Lymphocyte DNA damage precedes DNA repair or cell death after orthopaedic surgery under general anaesthesia

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Anaesthetics have gained a lot of attention for their potential mutagenic/carcinogenic effects. In the present study we have investigated the genotoxicity of the inhalation anaesthetic sevoflurane on DNA of lymphocytes isolated from 20 patients undergoing orthopaedic surgery. The genotoxicity of the anaesthetic was studied by assaying DNA damage, apoptosis, DNA repair enzyme activity and GSH content in peripheral lymphocytes before, 15 min after anaesthesia and 24 h after surgery. Lymphocytes isolated 15 min after anaesthesia showed an increase in oxidized purine and pyrimidine bases without DNA strand break formation. DNA strand breaks occurred on the first post-operative day, associated with an enhancement of DNA repair activity and a decrease in GSH. Formation of strand breaks could be the consequence of DNA repair activity. In fact, at 24 h after surgery most of the oxidized DNA bases were repaired. When DNA damage was not repaired, activation of the cell cycle checkpoint protein p53 could lead to apoptosis. An altered redox status may contribute to lymphocytopenia due to an apoptotic event as a consequence of surgical trauma. The presence of apoptotic cells at 1 day after surgery could support the hypothesis that highly damaged peripheral lymphocytes are committed to undergo programmed cell death if the damage is not repaired. In conclusion, the actual risk from anaesthesia is presumably extremely small. However, these findings contribute to our understanding of the regulation of DNA damage/repair and cell death.

Introduction

DNA is continuously exposed to a variety of biological, chemical and physical factors which may alter its structure, leading to mutations and, consequently, modifying its function in vivo (Halliwell, 1994). Among the exogenous compounds, anaesthetic gases, commonly used in general anaesthesia procedures, have attracted a lot of attention recently for their potential mutagenic/genotoxic effects. Experimental and epidemiological studies suggest that genotoxic effects can arise from inhalation anaesthetics (Baden and Simmon, 1980; Sardas et al., 1992).

In fact, genetic damage has been observed in operating room personnel exposed to trace concentrations of anaesthetic gases (Sardas et al., 1998a). Among anaethetics, sevoflurane (SVF) is a potent new inhalation anaesthetic agent widely used in clinical practice. Though the potential mutagenicity of SVF has not been fully understood, in vitro studies carried out on SVF and its parent molecules halothane and isoflurane (ISF) demonstrated that these anaesthetics were able to induce significant DNA damage (Sardas et al., 1998b; Jaloszynski et al., 1999; Karabiyik et al., 2001). The mechanism by which polyfluorinated anaesthetics induce DNA damage remains unclear. If halothane or ISF react directly with DNA, the most feasible alkali-labile modification may be alkylation at the N-7 position of purines. Another explanation is that inhalation anaesthetics undergo a residual metabolic oxidation or reduction, giving rise to reactive products. Among known SVF metabolites, fluormethyl 2,2-difluoro-1-(trifluoromethyl) vinyl ether is an alkylating agent (Eger et al., 1997; Yamakage et al., 2000). Radical-mediated reactions may also be involved in DNA damage induction. In fact, it is known that free radical species are capable of directly attacking DNA. Reactive oxygen species (ROS) induce a variety of lesions in DNA, including abasic (AP) sites, DNA strand breaks and oxidized bases (Epe, 1995). Among guanine modifications, 7,8-dihydro-8-oxoguanine (8-oxoGua) is well known, and the lesion has a distinct mutagenic potential, giving rise to GC→TA transversion (Cheng et al., 1992; Moriya, 1993). Such a modification is frequently detected in the RAS oncogene (Bos, 1988) and in the TP53 tumour suppressor, which represents a possible mechanism of tumour initiation by ROS (Takahashi et al., 1989). Since oxidative DNA damage is subject to a specific repair, the steady-state levels in cellular DNA reflect the balance between generation and removal of the lesions (Breimer, 1991; Collins and Horvathova, 2001). Lesion generation is modulated by cellular antioxidant systems implicated in the prevention of DNA oxidation, counteracting and neutralizing reactive radicals (Collins, 1999; Tomasetti et al., 2001). Among antioxidant systems, glutathione is found in cells mainly in its reduced form in concentrations that vary between 1 and 5 mM (Deneke and Fanburg, 1989). It acts as a scavenger of free radicals and regulates the redox status of many other cellular substances, thus playing an essential role as a reducing cofactor for detoxification of lipid and organic peroxides (Shi et al., 1994). In addition, a decrease in GSH level and an ensuing increase in ROS during the apoptotic process have been reported by several groups, suggesting that the level of GSH also reflects changes in the cell function and environment (Buttke and Sandstorm, 1994; Kondala and Chandrima, 2000). Despite the presence of antioxidants capable of scavenging endogenous and exogenous free radicals, DNA damage still occurs, although cellular repair processes deal with most of the damage (Loft and Poulsen, 1999).
1996). Unrepaired, modified bases, if present during DNA replication, may result in misincorporation and thus could be an important source of mutations in somatic cells (Dreher and Junod, 1996).

In order to evaluate the genotoxic effect of general anaesthesia maintained with SVF, we studied DNA single-strand break (SSB) formation and purine and pyrimidine oxidation in lymphocytes of subjects before/after general anaesthesia and 1 day after surgery.

Oxidative DNA damage was assessed using a modification of the single cell gel electrophoresis (SCGE or Comet) assay, a very sensitive method for the evaluation of DNA damage in individual cells (Collins et al., 1993; Angelis et al., 1999).

To determine whether DNA oxidative damage is repaired or followed by cell death, we assessed both the ability of lymphocytes to repair DNA damage and apoptosis.

To evaluate whether GSH plays a role in preventing or modulating oxidative DNA damage, we assessed GSH levels in both plasma and lymphocytes of the subjects.

Materials and methods

Patients

The study was performed in accordance with the Helsinki declaration and was approved by the local medical ethical committee. We enrolled 20 patients (11 male, 9 female, 18–50 years of age), scheduled for hip arthroplasty. All the patients provided written informed consent at a pre-operative visit. Enrolled patients underwent general anaesthesia met either the Class I (including normal healthy patients, no disease other than surgical pathology, no systemic disturbances) or Class II (patients with mild systemic disease, systemic disturbances due to surgical condition) criteria of classification of the American Society of Anesthesiologists, and were chosen among non-smokers to limit confounding factors. During the surgery (120–180 min), electrocardiogram, blood pressure (systolic, diastolic and mean), heart rate and peripheral oxygen saturation (SpO2) were monitored.

General anaesthesia procedure

Propofol (2 mg/kg) and atracurium (0.5 mg/kg) were administered i.v. to induce general anaesthesia, which was maintained with 2% SFV in O2/H2O in the ratio 1:1. The duration of SFV administration ranged from 120 to 160 min. N2O was not used for any patient in order to avoid possible additional DNA damage.

Reagents

NADPH, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), yeast glutathione reductase, low and high melting point agarose and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI), annexin V–FITC and propidium iodide were purchased from Sigma Chemical Co. (St Louis, MO). Ficoll-Paque research grade medium was obtained from Amersham Pharmacia Biotech AB (Uppsala, Sweden). Endonuclease III (endo III) and formamidopyrimidine glycosylase (Fpg protein) were generous gifts from Andrew R. Collins (Rowett Research Institute, Aberdeen, UK). (R)-1-[(10-chloro-4-oxo-3-phenyl-4H-benzo[a]quinolin-1-yl)carbonyl]-2-pyrolidinemethanol (Ro 19-8022) was a generous gift from Hoffmann-La Roche (Basel, Switzerland). RPMI 1640 medium and foetal calf serum (FCS) were purchased from ICM Biomedical (Irvine, UK).

Sampling and lymphocyte isolation

Whole blood (10 ml) was collected into EDTA tubes before inducing general anaesthesia at 15 min after anaesthesia induction (t1) and 24 h after surgery (t2). Blood samples, collected at the t0, t1 and t2 time points, were immediately centrifuged at 3000 g for 15 min and the plasma obtained stored at −80°C for the analysis of GSH. Buffy coat was removed, placed in a 15 ml Falcon tube and suspended in 4 ml of RPMI 1640 medium. The suspension was then layered onto 4 ml of Ficoll–Plaque medium and centrifuged at 1000 g (20°C, 30 min). After centrifugation, the cloudy layer was collected and placed in a 15 ml Falcon tube, filled with phosphate-buffered saline (PBS), pH 7.4, and centrifuged at 1000 g (20°C, 15 min). After removing the supernatant, the pellet was re-suspended in RPMI 1640 medium supplemented with 10% FCS, counted and the cells assayed for viability by using trypan blue exclusion method. Aliquots of 5.0 × 106 cells were assessed for their total GSH cellular content, 5.0 × 106 cells were assayed for DNA repair enzyme activity, 0.5 × 106 cells for annexin V–FITC assay and 2.0 × 106 cells were suspended in freezing medium (FCS plus 10% DMSO) and stored at −80°C until further use. For the evaluation of DNA oxidative damage and of late apoptosis, human lymphocytes were defrosted, washed with 20 vol PBS, pH 7.4, counted and assayed for viability before starting the Comet assay.

Alkaline Comet assay

DNA breaks and oxidized purine and pyrimidine bases were measured using a modification of the Comet assay described elsewhere (Collins et al., 1993). Briefly, lymphocytes were embedded in agarose on a microscope slide, lysed with Triton X-100 and 2.5 M NaCl to produce nucleoids and treated with 0.3 M NaOH/1 mM EDTA before electrophoresis in this solution. During electrophoresis, DNA moves towards the anode, and this is more significant if breaks are present (as superrichs in the tightly packed nucleoids are relaxed under this condition). The relative amount of DNA appearing in the tail of the comet (visualized by fluorescence microscopy after staining with DAPI) is linearly related to DNA break frequency. The assay can be used to detect oxidized bases in addition to DNA breaks, by including an extra step, in which nucleoids in the gel are digested with a repair endonuclease specific for oxidized pyrimidines (endo III) or recognizing altered purines, including 8-oxoGua (Fpg protein). DNA breaks, with or without enzymatic treatment, were estimated as arbitrary units (au). Oxidized purine and pyrimidine bases were calculated by subtracting the value without enzyme incubation (i.e. DNA strand breaks) from the value with enzyme incubation. The extent of DNA migration was evaluated by visual scoring by an independent observer. Comets were classified and assigned to five categories (0–4) according to the extent of DNA migration. The classification was carried out on the basis of the comet appearance (i.e. tail length, head diameter and intensity) as described elsewhere (Collins et al., 1993; Angelis et al., 1999). The number of comets counted on each slide was 100. Each sample was analysed in duplicate and the value of oxidative damage was expressed in au (range 0–400).

DNA repair enzyme activity

The activity of DNA repair enzymes was assessed in cellular extracts of lymphocytes obtained from each donor using a modification of the Comet assay (Collins et al., 2001). The Comet assay has been modified to measure the ability of a simple subcellular extract of lymphocytes to carry out the initial step of repair, i.e. incision on a DNA substrate carrying specific lesions (oxidized bases) introduced by visible light in the presence of a photosensitizer. The method consisted of incubation of a cellular extract with a DNA substrate containing specific DNA damage. The enzyme repair activity was evaluated as the capacity of human lymphocyte extract to repair the oxidized bases introducing DNA breaks detected by the Comet assay. Lymphocytes (5.0 × 10⁶) isolated from venous blood of individual subjects before anaesthesia, after 15 min of induction and at 24 h after surgery were washed in 3× diluted extraction buffer A (45 mM HEPES, 0.4 M KCl, 1.0 mM EDTA, 1 mM DTT and 10% v/v glycerol adjusted to pH 7.8 with KOH) and centrifuged (700 g, 5 min, 4°C). The supernatant was removed and the pellet resuspended with buffer A to 10⁶ cells/ml. The suspended cells, divided into 50 μl aliquots, were frozen in liquid nitrogen and stored at −80°C until use. Prior to assay, 50 μl of cell suspension was supplemented with 12 μl of buffer A containing 1% Triton X-100 and the cell lysate was centrifuged at 14 000 g for 5 min at 4°C to remove nuclei and cell debris. The supernatant was diluted with 4 vol of reaction buffer B containing 2.5 mM ATP, 10 μg/ml creatine phosphokinase, 0.18 mM GTP, 18 mM creatine phosphophatase, in HEPES buffer supplemented with 0.25 mM EDTA, 2% glycerol and 0.3 mg/ml BSA, pH 7.8, and kept on ice until use. The substrate was prepared from fresh lymphocytes obtained from volunteers. Lymphocytes isolated from venous blood by standard centrifugation on a density gradient were washed twice with ice-cold PBSG (PBS plus 0.1% glucose) and incubated in a 60 mm dish with 100 nM Ro 19-8022 photosensitizer (Hoffman-La Roche) in 2 ml of PBSG. The cells were irradiated for 2 min on ice at 330 nm from a 1000 W tungsten halogen lamp. The cells were washed twice with PBSG and divided into aliquots of 4.0 × 10⁴ and centrifuged for 3 min at 200 g at 4°C. The cell pellet was suspended in 160 μl of low melting point agarose at 40°C and two aliquots of 80 μl were placed on a microscope slide pre-coated with normal electrophoresis grade agarose in a 1% solution. Glass coverslips were placed on the gel, which were left to set at 4°C and then placed in lysis solution (2.5 M NaCl, 0.1 M Na2EDTA, 10 mM Tris made pH 10 with NaOH, and 1% Triton X-100) for 1 h at 4°C. The slides were immersed in three changes of buffer B and 50 μl of cell extract was added to each gel, which was covered with a coverslip and incubated for 20 min at 37°C in a humid chamber. Slides were then placed in an electrophoresis tank and immersed in 0.3 M NaOH, 1 mM Na2EDTA for 40 min before electrophoresis at 25 V for 30 min. After neutralization with 0.4 M Tris–HCl, pH 7.5, comets were stained with DAPI and examined by fluorescence microscopy and analysed as described above. Slides incubated with 50 μl of buffer B or with 50 μl of Fpg protein were used as negative and positive controls, respectively.

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Apoptosis detection

Annexin V–FITC assay. Early apoptosis was quantified using the annexin V–FITC method which detects the phosphatidylserine externalized in the early phases of apoptosis. Briefly, $0.5 \times 10^6$ cells were suspended in $0.5 \text{ ml}$ of binding buffer (10 mM HEPES, 140 mM NaCl, 5 mM CaCl$_2$, pH 7.4), incubated for 20 min at room temperature with $2 \mu l$ annexin V–FITC, supplemented with $10 \mu l$ of propidium iodide ($10 \mu g/\text{ml}$) and finally analysed by flow cytometry (FACS; Coulter).

Comet assay. Late apoptosis was detected as DNA fragmentation using the neutral Comet assay (Reiss and Rutz, 1999). Cells were sandwiched between thin layers of agarose on a microscope slide, lysed at neutral pH, electrophoresed and stained with silver stain. The neutral Comet assay distinguishes apoptotic from non-apoptotic cells on the basis of their characteristic signature or DNA fragmentation pattern (Olive et al., 1993). Briefly, cells ($1.0 \times 10^6$) were suspended in $160 \mu l$ of low melting point agarose (1% solution in PBS) at $40^\circ C$ and two aliquots of $80 \mu l$ were placed on a microscope slide pre-coated with normal electrophoresis grade agarose in a 1% water solution. Glass coverslips were placed on the gels, which were left to set at $4^\circ C$ and than placed in lysis solution (1 M lithium chloride, 1 M urea, 10 mM EDTA, 50 mM Tris base, 2% SDS, pH 7.3) containing $60 \mu g/\text{ml}$ proteinase K and incubated at $50^\circ C$ for 1 h. After lysis, the slides were washed twice for 20 min in TAE running buffer (0.04 M Tris–acetate, 0.01 M EDTA, 0.02 M glacial acetic acid), placed in an electrophoresis tank and immersed in TAE running buffer for electrophoresis at $22 \text{ V}$ for 5 min. Following electrophoresis, the slides were fixed in absolute ethanol and then dried overnight. Comets were visualized by the silver stain method (Reiss and Rutz, 1999). Apoptotic cells form structures with large fan-like tails and small heads, whereas normal or less critically damaged cells form smaller structures with large heads and narrow tails of varying lengths.

Determination of glutathione

The concentration of total glutathione was assessed using the enzymatic recycling assay as described by Tietze (1969). Briefly, the cell pellets or $10 \mu l$ of blood were suspended in ice-cold cell lysis buffer (10 mM Na$_2$HPO$_4$, 5 mM EDTA, 0.1% Triton X-100, pH 7.5). The samples were stabilized by addition of 10% sulphosalicylic acid. After centrifugation at 13 000 $g$ for 15 min, the supernatant was analysed for GSH. For analysis, 25 $\mu l$ of cell lysate or diluted blood was added to 300 $\mu l$ of 0.2 mM NADPH, 0.6 mM DTNB and 0.1 U/ml glutathione reductase in 10 mM sodium phosphate buffer with 5 mM EDTA (pH 7.5). Absorbance at 405 nm was recorded at 1 min intervals for 6 min using an automated Cobas Fara centrifugal analyser (Beckman).

Statistical analysis

Data are shown as means $\pm$ SD. For the Comet assay, two slides were prepared for each sample. The comet scores of individual subjects, were analysed by the Kruskal–Wallis test to assess the homogeneity among individual slides. Differences in the extent of DNA damage between the groups examined were analysed using the Kolmogorov–Smirnov test. A repeated measures ANOVA test was performed to compare glutathione concentration before and after anaesthesia. The correlation analysis was evaluated by the Pearson $r$ coefficient and values with $P < 0.05$ were considered statistically significant.

Results

Inhalation anaesthesia induces DNA oxidative damage

The alkaline Comet assay was applied to quantify the number of modifications sensitive to Fpg protein and endo III, as well as DNA SSBs. The analysis of lymphocytes showed that general anaesthesia induced base modifications recognized by Fpg protein and endo III within 15 min ($t_1$), whereas no formation of DNA strand breaks occurred (Figure 1). However, SSBs showed a subsequent increase, reaching their highest...
value at 24 h after surgery ($t_2$). At 24 h we observed a decrease in oxidized pyrimidine and purine levels compared with those seen after 15 min of induction; the levels at 24 h were not different from the levels before anaesthesia. The flow cytometer analysis showed that the first signs of apoptosis occurred 24 h after surgery, whereas no DNA fragmentation (late apoptotic cells) was observed (Figure 2A and B).

**General anaesthesia affects DNA repair activity**

Lymphocyte extracts collected at the $t_0$, $t_1$ and $t_2$ time points were incubated with a substrate consisting of DNA with oxidized purine bases obtained from lymphocytes previously exposed to Ro 19-8022 plus light. Upon irradiation Ro 19-8022 produces singlet oxygen, which induces purine modifications, i.e. 8-oxoGua, in high excess over SSBs. Oxidized purines are the specific substrate of Fpg protein, which introduces breaks at sites of oxidized DNA bases and the number of DNA incisions reflects the enzyme activity. As shown in Figure 3, DNA repair activity increased at 24 h after surgery ($26 \pm 8$ versus $44 \pm 14$ au, $P < 0.05$). Interestingly, a positive correlation was observed between DNA repair activity and DNA SSB formation ($r = 0.84$, $P < 0.05$; Figure 4A), measured at the $t_0$, $t_1$ and $t_2$ time points. Conversely, an inverse correlation was observed between oxidized purine and pyrimidine bases and the relative DNA repair activity ($r = 0.65$ and $0.55$, $P < 0.05$, respectively; Figure 4B and C).

**General anaesthesia causes a decrease in blood and intracellular glutathione**

Oxidative DNA damage may derive from an imbalance between antioxidants and pro-oxidants, in which the equilibrium is shifted in favour of oxidizing agents. Glutathione is a tripeptide whose antioxidant activity is well recognized in different biological systems, and its level may reflect the cellular oxidative status. Several studies indicate that changes in blood GSH may provide a measure of oxidative stress in vivo. Therefore, we tested the concentration of glutathione in both blood and in lymphocytes. A decrease in GSH level was observed in blood and in lymphocytes of patients, reaching the lowest concentration 24 h after anaesthesia ($t_2$), $(1.52 \pm 0.40$ versus $0.81 \pm 0.56$ µM, $P < 0.05$, and $29.0 \pm 5.7$ versus $22.0 \pm 5.1$ pmol/10⁶ cells, $P < 0.05$, respectively; Figure 5).

**Fig. 3.** DNA repair activity of lymphocyte extract before anaesthesia ($t_0$), 15 min after induction ($t_1$) and 24 h after surgery ($t_2$). Data were obtained from 10 subjects and are expressed as means ± SD. The asterisk denotes statistical difference in values at the $t_0$ versus $t_2$ time points at $P < 0.05$.

**Fig. 4.** Correlation between DNA repair activity and single-strand breaks (A) or oxidized purine (B) or pyrimidine (C) bases. The correlation is calculated by including data for DNA repair and DNA damage collected before anaesthesia ($t_0$, open circles), 15 min after induction ($t_1$, solid triangles) and 24 h after surgery ($t_2$, solid circles) from 10 subjects ($n = 30$).

**Fig. 5.** GSH content in blood and in lymphocytes isolated from subjects before anaesthesia ($t_0$), 15 min after induction ($t_1$) and 24 h after surgery ($t_2$). Results are expressed as means ± SD of values obtained from 20 subjects. The asterisk denotes statistical difference in values before anaesthesia ($t_0$) versus those 24 h after its cessation ($t_2$) at $P < 0.05$. 

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Discussion

The possibility of a health hazard resulting from exposure to anaesthetic agents has been discussed extensively during the last decade. Many of the published cytogenetic data on the effects of anaesthetic gases on humans concern chromosome aberrations and sister chromatid exchange (Sardas et al., 1992). Among inhalation gases, SVF is widely used in anaesthetic practice, although it was not used until 1993 due to questions regarding its metabolism to inorganic fluoride and the extent of degradation of SVF in the presence of CO₂ absorbents (Fink et al., 1992). It is known that SVF undergoes dehydrofluorination by soda lime and barium hydroxide lime in the anaesthesia apparatus and that this leads to the formation of small quantities of several degradation products such as fluoromethyl 2,2-difluoro-1-(trifluoromethyl) vinyl ether (compound A) (Eger et al., 1997; Yamakage et al., 2000). No clinical data directly suggest that SVF or compound A is linked to mutagenicity or carcinogenicity. However, the findings of Eger et al. (1997) demonstrated that the degradation product vinyl ether is an alkylating agent producing an increased frequency of sister chromatid exchanges in exposed Chinese hamster ovary cells. Several studies were carried out on genetic damage (White et al., 1979; Husum et al., 1984; Eger et al., 1997), whereas few data are available about the capacity of anaesthetic gases to generate oxidative DNA damage. The formation of DNA SSBs was observed by Karabiyik et al. (2001) in lymphocytes of patients that underwent general anaesthesia induced by SVF and ISF after 2 h induction. The observed high levels of DNA oxidative damage began to decrease in the post-operative period, reaching normal values on the third post-operative day. The authors attributed the decrease in DNA damage to DNA repair activity of the cells. However, it should be noted that the restorative processes observed in lymphocytes during post-operative days could be determined by other mechanisms that do not involve DNA repair, such as cell turnover, that would reduce the number of breaks. Inhalation anaesthetics are known to affect the immune system and to cause peripheral lymphocytopenia in association with surgical stress (Rem et al., 1980; Oka et al., 1996). Several studies reported that both SVF and ISF induce apoptosis in human peripheral lymphocytes in a dose- and time-dependent manner in vitro (Matsuoka et al., 2001) and that apoptosis is strictly associated with over-production of ROS (Delogu et al., 2001). Moreover, it has been reported that the rate of cellular apoptosis is strongly enhanced during the early post-operative period among lymphocytes isolated from patients undergoing elective surgery under general anaesthesia (Delogu et al., 2001). However, it is difficult to assess in vivo whether inhalation anaesthetics or surgical stress are the main cause of the lymphocyte damage and cell apoptosis. To evaluate whether DNA damage was associated with anaesthesia and not with surgery, we detected DNA SSBs and oxidized purine and pyrimidine bases in lymphocytes after 15 min anaesthesia induction before surgery. Despite the fact that general anaesthesia was induced using propofol, whose antioxidant activity has been reported by different authors (Allauchiche et al., 2001; Tsuchiya et al., 2001, 2002), we observed oxidation of purine and pyrimidine bases recognized by the Fpg protein and endo III, which was observed as early as 15 min after induction. Accumulation of oxidized bases has been implicated in a number of degenerative diseases and has also been considered a pathogenic event in cancer (Moriya, 1993; Collins, 1999). Whereas some oxidized bases can be removed quickly by excision, others are removed slowly, probably due to different activities of repair enzymes. The damage to purine and pyrimidine bases was completely removed at 24 h after surgery and the low number of oxidized bases observed was associated with enhanced DNA repair activity measured directly on lymphocyte extracts. DNA SSBs were not observed after anaesthesia induction but high numbers of DNA breaks appear on the first post-operative day. A positive correlation between DNA SSB formation and DNA repair activity was observed. The repair of oxidized bases by endonucleases occurs in two steps: first, removal of the oxidized bases as free bases, second, cleavage of the phosphodiester bonds 3’ and 5’ of the resulting AP site (Janssen et al., 1993). Since the alkaline Comet assay detects strand breaks as well as alkali-labile sites, including abasic sites, we can hypothesize that the high level of DNA breaks might reflect the high repair activity with AP intermediate formation. Furthermore, we point out that the inverse relationship between repair and oxidized bases supports the hypothesis that there is a steady-state level of oxidized bases determined by the rate of repair, so that the higher the repair rate the lower the level of base damage, a logical but not inevitable relationship.

However, the alkaline Comet assay cannot distinguish between dead cells and cells with heavily damaged DNA but still able to recover (Henderson et al., 1998). Thus, we used the annexin V–FITC and neutral Comet assays to detect apoptotic and non-apoptotic cells on the basis of their characteristic signature or DNA fragmentation patterns. Early events of apoptosis were detected 24 h after surgery, although we did not observe genomic DNA fragmentation, which is associated with late apoptosis. The formation of DNA SSBs on the first post-operative day could be related to an early phase of cell death. It is clear that surgical stress is associated with a greatly enhanced burst of oxidative activity of neutrophils which, during the anti-inflammatory response, leads to further production of ROS (Simms and D’Amico, 1997). The latter are probably the key factors responsible for the accelerated apoptosis seen in lymphocytopenia. There is evidence that cells committed to undergo apoptosis exhibit an altered oxidation–reduction status. Hence, the plasma levels of oxidized substances have been considered to be reliable markers of the severity of the oxidative stress associated with surgical trauma (Cono et al., 1995; Lases et al., 2000).

The observed decrease in GSH in both blood and lymphocytes was significant on the first post-operative day, suggesting that the normal balance between pro-oxidant and antioxidant substances was transiently altered in favour of the former as a result of surgical trauma, rather than anaesthesia induction. In conclusion, general anaesthesia causes oxidation of purine and pyrimidine bases but the DNA damage is removed in the presence of an efficient repair system. The high number of DNA strand breaks observed on the first post-operative day could reflect the high rate of DNA repair. Surgical trauma induces oxidative stress, as observed by a decrease in GSH level in both lymphocytes and blood 24 h after surgery. An unbalanced cellular redox state is associated with a transient increase in apoptosis. Thus, we can hypothesize that highly damaged peripheral lymphocytes are committed to undergo programmed cell death if the damage is not repaired.
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References


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