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The effect of surgery and anesthetic agents on granulocyte-chemiluminescence in whole blood

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Summary. The effect of anesthesia and major abdominal surgery on zymosan-induced chemiluminescence (CL) of neutrophil granulocytes was evaluated. CL was measured in diluted whole blood taken at distinct intervals within the perioperative period. In addition, blood samples from healthy volunteers were supplemented with ether and halothane to investigate the in vitro effect of these agents. The phagocytosis-induced CL was not found to be depressed by anesthesia and surgery. Only at supranarcotic concentrations was CL reduced. Surgery and anesthesia, therefore, do not appear to impair this defense system significantly under the conditions of this investigation.
Key words. Chemiluminescence; anesthesia; surgery, abdominal; granulocytes; perioperative period; immunological resistance.

Introduction

The question of impaired immunological resistance following general anesthesia and surgery, and its biological significance, has been raised frequently. To date, the influence of anesthesia and surgery on the humoral-phagocyte defense system is still a subject of controversy. The phagocytosis-induced chemiluminescence (CL) of polymorphonuclear leukocytes (PMN) in whole blood depends on both the humoral and the cellular components of the phagocyte defence system[1,2]: Opsonization of particulate matter or activation of the complement cascade (humoral factors) can lead to the activation of the PMN-membrane (cellular factors). This in turn results in the production of activated oxygen species (O_2^-, $\cdot OH$, $^1O_2$, H_2O_2) which mediate the PMN-killing function and the generation of CL. Although the biological significance of CL is not yet understood it can be used to measure these functionally important oxygen products of PMN-activation. CL measurements in whole blood samples make it possible to follow the activity of the humoral-phagocyte system under nearly physiological conditions, because they reflect the in vivo interaction of cellular and humoral factors.

In the present study, the influence of general anesthesia and limited surgical stress on the humoral-phagocyte defense system was investigated by phagocytosis-induced CL-measurements. CL in diluted whole blood samples was determined before and several times during and after surgery. In addition to that, CL was measured in whole blood samples from healthy volunteers, equilibrated in vitro with volatile anesthetic agents (halothane, ether).

Methods

Chemiluminescence-assay. The reaction mixture contained in a final volume of 0.5 ml heparinized whole blood (0.1 ml), Dulbecco's modified Eagle's medium (0.4 ml), luminol (20 µg, 0.23 mM) and zymosan (500 µg). CL was measured at 37 °C in a photon counter (Biolumat, model LB 9700 or 6-channel-Biolumat LB 9505, Berthold, Wildbad, FRG) over a 30-min period after the initiation of the reaction by non-preopsonized zymosan. The mean activity during this period expressed as counts per minute (cpm) was related to both units of whole blood (whole blood (WB) activity: cpm/µl WB) and to the number of polymorphonuclear granulocytes (PMN) in the system (specific activity: cpm/1000 PMN). Details of chemicals, media and procedure were as published previously[2].

In vitro experiments. For ether and halothane experiments, the usual polystyrene vials were replaced by inert glass vials which were dark-adapted 2 h prior to the experiment.

For the ether experiments, six samples were simultaneously supplemented with varying concentrations of liquid ether to give final concentrations from 0 to 5 vol% ether. The calculations were based on the following figures: Minimal alveolar concentrations for anesthesia, MAC, 1.92 vol%, lambda blood/gas 12.0 and 1 ml liquid ether substituting for 233 ml gaseous ether[3]. Following incubation of the diluted and ether-supplemented sample for 10 min at 37 °C, phagocytosis was initiated by the addition of zymosan and the reactions were simultaneously followed by the 6-channel photon counter.

For the halothane experiments, two single-channel photon counters were used. The reference counter was left under room conditions, whereas the second counter was placed in a closed box with three lines; the first line to fill the box with a defined halothane-oxygen mixture, the second to measure the halothane concentration (EMMA Engström Analyzer, Engström, Bromma, Stockholm) and the third as an overflow. After equilibration of the box and the photoncounter with 1 and 3 vol% halothane in 100% oxygen for 30 min, the diluted blood sample was introduced and incubated for 30 min. The reaction was then initiated simultaneously in both the reference and the halothane-incubated photoncounters by the addition of zymosan. The CL measured in the reference counter was referred to as the 100% standard.

CL measurements during anesthesia and surgery. In 10 surgical patients blood samples taken at the following points of time (1–6) were immediately processed according to the standard CL-assay:

1) 1 h prior to surgery, 2) 10 min after induction of anesthesia, 3) 20 min after induction of anesthesia, 4) 1 h after skin incision, 5) 1 h after the end of the operation, and 6) 24 h after the operation.

At each point of time, measurements of CL, leukocyte and differential white blood cell counts were performed (Coulter Super S, Wright's stain). CL was expressed as whole blood activity and specific activity.

To summarize the results for the 10 individuals, each initial CL-value (point of time 1) was taken as 100% and the following values were expressed relative to the 100% reference. The results were summed and expressed as mean and standard deviation. The statistical tests used are indicated in the text.

Results

In vitro effect of halothane and ether. Incubation of whole blood with 1 vol% halothane did not reduce the CL response to zymosan. At 3 vol% CL was significantly diminished (78+21% of the respective controls; χ+SD; $n = 8$; Wilcoxon-Mann-Whitney U-test: $p < 0.05$).

For ether, a significant inverse dose-response relationship between the concentration applied and the resultant CL response to zymosan was found (fig. 1). 25% inhibition was observed at approximately 2 vol% ether, 50% inhibition at about 5 vol% ether.

Effect of general anesthesia and surgical stress on CL in whole blood. The diagnoses, operative procedures and type of anesthesia of the 10 patients examined are listed in the table. Two representative examples of the perioperative CL course are shown in figures 2 and 3: One

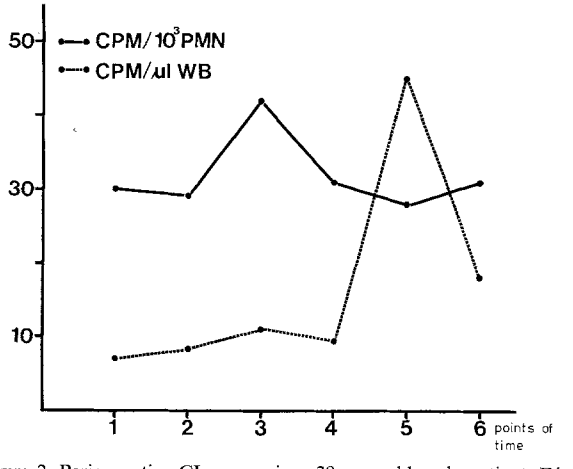

Figure 2. Perioperative CL course in a 39-year old male patient. Diagnosis: Non-Hodgkin lymphoma. Operation: Staging-laparotomy, duration: 4 h. Anesthesia: Opiate: fentanyl. Sedative: flunitrazepam, thiopental. Inhalation anesthetics: N_2O/O_2, halothane (0.5–1%).

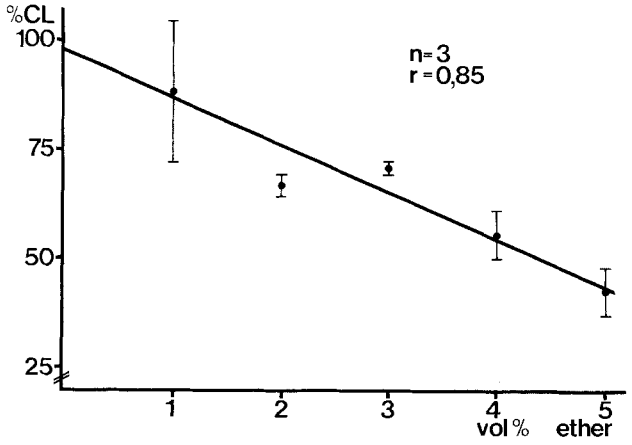

Figure 1. Effect of ether on zymosan-induced CL in vitro. Linear regression analysis.

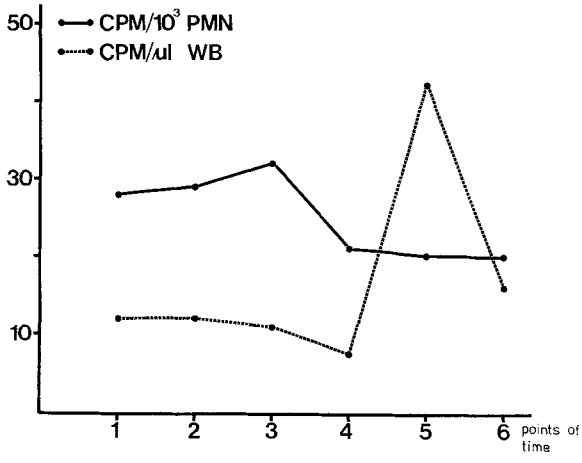

Figure 3. Perioperative CL course in a 48-year old male patient. Diagnosis: Ulcus duodeni. Operation: Proximal selective vagotomy. Anesthesia: Opiate: morphin. Sedative: flunitrazepam, thiopental. Inhalation anesthetics: N_2O/O_2.

curve represents a patient with malignant (fig. 2), the other with benign disease (fig. 3). As in these examples, no characteristic difference in the perioperative CL course of patients with benign and malignant disease was detected. Neither were there characteristics specific for a type of general anesthesia (halothane+N_2O cp., fig. 2; N_2O cp., fig. 3).

In summary, the specific CL activity (counts per 1000 PMN) was not found to be depressed at any time of the observation (fig. 4). On the contrary, the specific CL activity climbed to a statistically significant peak 20 min after induction of anesthesia (fig. 4, point of time 3). This point of time was immediately prior to the skin incision.

The whole blood activity (counts per µl of whole blood) remained constant during anesthesia and operation but reached a statistically significant peak 1 h after the operation. This reflected the trauma-induced leukocytosis.

Discussion

In this study general anesthesia and surgical stress were not found to depress the oxygen dependent granulocyte function as expressed by the zymosan induced and luminol amplified CL in 1:5 diluted whole blood. The in vitro experiments supported this result, since neither of the anesthetic agents examined (ether, halothane) impaired the granulocyte function at concentrations used for anesthetic purposes. Only at higher concentrations (3 vol% halothane, 2 vol% ether) did a suppressive effect on CL become clear.

One substantial difference between the in vitro and in vivo experiments was the presence of the volatile anesthetic agent: All samples taken during anesthesia were equilibrated against ambient air throughout the time of transport and processing, so that the anesthetic gases were eliminated at the time of the CL measurements. The in vivo experiments, therefore, could only detect long-acting changes induced by the anesthetic agents and the operation. On the contrary, in the in vitro experiments the volatile anesthetic agents were present in effective concentrations throughout incubation and CL measurements. Therefore, in these experiments it should be possible to detect also short lasting changes of granulocyte function, which are reversed immediately following wash-out of the anesthetic gas. As a result, no significant impairment of granulocyte function was observed either in the in vivo or in the in vitro experiments at gas concentrations within the clinical range.

In principal, these findings are in agreement with the literature: Investigating in vitro effects of volatile anesthetic agents on granulocyte function, Graham reported in 1911 that a significant suppression of opsonization and phagocytosis required an ether-concentration of 2 vol% or more, whereas the effects of 1 vol% were minimal[5]. Cullen[6] microscopically examined phagocytosis of latex beads and the concomitant reduction of nitroblue tetrazolium (NBT) by granulocytes exposed to halothane and nitrous oxide; under the conditions of the experiment (30 min incubation, halothane up to 2.5%, N_2O 80%) no significant effects were observed.

The data of Lippa[7], however, seem to disagree with these and our own results: If isolated granulocytes were stimulated in a serum-free medium by phagocytosis of latex particles both halothane and ethrane markedly reduced the granulocyte CL even at very small concentrations (e.g. CL 50% at 0.3 vol% halothane).

The assay used by Lippa[7] was different from that in our experiments, in which the granulocytes were activated in 1:5 diluted whole blood and phagocytosis was induced by zymosan. Contrary to latex, zymosan is known to activate the alternative complement pathway. Neutrophil activation in the whole blood system might

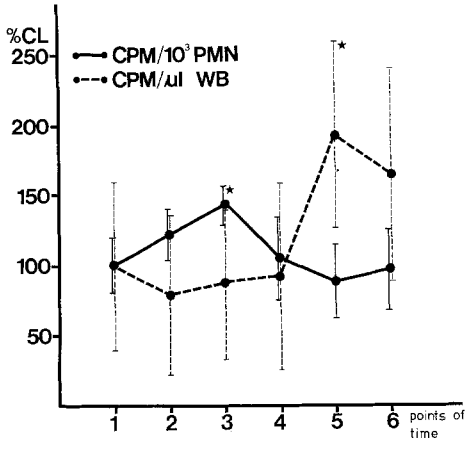

Figure 4. Summary of the perioperative CL course in 10 patients. The graph gives mean and SD at each point of time (1–6). Differences between the preoperative (point time 1) and the subsequent measurements (2–5) were analyzed using the wilcoxon matched pairs test. To reduce the incidence of type I errors consequent to multiple comparisons, the critical probability level was computed by the Bonferroni-Holm method, as the ratio of $a/k-(i-1)$, where a equals the error rate ($p < 0.05$), k equals the number of tests ($k = 5$) and i corresponds to the order of the test ($1 < i < 5$). * = $p < 0.05$.

Diagnoses, operative and anesthetic procedures in the ten patients examined for perioperative CL-activity

No.	Diagnosis	Operation	Sedatives	Opiates	Volatile anesthetics	Epidural anesthesia
1	Duodenal ulcer	Proximal gastric vagotomy	Flunitrazepam thiopental	Morphine	N_2O/O_2	–
2	Cholelithiasis, pancreatitis	Cholecystectomy	Thiopental althesin	Fentanyl	N_2O/O_2	+
3	Renal tpl.-rejection	Transplant-nephrectomy	–	–	N_2O/O_2 halothane	–
4	M. Crohn	Ileocoecal resection	Flunitrazepam thiopental	Fentanyl	N_2O/O_2	–
5	Pancreatic carcinoma	Cholecystojejunostomy	Thiopental	Fentanyl	N_2O/O_2	–
6	Pancreatic carcinoma	Exploratory laparotomy	Droperidol	Fentanyl	N_2O/O_2 ethrane	–
7	Hepatocellular carcinoma	Cholecystojejunostomy	Thiopental	Fentanyl	N_2O/O_2	–
8	Hodgkin lymphoma	Staging laparotomy	Flunitrazepam	Fentanyl	N_2O/O_2	+
9	Non-Hodgkin lymphoma	Staging laparotomy	Flunitrazepam thiopental	Fentanyl	N_2O/O_2 halothane	–
10	Colonic carcinoma	Hemicolectomy	Thiopental	Fentanyl	N_2O/O_2 ethrane	–

therefore be mediated by C3b-receptors and this mechanism could be less affected by anesthetic agents than the phagocytosis of nonopsonized matter used by Lippa.

Another potentially critical difference between the various methods is the presence or absence of serum: Lippa[7] described a long-lasting and profound decrease of latex-induced CL even after a short contact of low concentrations of the anesthetic agent (halothane, ethrane) with the isolated granulocytes in the absence of serum. Welch[8] found that luminol-dependent CL during exposure of isolated neutrophils to halothane in vitro was depressed, but only at concentrations of 2 or 3 vol%, whereas at 1 vol% halothane no change was observed. However, using 1:5 diluted whole blood as reported in this investigation, 3% halothane was the minimal concentration to effectively reduce the zymosan induced CL. This may suggest a protection of granulocyte function against the effects of anesthetic agents by serum factors. Under these conditions concentrations of anesthetics exceeding the clinical range were required to inhibit phagocytosis and CL.

Investigations of granulocytes obtained during anesthesia and surgery are difficult to interpret: By the time of measurement, volatile anesthetic agents will be washed out and this will only permit the detection of long-lasting changes. Moreover, primary effects of anesthesia and surgery will interfere with secondary metabolic and neurohormonal influences on the phagocytes. This multiplicity of interrelated factors might be responsible for differences in results:

Investigating the effect of surgery upon monocytes Everson[9] distinguishes between patients with benign and malignant disease: In patients with benign disease, phagocytosis of latex beads by monocytes was found to be stimulated 24 h postoperatively. This activity returned to normal another 24 h later. In patients with malignant disease there was no increase in phagocytosis either 24 nor 28 h postoperatively, and also 1 h postoperatively the rate of phagocytosis did not differ from the preoperative value.

Oladimeji et al.[10] investigated the influence of surgery on monocyte lysozyme production. They report elevated lysozyme production 6–48 h postoperatively. At 10 days postoperatively they again found normal values.

Neutrophil function during and immediately after surgical trauma was also investigated upon by Wandall and Binder[11]. In 10 patients with herniotomy they found a depressed chemotactic response, but unaltered phagocytosis and NBT-reduction, during anesthesia. 24 h after the operation, the rate of phagocytosis was increased ($p < 0.01$) and NBT-reduction was reduced (ns).

These results partially agree with findings of El-Mallem[12] reporting normal phagocytosis of Candida by neutrophils during a 10-day period after the operation. However, the microbicidal activity against the ingested Candida was significantly decreased, probably as a result of partial exhaustion of the neutrophils' myeloperoxidase (MPO) content. The latter result appears somewhat contradictory to our own finding of an unchanged level of luminol-CL during and after operation, because luminol-CL depends very much on the MPO content of the neurtrophil[13]. However, the 24% postoperative reduction in MPO content[12] could well be critical for the ability to kill Candida but not for the generation of luminol-CL.

A different pattern of response to the anesthesia was reported by Cullen[14]. They observed a slight but statistically significant decrease of the latex-phagocytosis induced NBT-reduction in a serum suspension of neutrophils obtained during anesthesia with halothane-N_2O. The blood samples were taken immediately prior to surgery, so that the effect should be attributable to anesthesia. As the authors claim to have excluded a direct effect of the anesthetic on the leukocytes in their in vitro investigations quoted above[6], nonspecific stress effects or the elevation of serum corticosteroid or catecholamine levels remain as speculative inhibiting factors of granulocyte function.

Correspondingly, Lippa[7] purified granulocytes during surgery and halothane-anesthesia (concentration 1%) but observed in seven patients a 59.9+18.2% inhibition of latex induced CL (mean ± SD). Again, this assay was carried out in a serum-free medium.

A third pattern of results has been described by Conroy[15] in patients undergoing cardiopulmonary bypass: Latex stimulated CL was measured in 1:10 diluted whole blood and in a suspension of isolated neutrophils before, during and immediately after surgery. Both neutrophil- and whole blood-CL (specific activity) were doubled during and after surgery compared to the respective preoperative values. There was, however, no significant correlation between neutrophil and whole blood-CL. In comparison to the literature quoted above these results may reflect the influence of operative stress (i.e. cardiopulmonary bypass vs abdominal surgery). Moreover, they emphasize the difference between CL of whole blood and of isolated leukocytes or neutrophils (lack of correlation between the two methods).

If CL measurements are performed to estimate the oxygen-mediated microbicidal capacity of granulocytes under the influence of anesthesia and surgery, the assay should reflect physiological conditions. CL of isolated cells can only detect the capacity of the cellular component. The cellular activity, however, may be significantly modified by the interaction of serum factors, cells other than neutrophils and the variables of the experiment (e.g. concentration of anesthetics, stress, etc.). This has been demonstrated by simultaneous measurements of CL of isolated leukocytes and of whole blood which failed to correlate[15]. The determination of granulocyte activity in whole blood is based on both humoral and cellular factors. Therefore, this activity appears to reflect best the in vivo activity of the granulocytes.

In summary: In whole blood samples from volunteers, equilibrated with volatile anesthetics at clinical concentrations as well as in the group of patients undergoing elective major general surgery the phagocytosis-induced granulocyte CL was not found to be depressed. CL seems to be reduced in these patients only at supranarcotical concentrations or in the absence of serum factors. Therefore, surgery and anesthesia do not seem to impair this important defense system in patients undergoing major general surgery.

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Short Communications

Proline accumulation as a reliable indicator of monocarpic senescence in rice cultivars[1]

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3 February 1984

Summary. Proline content proportionally increased with leaf age as well as during aging in darkness of excised leaf segments collected from the flag, second and third leaves of the Jaya cultivar of rice. BA significantly suppressed, whereas ABA augmented, the rise of proline level in the leaf segments. Proline also increased in the attached flag, second and third leaves of all the 4 rice cultivars with the progress of reproductive development but the pattern of its accumulation was non-sequential in Jaya and Ratna and sequential in Masuri and Kalojira. Although proline accumulation was retarded and enhanced by treatment with BA and ABA, respectively, as foliar sprays, the mode of proline accumulation (non-sequential or sequential) remained unaltered.
Key words. Oryza sativa; rice cultivar; proline accumulation; benzyladenine; abscisic acid; senescence, monocarpic.

Several workers[2–6] have reported the striking increase in proline content in water stressed plants. Recently, it has been demonstrated that proline also accumulates during senescence of excised rice leaves[7,8]. However, relatively little attention has been paid to the proline accumulation during senescence of intact attached leaves, particularly during monocarpic senescence.

It was reported earlier from this laboratory that there are varietal differences in the pattern of monocarpic leaf senescence in rice[9–11]. Thus, for example, the cultivars Jaya and Ratna exhibited a non-sequential mode of senescence where the young flag leaf senesced earlier than the old second leaf; but the cultivars Masuri and Kalojira manifested a sequential mode of senescence where the leaves senesced in a chronological sequence. The present communication describes the pattern of changes in proline content in aging excised leaf segments as well as in intact attached leaves of 4 rice cultivars during the progress of reproductive development. In addition, the effects of benzyladenine (BA) and abscisic acid (ABA), the inhibitor and promoter respectively of rice leaf senescence[12], on proline accumulation in both excised and intact attached leaves during senescence were also analyzed.

Seeds of 4 cultivars of rice (*Oryza sativa* L.) viz., Ratna, Jaya (both dwarf and photoperiod insensitive) Masuri and Kalojira (both tall and photoperiod sensitive) were collected from the Crop Research Farm of Burdwan University. 30-day-old seedlings raised from these seeds were transplanted with 1 seedling per hill at a spacing of 25 × 30 cm in 1-m^2 plots.

Experiments were carried out with excised leaf segments induced to age in the dark, and also with the 3 uppermost attached leaves of the 4 rice cultivars during the progress of reproductive development. In one set of experiments, the effects of benzyladenine (0.5×10^{-3} M) and abscisic acid (10^{-4} M) were examined on changes in the proline content in excised leaf segments collected from the flag and second and third leaves from the tops of 4 rice cultivars when they entered into the reproductive phase. Leaf segments weighing 1 g were placed in sterile petri dishes containing 30 ml of water, BA or ABA solu-