THE ROLE OF LOCAL BLOOD FLOW INTENSITY, BLOOD RHEOLOGICAL PROPERTIES AND FREE RADICALS IN DEVELOPMENT OF LOCAL HYPERTHERMIA-INDUCED MORPHOLOGICAL CHANGES IN CEREBRAL TISSUE OF THE RAT

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OBJECTIVE

In regard to central nervous tissue, there are some discrepancies in published data concerning irreversible damages induced by local hyperthermia. We tried to obtain more specific data pertaining to sensitivity of cerebral tissue to hyperthermia treatment, its immediate effect, manifested by histological changes and the role of local blood flow, blood rheological properties, and the possible role of free radicals in development of mentioned changes.

RESEARCH DESIGN AND METHODS

Through the small cranial window local area of cerebral surface was irrigated by artificial cerebrospinal fluid heated up to the temperature necessary for achievement of stable level of temperature on the brain surface (41, 43 or 45^{0}C). Serial brain coronal sections 50 μm thick stained with Azure-Eosin were analyzed under light microscope. The local cerebral blood flow was measured by thermal clearance method. Blood rheological properties were changed by injection of high molecular weight Dextran T-500 and free radicals existence was controlled by Dimethyl sulfoxide injection.

RESULTS

At 41^{0}C just superficial lesions of the cerebral cortex and a few cases of thrombosed cerebral microvessels have been observed. The rise of temperature on 2^{0}C resulted in very severe lesions of cerebral tissue; the layered structure of the cerebral cortex in the central parts of the hyperthermia–induced lesions was impaired. The highest temperature (45^{0}C) caused complete destruction of the layered structure of the cortex in the area of hyperthermic exposure, numerous areas with lost neurons and thrombosed cerebral vessels with perivascular accumulation of erythrocytes were revealed. Injection of Dextran T-500 resulted in deterioration and injection of Dimethyl sulfoxide in significant improvement of histological changes in cerebral tissue when 43 or 45^{0}C hyperthermia is used. Bi-phase alterations of local cerebral blood flow have been revealed at 43^{0}C hyperthermia - well pronounced hyperemia in first stage of heating is followed by remarkable ischemia on the late stage.

CONCLUSION

High sensitivity of cerebral tissue to hyperthermic exposure even at a temperature of 41^{0}C has been confirmed. We can consider cerebrovascular thrombosis as one of the most significant complication of brain hyperthermia. In case of deteriorated blood rheological properties hyperthermia-induced cerebral lesion is more remarkable.

Administration of antioxidants, scavengers of free radicals can partially lessen hyperthermia induced cerebral lesion.

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The clinical use of hyperthermia for cancer treatment was increasingly accepted during past decades and today there is well established fact that the hyperthermia pre-treatment at temperatures between 40-44^{0}C enhances the therapeutic effect of radiotherapy or chemotherapy (Van der Zee, 2002). In this temperature range due to the difference in characteristics of normal and tumor tissue, tumor cell killing is achieved. Most biological tissues, with the exception of the central nervous tissue, are tolerant to hyperthermia treatment and can survive at a temperature of up to 44^{0}C (Fajardo, 1984). In regard to central nervous tissue, there are some discrepancies in published data concerning irreversible damages that were found after treatment: at 42-42.5^{0}C (Sminia et al, 1994), at 43.1^{0}C (el-Sabban, Fahim, 1995) at 43.9^{0}C and greater (Fike et al., 1991). And, what is more, Matsumi et al (1994) have showed no obvious irreversible changes in monkeys normal brain tissue at 44^{0}C and below in case of non-survival experiment, and just in survival experiment (animals were sacrificed 7 days after the treatment) cerebral areas heated at 44^{0}C or above coagulative necrosis developed, the authors suggesting that the safety limit for brain hyperthermia is 43^{0}C for 60 min.

Analysis of experimental data received on dogs (Harris et al., 1962; Lyons et al., 1984; Sneed et al., 1986; Fike et al 1991; Eddy et al., 1992; Ikeda et al., 1994), cats (Samaras et al., 1982; Britt et al., 1983; Lyons et al., 1986) and rabbits (Silberman et al., 1982) using different techniques for brain local hyperthermia allowed P. Sminia and M. Hulshof (1998) conclude that maximum tolerable heat dose is 42-42,5^{0}C for 40-60 min or 43^{0}C for 10-30 min. Effects of hyperthermia were expressed immediately or within a few days after treatment. The most recent review on effect of local hyperthermia on the cerebral nervous tissue was published by J. Haveman et al (2005).

It is accepted that hyperthermia-induced damages in central nervous tissue are mostly conditioned by thrombosis and arteriolar constriction [el-Sabban, Fahim, 1995]. The microcirculation in its turn in many aspects is conditioned by rheological properties of blood. One of the most significant rheological parameter of blood is its viscosity, which depends on RBC aggregation and deformability, haematocrit, blood temperature and others. If by some reason either haematocrit increased or fibrinogen and immunoglobulin rose, or there is a hypothermic condition, or increased aggregation of erythrocytes or changes in their deformability, or all named factors take place simultaneously, hyperviscosity of blood is observed. Increased viscosity results in a slowing down of blood flow, stagnation of its constituents and in ischemia [Larcan, Stoltz, 1983]. Decrease in regional cerebral blood flow (rCBF) and regional cerebral metabolic rate of oxygen were observed in the elderly (66.6+4.6 years old) with increased blood viscosity as a result of various kind of polycythemia and erythrocytosis [Shikatura, Kubota , Tamura, 1993]. Plasma viscosity must also be taken into consideration. Special investigation devoted to clarification of relationship between plasma viscosity and cerebral blood flow [Tomiyama et al., 2000] has shown that rCBF more closely follows changes in plasma viscosity rather than whole blood viscosity. Authors believe that plasma viscosity may be the more important factor in controlling cerebral blood flow.

In accessible for us literature we did not find any data concerning changes in hyperthermia-induced damages of cerebral tissue under different rheological properties of blood.

Besides above-mentioned hyperthermia has a significant influence on cerebral metabolism. The release of excitatory neurotransmitters and oxygen free radicals causes more extensive blood-brain barrier breakdown {Arboix 2005]. It has been hypothesized that hyperthermia promotes oxygen-centered free radicals formation in cells. By means of electron paramagnetic resonance spin trapping Flanagan et al. (1998) received direct evidence for free radicals generation during

hyperthermia in intact functioning cells. This finding indicate that heat increases the flux of cellular free radicals and support the hypothesis that increased generation of oxygen-centered free radicals and the resultant oxidative stress may mediate in heat-induced cellular damage [Flanagan et al., 1998].

Taking into account all above-mentioned, we tried to obtain more specific data pertaining to sensitivity of cerebral tissue to hyperthermia treatment and its immediate effect, manifested by histological changes, and the role of local blood flow, blood rheological properties, and the possible role of free radicals in development of mentioned changes.

MATERIALS AND METHODS

Non-survival experiments were performed on pathology free adult mail Wistar rats weighing 250-300g. Before the experimental sessions all rats were housed in temperature and light controlled rooms with standard rodent chow and water available ad libitum.

All experimental protocols were performed in compliance with NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Publication 86-23) and were approved by the institutional Animal Care and Use Committee for the Beritashvili Institute of Physiology, Georgian Academy of Sciences.

Each rat was anesthetized by 0.15ml/100g body weight IP injection of 4% chloral hydrate solution. After catheterization of right femoral vein animal was mounted in a stereotaxic apparatus. The skull was exposed and about 3mm hole was drilled in the parietal bone (right or left). The dura mater was carefully retracted, and a thermistor bead probe for thermal pulse delivery and recording of local blood flow by means of thermal clearance method [Xu et al., 1998; Zhu et al., 2005] was dipped into the sensory-motor area of the cerebral cortex at a depth of 0.5 mm. In parallel with the temperature probe a silicon tube (0.5 mm internal diameter) connected to a peristaltic pump was lowered to the cerebral surface (Figure 1).

After testing the thermistor probe it was connected to the thermistor data acquisition block of polygraph “MX-01" (USSR). The silicon tube, lowered to the brain surface, was connected to the outlet of one-channel peristaltic pump (MMC, Czechoslovakia). The pump’s inlet, via polyethylene catheter, was connected to an ultra-thermostat’s reservoir, filled with artificial cerebrospinal fluid heated up to the temperature necessary for achievement of stable level of temperature on the brain surface (either 37, 41, 43 or 45 degrees Celsius).

Rectal temperature was measured with a thermocouple probe, connected to the second channel of temperature measuring block of polygraph, and maintained on 37^{0}C by a feedback-controlled infrared lamp.

Temperature in the room was maintained near 23^{0}C.

After completion of the surgical procedure and placing the thermistor probe and silicon tubing in the craniotomy location, controlled hyperthermic exposure was applied regionally by irrigating the cerebral surface with cerebrospinal fluid heated in the thermostat reservoir up to the desired temperature.

In the first series of experiments the temperature of the artificial cerebrospinal fluid in normothermic (control) Group 1 of animals (6 rats) was maintained on the level of 37^0C. In the following three groups (6 animals in each) the temperature of the artificial cerebrospinal fluid correspondingly was 41 (#2 Group), 43 (#3 Group), and 45 (#4 Group) degrees Celsius.

In the second series of experiments similar to the first series, 4 groups of animals (#5 – normothermic, #6 – 41, #7 – 43 and #8 – 45^0C) 15 minutes prior to heating and 15 minutes after its beginning 1ml of 10% high molecular weight Dextran T-500 (Pharmacia, Sweden) was administered i/v. In order to maintain systemic arterial and venous pressure levels close to the normal, the same amount of blood was withdrawn.

In the third series of experiments 4 groups of animals (#9 – normothermic, #10 – 41, #11 – 43 and #12 – 45^0C) 15 minutes prior to heating 0.3 ml/100g body weight 5% solution of Dimethyl sulfoxide (DMSO) – well-known scavenger of free radicals was administered i/v.

The composition of the artificial cerebrospinal fluid (in mmolls/L) was the following:

NaC1 - 118.0; KCI - 4.7; $NaHCO_3$ -14.9; KH_2PO_4 - 1.18; $MgSO_4.7H_2O$ -1.17;

$CaC1_2H_2O$ - 2.5; Glucose - 11.0. Permissible changes in pH of this solution were in the range of 7.35 - 7.45.

Duration of the brain local hyperthermic exposure in all experimental series was 60 minutes.

Before the temperature exposure in every animal the baseline local cerebral blood flow (lCBF) was measured three times (with 5 minutes interval). For each temperature grade lCBF was measured before (5-10 minutes after beginning of heating) and after (20-30 minutes after beginning of heating) reaching the steady state level in brain tissue temperature. For recording of "thermal clearance" curve in each measurement, 3 seconds duration heating pulse was delivered to the cerebral cortex tissue and the temperature "clearance" was recorded till to reaching the temperature level prior to heating pulse.

After completion of each experimental session, the animal was deeply anesthetized and transcardially perfused with heparinized saline followed by 10% formalin solution. The brain was removed and stored for subsequent histological processing.

Serial brain coronal sections 50 μm thick were prepared throughout the extent of hyperthermic lesions, mounted on glass slides and later stained with Azure-Eosin. The area (mm^2) of injured tissue and character of histological changes on coronal sections' was determined under light microscope using an ocular micrometer and the volumes (mm^3) were calculated by summing of injured areas of all sections and multiplying by the interval thickness between sections (Kim et al, 1996).

The Students' t-test was used to examine the effect of different temperature exposure on local cerebral blood flow and the geometric dimensions of damaged areas of brain tissue. A value of $P<0.05$ was considered to be significant.

RESULTS

NORMOTHERMIC GROUPS (#1, 5 AND 9). Data were analyzed from all series of experiments (18 rats). In each of them visual (macroscopically) and histological examination confirmed the absence of changes to 37^0C temperature exposure lasting 60 minutes (Figure 2). An average level of local blood flow in cerebral cortex was 61.5±3.2 ml/100g/min.

410C HYPERTHERMIC GROUPS (#2, 6 AND 10). None of the visible alterations and differences from the control has been found during visual examination of the cerebral surface of these groups of animals after completion of a 60-minute hyperthermic (41^0C) exposure.

In the animals from first series of experiments (#2 Group) changes on brain histological slices (Figure 3) were well pronounced. The average area of the lesion was 1.2±0.15mm^2 and the average volume of damaged tissue – 36.5±7.4 $mm^{3.}$ Individual thrombosed vessels were found mainly in the 2nd and 3rd layers of cerebral cortex and very seldom in 5th and 6th layers. The hyperthermia-induced lesion has semicircle form with clear delineated light outlines in the 2nd and 4th layers. The layered structure of the cerebral cortex is poorly disturbed and neuron disorientation is negligible, although the cell edges are significantly modified.

In this series of experiments hyperplasia and pycnosis of the cells occurred in the 3rd layer of the rats' cerebral cortex and most of them in pyramidal neurons.

In the animals from second series of experiments which before onset of hyperthermia received Dextran T-500 pretreatment (#6 Group) the average area of the lesion were 3.3±0.4mm^2 (Figure 4) and the average volume of damaged tissue – 100.3±12.6mm^3. Individual thrombosed vessels were found mainly in the II and III layers of cerebral cortex and very seldom in V and VI layers. The layered structure of the cerebral cortex is disturbed mildly and neuron disorientation is negligible, although the cell edges are significantly modified.

In this series of experiments hyperplasia and pycnosis of the cells occurred in the III layer of the rats' cerebral cortex and most of them in pyramidal neurons.

In the animals from third series of experiments which before onset of hyperthermia received Dimethyl sulfoxide (DMSO) pretreatment (#10 Group) the average area of the lesion were 0.78±0.11mm^2 (Figure 5) and the average volume of damaged tissue – 23.4±6.2mm^3. The layered structure of the cortex practically is not impaired. The damaged part of the tissue is not surrounded by a penumbra zone and disoriented neurons were not observed. There are some partially pycnotic and hyperplasic neurons in the second and third cortical layers and the density of their distribution is relatively high. The cell's shape, as well as nucleus and nucleolus are well recognizable.

430C HYPERTHERMIC GROUPS (#3, 7 AND 11). The cerebral cortex surface in locations of 43^0C hyperthermic exposure similar to the previous groups did not show the color changes or any other macroscopically visible alterations.

In #3 group of rats the hyperthermia-induced lesion's average area in the central coronal sections was 3.54 ± 0.8mm^2 and the calculated average volume of the damaged tissue 106.2 ± 11.3$mm^{3.}$ The

form of lesion on coronal sections of the brain was modified semicircle (Figure 6A). In the central part of the lesion thrombosed arterioles and capillaries were found and usually located up to the 3rd layer of cerebral cortex. Perivascular accumulation of erythrocytes was rare.

The layered structure of the cerebral cortex in the central parts of the hyperthermia –induced lesions was impaired (Figure 6B). In the 3rd layer some pyknotic neurons with difficult recognizable nucleus and nucleolus were observed. In this layer of the cortex neurons were distributed with very high density and loss of neurons was not observed.

The 4th layer of the cerebral cortex in this group of animals was the most damaged. The neurons in this layer were hyperplasic and the density of their distribution is very low. The light border between the 4th and 5th layers of the cerebral cortex formed because of heavy neuron loss was easily recognizable on the brain coronal slices. As a result of hyperthermic exposure pyramidal neurons mainly survived in the 4th layer of cerebral cortex. Stellate cells with identifiable soma with outgoing dendrites; nucleus and nucleolus were seldom found.

In the 5th layer of cerebral cortex neurons appeared disoriented around the area of lesion. Pycnosis of the neurons in this layer is less pronounced in comparison with the neurons of the 3rd layer but the neurons of this layer are more disoriented. It is very difficult to determine their type. The 6th layer of the cerebral cortex in this series of experiments was showed some medium hyperplastic neurons.

In #7 group of Dextran T-500 pretreated animals (Figure 7) the average area of the lesion was $5.9\pm0.7mm^2$ and the average volume of damaged tissue – $177.8\pm16.5mm^3$. The layered structure of the cerebral cortex in the central parts of the hyperthermia–induced lesions was impaired**.** In III layer some pycnotic neurons with difficult recognizable nucleus and nucleolus were observed. In this layer of the cortex neurons were distributed with very high density and loss of neurons was not observed. IV layer of the cerebral cortex was significantly damaged. The neurons in this layer are hyperplasic and the density of their distribution is very low. In V layer of cerebral cortex neurons appeared disoriented.

In #11 group of DMSO pretreated animals (Figure 8) the average area of the lesion was $1.2\pm0.13mm^2$ and the average volume of damaged tissue – $36.1\pm6.9mm^3$. In the first and second layers of cerebral cortex some thrombosed arterioles and capillaries were found. The cells in III-V layers are pycnotic (mostly in III layer). The 4-th layer is involved in penumbra zone; here neurons are partially pycnotic and disoriented. As a whole, the layered structure of the cortex is preserved.

450C HYPERTHERMIC GROUPS (#4, 8 AND 12). After completion of local hyperthermic exposure lasting 60 minutes all animals brain surface of ***#4 group*** on the exposed location had a rose color. The same changes in color at a depth of about 2mm were observed on the hyperthermia-damaged brain 50 μm thick coronal sections stained with Azure-Eosin. On the coronal section the hyperthermia-induced lesions look like a modified semicircle with an average area in the central sections equal to 6, 13 ± 0,21mm² (Figure 9A). The calculated volume of the hyperthermia-induced lesions in brain tissue for this group of rats was 183.6 12.4mm².

In the central part of the hyperthermia lesion numerous thrombosed arterioles and capillaries were revealed, with perivascular accumulation of erythrocytes, mostly they were found up to the 4th

cortical layer. In the area of lesion the layered structure of the cortex was significantly impaired and a loss of neurons (including the 4th layer) could be seen (Figure 9B).

The damaged (practically burned-out) part of the tissue is surrounded by a well-pronounced penumbra zone with disoriented neurons. The cell's edges in both focus of lesion and the penumbra area changed forms and identification of the type of cells was difficult. Many of the neurons were swollen although the edge and nucleolus were still distinguishable.

Cell lesions in the perifocal, penumbra zone are less defined when compared with those in the central part of the hyperthermic injury in all cortical layers.

IN #8 GROUP of Dextran T-500 pretreated animals (Figure 10) the average area of the lesion was 10.21±1.3 mm^2 and the average volume of damaged tissue – 315.7±26.3 mm^3.

In the central part of the hyperthermia lesion numerous thrombosed arterioles and capillaries were revealed, with perivascular accumulation of erythrocytes, mostly they were found up to the VII cortical layers. In the area of lesion the layered structure of the cortex was significantly impaired. The damaged part of the tissue is surrounded by a well-pronounced penumbra zone with disoriented neurons. Many of the neurons were swollen although the edge and nucleolus were still distinguishable.

IN #12 GROUP of DMSO pretreated animals (Figure 11) the average area of the lesion was 5.2±0.7 mm^2 and the average volume of damaged tissue – 156.5±11.4 mm^3. In the damaged area of the cortex thrombosed arterioles and capillaries are sharply defined and they are observed up to 6-th layer. The density of their distribution diminished in lower layers. Heavy neuron loss was easily recognizable on sufficiently great areas of the brain coronal slices. Some hyperplasic cells with changed shape have also been revealed.

Statistical data concerning immediate morphometrical changes in cerebral tissue, induced by local hyperthermic exposure for all described experimental conditions are summarized in Table 1 and graphically are presented in Figures 12 and 13.

Hyperthermia-induced alterations of local cerebral blood flow in normal rats as well as in Dextran T-500 and Dimethyl sulfoxide pretreated rats are summarized in Table 2. Percentages of these alterations in each series of experiments are presented on the figures 14-16.

DISCUSSION

Significant theoretical and practical experience in the application of local hyperthermia in cancer clinic and experimental studies has been accumulated.

There are several different technologies for induction of local hyperthermia and their usage varies depending on the character of a given task.

In our study we have chosen one of the simplest ways for induction of local cerebral hyperthermia – a simple cranial window assembly and delivery therein of artificial cerebrospinal fluid heated up to the desired temperature. It is evident that this methodical approach could not be used in clinical practice, but for accomplishing our task –revealing the temperature threshold and cerebral tissue sensitivity to temperature exposure and some contributory or impedimental factors for hyperthermia-induced cerebral lesions in an experimental setting, we consider this method as the most adequate, reliable and simple way for induction of local cerebral hyperthermia without significant side effects due to the technique.

The main goal of local hyperthermia treatment is affecting tumor tissue causing apoptosis or necrosis depending on the level of temperature and duration of hyperthermic exposure. It turned out, that in tumor tissue most pronounced apoptosis is observed at prolonged hyperthermia exposure (Toyota et al, 1998). But comparison of results of long-lasting (6 hours) low temperature (40^0C) hyperthermia in combination with chemotherapy, during procedure of short-term, high temperature hyperthermia most pronounced apoptosis in tumor tissue has been revealed (Toyota et al, 1998).

In our early experimental studies of local hyperthermia effects on rabbit's cerebral tissue, microwave induced local hyperthermia caused remarkable changes in cerebral blood flow manifested by increase of blood flow rates at the beginning of hyperthermia, and slump decreasing of blood flow rates after raising the brain temperature upwards of 43^0C (Bicher, Mitagvaria, 1980; Mitagvaria, Bicher, 1984).

On the same animal species in conditions of whole body hyperthermia doubling of cerebral blood flow intensity has been observed at the core body temperature 43^0C; 2.5 times increasing of cerebral blood flow at 44^0C and 3.5 times increasing at 45^0C. At the same time augmentation of tissue oxygen partial pressure and pH have been observed (Yamada, 1989).

In similar experiments carried out on canine brain (60 minutes duration 42^0C whole body hyperthermia) microscopically investigation of the brain tissue did not reveal any damaged brain area (Takahashi, et al, 1999). However, under slightly less temperature (41.8^0C) increase in oxygen transport and consumption in tissue have been revealed (Kerner et al, 1999).

During whole body hyperthermia statistically significant increase of oxygen saturation in arterial blood, arterial-venous difference in oxygen partial pressure and the level of venous blood PCO2 were observed. At the same time, decrease of oxygen saturation and pH in venous blood has also been observed (Hall et al, 1999). All these data testify that hyperthermia caused cellular hypoxia in visceral tissue. Hypoxia and decrease of pH has been credited by some authors as causing the anti-tumor effects of hyperthermia (Madden et al, 1990; Van der Zee et al, 1989).

Whatever the nature of hyperthermia's positive effect on tumor tissue and increase of its sensitivity to chemo-and radiotherapy, it is very important to ascertain normal tissue safety and the absence of irreversible damage. From this point of view special attention has to be paid to the central nervous tissue thermotolerance and sensitivity for hyperthermia [Sminia et al., 1990; Haveman et al., 2005]. As it already has been underlined above, there is a wide discrepancy concerning the temperature sensitivity of brain tissue using simple methodology. Our experiments seem to indicate irreversible damage to brain tissue at temperature close to those clinically used**.**

As we can see (Table 1) all three experimental levels of temperature (excepting the control group) caused different pronounced histological changes in the brain tissue of rats. Depending on temperature hyperthermic lesions vary from group to group of animals. At 41^0 we can observe just superficial lesions of the cerebral cortex penetrating 2, and very seldom 3 cerebral layers. Only a few cases of thrombosed cerebral microvessels have been observed in this group of animals.

The rise of temperature on 2 degrees of Celsius resulted in very severe lesions of cerebral tissue. Morphological changes are well pronounced and numerous thrombosed vessels are revealed.

The highest temperature (45^0C) caused most severe hyperthermic lesion of the cerebral tissue – complete destruction of the layered structure of the cortex in the area of hyperthermic exposure, numerous areas with lost neurons and thrombosed cerebral vessels with perivascular accumulation of erythrocytes were revealed.

Our results confirmed very high sensitivity of cerebral tissue to hyperthermic exposure even at a temperature of 41^0C and this finding suggests the need for very quick preventive actions if core body temperature for some reason (during sickness or changes of environmental temperature) is rising above 41^0C.

One of the most significant reasons for so extensive damage of nervous tissue under hyperthermic exposure is the formation of micro thrombi and occlusion of cerebral vessels. Stoppage of cerebral blood flow in the zone of vascular occlusion decreases of temperature clearance from the exposed area of the brain and that causes increase in temperature and aggravates the destructive action of hyperthermia.

In accordance with results of our experiments in the first stage of heating up to 43^0C lCBF sharply increases but later (on 20-30 minutes of heating) it falls down. At the earliest stage of 45^0C heating the level of lCBF is extremely low and then it stops. This kind of lCBF dynamic is more pronounced in Dextran T-500 pretreated animals. In this case decrease of lCBF on the second stage of 43^0C heating is sharply defined and in series of experiments with 450C local blood flow in temperature exposed area of cortex is not measurable at all. Injection of free radicals scavenger Dimethyl Sulfoxide gives an opposite to Dextran T-500 effect – late stage of 430C and first stage of 450C are slightly better, but in late stage of 45^0C lCBF still is not measurable.

In normal conditions the brain tissue temperature depends on local heat production, (a corollary of metabolic activity)**,** rate of cerebral blood flow and the temperature of the blood (Rossi et al, 2001). The effect of local cerebral hyperthermia on responses of pial microvessels of the mouse was investigated by F. El-Sabban and M. Fahim (1995). At the end of 50 minutes hyperthermic exposure (43.1^0C)**,** arterioles attained a constriction of 37% and thrombus formation was massive enough to occlude fully the microvessels. The same authors during hyperthermia have demonstrated numerous platelets in association with scattered red blood cells and occasional white blood cells in

a close proximity but not adhered, to the endothelial wall of hyperthermic brains. The site of platelet aggregation in both venules and arterioles was accompanied by focal endothelial lucency land denudation, vacuole formation, luminal membrane rupture and swelling of the nuclear envelope (Fahim, el-Sabban, 1995).

It is known that there is no independent, direct effect of dextran on vascular tone, but high molecular weight dextran can cause erythrocyte aggregation and affect blood flow [Tomiyama, Brian, Todd, 2000]. In experimental study Chen et al. (1989) have demonstrated fourfold rising of plasma viscosity while apparent blood viscosity was increased about twofold after administration of high molecular weight dextran (mol wt 500,000, 20% wt/vol). Same authors suggest that dextran-induced hyperviscosity leads to a compensatory vasodilatation in several vital organs [Chen et al., 1989].

Erythrocyte aggregation is modified in certain conditions; it tends to occur at the very low shear rates encountered in the venous circulation, where most thrombi occur [Freyburger et al., 1996]. As already has been mentioned besides increased aggregability of red blood cells number of other factors can contribute to rise of blood viscosity: increased haematocrit (polycythaemia), increased serum proteins, drop in temperature, impaired erythrocyte deformability due to various acquired or inherited disorders of red cell membrane or cytoplasm [Larcan, Stoltz, Gaillard, 1981]. Administration of Dextran T-500 (used in our experiments) leads to increase of erythrocyte aggregability index about twofold [Mantskava M., 2003]. When erythrocyte aggregation develops, the normal blood flow structuring inside of microvessels becomes inevitably disordered. This disturbs the blood rheological properties and results in a local slow down to a full stop of flow, even though the microvessel lumina and the pressure gradient along their course remain preserved [Mchedlishvili, Maeda, 2001].

Erythrocyte aggregation has been shown to affect venous vascular resistance and has been suggested to play a role in determining microcirculatory hemodynamics [Baskurt, Farley, Meiselman, 1997]. This phenomenon was found to be associated with different cardiovascular risk factors such as hypertension, hyperlipoproteinemia and smoking, miocardial ischemia, thromboembolic states, retinal venous occlusion and others [Hadengue et al., 1998].

To summarize all above-mentioned we can conclude that increased (by any reason) viscosity of blood can slow down blood flow and create favorable conditions for thrombosis especially in venous system but hyperthermic exposure in such cases presumably must hinder thrombogenic activity with simultaneous acceleration of fibrin formation [Pivalizza et al., 1999].

The study of blood flow response to changes in local tissue temperature has revealed that "blood flow acts as a feedback of local tissue temperature in a closed control system" [Xu, Zhu, Holmes, 1998].

The results of our experiments demonstrated, that the role of free radicals also has to be taken in account. As already has been mentioned, heat increases the flux of cellular free radicals [Flanagan et al., 1998]. It is well known that they are involved in the formation of thrombosis [Hillbom, 1999]. Ischemic condition that, as we have seen, develops in the late stage of 43^0C hyperthermia and in higher temperature also contributes in generation of free radicals and thereby promotes to development of vascular thrombosis. Using of Dimethyl Sulfoxide (DMSO) – an antioxidant, a scavenger of free radicals did not gave significant improvement in case of 41^0C hyperthermia, but in experiments with 43 and 45^0C notable improvement was observed in both: level of local cerebral

blood flow (less pronounced hyperthermic ischemia) and in smaller size of hyperthermia-induced cerebral lesion.

These observations give a good reason to consider cerebrovascular thrombosis as one of the most significant complication of brain hyperthermia. In case of deteriorated blood rheological properties hyperthermia-induced cerebral lesion is more remarkable. Administration of antioxidants, scavengers of free radicals can partially lessen hyperthermia induced cerebral lesion.

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LEGENDS

Fig. 1. Design of Experiment. Temperature of Artificial Cerebrospinal Fluid (ACSF) was maintained by Ultra-thermostat (UT) on 370C (Control Group), 41, 43 and 450C (respectively in the following three series of experiments). Core body temperature (measured rectally) was maintained on 370C by feedback controlled Infrared Lamp. ACSF heated up to desire temperature irrigates rats' brain surface during 60 minutes by means of peristaltic pump. The temperature on the cerebral surface and thermal clearance was measured by means of thermistor bead probe.

Figure 2. The absence of changes in sensory-motor cortex of rats' brain to lasting 60 minutes 370C temperature exposure. A – magnification: x15; B – framed area from the picture A, magnification: x40.

Figure 3. Sensory-motor cortex of the rats' brain; 60 minutes hyperthermia (410C); Arrows show the clear-cut edge of damaged tissue. A – magnification: x15; B – framed area from the picture A, magnification: x40.

Figure 4. Sensory-motor cortex of the Dextran T500 pretreated rats' brain; 60 minutes hyperthermia (410C); Arrows show the clear-cut edge of damaged tissue. A – magnification: x15; B – framed area from the picture A, magnification: x40.

Figure 5. Sensory-motor cortex of the Dimethyl sulfoxide (DMSO) pretreated rats' brain; 60 minutes hyperthermia (410C); Arrows show the clear-cut edge of damaged tissue. A – magnification: x15; B – framed area from the picture A, magnification: x40.

Figure 6. Sensory-motor cortex of rats' brain; 60 minutes hyperthermia (430C); Arrows show the clear-cut edge of damaged tissue. A – magnification: x15; B – framed area from the picture A, magnification: x40.

Figure 7. Sensory-motor cortex of the Dextran T500 pretreated rats' brain; 60 minutes hyperthermia (430C); Arrows show the clear-cut edge of damaged tissue. A – magnification: x15; B – framed area from the picture A, magnification: x40.

Figure 8. Sensory-motor cortex of the Dimethyl sulfoxide (DMSO) pretreated rats' brain; 60 minutes hyperthermia (430C); Arrows show the clear-cut edge of damaged tissue. A – magnification: x15; B – framed area from the picture A, magnification: x40.

Figure 9. Sensory-motor cortex of rats' brain; 60 minutes hyperthermia (450C); Arrows show the clear-cut edge of damaged tissue. A – magnification: x15; B – framed area from the picture A, magnification: x40;

Figure 10. Sensory-motor cortex of the Dextran T-500 pretreated rats' brain; 60 minutes hyperthermia (450C); Arrows show the clear-cut edge of damaged tissue. A – magnification: x15; B – framed area from the picture A, magnification: x40.

Figure 11. Sensory-motor cortex of the Dimethyl sulfoxide (DMSO) pretreated rats' brain; 60 minutes hyperthermia (450C); Arrows show the clear-cut edge of damaged tissue. A – magnification: x15; B – framed area from the picture A, magnification: x40.

Figure 12. Temperature dependent changes in volume of hyperthermia-induced cerebral lesions in Normal, Dextran T-500 and Dimethyl Sulfoxide (DMSO) pretreated rats.

Figure 13. Percentage of hyperthermia-induced local blood flow (lCBF) changes in cerebral cortex of normal rats. lCBF was measured on 5-10 and 20-30 minutes after beginning of heating.

Figure 14. Percentage of hyperthermia-induced local blood flow (lCBF) changes in cerebral cortex of Dextran T-500 pretreated rats. lCBF was measured on 5-10 and 20-30 minutes after beginning of heating.

Figure 15. Percentage of hyperthermia-induced local blood flow (lCBF) changes in cerebral cortex of Dimethyl Sulfoxide (DMSO) pretreated rats. lCBF was measured on 5-10 and 20-30 minutes after beginning of heating.