

SYSTEM OF COMPUTER ANALYSIS AND EVALUATION OF CEREBRAL MORPHOLOGICAL CHANGES AFTER HYPERBARIC OXYGEN THERAPY OF EXPERIMENTAL CARBON MONOXIDE INTOXICATION IN RATS

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ABSTRACT

The method for computer analysis and evaluation of the morphological cerebral changes after hyperbaric oxygen therapy (HOT) in experimentally CO-intoxicated rats allows the verification of the effectiveness of HOT regimen applied in practice. The results from this method coincide to a great extent with those achieved by means of other two methods, i. e., of laboratory analysis and clinical observation. HOT gives an opportunity for a rapid and efficient treatment. The most efficient regimens of HOT that we recommend are the following: low levels of intoxication - NH-b, medium levels - NH-2, and severe levels - NH3.

Key words:computer analysis, carbon monoxide intoxication, hyperbaric oxygen therapy, brain morphology, rats

INTRODUCTION

Lower oxygen concentration in tissues, hypoxia, takes a significant place in the genesis of many diseases one of the most important of which is the acute carbon monoxide (CO) intoxication. CO blocks the oxygen-transporting function of hemoglobin by forming the carboxyhemoglobin combination. It results in acute hypoxemia and later hypoxia which leads to a lethal outcome. Giving oxygen to the affected subject is an option from the variety of methods, but the results from the oxygen therapy under normal pressure are unsatisfactory. Introducing the hyperbaric oxygen therapy (HOT), i.e. using hyperbaric oxygen achieved by inhaling oxygen with over 1 atm pressure allows the significant supply of oxygen to the hypoxic cells and tissues to increase by its salvation in the plasma. The priority of the HOT has been undoubtedly proved because the dissociation of carboxyhemoglobin is much faster than under normal conditions. In a short time HOT allows elimination of the hypoxia, normalization of the disturbed metabolism and achievement of a quick recovery. The oxygen needs strict dosing so a maximum healing effect could be achieved and an oxygen intoxication be avoided. The doses of hyperbaric oxygenation are specified by the HOT that includes maximum pressure and duration of oxygen supply. Until now there were no differentiated regimens of HOT for treatment of different levels of CO intoxication - low, modest, severe, and fulminant. One of the reasons is the lack of a system for rapid automatic analysis of the results for the applied treatment.

The purpose of the present work is by using of system for computer analysis and evaluation of morphological cerebral changes to study some new and effective regimens of HOT for single treatment of acute intoxications with CO according to the levels of toxemia in order to ensure an optimal treatment and a faster rehabilitation.

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MATERIAL AND METHODS

During one-year period an experimental study was carried out on test animals intoxicated with CO at different levels and treated with different HOT regimens according to the intoxication level. According to the world experience for the experiment 124 Wistar white rats were used selected to meet the following requirements: gender - male; weight - 250-300g. We strictly followed all the ethical standards for work with test animals and biological materials, and we kept all the safety measures for working with toxic gases and high-pressured vessels.

Two methods for oxygen treatment were used: normobaric and hyperbaric therapy. Normobaric therapy (NOT) - a method of treating the animals with oxygen with normal pressure of 1 atm (ATA).

Hyperbaric therapy (HOT) - the effect on intoxicated animals of a few chosen regimens of HOT was tested for each level of intoxication.

The intoxicated animals were treated with some HOT regimens: one or two are developed by us and one or two are foreign divers' regimens. For developing our regimens some data was used from the divers' regulations - 90 (2) and oxygen decompression tables of British Royal Navy (3) which were similar to the US Navy (11). A total of 32 rats were intoxicated at a low level, 32 - at a medium, 52 - at a severe one while 8 were left for healthy controls. The groups of test animals for each level of intoxication according to the applied treatment are shown on Table 1. Before the test samples were taken the animals were anaesthetized with Ketamin of 100mg/kg b.w. i.m. Perfusion of the tissues has been made through the left ventricle with glutaraldehyde. The injection of the fixing agent was slow with speed of 1ml/min. The effectiveness of the perfusion was evaluated by the paling of tissues, fibre trembles of the skeleton muscles and appearance of the glutaraldehyde on the cut areas.

Computer analysis and evaluation of the morphological changes

The whole brain was taken and put in 10% neutral formalin The test material absorbed histowax and frontal plane sections were prepared of the upper surface of the corpus callosum with width of the section - sum stained with hematoxylin-eosin The observations were made with BX-50 OLYMPUS microscope. The correlation between the pericellular edema zone and the whole area was measured. From each rat two test materials were examined. From each test material three images were chosen and from each three calculations by computer analysis were made (there were 18 results of each rat). The computer analysis was made by using Micro Analysis -1.1 software program for microscope image analysis of Chris Soft, Bulgaria.

Table 1. Allocation of the test animals according to the level of intoxication and type of treatment

low level			medium level			severe level		
group	number	treatment	group	number	treatment	group	number	treatment
I	8	air	I	8	air	I	8	air
II	8	NOT	II	8	NOT	II	8	NOT
III	8	HOT /NH-la/	HI	8	HOT /NH-2/	III	8	HOT /NH-3/
IV	8	HOT /NH-lb/	IV	8	HOT /US-5/	IV	8	HOT /US-6/
						V	8	HOT /DS-90/
						VI	8	HOT /NH-lb/

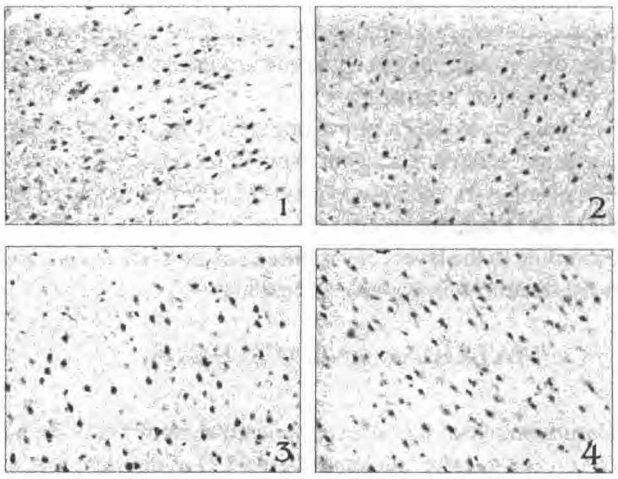

Fig.1 Cerebrum of rat-intoxication with CO (low level): 1-first group(air); 2-second group(HOT); 3-third level(HOT-MMA-MH); 4-fourth group (HOT-MMa-MH-1b0-stained with HE,x200

RESULTS AND DISCUSSION

The morphological investigations at this stage of work undoubtedly show and prove the marked antiedematous effect of HOT. With all the three levels of intoxication some common regularities about the effectiveness of the applied HOT to the brain edema were observed. Differences concerning the qualities of the HOT regimens were reported, too. For each of the three levels the following characteristics were typical:

Low level - a significant decrease of the cerebral edema with a high level of authenticity ($p<0,001$) is reported from the treatment of the intoxicated rats with both applied regimens HOT- NH-1a and NH-lb. With the group of animals treated with NOT a significant decrease of the brain edema is also reported, but with a lower level of reliability ($p<0,05$). Therefore, the priority of the treatment with hyperbaric oxygen is more significant than the one with

normal pressure oxygen for the treatment of cerebral edema (Fig. 1, Table 1).

Medium level - antiedematous effect with significantly high level of reliability ($p<0,001$) compared to the control group is reported for the rest three groups. The comparison between them with the t-standard of Student-Fischer shows greater influence over the vrain edema with the third group of animals (t-standard - 30,8) treated with HOT of regimen NH-2. Both applied regimens of HOT have equal values of maximum work pressure - 0,18mPa and differ from the length of breathing oxygen under this pressure (higher for NH-2). Therefore, we accept that at this level of intoxication as well as at the low level the defining factor of antiedematous effect is the maximum work pressure of HOT regimen.This statement is confiemed by the fact that with the US Navy table-5 regimen for breathing oxygen pressure of 0,09mPa is used in addition. Although there is no such stage with regimen NH-2 better results are reported (Fig. 2).

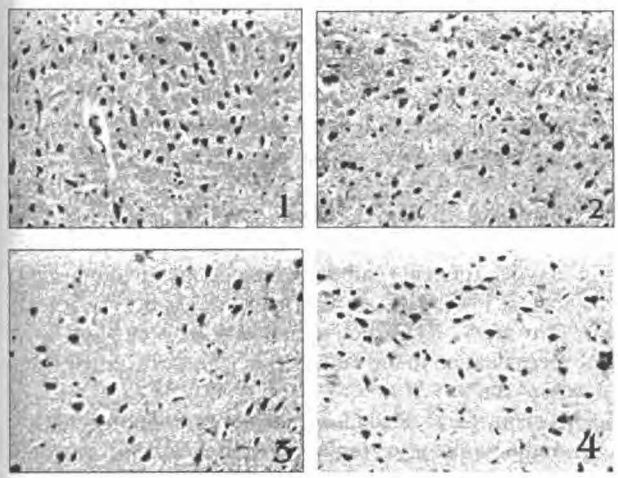

Fig.2. Cerebrum of rat-intoxication with CO (medium level): 1-first group(air); 2-second group (HOT); 3-third group (HOT-MMA-MH-2); 4-fourth group(HOT -US Navy-5)-stained with HE, x200

Severe level - when comparing the morphological results of the treatment with those levels of intoxications the differences in affecting the brain edema, according to the qualities of the HOT regimens, are most distinct. Four regimens of HOT with different characteristics were used. Statistically, the effectiveness on cerebral edema is most significant with the treatment of intoxicated animals with NH-3 regimen and US Navy table-6 ($p<0,001$). After applying the other two regimens - diving service (DS)-90 and NH-1b a significant difference from the control group is also reported, but it is less significant ($p<0,01$). Lack of significance is reported only with the second group of test animals treated with HOT.

With this level of intoxication by computer software processing it is most clearly visualized and projected how the significant perivascular and pericellular zones of brain edema decrease progressively with the applying of long-lasting regimen of HOT. At the same time it is registered that with severe level of intoxications it is of no such significance if the regimen is 170 or 285min long (it is important that it is not shorter than 60 min). Probably, after the 60th min of HOT a process of vasoconstriction of the cerebral arterioles and resorption of edema liquids starts (Table 2, Fig. 3). Final results of the computer processing of light microscope images concerning the percentage correlation of the cerebral edema area compared to the whole area are represented on Table 2 for low, medium, and severe levels of intoxication, respectively.

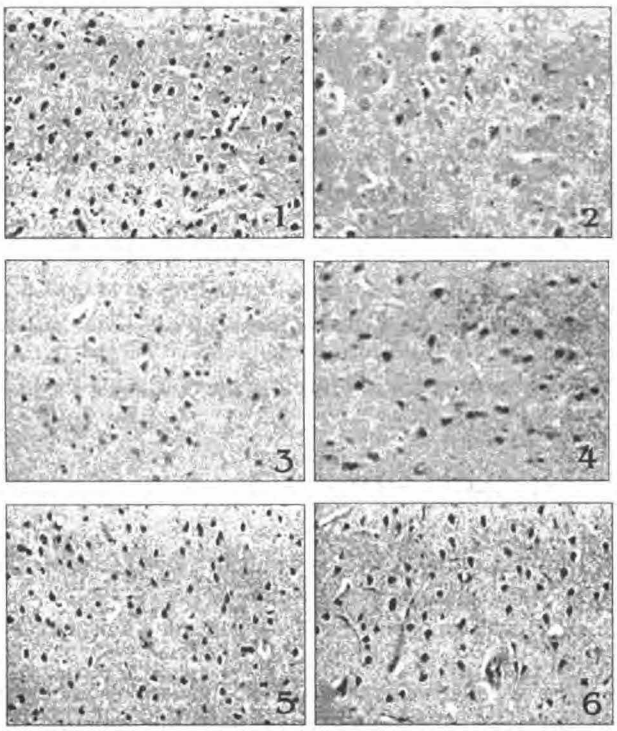

Fig.3. Cerebrum of rat-intoxication with CO (severe level): 1-first group(air); 2-second group (HOT0); 3-third group (HOT-MMA-MH-3); 4-fourth group (HOT-US Navy-6); 5-fifth group (HOT-diving service DS-90); sixth group (HOT-MMa-MH-1b)-stained with HE ,x200

Our results from the application of this method to a great extent coincide with those achieved by using the other two methods - laboratory analysis and clinical observation (1). Based on the computer analysis of the morphological changes after intoxication with CO we recommend the use of the following regimens: for low levels, most appropriate regimen is NH-1b, for medium levels - NH-2, and for severe levels - NH-3. Very close results are obtained after applying: NH-1a for low levels, US Navy table-5 for medium and US Navy table-6 for severe intoxication levels. There are similar results in our regimens and the US divers' recompression tables (US Navy table 5 and 6). That is a reason for applying both regimens for treating severe intoxication with CO. Since this work is for optimal plans we accept that the described regimens are most suitable for the required conditions. They are significantly shorter than the US ones and that is the reason they are well-accepted.

Table 2. Results in percentages for the applied regimens for the different levels of intoxication

low level			medium level			severe level		
regimen	group	% of cerebrum edema	regimen	group	% of cerebrum edema	regimen	group	% of cerebrum edema
air	I	4.77 ±0.82	air	I	5.93 ±0.37	air	I	6.59 ±1.30
NOT	II	4.28 ±0.89	NOT	II	4.92 ±1.054	NOT	II	5.41 ±1.34
HOT /NH-la/	III	2.65 ±0.33	HOT /NH-2/	III	3.19 ±0.34	HOT /NH-3/	III	3.82 ±0.40
HOT /NH-lb/	IV	2.1 3±0.45	HOT /US-5/	IV	3.71 ±0.63	HOT /US navy-6/	IV	4.14 ±0.47
						HOT /DS-90/	V	4.48 ±1.33
						HOT /NH-lb/	VI	5.01 ±0.75

That makes them safer, since they are developed for patients but not for divers.

CONCLUSION

1. The treatment with NOT is with much lower level of effectiveness compared to that with HOT.
2. HOT gives an opportunity for quick and efficient treatment.
3. The most efficient regimens of HOT that we recommend are: for low levels of intoxication - NH-1b, for medium levels - NH-2, and for severe levels - NH-3.

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