**Application of neutrophils chemiluminescence test in medical diagnostics**

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**Abstract**

Presented work contains basic informations describing „luminescence” of cells which due to the use of luminophores is called chemiluminescence (CL). In the first part of the paper the author reviews the literature on environment in which the luminescence of neutrophiles occurs and briefly describes redox reactions within neutrophils which are called „oxidative burst”. The second part of the paper reviews the literature on neutrophils’ CL in selected diseases. The article contains informations on the first researchers in the field of luminescence dating from 1961 – Tarusow et al. – through the group of Italians (Colli, Facchini) to contemporary scientists as R. Allen, P. Stevens and K. van Dyke. Their research on the cellular luminescence contributed to the practical use of chemiluminescence test in clinical diagnostics (mostly in chronic granulomatous disease). The author cites the observations of others and her own on the usefulness of neutrophils’ CL test in the prognosis of duration and the prediction of complications in the course of severe infections: bacterial, parasitic and AIDS. The paper contains data on neutrophils’ CL in selected neoplastic diseases and the diseases of the respiratory system (including bronchial asthma and sarcoidosis). Various reports on free oxygen radicals in the pathology of the urinary tract and circulatory system are cited, where neutrophiles CL test allows to observe the severity of the process and the effects of the treatment. The work contains also informations about the metabolic potency of neutrophils in the course of certain self-aggressive disorders of the liver and during some surgical procedures.

**Key words:** Chemiluminescence, oxygen metabolism, neutrophils.

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**Introduction**

The ability of lighting is a characteristic trait of alive organisms on the organ, tissue or cell level. It is often called as a ultra-weak luminescence or even “cold lighting” [1, 2]. This phenomenon could appear in vivo spontaneously. The described situation is in need of the energy coming from some biochemic reactions, which product very active compounds as oxygenic and lipids radicals. All these processes could be accompanied by the luminescence (the light quantum emission). This luminescence, intensified by the substances increasing the intensity of lighting – luminophores, is called chemiluminescence (CL).

To make the exist of chemiluminescence the following conditions have to be done:

- an activated molecule must have the energetic levels with possibilities of moving from higher to the lower one with proper efficiency,
- the energy should be left in $10^{-14}$-$10^{-11}$ second time, not to go away as a thermic energy [1-3].

The total efficiency of chemiluminescence in vivo is about $10^{14}$-$10^{10}$ photones per second.

There are some factors light reducing: inhibitors of chemical reactions, cleaners of radicals, stoppers of reactions.

There are the following traits characterising chemiluminescence:

- the light intensity – depends on the quantum efficiency and the constant reaction speed,
- the light amount – the number of photones, which emissioned while the time unit, counting per field unit,
The NADPH-oxidase catalyzes the oxygen ($O_2$) reduction to the supraperoxide anion ($O_2^-$):

$$\text{NADPH} + 2O_2 \rightarrow \text{NADP}^+ + O_2^- + H^+$$

The supraperoxide anion is subject to be dismutated into a hydrogen peroxide and into a singlet oxygen:

$$\text{peroxide dismutase}\quad 2O_2^- + 2H^+ \rightarrow H_2O_2 + 'O_2$$

The hydrogen peroxide, in the presence of ferrous ions, could be a substrate in Fenton reaction leading to hydroxylic radical' production:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + 'OH$$

In the presence of chloride anions and of myeloperoxidase (MPO), releasing from azurophil granules of neutrophiles, the hypochloric acid is produced:

$$\text{MPO} \quad H_2O_2 + Cl^- + H^+ \rightarrow HOCl + OH^-$$

In the reaction with amine groups of proteins, HOCL produces toxic and more constant chloramines, however with a hydrogen peroxide - singlet oxygen ($'O_2$):

$$HOCl + H_2O_2 \rightarrow O_2 + H_2O + Cl^- + H^+$$

It is characterised by an increased uptake of oxygen, an increased oxygen utilization in pentose-monophosphoric cycle working with an enzymatic system described as NADPH oxidase, which is helped by (MPO) myeloperoxidase and at the end the free oxygenic radicals are produced (RT): supraphydroxylic and hydroxylic radicals, supraperoxide aniono-radical, an singlet oxygen [12, 13] (Fig. 1). RT have a very strong physical and chemical power.

It is possible thanks to the one out-of-pair electron present on the external orbit, which binds with other different electrons into a chemical bond and emits a light quantum. This phenomenon is one of main ways of pathogens destruction by neutrophils and is called an oxygen-dependent killing way. The reactions lead to RT- producing with luminophores' help (luminol, lucygenina) [14] could be measured by different instruments for instance – scintillation counter β working on one photocopier, – Polon or a special constructed-luminometer. The RT are responsible for different destructions of cells, tissues or organs (Fig. 2) [15- 17].

The possibility of an intermediate estimation of the ability to RT-production using chemiluminescence, gave us many informations about granulocytes and their role, a role of RT in plenty diseases’ pathology, allowed us to find them, to observe their dynamic changes and treatment results [18, 19].

**Immunodeficiencies**

The neutrophils luminescence measurement was a real breakthrough in diagnostics of a Chronic Granulomatous Disease (CGD). The defect of catalazo-positive patogens killing is the source of the disease and might deal with the lack of one of NADPH oxidase subparts in a cell membrane or in plasma (or its abnormal activation) [20]. There is no spontaneous or stimulated chemiluminescence (CL) in patients with chromososomeX-linked CGD. The decreased chemiluminescence (CL) is observed in mothers of CGD children, what could exists as its marker. The CGD diagnosis might be done in pregnancy. Huu et al [21] used prenatal diagnostics in children with CGD-trait inheritance, during twentieth week of pregnancy and also showed very high sensitivity and specificity of that method.

Stevens et al. [22] as first, have used the methods of the luminol-dependent chemiluminescence to discover MPO deficit. They showed that CL in patients with MPO deficit was about 50% decreased than in healthy people. The use of stimulators: f-MLP (formyl-methionyl-leucyl-phenylalanine) or Zymosan (polypeptide produced from yeast) showed a higher CL than in healthy people. The situation was controversial indeed, especially there were no changes in a luminol-dependent CL of granulocytes after the MPO-inhibitors use.

The induction of an acid arachidic metabolism-dependent CL, which is the possible way to produce some substracts for cells’ luminescence, is a suppose cause of this situation.
Infections

There are many researches deal with CL use and role to predict some viral or bacterial infections, also help to expect the degree of its severity in definite patient [23-25].

The bacterial infections

Our own papers [26] showed an decreased CL of neutrophiles in children with frequent pneumonias, comparing to children who suffer from pneumonias from time to time. There was also observed a very slow recovery in children with a low activity of neutrophils at the onset. Zabuska-Jabłońska et al [27], also Zgliczyński et al [28] observed the same lower values of neutrophils’ CL in adults with recurrent pneumonias. The decreased CL was described in children with recurrent respiratory tract infections [29]. The low values of neutrophils’ CL in children with prolonged or chronic pneumonias were noticed what obliged to intensify the treatment. Werner et al [30] showed an exact increasing of CL in children from cities with bigger pollution.

Braun et al [31] showed the increased CL of neutrophils in patients during severe period of bacterial pneumonia, comparing to the patients with pulmonary oedema because of heart failure. Tekeuchi et al [31], also Adachi et al [32] described the higher CL of neutrophils, both: isolated and in full blood, in patients during a first period of pneumonia comparing to the values in healthy people. Through the treatment and the relieving of symptoms the CL collapsing was observed to the normal values in health. Very high values of CL were noticed during peritonitis.

AIDS

In 1994 there were many observations of decreased CL of neutrophils in patients with AIDS of different periods of disease [33]. The more severe phase the less active potential of neutrophils metabolism. The precised CL- decreasing was showed in patients with accessory Pneumocystis carinii invasion [34]. To intensity oxygenic metabolism of neutrophils and to make the immunological deficiency of the patient stronger there were used in vitro and in vivo G-CSF or IFN-γ with good results [35, 36].

Parasites’ invasions

The increased CL in patients with Plasmodium falciparum in severe phase of disease was observed [37, 38]. In some chronic cases the reduced CL was observed, what predisposed to prolonging the disease. There was no luminescence of neutrophils in trichinellosis [39].

Neoplasmal diseases

There were showed plenty of abnormalities dealing with the neutrophils metabolic activity in neoplasmal diseases. For instance the weak neutrophils response after LTB-4 and MMLP stimulation in patients with true polycythaemia comparing
Urinary system

To healthy people [40], there were no changes after PMA-stimulation (phorbol-12-miristate-13-acetate). It is a great evidence to proof a specific defect of a stimulated-CL. Itala et al. [42] described an abnormal (decreased) RT-production during infections in patients with lymphoblastic leukaemia. Cheze et al. [43] noticed a great low CL in patients with aplasia (panmyelophthisis). Within the time of disease remission they observed a slow CL increasing. Teramoto et al. [44] showed a CL-decreasing in patients with pulmonary infections in patients with lymphoblastic leukaemia. Cheze et al. [43] noticed a great low CL in patients with RA (rheumatoid arthritis). Asmsan et al. [47] showed the increased CL of separated granulocytes and of the full blood in patients with bronchial asthma. Teramoto et al. [48] showed the increased stimulated-CL in patients under second phase of pulmonary sarcoidosis comparing to the values in healthy people. That emission was also higher than of pulmonary macrophages. However, there was no correlation between CL and cells markers estimating the severity of the disease, although the authors recommended CL-test as one more examination to proof the sarcoidosis’ activity.

Asmsan et al. [47] showed the increased CL of separated granulocytes and of the full blood in patients with bronchial asthma. Teramoto et al. [48] showed the increased stimulated-CL in patients suffering from bronchial asthma and chronic obturative pulmonary (pulmonal) disease comparing to healthy people. The spontaneous CL was higher only in patients suffering from asthma. The same results were in our own researches in 1993-1994, which showed the increased CL in full blood, in children with asthma [49]. Li et al. [50] showed the increased CL in asthma, together with a collapse of an antioxidatic potential. That process was as more observed as more the exact decreasing of SOD was in the serum and as more higher the IgE-level was.

Respiratory tract diseases

There were showed a much increased rest-CL in patients suffer from ARDS (acute respiratory distress syndrome), comparing to the risk group of this disease and also to healthy people [45]. There were also noticed significant higher results of a stimulated-CL by PMA in ARDS patients than in the risk group or in healthy people. As the authors wrote this test might be very useful to predict the risk of ARDS.

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Liver diseases

Lunel et al. [65] showed much increased basic CL in patients with an alcoholic hepatitis (liver failure). After latex, Zymosan and PMA stimulation there was observed an increasing RT-generation and a higher CL of granulocytes in full blood. Okada et al. [54] had similar results of isolated neutrophils. Those informations are different than Pawlicki’s [55] ones and Lucci’s who noticed the increased rest CL of isolated neutrophils, while the stimulated CL was decreased. During haemodialysis a considerable decreasing of isolated neutrophils chemiluminescence was observed. Pawlicki et al. [56] showed a great number of superoxide anions measured by a reduction of cytochrom C in the same time as CL, what seems to be controversial to CL results. The authors explained that the fact is probably caused by RT-relieving from neutrophils out of the cell, because of granulocytes degranulation what provide to leaving for example MPO-responsible for CL. The observed RT-increasing could be caused by the complement activation and a direct contact of cells with dialysing membrane.

In patients with chronic pyelonephritis the decreased oxygenic metabolism of neutrophils and CL of these cells were observed [57].

Autoimmune diseases

The disturbed oxygenic metabolism of neutrophils leading to RT-over production plays a great role in rheumatoid arthritis (RA) [58]. There were observed the increasing of both spontaneous and stimulated luminol-dependent CL of neutrophils in full blood [59] and of neutrophils coming from the arthral liquid of patients with RA [60]. There were also higher spontaneous and stimulated CL of the granulocytes taken from healthy people and incubated with an arthral liquid from people suffer from RA. There was also higher CL of granulocytes in patients with progressive scleroderma [61]. There was observed an intensification of basic CL together with a progression of skin change. The stimulated CL was similar to value in healthy people. The authors suggested that a neutrophils preactivation begins in vivo in organism.

There were showed an intensive oxygenic metabolism of neutrophils and of course an increased CL in children suffer from rheumatoid arthritis [62]. The intensification of CL was observed coming together with an aggravation of the disease. Miesel et al. [63] did not notice any increased values of CL in patients with RA during remission time, but in an aggravation of the disease they observed the increased CL coming with TNF-α elevation. These authors also observed, in another work, up to 8-times intensified CL of neutrophils which was caused by their phagocytic activity, comparing to healthy people.

Arntchold et al. [64] also showed the increased native CL in patients with RA comparing to healthy people.
a significant weaker luminescence of neutrophils than observed in healthy people. Iushuk et al. [66] made a proof of the CL-test usefulness in a differential diagnostics between a viral hepatitis and bacterial one, leading on own observations. They showed the decreased oxygenic metabolism of granulocytes (luminol-dependent CL) in patients with an acute viral hepatitis comparing to CL in patients with Yersinia enterocolitica during its severe phase. Uehara M. et al. [67] noticed a significant decreased CL of neutrophils in patients suffer from a liver cancer and a liver fibrosis in confrontation with healthy people. The low values of CL were also observed during some chronic hepatitis, but the differences were not so clear as in liver cancer.

The surgical procedures

The meaningful collapsing of granulocytes oxygenic metabolism was also observed in some patients after splenectomy and after cholecystectomy too, but in a smaller degree [68]. Sakamoto et al [69] showed the increased CL of neutrophils in patients after the urologic operations through abdomen on the contrary to those through an endoscopy.

The others scientists showed a negative influence of lidokaine on CL of neutrophils both in vivo and in vitro [70]. The granulocytes of healthy people were treated by thiopental and had the significant decreased CL which was stimulated by Staphylococcus aureus and Escherichia coli [71]. Heberer et al. [72] noticed quite different results, showing no influence of analgesia and of severe abdominal operations on CL in full blood.

The ischaemic diseases

There were showed decreased values of CL in patients with an acute phase of myocardic ischaemia and CL increasing during a reperfusion period [73, 74]. Kowalski et al. [75] described the correlation between an increased CL of neutrophils and a decreasing of C3c, C5 of complement and also their haemolytic activity during an unstable ischaemic angina. Takeshita et al. [76] showed an intensified oxygenic metabolism of neutrophils tested by CL in patients with ischaemic angina. They investigated whether increased PMN activity in the peripheral blood is a marker for high-grade coronary artery stenosis in patients with angina pectoris [77]. Hansen et al. [78] showed on contrary, lower values of CL in patients with infarct, cured by streptokinase.

In spite of many observations and uses, chemiluminescence is not a specific method of diagnosis (except CGD) for any disease. We could observe its intensity and also its impairment in plenty diseases like we observe erythrocytes sedimentation and CRP-protein elevation or its reduction. Chemiluminescence is a very useful diagnostic test what helps in estimating the results of the treatment, the inflammatory progression and the degeneration, where there are any free-radicals reactions. CL is a fast, simple laboratory test used more and more often by many scientists and physicians.

References

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