (ICU) and were monitored with a bladder catheter until the first postoperative day. Intubation was only maintained if the patients had a central tempera-

ture below 35.5°C, and if so, the patients received 100-150 mg/hour of propofol and 0.05-0.1 mg of fentanyl for continuous sedation during intubation. All other patients received either 0.05-0.1 mg of fentanyl or 2.5 to 10 mg of morphine at the end of surgery. Postoperatively, the patients received 4 g per day of paracetamol and 1200 mg per day of gabapentin. Furthermore, the epidural analgesic regimen comprised of 50 μg/ml morphine and of 2.5 mg/ml bupivacaine with an infusion rate of 4 ml/hour, was continued for 3 days postoperatively.

In study III, the standard French technique (surgical technique where the surgeon is standing between the legs of the patient) was used in patients undergoing laparoscopic cholecystectomy. A continuous intra-abdominal pressure of 12 mmHg was kept during surgery. The anaesthetic care was standardized. It was induced either by 3-5 mg/kg of thiopental or by 2 mg/kg of propofol and maintained with 5 mg/kg/hour of propofol and 0.5 μg/kg/min of remifentanil. 0.6 mg/kg of rocuronium was used for muscle relaxation. At the end of the surgery, patients were given 0.5 mg of glycopyrron and 2.5 mg of neostigmine, 30 mg of ketorolac and 2 μg/kg of fentanyl or 0.2 mg/kg of morphine. During operation, all patients received 160 mg gentamicin as prophylactic antibiotic and 500-1000 ml of isotonic saline. Bupivacaine with a dose of 5 mg/ml was installed intradermally and preperitoneally at each trochar incision (a total of 28 ml was used). In the immediate postoperative period, the patients received 2.5 to 10 mg of morphine and 10 mg of ketorolac intravenously for rescue analgesia. A preoperative dose of 4-8 mg of dexamethasone-21-dihydrogenphosphate for nausea prophylaxis. At the end of the anaesthesia, the patients received 4 mg of ondansetron. Continued nausea was treated with 0.625 mg of dehydrobenzperidol or 4 mg of ondansetron. For postoperative pain, all patients received 4 g per day of paracetamol and 1200 mg per day of ibuprofen for three days.

Study medication

The study medication, melatonin for infusion, was chromatographically pure (Helsinn Chemical Co, Biasca, Switzerland), and was tested for sterility according to the European Pharmacopoeia requirements. Melatonin was dissolved in either a 2:23 mixture (study II) or a 2:98 mixture (study III) of ethanol-physiologic saline and infused intraoperatively as a continuous infusion starting from the first incision. In studies I and II, melatonin or placebo (a mixture of ethanol-physiologic saline) was infused for 2 hrs starting from the first incision, whereas the medication in study III was infused for 30 min starting from the first incision.

In study I [44], the patients received 10 (n =2), 30 (n=2) or 60 (n=2) mg melatonin intravenously in the intraoperative phase and 10 mg orally for three nights after surgery. In study II the patients received either 50 mg melatonin or placebo as infusion, whereas the patients in study III received either 10 mg melatonin or placebo as infusion. Additionally, the patients in study II received either 10 mg melatonin (Penn Pharmaceutical Services Limited, Tredegar, Gwent, Wales) or 10 mg placebo (lactose) orally the first three nights after surgery at 22:00 hr.

Malondialdehyde

The products produced by the interaction of oxidants with lipids (lipid peroxidation), proteins and DNA, are used as indicators for oxidative stress response. Two methods have been widely used for assessment of lipid peroxidation: the determination of malondialdehyde (MDA) and the detection of F2-isoprostanes [45].

Measurement of MDA is relatively simple in a clinical setting [46-48]. There are different methods for analyses of MDA. The method of choice is based on high-performance liquid chromatography (HPLC) with fluorescence detection [46, 49, 50]. MDA reacts with thiobarbituric acid and gives a pink and fluorescent chromogen that can be assessed by colorimetry at 532 nm or by fluorimetry with excitation at 515 nm and emission at 553 nm. The colomerimetric assay for MDA is considered to be the best method to use in clinical conditions aiming to evaluate the involvement of oxidative stress and to assess the effect of pharmacological treatments [46-48].

In studies I-IV, blood samples for MDA analysis were centrifuged immediately after collection for 2 min at 14.000 rpm. 100 μl plasma was placed in a 0.6 ml tube and stored at -80°C until analyses with HPLC with fluorescence detection46.

Ascorbic acid (vitamin C) and dehydroascorbic acid

Ascorbic acid (AA) and dehydroascorbic acid (DHA), an oxidized form of ascorbic acid, are valuable biomarkers of oxidative stress [51]. AA is a potent intra- and extracellular antioxidant scavenging superoxide, hydroxyl and peroxyl radicals and reacting with hypochlorite and singlet oxygen [52].

In our studies, plasma samples for AA and DHA analysis were collected and handled as soon as possible (within 5 min) to avoid redistribution of AA from plasma to erythrocytes and oxidation of AA [53]. Given that AA rapidly oxidizes at neutral or alkaline pH, an aliquot of our samples was immediately acidified by directly mixing the samples with 10% meta-phosphoric acid containing 2 mM disodium EDTA54. We removed the precipitate by centrifugation and froze the samples on dry ice and kept them at -80°C until analyses with HPLC with coulometric detection [51,55]. HPLC technique is commonly used and has the advantage of allowing detection of both vitamin C and its degradation products [45]. Total ascorbic acid (TAA) was measured after reduction with tris[2-carboxyethyl]phosphine hydrochloride. Because of no existent method to directly and sensitively detect DHA, the concentration of DHA was calculated by subtraction of AA concentration from TAA [51].

Alfa tocopherol

Alfa tocopherol (AT) is the main liposoluble antioxidant in humans, exhibiting its antioxidant properties in lipoproteins [45] and membranes [46]. AT mainly scavenges peroxyl radicals ($RO_2^{\bullet}$) produced lipid peroxidation. AT is separated by paper-, thin layer- or gas-chromatography or by HPLC. Paper- or thin layer-chromatography is not used in routine. Gas chromatography is only used for pharmaceutical assays. HPLC is a widely used method[45]. In our study, the HPLC technique with amperometric detection was used to analyze AT as described previously [56].

Interleukin-6

Surgical injury induces hemodynamic, metabolic and immunologic alternations resulting in production of endogenous mediators referred to as cytokines. The latter are polypeptides or glycoproteins produced by different cell types at the site of injury and by systemic immune cells such as T-lymphocytes and macrophages. Cytokines regulate the production and activity of other cytokines, which may either augment (proinflammatory) or attenuate (anti-inflammatory) the inflammatory response. Cytokines usually direct the inflammatory response to sites of infection and injury, and promotes wound healing. However, exaggerated production of proinflammatory cytokines from the local site of injury can

manifest systemically as e.g. hemodynamic instability [2]. Persistently increased proinflammatory cytokine responses are believed to contribute to multiple organ failure and mortality [2].

When operating, the interleukin (IL-6) levels in the circulation are detectable from 60 minutes from the first incision, with a peak between 4 and 6 hours, and can persist for as long as 10 days. IL-6 levels appear to be proportional to the extent of tissue injury during the operation, more than to the duration of the surgical procedure itself [2, 57]. IL-6 is a major pro-inflammatory cytokine that induces an acute phase response [58].

Blood samples collected for IL-6 analyses were centrifuged and the serum was stored at -20°C until analysis. Enzyme-linked immunosorbent assay (ELISA, Quantikine HS, High Sensitivity, R&D Systems Ltd., Abingdon, Oxon, UK) was used to analyze the samples. The ELISA method has high specificity and sensitivity for IL-6 analysis [59].

C-reactive protein

C-reactive protein (CRP) is mainly produced by hepatocytes under the control of IL-6 as part of the non-specific acute-phase response to tissue damage, infection, inflammation and malignant neoplasia [60]. CRP is also produced by vascular smooth muscle cells and endothelial cells, and in human atheroma [61]. A rise of CRP may be detected within 6 to 10 hours after surgery [60], and it may increase by as much as 4000-fold at the peak of the acute-phase response [62]. Blood samples for CRP were collected aseptically in tubes containing lithium heparin and were tested within 24 hrs of collection. The requested sample volume for the assay was 100 µl. For analysis the sample was mixed with latex particles coated with monoclonal anti-CRP antibodies. An immunoturbidimetric assay (Cobas Integra 400, Roche Diagnostics A/S, Hvidovre, Denmark) was used to assess CRP, with a lower detection limit of 0.71 mg/l.

Aldrete sedation score

At admission to the intensive care unit, the sedation for all patients was recorded by using Alderete standard sedation criteria [63]. The latter is a 3-point scale ranging from awake and lucid to sleeping and/or not possible to awake.

Blood pressure and pulse rate level

All patients were monitored with non-invasive blood pressure and pulse rate level assessments. The assessments were performed pre- and postoperatively. Furthermore, the same measurements were performed every 15 min in the intraoperative phase.

STASTICAL CONSIDERATIONS

Data in studies I [44] to III are reported as mean (SD) unless stated otherwise. Data in study IV are reported as mean (SE). Friedman's test was used in studies I [44] and IV for repeated measures analysis for non-parametric data. Kolmogorov-Smirnof's test was used in studies II to IV to check normality of distribution. In studies II and III, Chi squared test was used for categorical variables, Fisher`s exact test for dichotomous variables and Bonferroni correction was made to compensate for multiple testing. Additionally, the independent samples t-test was used for intergroup comparsions and the paired-samples t-test was used to assess the intragroup differences. In study II, Mann-Whitney U-test was used in intergroup analysis, when data was not normally distributed, and Spearman's test was used for correlation analysis. Repeated measures ANOVA with Bonferroni correction for multiple testing was used in study IV for parametric data. For all studies, a P-value less than 0.05 was considered statistically significant. SPSS version 17.0 (SPSS Inc., Chicago, USA) was used for data analysis.

ETHICAL CONSIDERATIONS

Studies I [44]-III were conducted according to the principles in the Helsinki Declaration, and were approved by the Regional Ethics Committee, the Danish Medicines Agency and the Danish Data Protection Agency. Furthermore, the studies were monitored by standards defined by the Good Clinical Practice Unit (GCP-unit) at the Copenhagen University Hospital. GCP is an international quality standard that is provided by the International Conference on Harmonisation (ICH). Furthermore, studies II and III were registered at www.clinicaltrials.gov with registration numbers NCT00315926 (study II) and NCT00311259 (study III).

All patients gave oral and written informed consent before inclusion. The patients were asked about side effects possibly caused by project medicine (melatonin), and the answers were registered in accordance with the study protocols.

In the primary application for approval from the Regional Ethics Committee, the use of 100 mg melatonin as an intravenous dose in study II and 25 mg intravenous in study III was rejected due to safety considerations. The protocol was therefore revised an after reduction of the doses to 50 and 10 mg melatonin respectively the approval was granted. An independent monitoring committee including two senior general surgeons and one senior anaesthesiologist were involved in case of any serious complications or death for evaluation of the patient course and the possible relation of the event to melatonin.

RESULTS

Main results from study I

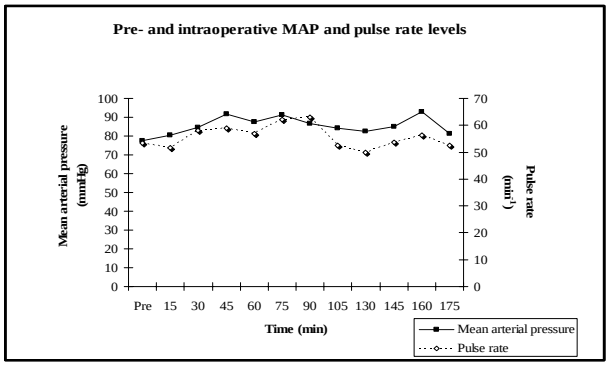
Six patients were enrolled in the study. We reported a significant reduction in the level of MDA after major vascular surgery compared with before. The concentrations of A and the oxidized form of AA decreased significantly, whereas the changes of DHA were non-significant. IL-6 increased in the intraoperative period, whereas a rise in CRP level was observed after the first postoperative day with a maximum increase in CRP on day 3. Changes in body temperature and mean arterial pressure (MAP) were non-significant (Figures 3 and 4).

Administration of up to 60 mg of intravenous melatonin during major vascular surgery was safe and without a significant impact on intra- or postoperative hemodynamic parameters.

Main results from study II

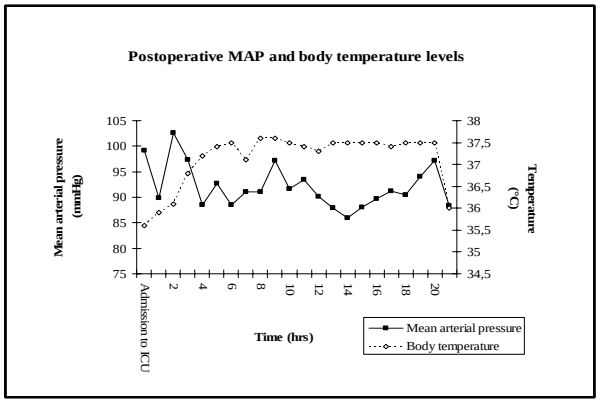
52 patients were enrolled whereof 50 patients completed the study (Figure 5). No differences were observed with regards to demographic variables and baseline characteristics (Table 1). Also, no differences were found regarding duration of anaesthesia, surgery and ischaemia periods. Loss and replacement of fluids and blood were comparable for both groups without any statistical significant differences. There were significantly more female patients in the placebo group (Fisher's test, $P = 0.047$) MDA concentrations were not significantly different between groups during the study period (independent-samples t-test, $P > 0.05$ at all measurement points). We observed a significant decrease in MDA values in the whole patient group at 5 min, 6 hrs and 24 hrs after reperfusion (paired-samples t-test, $P < 0.001$, $P < 0.001$ and $P < 0.001$ respectively) (Figure 6). No differ ences between groups were observed in AA (independent-samples t-test, $P > 0.05$ at all measurement points). Plasma AA was reduced at 5 min, 6 hrs and

Figure 3:



Assessed levels of mean arterial blood pressure (MAP) and pulse rate in the intra- and postoperative phases measured immediately before incision and then 175 min thereafter.

Figure 4:



Assessed levels of mean arterial blood pressure (MAP) and body temperature in the postoperative phase measured immediately after surgery at admission to the ICU and then 21 hours thereafter. Date are given as mean (SD).

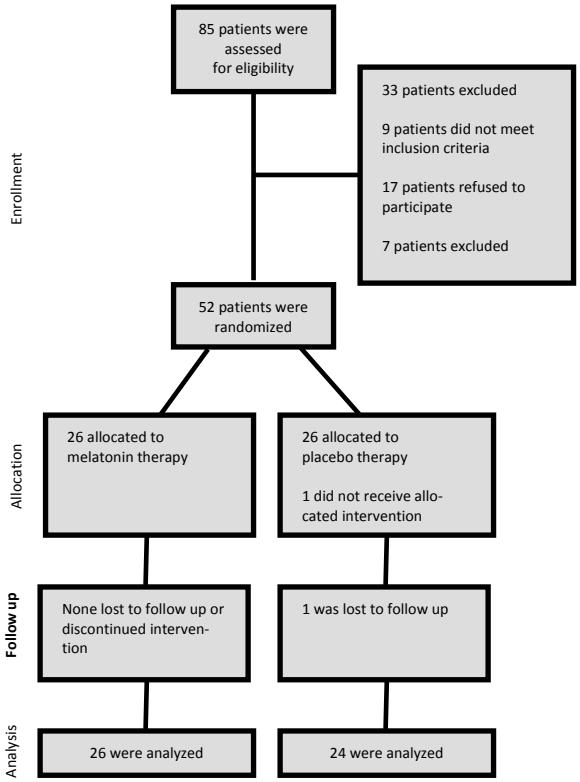
24 hrs after reperfusion in the whole patient group (paired-samples t-test, P < 0.001, P < 0.001 and P < 0.001 respectively) (Figure 7). There were no significant differences in DHA between the two groups (P > 0.05 at all measurement points). Plasma DHA levels for the whole patient group increased significantly at 5 min after reperfusion (paired-samples t-test, P = 0.001) but was not normalized on the rest of the measurement points (Figure 8). Plasma CRP concentrations were significantly decreased at 5 min and increased for all measurements at 24, 48, 72 and 96 hrs after reperfusion (paired-samples t-test, P < 0.001 for all measurement points) for the whole patient group but no intergroup differences were demonstrated (independent-samples t-test, P > 0.05) (Figure 9).

Aldrete sedation score for the two groups at arrival in the ICU was not significantly different, and there were no significant differences in the reported side effects between the two groups (Table 1).

Main results from study III

44 patients were enrolled whereof 41 patients completed the study (Figure 10). No differences were observed with regard to demographic variables and basic characteristics between the groups. Duration of anaesthesia, surgery and insufflation was not different between the groups (Table 2).

Figure 5:



CONSORT diagram for study II.

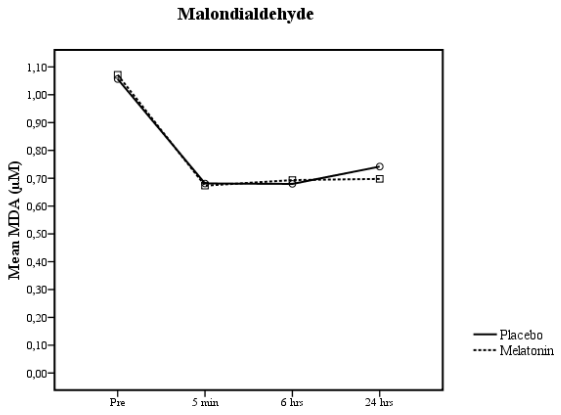
Table 1:

	Placebo group (n=24)	Melatonin group (n=26)	P-value
Age (years)	65 (11)	66 (10)	0.907
Gender (male/female)	15/9	23/3	0.047
BMI (kg/m^2)	25 (4)	26 (4)	0.462
ASA (I/II/III)	0/16/8	1/20/5	0.357
Surgery duration (min)	164 (43)	158 (33)	0.599
Anaesthesia duration (min)	284 (27)	273 (30)	0.169
Ischaemia duration* (min)	64 (22)	68 (24)	0.597
Fluid loss (ml)	696 (576)	700 (433)	0.979
Blood loss (ml)	1802 (1030)	2097 (1479)	0.409
Fluid replacement (ml)	4358 (1246)	4735 (1273)	0.286
Blood replacement (ml)	1192 (995)	1413 (1501)	0.535
Blood pressure the day before surgery			
Systolic pressure (mmHg)	145 (19)	149 (19)	0.396
Diastolic pressure (mmHg)	84 (16)	89 (15)	0.260
Intraoperatively			
Pulse rate level (bpm)	68 (8)	72 (9)	0.058
Mean arterial pressure (mmHg)	73 (6)	74 (7)	0.552
Postoperatively			
Mean arterial pressure (mmHg)	83 (10)	80 (8)	0.248
Temperature (ºC)	37.1 (1.1)	37.4 (0.8)	0.277
Aldrete sedation score (0/1/2/3)	9/7/2/6	10/10/1/5	0.629
Side effects			
Headache	1	0	1.000
Confusion	0	3	1.000
Depression	0	1	1.000
Dizziness	1	5	1.000

*Basic demographics and characteristics. ASA: American Society of Anesthesiologist physical status classification; BMI: body mass index. Data are shown as mean (SD). Bpm: beats per minute. *: duration of aortic cross-clamping. Postoperatively: Mean arterial blood pressure and temperature assessments until 24 hrs postoperatively.*

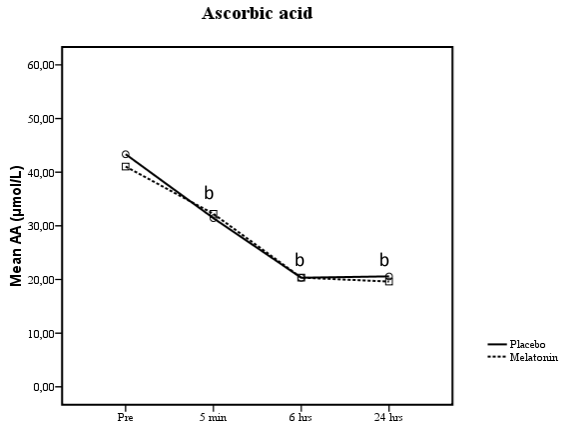
MDA levels in the melatonin group were significantly lower 24 hours after surgery compared with the placebo group (independent samples t-test, P = 0.013) (Figure 2). No differences were observed between the two groups during the other measurement points regarding MDA. Plasma levels of MDA in the whole group were significantly lower at 5 min and 24 hrs after desufflation

Figure 6:



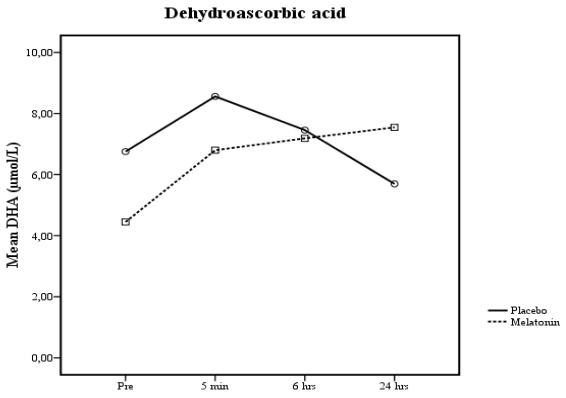
Measured levels of mean malondialdehyde (MDA) for the placebo and melatonin group i n the pre-, intra- and postoperative phases. Pre: preoperatively; 5 min: 5 min after clamp removal (recirculation of the first leg); 6 hrs: 6 hrs after clamp removal (recirculation of the first leg); 24 hrs: 24 hrs after clamp removal (recirculation of the first leg). b: P < 0.05 for intragroup comparisons.

Figure 7:



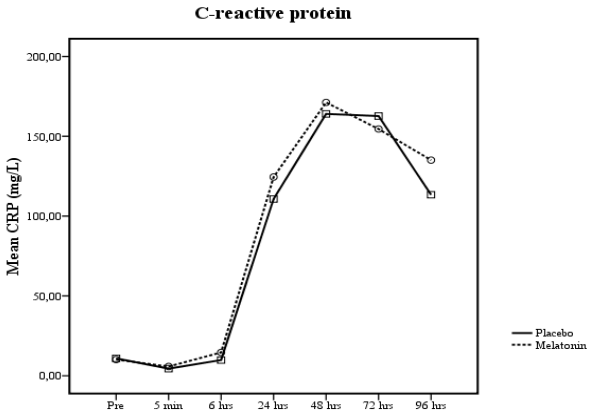
Measured levels of mean ascorbic acid (AA) for the placebo and melatonin group in the pre-, intra- and postoperative phases. Pre: preoperatively; 5 min: 5 min after clamp removal (recirculation of the first leg); 6 hrs: 6 hrs after clamp removal (recirculation of the first leg); 24 hrs: 24 hrs after clamp removal (recirculation of the first leg). b: P < 0.05 for intragroup comparisons.

Figure 8:



Assessed levels of mean dehydroascorbic acid (DHA) for the placebo and melatonin group in the pre-, intra- and postoperative phases. Pre: preoperatively; 5 min: 5 min after clamp removal (recirculation of the first leg); 6 hrs: 6 hrs after clamp removal (recirculation of the first leg); 24 hrs: 24 hrs after clamp removal (recirculation of the first leg). b: P < 0.05 for intragroup comparisons.

Figure 9:



Measured levels of mean malondialdehyde C-reactive protein (CRP) for the placebo and melatonin group in the pre-, intra- and postoperative phases. Pre: preoperatively; 5 min: 5 min after clamp removal (recirculation of the first leg); 6 hrs: 6 hrs after clamp removal (recirculation of the first leg); 24 hrs: 24 hrs after clamp removal (recirculation of the first leg); 48 hrs: 48 hrs clamp removal (recirculation of the first leg); 72 hrs: 72 hrs clamp removal (recirculation of the first leg); 96 hrs: 96 hrs clamp removal (recirculation of the first leg). b: P < 0.05 for intragroup comparisons.

(paired-samples t-test, P < 0.0001 and P = 0.006 respectively) (Figure 11).

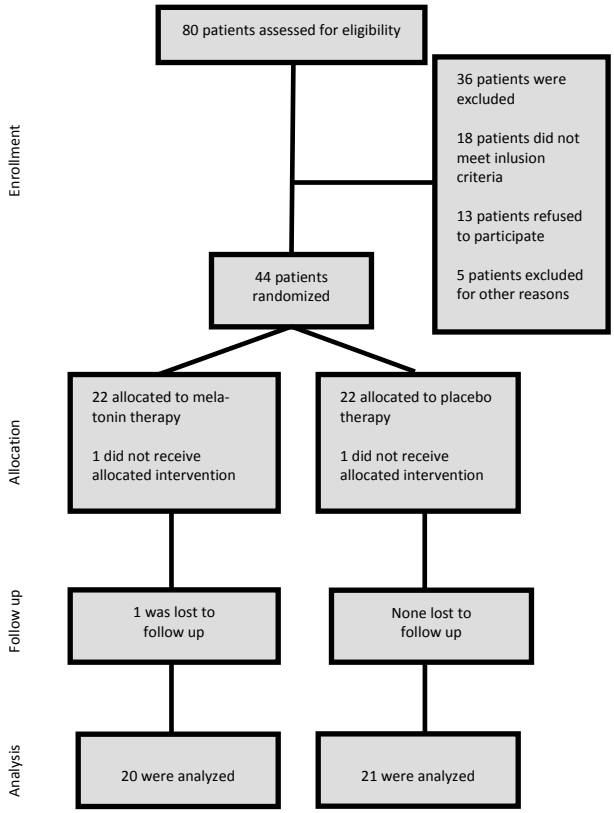
There were no significant differences between the melatonin and placebo group for AA or DHA at any of the postoperative measurement points (independent samples t-test, P > 0.05 for all measurement points). AA levels for the whole group were significantly changed at 5 min, 6 hrs and 24 hrs after desufflation (paired-samples t-test, P = 0.004, P < 0.001 and P < 0.001 respectively) (Figure 12).

DHA concentrations did not change during the measurement period (paired-samples t-test, P > 0.05). There were no significant differences between the two groups in the postoperative concentrations of CRP (independent samples t-test, P > 0.05 for all measurement points). CRP concentrations in the whole patient group were significantly decreased at 5 min and increased 24 hrs after desufflation (paired-samples t-test, P < 0.001 and P < 0.001, respectively) (Figure 13). Pre- and intraoperative diastolic blood pressures were significantly lower in the melatonin group compared with the control group (independent samples t-test, P = 0.033 and P = 0.006 respectively). We observed no significant differences between the care unit (Chi squared test, P = 0.446) and no differences in side effects (Table 2).

Main results from study IV

Ten units of whole blood were analysed aseptically on storage days 1, 3, 7, 14, 21, 28 and 35 days (T1-T7), respectively. MDA, groups regarding pre-, intra- and postoperative systolic blood pressure levels, and in the postoperative diastolic blood pressures (independent samples t-test, P > 0.05 for all measurement points). Furthermore, no differences were observed regarding sedation score for both groups at arrival in the postanesthesia AA

Figure 10:



CONSORT diagram for study III.

Table 2:

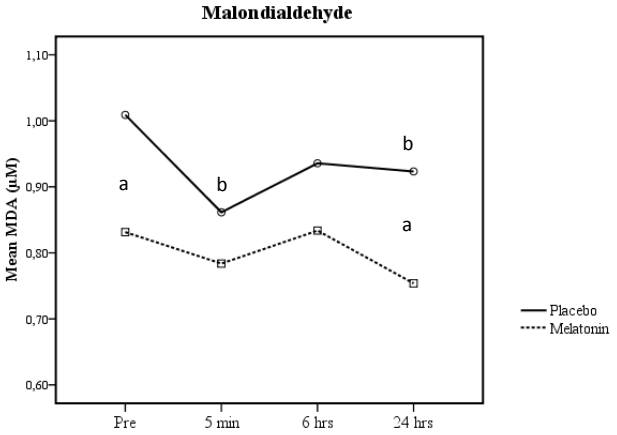
	Placebo group (n=21)	Melatonin group (n=20)
Age (years)	52 (14)	44 (14)
BMI (kg/m^2)	26 (4)	27 (5)
ASA (I/II)	14/7	8/12
Surgery duration (min)	79 (40)	78 (44)
Anaesthesia duration (min)	125 (37)	135 (45)
Insufflation duration (min)	66 (40)	62 (42)
Fluid replacement (ml)	1100 (400)	1100 (300)
Erythrocyte volume fraction (1.00)	0.39 (0.02)	0.40 (0.03)
Postoperative care unit stay (min)	168 (76)	146 (59)
Aldrete sedation score (0/1/2/3)	10/10/1/0	8/13/0/0
Side effects		
Dizziness	0	2
Headache	0	2
Confusion	0	0
Depression	1	0

Demography and characteristics. ASA: American Society of Anaesthesiologist physical status classification. BMI: body mass index. Data are presented as mean (SD). There were no significant differences between groups.

and DHA levels changed significantly during the period (repeated measures ANOVA, P < 0.001 for all three variables) (Figures 14, 15 and 16). AT levels did not significantly change in the measurement period (P = 0.087). IL-6 and YKL-40 levels changed significantly during the measurement period (Friedman test, P = 0.004, and repeated measures ANOVA, P < 0.001). Initially, we observed a significant decrease in MDA levels (T1-T2) (P = 0.008), and then a significant increase (T2-T7) (P = 0.001) (Figure 14).

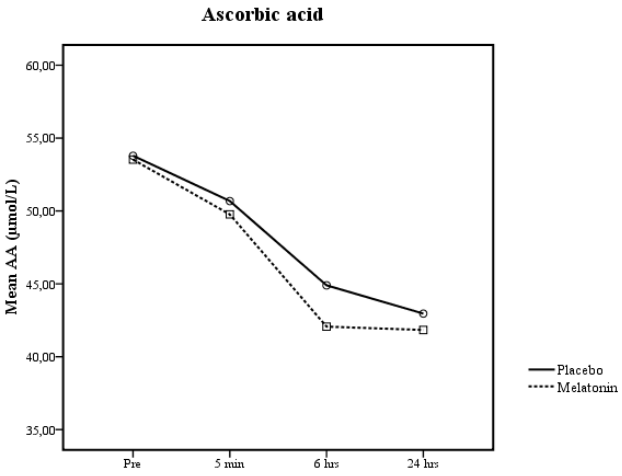
AA levels increased significantly between T1 and T5 (P < 0.001), and then declined significantly between T5 and T7 (P = 0.028) (Figure 15). DHA levels increased significantly between T2

Figure 11:



Assessed levels of mean malondialdehyde (MDA) for the placebo and melatonin group in the pre-, intra- and postoperative phases. Pre: preoperatively. 5 min: 5 min after desuflation. 6 hrs: 6 hrs after desuflation. 24 hrs: 24 hrs after desuflation. a: P<0.05 for intergroup comparisons. b: P<0.05 for intragroup comparisons.

Figure 12:



Measured levels of mean ascorbic acid (AA) for the placebo and melatonin group in the pre-, intra- and postoperative phases. Pre: preoperatively. 5 min: 5 min after desuflation. 6 hrs: 6 hrs after desuflation. 24 hrs: 24 hrs after desuflation. P>0.05 for all intergroup comparisons. b: P<0.05 for intragroup comparisons.

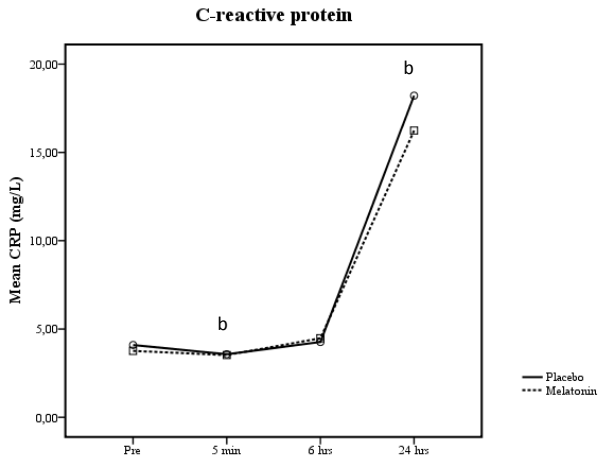
and T7 (P < 0.001) (Figure 16). YKL-40 levels significantly increased from T1 to T4 (P = 0.025) and from T5 to T7 (P = 0.037) (Figure 17).

DISCUSSION

Summary of results

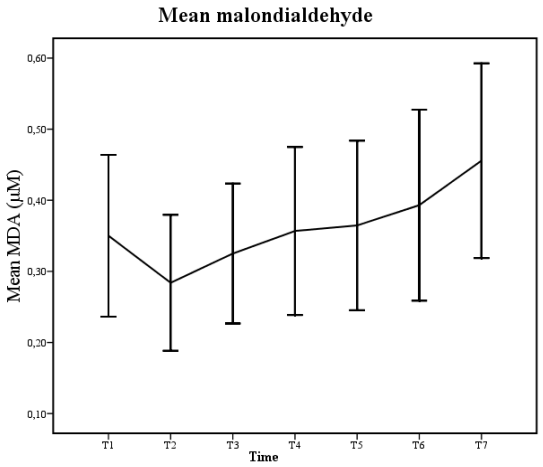
In study I [44], we found that administration of up to 60 mg of intravenous melatonin during major vascular surgery was safe and without a significant impact on intra- or postoperative hemodynamic parameters.

Figure 13:



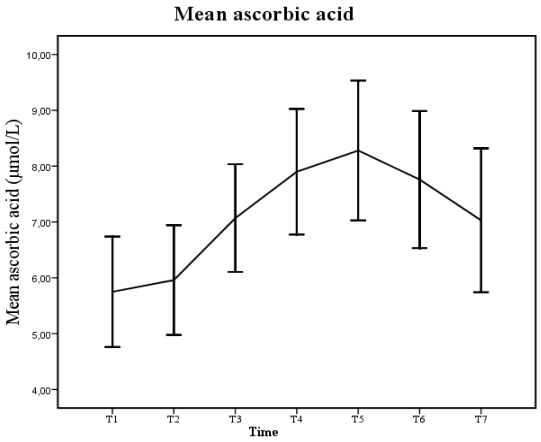
Assessed levels of mean C-reactive protein (CRP) for the placebo and melatonin group in the pre-, intra- and postoperative phases. Pre: preoperatively. 5 min: 5 min after desufflation. 6 hrs: 6 hrs after desufflation. 24 hrs: 24 hrs after desufflation. P>0.05 for all intergroup comparisons. b: P<0.05 for intragroup comparisons.

Figure 14:



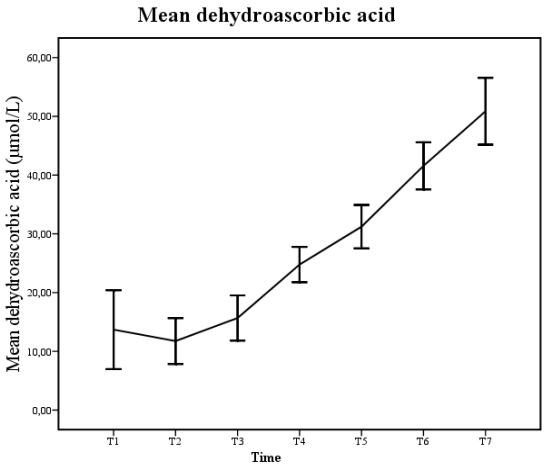
Mean (±SE, bars) values of MDA during the storage period. T1, T2, T3, T4, T5, T6, and T7 are storage day 1, 3, 7, 14, 21, 28 and 35, respectively. Repeated measures ANOVA and Friedman's tests were used. MDA, malondialdehyde. MDA changed significantly during the storage period (repeated measures ANOVA, P < 0.001).

Figure 15:



Mean (±SE, bars) values of ascorbic acid during the storage period. T1, T2, T3, T4, T5, T6, and T7 are storage day 1, 3, 7, 14, 21, 28 and 35, respectively. Repeated measures ANOVA and Friedman's tests were used. ASC changed significantly during the storage period (repeated measures ANOVA, P < 0.001).

Figure 16:



Mean (±SE, bars) values of dehydroascorbic acid during the storage period. T1, T2, T3, T4, T5, T6, and T7 are storage day 1, 3, 7, 14, 21, 28 and 35, respectively. Repeated measures ANOVA and Friedman's tests were used. DHA changed significantly during the storage period (repeated measures ANOVA, P < 0.001).

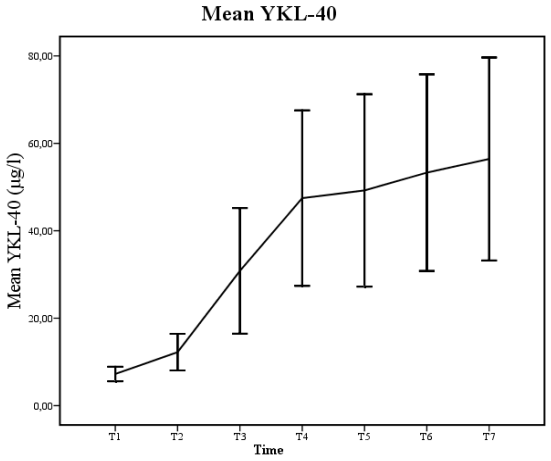
In study II, we found no significant differences in biochemical oxidative and inflammatory stress markers between the melatonin and placebo group, but that the surgical intervention produced a significant oxidative and inflammatory stress response. Melatonin was well-tolerated with no significant side effects compared with placebo intervention. In study IV, we found significant time-dependent changes in oxidative and inflammatory markers in stored non-filtered SAG-M blood.

Ischaemic-reperfusion injury during minimal invasive surgery

Laparoscopic surgery has become a standard procedure for symptomatic cholelithiasis because of clinical advantages that are believed to result from minimized surgical trauma, and therefore with a reduced acute phase response. In spite of that, there are few experimental studies revealing various adverse effects and complications of the laparoscopic method [64, 65].

Factors of importance for changes in intra-abdominal blood flow during laparoscopic operations are intra-abdominal insufflation of carbon dioxide (CO_2), increased intra-abdominal pressure (IAP) and the patient's position (e.g. reverse Trendelenburg). Insufflation of the peritoneal cavity with CO_2 during surgery increases the IAP, and thereby induces splanchnic vasoconstriction. The latter seems to result in decreased inferior caval, renal, and portal venous blood flow leading to splanchnic ischaemia [66-70]. Desufflation at the end of operation normalizes the intraabdominal pressure and splanchnic blood flow. The insufflation and

Figure 17:



Mean (±SE, bars) values of YKL-40 during the storage period. T1, T2, T3, T4, T5, T6, and T7 are storage day 1, 3, 7, 14, 21, 28 and 35, respectively. Repeated measures ANOVA and Friedman's tests were used. YKL-40 significantly changed during the measurement period (Friedman test, P = 0.004, and repeated measures ANOVA, P < 0.001).

desufflation appears to represent an ischaemia-reperfusion model, which is considered to increase free radical production leading to oxidative stress and damage [71-78]. As a consequence of pneumoperitoneum, the reduced venous return, increased systemic vascular resistance may induce cardiovascular changes resulting in decreased stroke volume and cardiac output [64, 65].

The ischaemia-reperfusion phenomenon has only been validated in few studies [71-78]. The effect of different IAP on lipid peroxidation and protein oxidation during laparoscopic cholecystectomy has been evaluated in 24 patients randomized to either 10 or 15 mmHg of IAP [71]. Serum protein carbonyls and thiobarbituric-acid-reactive substances (TBARS) levels increased, whereas protein sulfhydryl levels decreased in both groups, indicating no differences in oxidative stress response between 10 and 15 mmHg of IAP. Bickel et al [72] used an intermittent sequential pneumatic compression (ISPC) device to evaluate oxidative stress during laparoscopic cholecystectomy. The ISPC device was shown to improve cardiac output and visceral perfusion during pneumoperitoneum [79]. Twenty patients were enrolled in a randomized prospective controlled study and divided into 2 groups, whereas first group consisting of 10 patients used the ISPC device together with creation of pneumoperitoneum. They reported a significant increase of lipid peroxidation in the control group compared with the group using ISPC, indicating a decreased oxidative stress response due to improved cardiac output and visceral perfusion.

Whereas intestinal circulation alterations of hollow organs such as stomach and bowel, and of solid organs such as liver, spleen, pancreas and kidneys are transitory effects restricted to the perioperative period, metabolic and immunologic effects and the stress response are prolonged into the postoperative course. Despite the clinical effects of these alterations are still under debate, the majority of patients undergoing various laparoscopic procedures do not exhibit any increased morbidity.

Ischaemic-reperfusion injury during major vascular surgery

Abdominal aortic aneurysm (AAA) is characterized by localized structural deterioration of the aortic wall, leading to progressive aortic dilation [80]. AAA is associated with infiltration of inflammatory leukocytes [81, 82], which may play a key role in the degeneration of the elastic media promoting aneurismal remodelling of the aortic wall [82]. The inflammatory response is believed to promote aneurysm formation through enhanced production of ROS contributing to tissue destruction [83]. ROS seems to activate matrix metalloproteinases leading to degeneration of the elastic media, and to induce apoptosis of vascular smooth muscle cells leading to the disease process [84, 85].

AAA repair with aortic cross-clamping results in ischaemia-reperfusion injury leading to the initiation of a systemic inflammatory syndrome, which is the first step to multiple-organ failure and death [86, 87]. During reperfusion, a massive production of ROS reacts with unsaturated fatty acids within the phospholipids bilayer of the cell membrane resulting in lipid peroxidation causing tissue injury after ischaemia-reperfusion injury [83, 88]. Aortic aneurysm surgery is also associated with complications such as myocardial infarction, pulmonary oedema, arrhythmias and diminished cardiac output with increased risk of cardiovascular death in the postoperative period [10, 80].

Hafez et al [10] examined oxidative stress and myocardial injury in relation to aortic aneurysm repair. They included 44 patients undergoing AAA repair and thoracoabdominal aortic aneu rysm (TAAA) repair. Plasma samples collected at incision, aortic crossclamping, and reperfusion and 1, 8, and 24 hours thereafter were analyzed for the myocardial specific protein TpT, total antioxidant status and lipid hydroperoxides. Patients undergoing TAAA had a significant drop in total antioxidant status, and high levels of lipid hydroperoxides and TpT indicating a correlation of oxidative stress with the release of TpT. Similarly, Cornu-Labat et al [9] assessed levels of antioxidant capacity and the degree of systemic inflammatory response to evaluate the severity of pulmonary injury in patients undergoing elective aortic surgery. Twenty-four patients were included and monitored with chest radiography and arterial blood gases pre- and postoperatively to quantify the degree of pulmonary edema and pulmonary dysfunction. Postoperatively, 15 patients had pulmonary edema and lower total antioxidant capacity than in patients without pulmonary edema. They suggested an association between total antioxidant capacity and the degree of pulmonary edema, and that antioxidant supplementation may favourably impact the severity of systemic inflammatory response following ischaemia-reperfusion injury.

In 30 surgical and 30 medical patients admitted to the intensive care unit (ICU), the markers of oxidative damage (MDA and F2 isoprostanes) and antioxidant protection (total antixoxidant status, antioxidant gap, AA and the enzymes GPx and SOD) were compared with clinical scores and outcome, to evaluate the impact of oxidative stress on survival [89]. At the time of admission, the clinical markers (clinical scoring, Physiology and Chronic Health Evaluation II score, multiple organ dysfunction score and sepsis rating) and oxidative damage markers were significantly higher in non-survivors. Similarly, oxidative damage markers significantly correlated with multiple organ dysfunction scores at admission. Mishra et al [90] concluded that high levels of oxidative stress may be associated with poor outcome in critically ill patients, and may be a prognostic indicator.

Structural and functional changes in stored Red Blood Cells

For patients undergoing major surgical intervention, in our case in major aortic surgery, transfusion of blood in the intraoperative phase may be vital. Nevertheless, considerable evidence suggests that transfusion increases the risk of serious complications and

mortality [91-93]. It has been suggested that the frequency of adverse reactions to transfusions are dependent on storage time – the longer the storage the higher the frequency [94-97]. During storage, red blood cells (RBCs) undergo structural and functional changes that may decrease function and viability after transfusion [98-101]. These biochemical and biomechanical changes include a depletion of ATP [102] and 2,3-DPG [102], membrane phospholipid vessiculation103 and loss103, protein oxidation [104, 105] and lipid peroxidation [106, 107]. Oxidative damage, including lipid peroxidation, is a plausible mechanism contributing to the loss of deformability [104-107]. The loss of deformability may compromise microvascular flow of stored RBCs [101].

In a study by Dumaswala et al [99] a time-dependent increase in protein clustering and MDA were observed in stored RBCs. They also reported a time-dependent decline in GSH, GSH-Px activity with a concomitant increase in extracellular GSH, cysteine and homocysteine. These changes indicate oxidative modifications of membrane lipids and proteins, and a reduction in the activity of the antioxidant defence systems with a possible resulting destabilization of the membrane skeleton and thereby compromised RBC survival. It has also been reported that time-dependent accumulation of inflammatory bioactive substances in stored blood may increase the risk of posttransfusion complications [98, 100]. However, the exact mechanisms are not finally understood.

In study IV, we evaluated the quality of buffy-coat reduced RBCs by assessing biomarkers of oxidative and inflammatory stress during a storage period of 35 days. With this study we wanted to evaluate in which extent possible changes in stored blood could explain the course of lipid peroxidation products and inflammatory bioactive substances during studies I and II. In study IV, we reported a significant change in the levels of MDA, AA, DHA, IL-6 and YKL-40. The clinical significance of these findings may be serious. Our data suggest a possible rationale behind the observation that aging blood products may increase the risk of complications following surgery and blood transfusion. The oxidative stress response with reduction of antioxidants in study I and II might be affected by the changes observed in a random selection of stored blood. Because of study limitations including lack of control for confounding factors such as gender, age, smoking, alcohol consumption and morbidity, our findings should be further examined in a larger controlled clinical trial.

Ther surgery, anaesthesia and melatonin triade

Disturbances in circadian rhythms in relation to surgery seems to be associated with postoperative fatigue, pain, reduced general well-being, cognitive dysfunction, and cardiovascular morbidity.

In relation to surgery, plasma melatonin levels have been investigated in patients undergoing benign gynaecological surgery [108, 109], surgery for neoplastic disease in elderly patients [110], minor abdominal surgery [111], minor orthopaedic surgery [112], major abdominal surgery [113-116] and coronary artery by-pass grafting [117].

Cronin et al [108] found reduced melatonin secretion the day after gynaecological surgery probably contributing to postoperative sleep disturbances. Similarly, Pontes et al [109] compared the concentrations of melatonin and the circulating TNF-α in women who gave birth by vaginal or caesarean section. They reported an increase in TNF-α after caesarean section correlating with a suppression of the nocturnal melatonin surge, which probably promotes a disruption of internal endogenous processes. In elderly patients undergoing surgery, an impaired rhythm of melatonin secretion seems to correlate with postoperative insomnia and sepsis [110]. In patients undergoing major abdominal surgery, Gögenur et al [113, 114] reported a disturbed circadian rhythmicity as assessed by aMT6s. They were unable to show an association between the diminished melatonin secretion with postoperative circadian disturbances and cognitive dysfunction. In patients undergoing esophagectomy for treatment of esophageal cancer and in whom intensive care unit psychosis is frequently observed, Miyazaki et al [116] reported an irregular pattern of melatonin circadian rhythm with an abnormal melatonin rhythm. In the same manner, elderly patients undergoing major abdominal surgery and developing postoperative delirium show abnormal melatonin secretion, purposing that a disturbed melatonin secretion may trigger delirium in elderly patients [115]. In patients undergoing coronary artery bypass surgery with cardiopulmonary bypass, Guo et al [117] reported a disturbed secretion pattern of melatonin during surgery and in the immediate postoperative period but not on postoperative day 2.

In patients undergoing laparoscopic cholecystectomy the timing of the major urinary melatonin metabolite, aMT6s, measured for 1 day before and 1 day after surgery was significantly delayed, and the amplitude of the metabolite significantly decreased postoperatively [111]. Patients undergoing minor orthopaedic operations and randomly receiving either spinal or general anaesthesia were monitored with aMT6s and saliva samples to clarify possible anaesthesia or surgery induced changes in the nocturnal secretion of melatonin and in the phase of the melatonin rhythm [112]. They reported a diminished melatonin secretion evaluated from the saliva samples and a decline in aMT6s excretion postoperatively. Karkela et al [112] could not find a significant difference in melatonin secretion between the spinal and general anaesthesia groups.

Reber et al [118] studied the differences in hormone profiles and responses during anaesthesia and the recovery period in 32 female patients undergoing gynaecological surgery. Reber et al [118] observed that patients receiving isoflurane anaesthesia had higher plasma levels of melatonin during the recovery period compared with patients who received propofol anaesthesia. Similarly, Arai et al [119] compared the effect of isoflurane and sevoflurane on the blood concentrations of melatonin in female patients. In patients receiving isoflurane, blood melatonin concentrations increased significantly, whereas melatonin decreased in the sevoflurane group. Fassoulaki et al [119] assessed the effect of sevoflurane as a single anaesthetic on plasma levels of melatonin during the first 24 hours postoperatively in patients scheduled for dilatation and curettage of the uterus. Sevoflurane did not influence plasma melatonin levels significantly.

In a prospective, randomized, double-blinded study including 200 healthy volunteers, Naguib et al [120] studied the effect of 0.2 mg/kg melatonin as premedication on the propofol and thiopental dose-response curves for abolition of responses to verbal commands and eyelash stimulation. Melatonin decreased the doses of both propofol and thiopental required to induce anaesthesia. In patients undergoing lower limb orthopaedic surgery plasma levels of melatonin is reported to be increased during administration of propofol as a continuous infusion [121].

Use of melatonin as antioxidant in non-surgical trials

Melatonin has sedative [122, 123], anxiolytic [122, 123], analgesic [121], antihypertensive [124-126], anti-inflammatory [41, 42, 127, 128], chronobiotic [129] and oncostatic effects [130]. Furthermore, in the last decade, melatonin has been reported to possess potent antioxidant properties [11, 30, 41-43, 128, 131-134].

Recent evidence illustrates the beneficial effects of melatonin in reducing tissue damage and reversing cardiac pathophysiology in models of experimental ischaemia/reperfusion. Cardiac arrhythmias in several animal models of experimental ischaemia/reperfusion are believed to be related to free radicals generated in the heart especially during the period of reperfusion. In vitro studies have used ligation of isolated coronary arteries for induction of regional ischaemia resulting in ventricular arrhythmias (VA) and ventricular fibrillation (VF) [133, 135, 136]. Treatment with melatonin infusion in relation to the ischaemia-reperfusion period greatly improved the recovery of contractile function as well as reduced the VA and VF [133, 135, 136] suggesting a cardioprotective effect of melatonin. Occlusion of the coronary artery in pinealectomized rats resulted in irreversible VF compared with the control group [137]. On the other hand, melatonin administration to pinealectomized rats seems to reduce the incidence of VF, and this reduction was attenuated when physiological concentrations of melatonin were administered [137]. Pharmacological concentrations of melatonin did not increase its beneficial effect on these arrhythmias [137]. In in vivo studies [132, 138] with ligation of coronary arteries, melatonin may improve survival rate by reducing VA and reducing infarct size resulting from ischaemia-reperfusion injury. In both studies, the cardioprotective effect of melatonin is supposed to be mediated by its antioxidant activity [132, 138].

The ability of melatonin to influence oxidative stress has been investigated in several human studies [11, 40-42, 44, 128, 139-142] including in our studies I-III. In a placebo-controlled study, Herrera et al [140] investigated the oxidative stress induced by the intravenous iron and recombinant human erythropoietin treatment of anaemia in patients with chronic renal failure and whether pre-treatment with melatonin (0.29 mg/kg) would have a beneficial effect. Melatonin treatment had no adverse side effects and prevented oxidative stress (measured by MDA, GSH and catalase) generated by the use of intravenous iron and erythropoietin. Epileptic children on valproic acid monotherapy were randomly allocated to receive either oral melatonin or placebo to compare the effect of melatonin on the antioxidant enzymes glutathione peroxidase and glutathione reductase[141]. The dose of melatonin was 6 mg for children weighing less than 30 kg, and 9 mg for children weighing more than 30 kg. In the melatonin group, increased glutathione reductase activity was observed indicating a possible neuroprotection of melatonin due to its antioxidant effect, and antiexcitotoxic and free radical scavenging properties within the central nervous system. Oxidative stress in patients with diabetes mellitus and long-term hyperglycemia seems to correlate with an inadequate response to oral hypoglycaemic agents even when insulin levels are sufficient [142]. The effect of melatonin and the antioxidant zinc on tissue response to insulin and the efficiency of drugs, e.g. metformin, were investigated in a placebo-controlled, double-blinded clinical trial [142]. 46 patients with type 2 diabetes mellitus were allocated into three groups. First group was treated with a single daily oral dose of melatonin (10 mg) and zinc (50 mg), second and third groups received melatonin and zinc with same doses supplied with either metformin or placebo. Both melatonin and zinc were given at bedtime for 90 days. Monitoring included fasting lipid profiles and albumin excretion in urine before initiating the treatment and after 30 and 90 days of treatment. Kadhim et al [142] observed improved lipid profile and decreased levels of albumin excretion. Furthermore, melatonin and zinc treatment in combination with metformin improved the tissue response to this hypoglycaemic agent.

Because of the implication of free radicals in the pathogenesis of neonatal sepsis, asphyxia, respiratory distress syndrome and theirs complications, newborns were treated with melatonin [40-42] to determine the antioxidant effect of melatonin and the clinical status. Treatment with melatonin was reported to reduce levels of lipid peroxidation [40, 41, 128] and proinflammatory cytokines 41, 42] probably resulting in improved morbidity and reduced mortality [40, 42, 128].

Free radicals produced within the follicles are believed to be a cause of poor oocyte quality. Therefore, Tamura et al [134] investigated the relationship between oxidative stress and poor oocyte quality and whether melatonin improves oocyte quality. As a biomarker of oxidative stress, intrafollicular concentrations of 8-hydroxy-2´-deoxyguanosine (8-OHdG) were measured. Antioxidant treatment with melatonin and vitamin E significantly decreased intrafollicular concentrations of 8-OHdG. 115 patients who failed to become pregnant in the previous in vitro fertilization and embryo transfer (IVF-ET) cycle were divided into two groups during the next IVF-ET procedure. 56 of these women were treated with melatonin (3 mg) orally at 22 hr from the fifth day of the previous menstrual cycle until the day of oocyte retrieval. In this group, melatonin treatment significantly improved the fertilization rate.

Amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative disease, is in 20% of cases associated with mutations in the gene for SOD [143]. Weishaupt et al [43] explored the effect of melatonin as a neuroprotective compound and antioxidant in cultured motoneural cells, in a genetic mouse model of ALS, and in a group of 31 patients with ALS. The patients were treated with chronic high-dose (300 mg/day) rectal melatonin for an observation period of up to 2 year. In cultured motoneuronal cells, melatonin attenuated glutamate-induced cell death. In the genetic mouse model of ALS, melatonin delayed disease progression and extended survival. In the clinical study involving patients with ALS, melatonin normalized levels of the circulating serum protein carbonyls, which is a surrogate marker of oxidative stress. Weishaupt concluded that there were no clinical efficacy data available, and that they only have laboratory data to suggest an efficacy of melatonin in human ALS.

Use of melatonin as antioxidant in surgical trials

Melatonin has only been used in few surgical trials namely in neonates with surgical malformations [11], in patients undergoing surgery for abdominal aortic aneurysm (studies I [44] and II) and in patients undergoing laparoscopic cholecystectomy (study III).

Gitto et al [11] determined the effects of melatonin on IL-6, IL-8, TNF-α and nitrite/nitrate (NOx) levels in newborns undergoing surgical intervention because of malformations. 5 newborns with surgical malformations and respiratory distress (3 newborns with congenital diaphragmatic hernia and 2 newborns with esophageal atresia with distal fistula) and 5 other newborns with isolated abdominal surgical malformations (2 newborns with anorectal malformation, 2 newborns with intestinal atresia and 1 newborn with meconium ileus) received 10 doses of melatonin (10 mg/kg) at defined time intervals for 72 hours starting within 3 hours after the end of the surgery. Melatonin was dissolved in a 1:50 mixture of ethanol-physiologic saline and each dose was administered intravenously over 2 hours. 10 other newborns requiring surgical intervention (3 anorectal malformations, 2 malrotations, 2 omphaloceles, 3 intestinal atresias) were recruited but were given an equal volume of placebo (a 1:50 mixture of ethanol-physiologic saline). Furthermore, 20 healthy newborns served as controls for basal levels of cytokines and NOx.

Blood samples were collected at the end of surgery, before melatonin or placebo treatment, and 24 hours, 72 hours, and 7 days after start of melatonin or placebo treatment, respectively. Gitto et al[11] reported significantly lower levels of IL-6, IL-8 and NOx after administration of melatonin suggesting a potent antioxidant effect of melatonin. Moreover, they observed a progressive reduction in clinical parameters of inflammation possibly indicating improvements in clinical outcome.

In study I [44], we included 6 patients undergoing aortic surgery to evaluate the safety of various doses of melatonin during and after surgery and to monitor the changes in biomarkers of oxidative stress and inflammation during the pre-, intra- and postoperative periods. Our study was the first study to administer melatonin in the perioperative period in an adult population. During the operation the patients received either 10 (n=2), 30 (n=2) or 60 (n=2) mg melatonin intravenously in the intraoperative phase and 10 mg orally for three nights after surgery. Intravenous administration of melatonin up to 60 mg during major aortic surgery was safe and without any unexpected events.

In study II, we included 52 patients undergoing elective aortic surgery randomized to receive either melatonin or placebo intervention to evaluate the effect of melatonin on surgical oxidative and inflammatory stress response. The patients were treated intraoperatively with intravenous infusion of either 50 mg melatonin or placebo as a continuous infusion for 2 hrs starting from the first incision. Furthermore, the patients were treated with either oral 10 mg melatonin or placebo the first three nights postoperatively. Treatment with melatonin did not affect the measured oxidative and inflammatory stress parameters.

In study III, we included 44 patients undergoing elective laparoscopic cholecystectomy. They were randomized to receive either melatonin or placebo intervention to evaluate the effect of melatonin on the oxidative and inflammatory surgical stress response. The patients were treated intraoperatively with intravenous infusion of either 10 mg melatonin or placebo as a continuous infusion for 30 min starting from the first incision. No overall effect of melatonin was observed on oxidative or inflammatory stress parameters.

Clinical implications of our studies

The safety of intravenously administered melatonin during surgery has never been investigated previously. In study I, we showed that administration of up to 60 mg of intravenous melatonin during aortic surgery in adult patients is safe without affecting intra- and postoperative hemodynamic parameters. In study II we also demonstrated in a randomized, placebo-controlled trial that infusion of 50 mg of melatonin intraoperatively is safe. Unfortunately, we could not find any effect of melatonin on the biochemical markers of oxidative and inflammatory stress response. Future clinical trials with similar methodologies are required to evaluate higher doses of melatonin with different administrations schedules and/or longer duration of infusion in relation to surgery.

Study III was designed to evaluate the effect of 10 mg melatonin on oxidative and inflammatory stress response in patients undergoing elective laparoscopic cholecystectomy. Contrary to the first and second studies, this study consisted of a homogenous population with no co-morbidity thus reducing the intrinsic noise and variability, and thus increasing the likelihood of observing a measurable effect of melatonin. We could however, not demonstrate an overall antioxidant effect of melatonin. Future clinical trials are required to compare the effect of higher doses of melatonin, and different administrations schedules with a longer duration of infusion or timing in relation to surgery on the surgical stress response.

We have also demonstrated in study II and III that there is a significant oxidative stress response after surgery with use of antioxidants in early after the first incision and throughout the first postoperative day. The use of antioxidants precedes the increase in inflammatory acute phase proteins indicating that the use of antioxidants may not be simply coupled to the inflammatory reactions during the surgical stress response. However, this should be further elucidated in trials where inflammatory cytokines known to increase early after surgical stress (e.g. IL-6) are used in conjunction with the measurement of antioxidants.

Study IV was designed to evaluate the quality of red blood cells by determining the concentration of biomarkers of oxidative stress and damage as well as markers of inflammation. We reported significant time-dependent changes in oxidative and inflammatory markers. The potential clinical implications may be significant giving a potential explanation for the observed correlation between post-transfusion morbidity seen when using older blood. These correlation should however, be investigated in the future.

Possible improvements

We showed in study I [44] that use of melatonin intravenously during surgery is safe. The obvious limitations of this study were the small sample size and lack of a control group. Due to difficulties in receiving ethical approval (this was the first study where melatonin was given intravenously to surgical patients) we could only use up to 60 mg of melatonin. Higher dosage of melatonin should be given during surgery to obtain a possible beneficial effect in terms of surgical stress markers or to increase the likelihood of a positive clinical outcome. Thus, in study II where we had a larger sample size and also a control group, the study could be improved by giving a larger dose of intravenous melatonin and possibly, inspired by the only other study on infants, the infusion time should be increased. The use of a higher dose of intravenous melatonin during surgery and a prolonged infusion time could also have been used in study III.

Another possible improvement in both studies I, II and III would be to control the actual intravenous concentration of melatonin after the administration of the drug having an objective measure for the effective intravenous dose, at different time points during the postoperative phase.

The clinical implications of disturbed melatonin secretion after surgery are still not known. Previous studies have shown that there exists a substantial disturbance in endogenous melatonin secretion after surgery. The implications of these disturbances for the postoperative oxidative and inflammatory stress response are not known. We did not control if there were circadian disturbance of melatonin secretion in the postoperative phase and whether this affected the postoperative oxidative and inflammatory stress response.

Study IV was limited to 10 units of blood, and we did not control for confounding factors such as gender, age, smoking, alcohol consumption, and morbidity.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

As described in the above melatonin has been investigated in several animal and in few human trials. Consistently, melatonin has been shown to have a substantial antioxidant, anti-inflammatory and a possible clinical effect with reduced morbidity and mortality. Surgery elicits an acute surgical stress response leading to postoperative morbidity and mortality. Inspired of

these studies, we established and performed three different human trials to evaluate the antioxidant effect of melatonin. Additionally, we evaluated the biochemical changes in stored blood. The main results of our studies were:

- Up to 60 mg of intravenous infusion of melatonin during surgery in adult patients is safe without affecting intra- and postoperative hemodynamic parameters.
- There is an oxidative stress response with use of antioxidants after both laparoscopic cholecystectomy and abdominal aortic aneurism surgery.
- Melatonin does not have an antioxidant or anti-inflammatory effect in relation to elective abdominal aortic aneurism surgery
- Melatonin does not have an antioxidant or anti-inflammatory effect in relation to elective laparoscopic cholecystectomy
- Significant time-dependent changes in oxidative and inflammatory markers occur in stored non-filtered SAG-M blood.

Based on the results of our studies and based on the substantial literature supporting an antioxidant effect of melatonin, future prospective human trials should be done to evaluate the effect of higher doses of melatonin or longer duration of melatonin infusion in the perioperative period. The biochemical changes observed during storage of blood should be elucidated in larger controlled trials to evaluate the clinical importance of these changes.

LIST OF ABBREVIATIONS

AA = Ascorbic acid	IL-8 = Interleukin-8
AAA = Abdominal aortic aneurysm	IVF-ET = In vitro fertilization and embryo transfer
ACTH = Adrenocorticotrophic hormone	LD_{50} = Lethal dose, 50%
ALS = Amyotrophic lateral sclerosis	MAP = Mean arterial pressure
aMT6s = 6-sulphatoxymelatonin	MDA = Malondialdehyde
ASA = American Society of Anaesthesiologist	MT = Melatonin
AT = Alfa tocopherole	$NO^{\bullet}$ = Nitric oxide
BMI = Body mass index	NOx = Nitrogen oxide
CO_2 = Carbon dioxide	$OH^{\bullet}$ = Hydroxyl radical
CRP = C-reactive protein	$ONOO^-$ = Peroxynitrite anion
DHA = Dehydroascorbic acid	$^1O_2$ = Singlet oxygen
ECG = Electrocardiographic	$O_2^{\bullet}$ = Superoxide anion radical
EDTA = Ethylenediaminetetraacetic acid	RBCs = Red Blood Cells
ELISA = Enzyme-linked immunosorbent assay	RNS = Reactive nitrogen molecules
GCP = Good Clinical Practice	ROS = Reactive oxygen molecules
GH = Growth hormone	$RO_2^{\bullet}$ = Peroxyl radicals
GPx = Glutathione peroxidise	SAG-M = Saline-adenine-glucose-mannitol
GSH = Glutathione	SD = Standard deviation
GSSG = Oxidized glutathione	SE = Standard error
H_2O_2 = Hydrogen peroxide	SOD = Superoxide dismutase
HPLC = High-performance liquid chromatography	TAA = Total ascorbic acid
IAP = Intra-abdominal pressure	TAAA = Thoracoabdominal aortic aneurysm
ICH = International Conference on Harmonisation	TNF-α = Tumour necrosis factor-alpha
ICU = Intensive Care Unit	TpT = Troponin T
IL-1 = Interleukin-1	VA = Ventricular arrhythmias
IL-6 = Interleukin-6	VF = ventricular fibrillation

SUMMARY

Surgical trauma elicits a well-known stress response involving activation of inflammatory, endocrine, metabolic and immunologic mediators. Exaggerated activation of surgical stress response can manifest systemically and hemodynamic instability, and metabolic derangement leading to multiple organ failure and mortality. Oxidative stress, a biological condition with generation of free radicals higher than the capacity of the systems to detoxify them, is believed to be an integrated part of the surgical stress response. The latter is believed to be associated with complications such as myocardial injury, sepsis, pulmonary oedema, kidney and liver failure and increased mortality.

Melatonin, the chief secretory product of pineal gland, is a highly efficient scavenger of free radicals, and seems to be more effective than other antioxidants to combat stress response. Melatonin has only been used in one human surgical trial, showing a modification of the oxidative stress response in neonates with surgical malformations. We hypothesized that administration of melatonin in relation to minor and major surgery would reduce oxidative and inflammatory stress. We also wanted to investigate whether transfusion products would be expected to influence our results in the major surgery group. We performed two randomized, placebo-controlled human trials to examine the effect of melatonin on oxidative and inflammatory stress response during and after minor (laparoscopic cholecystectomy) and major (abdominal aortic aneurism) surgery. Before initiating these trials, we carried a safety study to examine if intravenous administration of melatonin during major surgery was safe.

We concluded that administration of intravenous infusion of melatonin intraoperatively is safe. We showed that surgery is followed by a substantial oxidative and inflammatory stress. However, we could not show any antioxidant or anti-inflammatory effect of melatonin. We reported time-dependent changes in oxidative and inflammatory markers in stored non-filtered SAG-M blood.

REFERENCES

5. Kehlet H. Neural Blockade in Clinical Anesthesia 3rd ed. Philadelphia: Lippincott-Raven Publishers, 1998:129-75.
6. Lin E, Calvano SE, Lowry SF. Inflammatory cytokines and cell response in surgery. Surgery 2000;127:117-26.
7. Guirao X, Lowry SF. Biologic control of injury and inflammation: much more than too little or too late. World J Surg 1996;20:437-46.
8. Sheeran P, Hall GM. Cytokines in anaesthesia. Br J Anaesth 1997;78:201-19.
9. Desborough JP. The stress response to trauma and surgery. Br J Anaesth 2000;85:109-17.
10. Raeburn CD, Sheppard F, Barsness KA, Arya J, Harken AH. Cytokines for surgeons. Am J Surg 2002;183:268-73.
11. Desborough JP, Hall GM. Endocrine response to surgery. In: Kaufman L. Anaesthesia Review, Vol. 10. Edinburgh: Churchill Livingstone, 1993:131-48.
12. Lyons FM, Meeran K. The physiology of the endocrine system. Int Anesthesiol Clin 1997;35:1-21.
13. Cornu-Labat G, Serra M, Smith A, McGregor WE, Kasirajan K, Hirko MK, Turner JJ, Rubin JR. Systemic consequences of oxidative stress following aortic surgery correlate with the degree of antioxidant defenses. Ann Vasc Surg 2000;14:31-6.
14. Hafez HM, Berwanger CS, McColl A, Richmond W, Wolfe JH, Mansfield AO, Stansby G. Myocardial injury in major aortic surgery. J Vasc Surg 2000;31:742-50.
15. Gitto E, Romeo C, Reiter RJ, Impellizzeri P, Pesce S, Basile M, Antonuccio P, Trimarchi G, Gentile C, Barberi I, Zuccarello B. Melatonin reduces oxidative stress in surgical neonates. J Pediatr Surg 2004;39:184-9.
16. Grune T, Berger MM. Markers of oxidative stress in ICU clinical settings: present and future. Curr Opin Clin Nutr Metab Care 2007;10:712-7.
17. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet 1994;344:721-4.

18. Claustrat B, Brun J, Chazot G. The basic physiology and pathophysiology of melatonin. Sleep Med Rev 2005;9:11-24.
19. Reiter RJ, Calvo JR, Karbownik M, Qi W, Tan DX. Melatonin and its relation to the immune system and inflammation. Ann N Y Acad Sci 2000;917:376-86.
20. Bourne RS, Mills GH. Melatonin: possible implications for the postoperative and critically ill patient. Intensive Care Med 2006;32:371-9.
21. Bojkowski CJ, Arendt J, Shih MC, Markey SP. Melatonin secretion in humans assessed by measuring its metabolite, 6-sulfatoxymelatonin. Clin Chem 1987;33:1343-8.
22. Cajochen C, Zeitzer JM, Czeisler CA, Dijk DJ. Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. Behav Brain Res 2000;115:75-83.
23. Haimov I, Laudon M, Zisapel N, Souroujon M, Nof D, Shlitner A, et al. Sleep disorders and melatonin rhythms in elderly people. BMJ 1994;309:167.
24. Garfinkel D, Laudon M, Nof D, Zisapel N. Improvement of sleep quality in elderly people by controlled-release melatonin. Lancet 1995;346:541-4.
25. Herxheimer A, Petrie KJ. Melatonin for preventing and treating jet lag. Cochrane Database Syst Rev 2001;1:CD001520.
26. Brzezinski A. Melatonin in humans. N Engl J Med 1997;336:186-95.
27. Reiter RJ, Tan DX. What constitutes a physiological concentration of melatonin? J Pineal Res 2003;34:79-80.
28. Waldhauser F, Waldhauser M, Lieberman HR, Deng MH, Lynch HJ, Wurtman RJ. Bioavailability of oral melatonin in humans. Neuroendocrinology 1984;39:307-13.
29. DeMuro RL, Nafziger AN, Blask DE, Menhinick AM, Bertino JS, Jr. The absolute bioavailability of oral melatonin. J Clin Pharmacol 2000;40:781-4.
30. Vijayalaxmi, Thomas CR, Jr., Reiter RJ, Herman TS. Melatonin: from basic research to cancer treatment clinics. J Clin Oncol 2002;20:2575-601.
31. Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 2005;27:101-10.
32. Hardeland R, Reiter RJ, Poeggeler B, Tan DX. The significance of the metabolism of the neurohormone melatonin: antioxidative protection and formation of bioactive substances. Neurosci Biobehav Rev 1993;17:347-57.
33. Tan DX, Manchester LC, Reiter RJ, Qi WB, Karbownik M, Calvo JR. Significance of melatonin in antioxidative defense system: reactions and products. Biol Signals Recept 2000;9:137-59.
34. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. J Biomed Sci 2000;7:444-58.
35. Reiter RJ, Tan DX, Sainz RM, Mayo JC, Lopez-Burillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. J Pharm Pharmacol 2002;54:1299-321.
36. Reiter RJ, Tan DX. Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. Cardiovasc Res 2003;58:10-9.
37. Reiter RJ, Tan DX, Gitto E, Sainz RM, Mayo JC, Leon J, Manchester LC, Vijayalaxmi, Kilic E, Kilic U. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. Pol J Pharmacol 2004;56:159-70.
38. Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C. Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. Toxicol Sci 1999;50:271-9.
39. Barchas J, DaCosta F, Spector S. Acute pharmacology of melatonin. Nature 1967;214:919-20.
40. Sugden D. Psychopharmacological effects of melatonin in mouse and rat. J Pharmacol Exp Ther 1983;227:587-91.
41. Nordlund JJ, Lerner AB. The effects of oral melatonin on skin color and on the release of pituitary hormones. J Clin Endocrinol Metab 1977;45:768-74.
42. Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, Vohra S, Klassen TP, Baker G. Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: meta-analysis. BMJ 2006;332:385-93.
43. Anton-Tay F, Diaz JL, Fernandez-Guardiola A. On the effect of melatonin upon human brain. Its possible therapeutic implications. Life Sci I 1971;10:841-50.
44. Fulia F, Gitto E, Cuzzocrea S, Reiter RJ, Dugo L, Gitto P, Barberi S, Cordaro S, Barberi I. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin. J Pineal Res 2001;31:343-9.
45. Gitto E, Reiter RJ, Cordaro SP, La Rosa M, Chiurazzi P, Trimarchi G, Gitto P, Calabrò MP, Barberi I. Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: beneficial effects of melatonin. Am J Perinatol 2004;21:209-16.
46. Gitto E, Reiter RJ, Sabatino G, Buonocore G, Romeo C, Gitto P, Buggé C, Trimarchi G, Barberi I. Correlation among cytokines, bronchopulmonary dysplasia and modality of ventilation in preterm newborns: improvement with melatonin treatment. J Pineal Res 2005;39:287-93.
47. Weishaupt JH, Bartels C, Pölking E, Dietrich J, Rohde G, Poeggeler B, Mertens N, Sperling S, Bohn M, Hüther G, Schneider A, Bach A, Sirén AL, Hardeland R, Bähr M, Nave KA, Ehrenreich H. Reduced oxidative damage in ALS by high-dose enteral melatonin treatment. J Pineal Res 2006;41:313-23.
48. Kücükakin B, Lykkesfeldt J, Nielsen HJ, Reiter RJ, Rosenberg J, Gögenur I. Utility of melatonin to treat surgical stress after major vascular surgery--a safety study. J Pineal Res 2008;44:426-31.
49. Thérond P, Bonnefont-Rousselot D, Davit-Spraul A, Conti M, Legrand A. Biomarkers of oxidative stress: an analytical approach. Curr Opin Clin Nutr Metab Care 2000;3:373-84.
50. Lykkesfeldt J. Determination of malondialdehyde dithiobarbituric acid adduct in biological samples by HPLC with fluorescence detection: Comparison with UV-visible spectrophotometry. Clin Chem 2001;47:1725-8.
51. Archer S. Measurement of nitric oxide in biological models. FASEB J 1993;7:349-60.
52. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med 1990;9:515-40.
53. Gil L, Siems W, Mazurek B, Gross J, Schroeder P, Voss P, Grune T. Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. Free Radic Res 2006;40:495-505.
54. Chirico S. High-performance liquid chromatography-based thiobarbituric acid tests. Methods Enzymol 1994;233:314-8.
55. Lykkesfeldt J. Determination of ascorbic acid and dehydroascorbic acid in biological samples by high-performance liquid chromatography using subtraction methods: reliable

reduction with tris[2-carboxyethyl]phosphine hydrochloride. Anal Biochem 2000;282:89-93.
56. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr 1995;62:1315S-21S.
57. Löwik MR, Schrijver J, Wedel M. Vitamin C analysis in whole blood, plasma and cells using reduced glutathione as preservative (stabilizer): losses and redistribution. Int J Vitam Nutr Res 1991;61:43-5.
58. Tangney CC. Analyses of vitamin C in biological samples with an emphasis on recent chromatographic techniques. Prog Clin Biol Res 1988;259:331-62.
59. Lykkesfeldt J, Loft S, Poulsen HE. Determination of ascorbic acid and dehydroascorbic acid in plasma by high-performance liquid chromatography with coulometric detection--are they reliable biomarkers of oxidative stress? Anal Biochem 1995;229:329-35.
60. Sattler W, Mohr D, Stocker R. Rapid isolation of lipoproteins and assessment of their peroxidation by high-performance liquid chromatography postcolumn chemiluminescence. Meth Enzymol 1994;233:469-89.
61. Cruickshank AM, Fraser WD, Burns HJ, Van Damme J, Shenkin A. Response of serum interleukin-6 in patients undergoing elective surgery of varying severity. Clin Sci (Lond) 1990;79:161-5.
62. Chaudhary R, Aggarwal A, Khetan D, Dayal R. Cytokine generation in stored platelet concentrate: comparison of two methods of preparation. Indian J Med Res 2006;124:427-30.
63. Sachdeva N, Asthana D. Cytokine quantitation: technologies and applications. Front Biosci 2007;1:4682-95.
64. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805-12.
65. Venugopal SK, Devaraj S, Jialal I. Macrophage conditioned medium induces the expression of C-reactive protein in human aortic endothelial cells: potential for paracrine/autocrine effects. Am J Pathol 2005;166:1265-71.
66. Pepys MB. C-reactive protein fifty years on. Lancet 1981;317:653-7.
67. Aldrete JA, Kroulik D. A postanesthetic recovery score. Anesth Analg 1970;49:924-34.
68. Marathe US, Lilly RE, Silvestry SC, Schauer PR, Davis JW, Pappas TN, Glower DD. Alterations in hemodynamics and left ventricular contractility during carbon dioxide pneumoperitoneum. Surg Endosc 1996;10:974-8.
69. Galizia G, Prizio G, Lieto E, Castellano P, Pelosio L, Imperatore V, Ferrara A, Pignatelli C. Hemodynamic and pulmonary changes during open, carbon dioxide pneumoperitoneum and abdominal wall-lifting cholecystectomy. A prospective, randomized study. Surg Endosc 2001;15:477-83.
70. Jakimowicz J, Stultiëns G, Smulders F. Laparoscopic insufflation of the abdomen reduces portal venous flow. Surg Endosc 1998;12:129-32.
71. Schilling MK, Redaelli C, Krähenbühl L, Signer C, Büchler MW. Splanchnic microcirculatory changes during CO2 laparoscopy. J Am Coll Surg 1997;184:378-82.
72. Takagi S. Hepatic and portal vein blood flow during carbon dioxide pneumoperitoneum for laparoscopic hepatectomy. Surg Endosc 1998;12:427-31.
73. Rist M, Hemmerling TM, Rauh R, Siebzehnrübl E, Jacobi KE. Influence of pneumoperitoneum and patient positioning on preload and splanchnic blood volume in laparoscopic surgery of the lower abdomen. J Clin Anesth 2001;13:244-9.
74. Andersson L, Lindberg G, Bringman S, Ramel S, Anderberg B, Odeberg-Wernerman S. Pneumoperitoneum versus abdominal wall lift: effects on central haemodynamics and intrathoracic pressure during laparoscopic cholecystectomy. Acta Anaesthesiol Scand 2003;47:838-46.
75. Polat C, Yilmaz S, Serteser M, Koken T, Kahraman A, Dilek ON. The effect of different intraabdominal pressures on lipid peroxidation and protein oxidation status during laparoscopic cholecystectomy. Surg Endosc 2003;17:1719-22.
76. Bickel A, Drobot A, Aviram M, Eitan A. Validation and reduction of the oxidative stress following laparoscopic operations: a prospective randomized controlled study. Ann Surg 2007;246:31-5.
77. Kim ZG, Sanli E, Brinkmann L, Lorenz M, Gutt CN. Impact of dopamine and endothelin-1 antagonism on portal venous blood flow during laparoscopic surgery. Surg Endosc 2002;16:1292-6.
78. Gudmundsson FF, Viste A, Myking OL, Grong K, Svanes K. Effects of the aldosterone receptor antagonist potassium canrenoate on renal blood flow and urinary output during prolonged increased intraabdominal pressure (IAP) in pigs. Surg Endosc 2004;18:1528-34.
79. London ET, Ho HS, Neuhaus AM, Wolfe BM, Rudich SM, Perez RV. Effect of intravascular volume expansion on renal function during prolonged CO2 pneumoperitoneum. Ann Surg 2000;231:195-201.
80. Yagmurdur H, Cakan T, Bayrak A, Arslan M, Baltaci B, Inan N, Kilinc K. The effects of etomidate, thiopental, and propofol in induction on hypoperfusion-reperfusion phenomenon during laparoscopic cholecystectomy. Acta Anaesthesiol Scand 2004;48:772-7.
81. Koksal GM, Sayilgan C, Aydin S, Uzun H, Oz H. The effects of sevoflurane and desflurane on lipid peroxidation during laparoscopic cholecystectomy. Eur J Anaesthesiol 2004;21:217-20.
82. Manataki AD, Tselepis AD, Glantzounis GK, Arnaoutoglou HM, Tsimoyiannis EC, Stavropoulos NE. Lipid peroxidation and the use of emulsified propofol in laparoscopic surgery. Surg Endosc 2001;15:950-3.
83. Alishahi S, Francis N, Crofts S, Duncan L, Bickel A, Cuschieri A. Central and peripheral adverse hemodynamic changes during laparoscopic surgery and their reversal with a novel intermittent sequential pneumatic compression device. Ann Surg 2001;233:176-82.
84. Patel MI, Hardman DT, Fisher CM, Appleberg M. Current views on the pathogenesis of abdominal aortic aneurysms. J Am Coll Surg 1995;181:371-82.
85. Brophy CM, Reilly JM, Smith GJ, Tilson MD. The role of inflammation in nonspecific abdominal aortic aneurysm disease. Ann Vasc Surg 1991;5:229-33.
86. Shah PK. Inflammation, metalloproteinases, and increased proteolysis: an emerging pathophysiological paradigm in aortic aneurysm. Circulation 1997;96:2115-7.
87. Miller FJ Jr, Sharp WJ, Fang X, Oberley LW, Oberley TD, Weintraub NL. Oxidative stress in human abdominal aortic aneurysms: a potential mediator of aneurysmal remodeling. Arterioscler Thromb Vasc Biol 2002;22:560-5.
88. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen molecules produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 1996;98:2572-9.

89. Li PF, Dietz R, von Harsdorf R. Reactive oxygen molecules induce apoptosis of vascular smooth muscle cell. FEBS Lett 1997;404:249-52.
90. Norwood MG, Bown MJ, Sayers RD. Ischaemia-reperfusion injury and regional inflammatory responses in abdominal aortic aneurysm repair. Eur J Vasc Endovasc Surg 2004;28:234-45.
91. Bown MJ, Nicholson ML, Bell PR, Sayers RD. The systemic inflammatory response syndrome, organ failure, and mortality after abdominal aortic aneurysm repair. J Vasc Surg 2003;37:600-6.
92. Lindsay TF, Luo XP, Lehotay DC, Rubin BB, Anderson M, Walker PM, Romaschin AD. Ruptured abdominal aortic aneurysm, a "two-hit" ischaemia/reperfusion injury: evidence from an analysis of oxidative products. J Vasc Surg 1999;30:219-28.
93. Charlson M, Peterson J, Szatrowski TP, MacKenzie R, Gold J. Long-term prognosis after peri-operative cardiac complications. J Clin Epidemiol 1994;47:1389-400.
94. Mishra V, Baines M, Wenstone R, Shenkin A. Markers of oxidative damage, antioxidant status and clinical outcome in critically ill patients. Ann Clin Biochem 2005;42:269-76.
95. Robinson WP 3rd, Ahn J, Stiffler A, Rutherford EJ, Hurd H, Zarzaur BL, Baker CC, Meyer AA, Rich PB. Blood transfusion is an independent predictor of increased mortality in nonoperatively managed blunt hepatic and splenic injuries. J Trauma 2005;58:437-44.
96. Malone DL, Dunne J, Tracy JK, Putnam AT, Scalea TM, Napolitano LM. Blood transfusion, independent of shock severity, is associated with worse outcome in trauma. J Trauma 2003;54:898-905.
97. Taylor RW, Manganaro L, O'Brien J, Trottier SJ, Parkar N, Veremakis C. Impact of allogenic packed red blood cell transfusion on nosocomial infection rates in the critically ill patient. Crit Care Med 2002;30:2249-54.
98. Zallen G, Offner PJ, Moore EE, Blackwell J, Ciesla DJ, Gabriel J, Denny C, Silliman CC. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. Am J Surg 1999;178:570-2.
99. Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T, Blackstone EH. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008;358:1229-39.
100. Mynster T, Christensen IJ, Moesgaard F, Nielsen HJ, Danish RANX05 Colorectal Cancer Study Group. Effects of the combination of blood transfusion and postoperative infectious complications on prognosis after surgery for colorectal cancer. Br J Surg 2000;87:1553-62.
101. Leal-Noval SR, Jara-López I, García-Garmendia JL, Marín-Niebla A, Herruzo-Avilés A, Camacho-Laraña P, Loscertales J. Influence of erythrocyte concentrate storage time on postsurgical morbidity in cardiac surgery patients. Anesthesiology 2003;98:815-22.
102. Mynster T, Dybkjoer E, Kronborg G, Nielsen HJ. Immunomodulating effect of blood transfusion: is storage time important? Vox Sang 1998;74:176-81.
103. Dumaswala UJ, Zhuo L, Jacobsen DW, Jain SK, Sukalski KA. Protein and lipid oxidation of banked human erythrocytes: role of glutathione. Free Radic Biol Med 1999;27:1041-9.
104. Cintin C, Johansen JS, Skov F, Price PA, Nielsen HJ. Accumulation of the neutrophil-derived protein YKL-40 during storage of various blood components. Inflamm Res 2001;50:107-11.
105. Tinmouth A, Fergusson D, Yee IC, Hébert PC, ABLE Investigators; Canadian Critical Care Trials Group. Clinical consequences of red cell storage in the critically ill. Transfusion 2006;46:2014-27.
106. Raat NJ, Verhoeven AJ, Mik EG, Gouwerok CW, Verhaar R, Goedhart PT, de Korte D, Ince C. The effect of storage time of human red cells on intestinal microcirculatory oxygenation in a rat isovolemic exchange model. Crit Care Med 2005;33:39-45.
107. Brunauer LS, Moxness MS, Huestis WH. Hydrogen peroxide oxidation induces the transfer of phospholipids from the membrane into the cytosol of human erythrocytes. Biochemistry 1994;33:4527-32.
108. Wagner GM, Chiu DT, Qju JH, Heath RH, Lubin BH. Spectrin oxidation correlates with membrane vesiculation in stored RBCs. Blood 1987;69:1777-81.
109. Wolfe LC. The membrane and the lesions of storage in preserved red cells. Transfusion 1985;25:185-203.
110. Knight JA, Voorhees RP, Martin L, Anstall H. Lipid peroxidation in stored red cells. Transfusion 1992;32:354-7.
111. Knight JA, Searles DA, Clayton FC. The effect of desferrioxamine on stored erythrocytes: lipid peroxidation, deformability, and morphology. Ann Clin Lab Sci 1996;26:283-90.
112. Cronin AJ, Keifer JC, Davies MF, King TS, Bixler EO. Melatonin secretion after surgery. Lancet 2000;356:1244-5.
113. Pontes GN, Cardoso EC, Carneiro-Sampaio MM, Markus RP. Pineal melatonin and the innate immune response: the TNF-alpha increase after cesarean section suppresses nocturnal melatonin production. J Pineal Res 2007;43:365-71.
114. Leardi S, Tavone E, Cianca G, Barnabei R, Necozione S, Citone G, Simi M. The role of melatonin in the immediate postoperative period in elderly patients. Minerva Chir 2000;55:745-50.
115. Gögenur I, Middleton B, Kristiansen VB, Skene DJ, Rosenberg J. Disturbances in melatonin and core body temperature circadian rhythms after minimal invasive surgery. Acta Anaesthesiol Scand 2007;51:1099-106.
116. Kärkelä J, Vakkuri O, Kaukinen S, Huang WQ, Pasanen M. The influence of anaesthesia and surgery on the circadian rhythm of melatonin. Acta Anaesthesiol Scand 2002;46:30-6.
117. Gögenur I, Ocak U, Altunpinar O, Middleton B, Skene DJ, Rosenberg J. Disturbances in melatonin, cortisol, and core body temperature rhythms after major surgery. World J Surg 2007;31:290-8.
118. Gögenur I, Middleton B, Burgdorf S, Rasmussen LS, Skene DJ, Rosenberg J. Impact of sleep and circadian disturbances in urinary 6-sulphatoxymelatonin levels, on cognitive function after major surgery. J Pineal Res 2007;43:179-84.
119. Shigeta H, Yasui A, Nimura Y, Machida N, Kageyama M, Miura M, Menjo M, Ikeda K. Postoperative delirium and melatonin levels in elderly patients. Am J Surg 2001;182:449-54.
120. Miyazaki T, Kuwano H, Kato H, Ando H, Kimura H, Inose T, Ohno T, Suzuki M, Nakajima M, Manda R, Fukuchi M, Tsukada K. Correlation between serum melatonin circadian rhythm and intensive care unit psychosis after thoracic esophagectomy. Surgery 2003;133:662-8.
121. Guo X, Kuzumi E, Charman SC, Vuylsteke A. Perioperative melatonin secretion in patients undergoing coronary artery bypass grafting. Anesth Analg 2002;94:1085-91.
122. Reber A, Huber PR, Ummenhofer W, Gürtler CM, Zurschmiede C, Drewe J, Schneider M. General anaesthesia for surgery can influence circulating melatonin during daylight hours. Acta Anaesthesiol Scand 1998;42:1050-6.

123. Arai YC, Ueda W, Okatani Y, Fukaya T, Manabe M. Isoflurane increases, but sevoflurane decreases blood concentrations of melatonin in women. J Anesth 2004;18:228-31.
124. Naguib M, Samarkandi AH, Moniem MA, Mansour Eel-D, Alshaer AA, Al-Ayyaf HA, Fadin A, Alharby SW. The effects of melatonin premedication on propofol and thiopental induction dose-response curves: a prospective, randomized, double-blind study. Anesth Analg 2006;103:1448-52.
125. Caumo W, Torres F, Moreira NL Jr, Auzani JA, Monteiro CA, Londero G, Ribeiro DF, Hidalgo MP. The clinical impact of preoperative melatonin on postoperative outcomes in patients undergoing abdominal hysterectomy. Anesth Analg 2007;105:1263-71.
126. Acil M, Basgul E, Celiker V, Karagöz AH, Demir B, Aypar U. Perioperative effects of melatonin and midazolam premedication on sedation, orientation, anxiety scores and psychomotor performance. Eur J Anaesthesiol 2004;21:553-7.
127. Naguib M, Samarkandi AH. Premedication with melatonin: a double-blind, placebo-controlled comparison with midazolam. Br J Anaesth 1999;82:875-80.
128. Cagnacci A, Cannoletta M, Renzi A, Baldassari F, Arangino S, Volpe A. Prolonged melatonin administration decreases nocturnal blood pressure in women. Am J Hypertens 2005;18:1614-8.
129. Kitajima T, Kanbayashi T, Saitoh Y, Ogawa Y, Sugiyama T, Kaneko Y, Sasaki Y, Aizawa R, Shimisu T. The effects of oral melatonin on the autonomic function in healthy subjects. Psychiatry Clin Neurosci 2001;55:299-300.
130. Scheer FA, Van Montfrans GA, van Someren EJ, Mairuhu G, Buijs RM. Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension. Hypertension 2004;43:192-7.
131. Maestroni GJ. The immunotherapeutic potential of melatonin. Expert Opin Investig Drugs 2001;10:467-76.
132. Gitto E, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P, Cordaro S, Corona G, Trimarchi G, Barberi I. Effects of melatonin treatment in septic newborns. Pediatr Res 2001;50:756-60.
133. Scheer FA, Czeisler CA. Melatonin, sleep, and circadian rhythms. Sleep Med Rev 2005;9:5-9.
134. Reiter RJ. Mechanisms of cancer inhibition by melatonin. J Pineal Res 2004;37:213-4.
135. Ochoa JJ, Vílchez MJ, Palacios MA, García JJ, Reiter RJ, Muñoz-Hoyos A. Melatonin protects against lipid peroxidation and membrane rigidity in erythrocytes from patients undergoing cardiopulmonary bypass surgery. J Pineal Res 2003;35:104-8.
136. Lee YM, Chen HR, Hsiao G, Sheu JR, Wang JJ, Yen MH. Protective effects of melatonin on myocardial ischaemia/reperfusion injury in vivo. J Pineal Res 2002;33:72-80.
137. Tan DX, Manchester LC, Reiter RJ, Qi W, Kim SJ, El-Sokkary GH. Ischaemia/reperfusion-induced arrhythmias in the isolated rat heart: prevention by melatonin. J Pineal Res 1998;25:184-91.
138. Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, Taketani T, Matsuoka A, Yamagata Y, Shimamura K, Morioka H, Ishikawa H, Reiter RJ, Sugino N. Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. J Pineal Res 2008;44:280-7.
139. Szárszoi O, Asemu G, Vanecek J, Ost'ádal B, Kolár F. Effects of melatonin on ischaemia and reperfusion injury of the rat heart. Cardiovasc Drugs Ther 2001;15:251-7.
140. Lagneux C, Joyeux M, Demenge P, Ribuot C, Godin-Ribuot D. Protective effects of melatonin against ischaemia-reperfusion injury in the isolated rat heart. Life Sci 2000;66:503-9.
141. Sahna E, Olmez E, Acet A. Effects of physiological and pharmacological concentrations of melatonin on ischaemia-reperfusion arrhythmias in rats: can the incidence of sudden cardiac death be reduced? J Pineal Res 2002;32:194-8.
142. Sahna E, Parlakpinar H, Turkoz Y, Acet A. Protective effects of melatonin on myocardial ischaemia/reperfusion induced infarct size and oxidative changes. Physiol Res 2005;54:491-5.
143. Dreher F, Denig N, Gabard B, Schwindt DA, Maibach HI. Effect of topical antioxidants on UV-induced erythema formation when administered after exposure. Dermatology 1999;198:52-5.
144. Herrera J, Nava M, Romero F, Rodríguez-Iturbe B. Melatonin prevents oxidative stress resulting from iron and erythropoietin administration. Am J Kidney Dis 2001;37:750-7.
145. Gupta M, Gupta YK, Agarwal S, Aneja S, Kohli K. A randomized, double-blind, placebo controlled trial of melatonin add-on therapy in epileptic children on valproate monotherapy: effect on glutathione peroxidase and glutathione reductase enzymes. Br J Clin Pharmacol 2004;58:542-7.
146. Kadhim HM, Ismail SH, Hussein KI, Bakir IH, Sahib AS, Khalaf BH, Hussain SA. Effects of melatonin and zinc on lipid profile and renal function in type 2 diabetic patients poorly controlled with metformin. J Pineal Res 2006;41:189-93.
147. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med 2001;344:1688-700.