# Investigation of Human Cells to assess the toxicity of Disinfectants used for destroying microbes and viruses at the work place.

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*Comparison of Anolyte and sodium hypochlorite levels reveals higher toxicity of the latter. Its use as disinfectant is only justified if reliable means of respiratory tract protection are used.*

Chlorine-containing agents are the most commonly used disinfectants in medical facilities, as they possess a wide range of anti-microbe activity and swift action ability. Decontamination effect being the same, preference shall be given to disinfectants that are cheap, safe for medical stuff and have no harmful influence on the environment.
Of all inorganic chlorine-containing disinfectants the cheapest ones are sodium Hypochlorite and electrochemically activated Anolyte solution, produced by sodium chloride electrolysis. [1,2] Disinfecting effectiveness of Anolyte is higher than that of calcium hypochlorite [3], and even in small concentrations it completely destroys microbes and viruses [4]. Anolyte with active chlorine concentration of 300mg/l causes irreversible damage to the membrane of most microbes already in one minute [5] and is recommended for wide implementation in clinical and preventive medical facilities for disinfecting and sterilisation purposes [6].
Infusion of Anolyte into the stomachs of warm-blooded animals causes no changes in basic internal organs, has no mutagenous effect and does not trigger allergic reactions [4]. High oxidation potentials of Anolyte exceed physiological levels of +200mV to -–00mV [7]. However, potential resources and resistance of human cell membranes [4] are higher than microbe membrane resistance [5]. That is why enzymatic systems in cells and tissues remain intact in spite of Anolyte use.
Sodium hypochlorite contains more active chlorine [8] than Anolyte. During its synthesis more HCLO molecules are generated than during Anolyte synthesis. An excessive content of hypochloreous acid in sodium hypochlorite leads to generation of active oxygen, containing a lot of free radicals, from HCLO. Free radical oxidation of lipids is especially dangerous for erythrocytes and the process is worsened considerably by iron they contain [9]. It results in hemolysis.
The present work studies the intravital assessment of Anolyte and sodium hypochlorite action bon human erythrocytes and buccal epithelial cells.

In 38 studies we investigated the effect of Anolyte bon the blood of 21 practically healthy persons.
In 49 studies the effect of Anolyte and hypochlorite bon epithelial strata and isolated buccal epithelial cells of 18 practically healthy persons was studied. Altogether more than 2,500 cells were examined. In the course of examination we used the method of bio-kinetic study of living cells in bipolar field [10], and our own methods [11,12,13]; also, an intravital dyeing of cells with the help of vital dyes (methylen blue, neutral red) was carried out. A classic thesis of cytology concerning diffuse binding of vital dyes in cell degeneration [14,15] was used for describing destructive impact of substances studied.
In this work we used commercial sodium hypochlorite with initial concentration of 3,000mg/l and Neutral Anolyte, produced by STEL device with active chlorine concentration 300mg/l, all other values standard. Four-fold dilution of Anolyte and hypochlorite with distilled water proportionally diminished active chlorine concentration but did not cause considerable and significant alteration of Anolyte pH and ORP values. Kinetics measurements and all cell morphometry were conducted with the help of ocular microscope MOB-1-A. Everything was registered in photo- and video film. Statistical processing was done in conformity with student test.

Electro-kinetic properties of the cell as a whole and of its separate structures are determined by a difference in charges of particles in exterior and interior environment. Irregularity of charge ratio causes a change of vital activity and functioning of the cell. Changes of this kind may be induced by Anolyte solution or hypochlorite, as they both contain ions. As a living system, the cell is capable of adaptation to slight shifts in potential difference.
Because the cytolemma is the first one to come into contact with the medium, the change in its potential during the first few minutes is more notable than a potential change in nucleus membrane, which is manifested by different kinetics (amplitude) of cytolemma and nucleus oscillations. If the ion concentration of a studies medium is low (Anolyte, 75mg/l), nucleus kinetics may suffer no changes at al for a long time. It is explained by normal functioning of membranes in erythrocytes and epithelial cells, which prevents chlorine-containing ions from entering the cell, so there are negligle changes to electro-kinetic properties and no obvious morphological changes.

Major changes in ion compensation of the external environment result in disfunction of cell membrane penetrability. The contact between nucleus and chlorine containing ions diminishes its electro-kinetic properties and is usually accompanied by morphological lesions of the cell. We may cite as an example sharp decline of electrical activity of erythrocytes and epithelial cells in hypochlorite and Anolyte solutions with high concentrations of active chlorine, and morphological changes of erythrocytes in hypochlorite solutions (3,000mg/l, 1,500mg/l, 750mg/l). Erythrocytes turn into echinocytes (hypochlorite, 1,500mg/l and 750mg/l), and sometimes become fragmented (hypochlorite 3,000mg/l and 1,500mg/l). epithelial cells swell up (hypochlorite, 3,000mg/l, 1,500mg/l, 750mg/l) and their nuclei becomes more compact (hypochlorite 3,000mg/l).
It is explained by the fact that in Anolyte and hypochlorite solutions, chlorine containing ions, due to their small sizes and high concentration, enter the cell through its own transportation channels and disrupt the normal osmotic function; by the presence of free radicals and by oxidation of erythrocyte membrane lipids. The toxic effect of hypochlorite is less obvious in relation to epithelial cells as they have a lot of aerobe enzymes. HCLO dissociation and oxygen generation provide temporary activation of these enzymes. But high concentration of hypochlorite in due time suppresses enzymes, and epithelial cells swell.
Research shows that sodium hypochlorite causes erythrocyte hemolysis (Table 1).

Parameters of Anolyte and sodium hypochlorite solutions' activity in erythrocyte hemolysis.

| Name of solution | Study period, Min. | Number of living cells in an area unit |
|---|---|---|
| Sodium hypochlorite 3,000mg/l | 0 | 50 |
| | 5 | 10 |
| | 10 | 0 |
| Sodium hypochlorite 1,500mg/l | 0 | 47 |
| | 5 | 15 |
| | 10 | 0 |
| Anolyte 300mg/l | 0 | 12 |
| | 5 | 12 |
| | 10 | 12 |
| Anolyte 75mg/l | 0 | 19 |
| | 5 | 19 |
| | 10 | 19 |

The amount of living cells in Hypochlorite declines abruptly during 5-10 minutes, while in Anolyte there is no lesion of erythrocytes.
Adverse influence bon human blood is connected with a higher toxicity level of sodium hypochlorite (Table 2)

Erythrocyte kinetics in Anolyte and sodium hypochlorite solutions

| Medium | Study period, Min. | Presence (+) or absence (-) of hemolysis | Data of the experiment, μm |
|---|---|---|---|
| Hypochlorite 3,000mg/l | 0<br>5<br>10<br>15 | +<br>+<br>+<br>+ | 2 ± 0.09<br>0.65 ± 0.13<br>0.3 ± 0.25<br>0 ± 0.03 |
| Hypochlorite 1,500mg/l | 0<br>5<br>10<br>15 | +<br>+<br>+<br>+ | 2.9 ± 0.1<br>1.1 ± 0.17<br>0.7 ± 0.17<br>0 ± 0.04 |
| Hypochlorite 750mg/l | 0<br>5<br>10<br>15 | +<br>+<br>+<br>+ | 3.5 ± 0.15<br>1 ± 0.14<br>1.9 ± 0.25<br>0 ± 0.04 |
| Anolyte 300mg/l | 0<br>5<br>10<br>15 | -<br>-<br>-<br>- | 2.3 ± 0.015<br>1.7 ± 0.1<br>0.2 ± 0.1<br>0 ± 0.07 |
| Anolyte 75mg/l | 0<br>5<br>10<br>15<br>20 | -<br>-<br>-<br>-<br>- | 5 ± 0.1<br>5 ± 0.12<br>3.7 ± 0.17<br>2 ± 0.1<br>0 ± 0.01 |
| Saline solution Control | 0<br>5<br>10<br>15<br>20 | -<br>-<br>-<br>-<br>- | 5.3 ± 0.09<br>4.35 ± 0.1<br>3.4 ± 0.1<br>3.0 ± 0.1<br>0 ± 0.09 |

Differences in erythrocyte and epithelial cell reaction to disinfectants are very important if Anolyte and hypochlorite are used in water and air disinfecting or for clinical purposes.

Anolyte does not cause an increase in cytolemma penetrability. This helps preserve normal cell volume and nucleus-cytoplasm ratio, and also prevents cytolysis. Preservation of blood kinetics and viability of blood cells is an indication of physiological quality of Anolyte.
Even after two-fold dilution sodium hypochlorite causes epithelial cell swelling and swift total destruction of red blood cells (hemolysis0. This makes it possible to attribute hypochlorite with high concentrations of active chlorine to membrane blockers and hemolytic poisons.
A comparison of Anolyte and sodium hypochlorite toxicity levels proves sodium hypochlorite to be more toxic. Its use as a disinfectant is only justified if there is reliable respiratory tract protection available.
Considering the above, caution should be exercised in using even low concentrations sodium hypochlorite for intravenous injections.