NEUROPAT. POL., 1984, 22, 3
PL ISSN 0028-3894

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INFLUENCE OF INDOMETHACIN ON THE ULTRASTRUCTURAL PATHOLOGY OF THE BRAIN FOLLOWING TEMPORARY ISCHEMIA IN MONGOLIAN GERBILS (*MERIONES UNGUICULATUS*)

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It has been repeatedly demonstrated that, cerebral ischemia is followed by an increased content of various classes of prostaglandins in the brain tissue (Ruszczewski 1977; Gaudet, Levine 1979; Gaudet et al. 1980; Iannotti et al. 1981; Moscovitz, Coughlin 1981a; Bhakoo et al. 1982). This led to a supposition, that they may be, at least in part, responsible for the development of postischemic brain tissue alterations. Theoretically, the damaging effect of prostaglandins may result from either their vasoactive function and induction of intravascular thrombocytic aggregations or their destabilizing activity on the cell membranes of the nervous system, regardless unknown exact mechanism(s) of the latter. All elements of the above mentioned pathology were shown to follow cerebral ischemia. The other studies demonstrated the preventive effect of inhibition of the cyclo-oxygenase activity on the development of postischemic events in the brain, which may participate in the nerve tissue lesions (Furlow, Hallenbeck 1978; Moscovitz, Coughlin 1981b; Boulu et al. 1982; Bhakoo et al. 1982; Kägström et al. 1983). In most of the studies this effect was obtained with the use of indomethacin, a well known potent inhibitor of prostaglandin synthesis (Abdel-Halim et al. 1978).

Our previous studies (Mossakowski, Kwiatkowska-Patzer 1982, 1983), carried out on Mongolian gerbils in which cerebral ischemia was produced by bilateral occlusion of common carotid arteries for 30 min, showed that indomethacin applied before cerebral ischemia prevented neither postischemic venous hyperemia nor the appearance of secondary ischemic foci located in the border-line zones between watersheds of larger cerebral arteries. On the contrary, it obviously prevented the occurrence of small patchy ischemic foci, spread over the most of the brain grey structures in the postischemic period. Tissue lesions in indo-

methacin-treated animals as compared with those in untreated ones, appeared later, were less extensive and solely restricted to posterior hypothalamic, septal and dorso-lateral thalamic regions. The remaining brain structures, including cerebral cortex, white matter and basal ganglia were apparently normal. The aim of the present investigations was an electron microscope evaluation of early tissue lesions and their evolution in animals in which temporary brain ischemia was preceded by indomethacin treatment. The electron microscope examination included both areas of the brain which under light microscope were apparently normal and those which showed severe tissue alterations.

MATERIAL AND METHODS

The experiments were carried out on adult Mongolian gerbils of both sexes, weighing 55—70 g in which under light ether anesthesia both common carotid arteries were ligated for a period of 30 min. In all animals tracheostomy was performed, which was used in case of spontaneous respiration disturbances for artificial ventilation with room air through mechanical respiratory pump (Medipan, Poland). The full surgical procedure was presented previously (Kapuściński, Mossakowski 1983).

The experimental animals were divided into two groups. The first group of animals (n=9), 45 min prior to carotid ligation were given intraperitoneally a single injection of indomethacin (Merck-Sharp and Dohme Res. Lab. USA), dissolved in Krebs-Ringer solution in a dose of 10 mg/kg body weight. Indomethacin was prepared immediately before each experiment by dissolving powdered substance in 70% ethanol and then in 5 ml of CO_2-saturated Krebs-Ringer solution. The second group of animals instead of indomethacin were given intraperitoneal injection of the solvent. The animals of both experimental groups were sacrificed on 2, 4 and 6 hr after release of carotid arteries by intracardiac perfusion with 2 percent glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. Following removal from the skull the brains were washed in the same solution and then cut coronally into slices 1 mm thick. Tissue blocks (1 mm^3 in size) were taken from the frontal cortex, Ammon's horn, subcortical white matter, thalamus, septal area and posterior hypothalamus. The latter 3 blocks were cut in these regions in which light microscopy revealed tissue damage, resistant to indomethacin action (Mossakowski, Kwiatkowska-Patzer 1983). Tissue blocks were further postfixed for 1 hr in 2% osmium tetraoxide in 0.1 M cacodylate buffer, pH 7.2, dehydrated routinely in graded ethanol solutions and embedded in Epon 812. Ultrathin sections, cut on ultramicrotome, were counterstained on grids with uranyl acetate and lead citrate. The material was studied under the Jeol electron microscope JEM 7 A.

In order to visualize the blood vessels content in the examined material part of animals (3 in each experimental group) were sacrificed by decapitation. Fresh tissue blocks taken from the brain regions mentioned above, were fixed by immersion in 0.1 M glutaraldehyde in cacodylate buffer, pH 7.3 and then processed routinely to embedding in Epon 812.

Additional control groups of animals were used for assessment of possible direct influence of indomethacin and surgical procedure. Four animals with no surgery were given identical dose of indomethacin and then sacrificed in pairs after 2 and 6 h. In 4 others 45 min after indomethacin injection, sham-operation, consisting in bilateral isolation of common carotid arteries, was performed. Animals were sacrificed 2 and 6 h after operation. The last control group consisted of 4 gerbils in which sham operation was not preceded by indomethacin administration. The material from all control groups was processed in the same manner as that in experimental ones.

RESULTS

The electron microscope tissue lesions were found in both experimental groups, although they differed remarkably in the intensity and topographic distribution.

In animals, in which bilateral carotid artery ligation was not preceded by indomethacin administration, contrary to the previous light microscopic observations, grey structure alterations were present in all examined areas of the brain already in the earliest stages of the study. At the beginning they consisted in severe swelling of astrocytic cytoplasm involving both perikarya and cellular processes. In all areas of the brain, numerous astrocytes with remarkably swollen cytoplasm, either totally deprived of subcellular organellae or showing their abnormal aggregation were present (Fig. 1). Swollen mitochondria with peripherally displaced cristae, widened channels and cysterns of rough endoplasmic reticulum with reduced number of ribosomes and numerous dense bodies were seen amongst preserved cytoplasmic organellae. The same concerned astrocytic processes, either surrounding capillaries or lying free in the neuropil. Most of the other structural elements of neuropil were normal. In most instances the capillary walls were unchanged, only some of them revealing swelling of endothelial cells (Fig. 2). At that period of observation neuronal perikarya were either normal or showed ultrastructural alterations of minor intensity. They consisted in some mitochondrial swelling and dilatation of channels of rough endoplasmic reticulum (Fig. 2). Typical ischemic dark neurons, with electron dense hyaloplasm, severely distended channels of rough endoplasmic reticulum, and remarkably swollen mitochondria were rare. As a rule, they were accompanied by swollen glial satellite cells (Fig. 3).

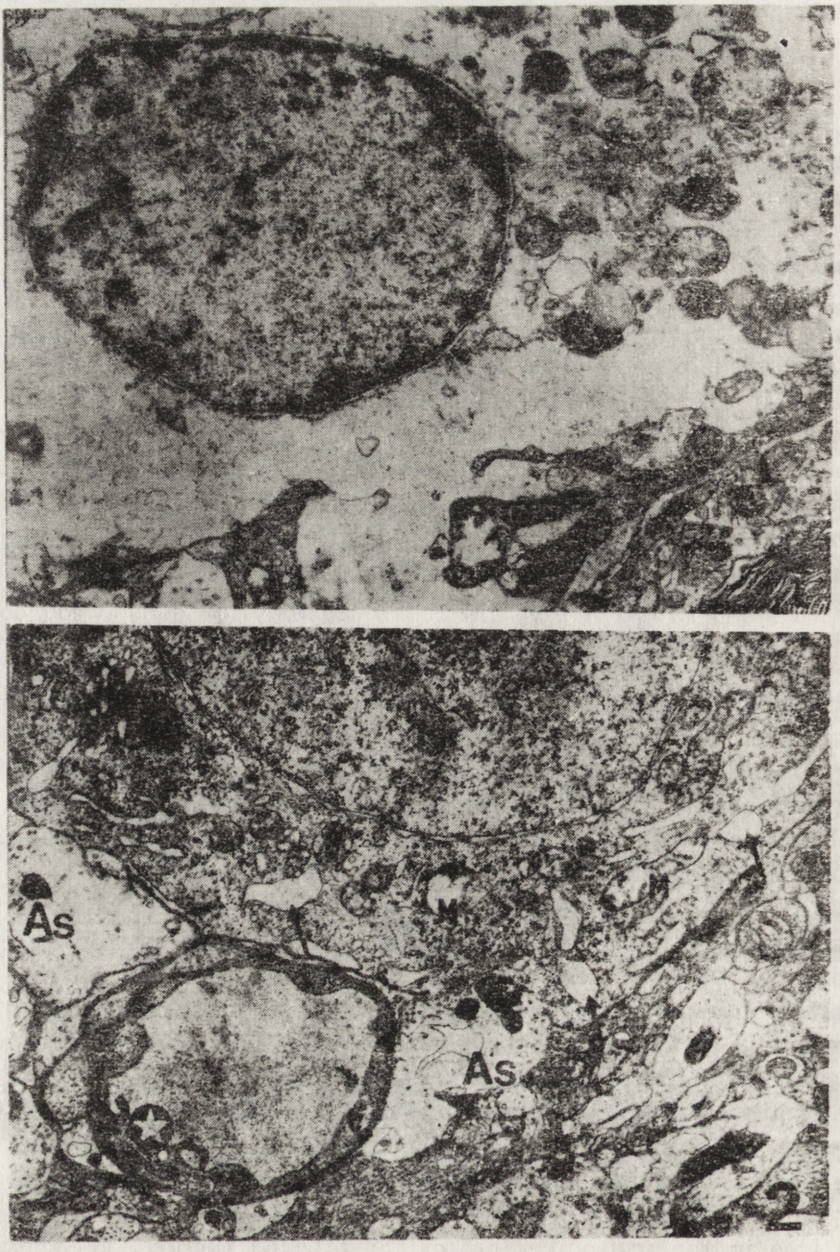


Fig. 1. Ischemia, 2 h survival. Hypothalamus. Fragment of astrocyte with remarkably swollen cytoplasm and reduced number of abnormal organelles grouped in perinuclear area. × 12 700

Ryc. 1. Niedokrwienie, 2 godz. przeżycia. Podwzgórze. Fragment obrzmiałej komórki gwiaździstej ze skąpą ilością zmienionych organelli cytoplazmatycznych zgrupowanych w okolicy przyjądrowej. Pow. 12 700 ×

Fig. 2. Ischemia, 2 h survival. Cerebral cortex. In the fragment of neuron (N) some mitochondria (M) are swollen and channels of rough endoplasmic reticulum dilated (arrows). Note swollen astrocytic processes (As) and endothelial cells (asterisk). × 6 000

Ryc. 2. Niedokrwienie, 2 godz. przeżycia. Kora mózgowa. We fragmencie komórki nerwowej (N) widoczne są obrzmiałe mitochondria (M) i poszerzone kanały szorstkiej siatki śródplazmatycznej (strzałki). Zwraca uwagę obrzmienie wypustek astrocytów (As) i komórek śródbłonka (gwiazdka). Pow. 6 000 ×

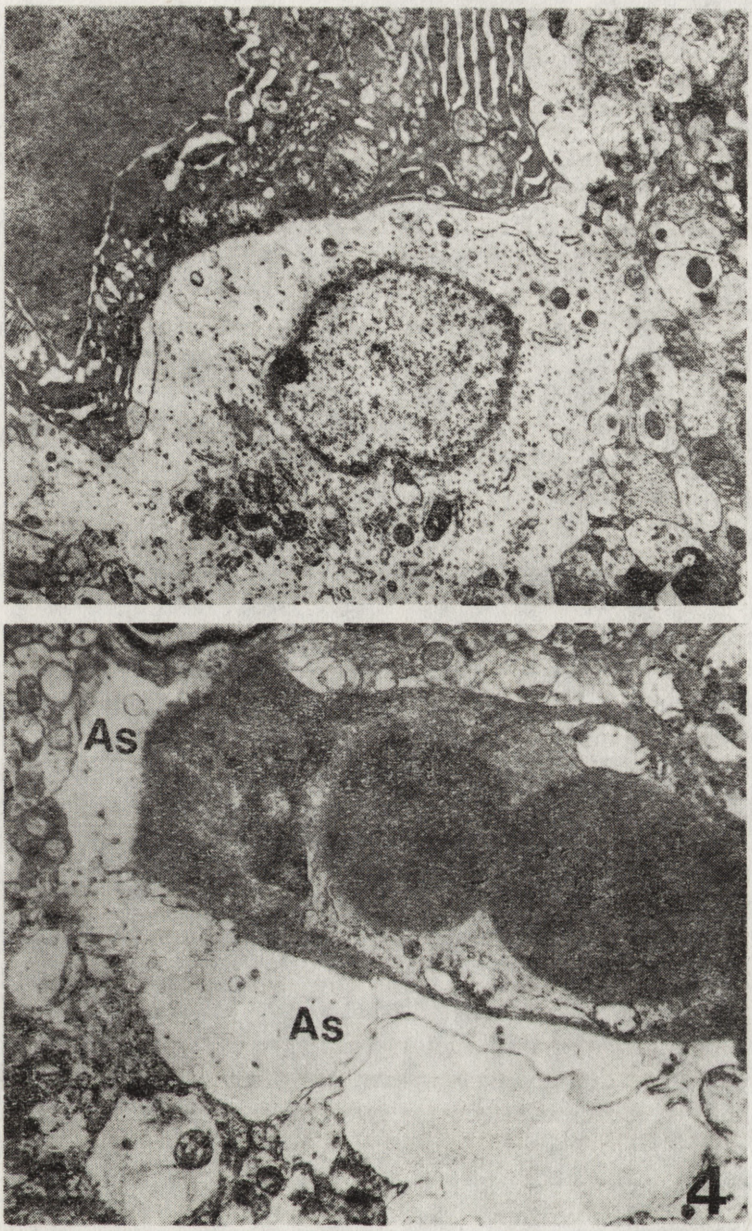


Fig. 3. Ischemia, 2 h survival. Cerebral cortex. Fragment of a typical ischemic dark neuron and swollen satellite glial cell with irregular aggregation of cytoplasmic organelles. × 5 700

Ryc. 3. Niedokrwienie, 2 godz. przeżycia. Kora mózgu. Widoczny fragment typowego ciemnego neuronu i obrzmiałej satelitarnej komórki glejowej z nieregularnymi skupieniami organelli cytoplazmatycznych. Pow. 5 700 ×

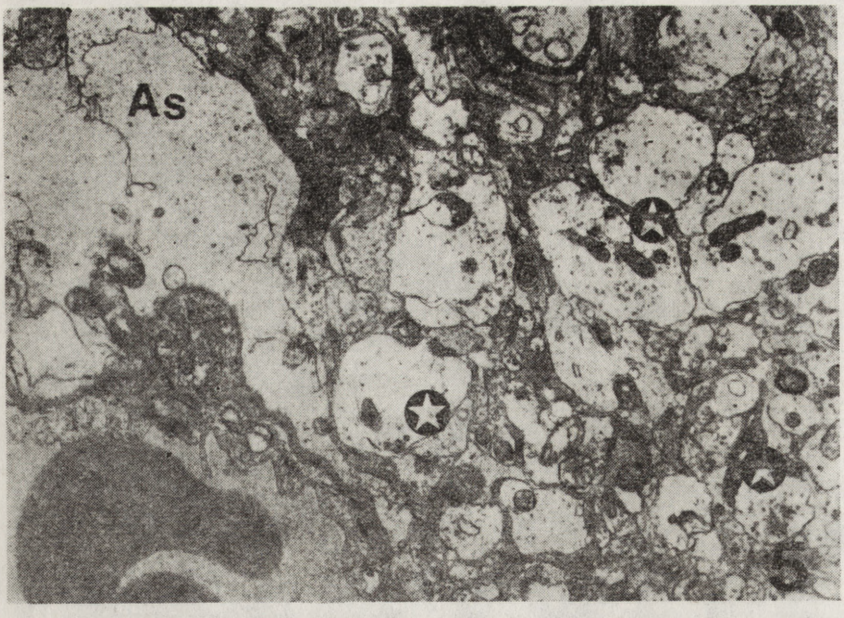
Fig. 4. Ischemia, 4 h survival. Cerebral cortex. Note extensively swollen perivascular astrocytic processes (As), totally deprived of cytoplasmic organelles. × 14 200

Ryc. 4. Niedokrwienie, 4 h godz. przeżycia. Kora mózgu. Bardzo znacznie obrzmiałe okołonaczyniowe wypustki astrocytów (As), całkowicie pozbawione struktur cytoplazmatycznych. Pow. 14 200 ×

Pathological changes intensified and become more widespread in the later period of observation, that is 4 h following the release of carotid arteries ligation. They were most severe in hypothalamic, septal and thalamic areas. Astrocytic processes surrounding capillaries were distended in a baloon-like fashion and their cytoplasm was totally deprived of any subcellular organelles (Figs 4, 5). The same concerned oligodendrocytes. Ultrastructural abnormalities involved neuropil elements such as axons, dendrites and synapses. Most of them were swollen, contained damaged mitochondria and widened cysterns and channels of either smooth or rough endoplasmic reticulum (Fig. 5). In most of the presynaptic bags, vesicles were abnormally dispersed. Great proportion of neurons showed severe ischemic alterations with cytoplasmic disintegration of varying intensity. On the 6th h after carotid ligation release, the above described changes were more intensive, evolving to full tissue necrosis (Fig. 6).

The white matter structures were also involved, starting from the earliest period of observation. Similarly as in the grey structures, pathological changes consisted in remarkable cytoplasmic swelling of both oligodendrocytes and astrocytes (Fig. 7). Perivascular astrocytic processes were most severely damaged. The membrane structures of numerous of them were entirely disrupted, leading to the situation, that capillaries with either swollen or normal endothelial cells were lying in empty spaces, containing some cellular residua (Fig. 8). Myelin sheaths were generally unchanged, while axons contained swollen mitochondria and vesicular structures originating probably from channels of smooth endoplasmic reticulum (Fig. 7). Neither intravascular clotting nor thrombocyte aggregations were found at any observation period.

Electron microscopy in indomethacin-treated animals showed an evident time-dependence and varied remarkably in different brain structures. In sections taken from the previously mentioned areas of hypothalamus, septum and dorso-lateral thalami the ultrastructural picture and its evolution did not differ essentially from those in untreated animals. There was severe astrocytic swelling, most intense around capillary walls, which were in many instances lined by greatly swollen endothelial cells (Fig. 9). The above changes intensified in time, involving other structures of neuropil (Fig. 10). Most of the neurons revealed typical ischemic alterations. Even those with less intensive lesions were surrounded by greatly swollen astrocytic processes and damaged nerve cell extensions and their endings (Fig. 11). Contrary to that, in the cerebral cortex and in Ammon's horn no ultrastructural abnormalities were seen in the earliest period of examination, that is 2 h following carotid release. In particular there was no astrocytic swelling both in the capillary surrounding and in the neuropil remote from the blood vessels (Fig. 12). Nerve cells were ultrastructurally normal. The same was



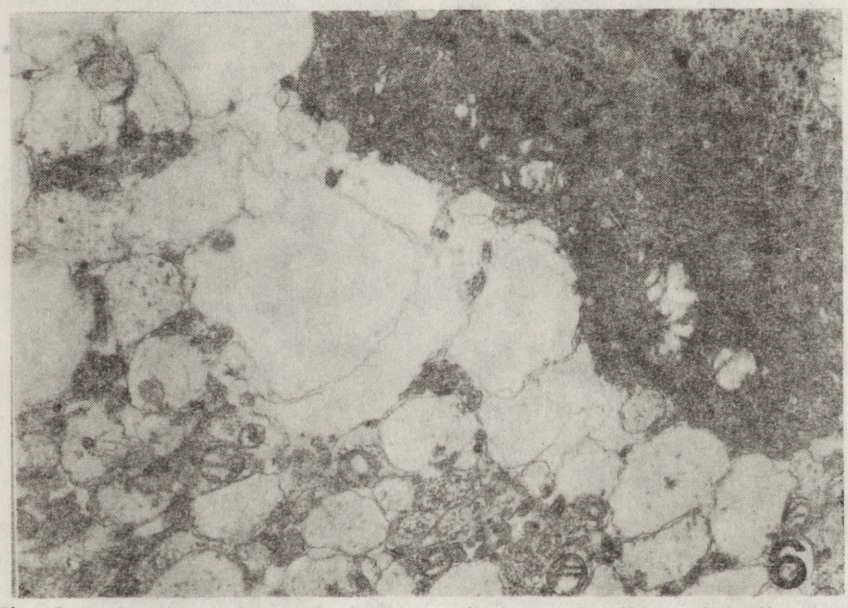
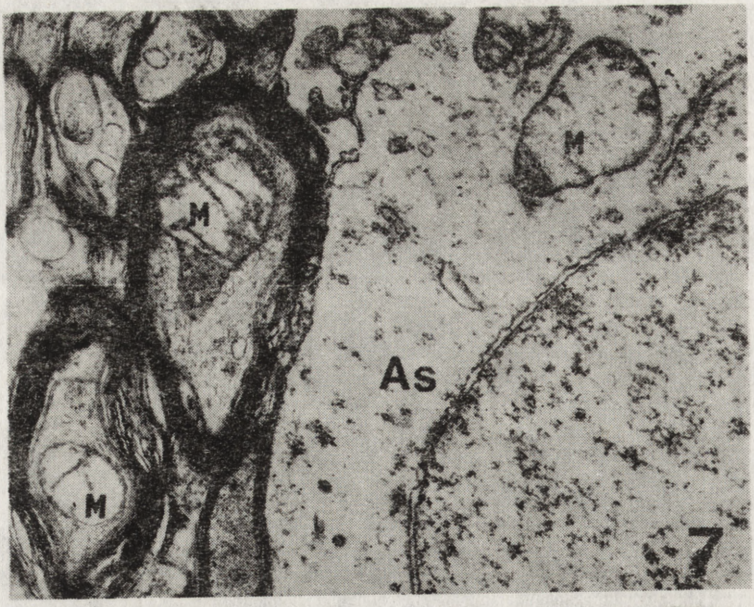


Fig. 5. Ischemia, 4 h survival. Ammon's horn. Swollen astrocytic processes (As) with reduced number of cytoplasmic organelles and swollen mitochondria. In neuropil adjacent to the blood vessels synapses (asterisks) with swollen axoplasm, irregularly distributed vesicles and small, shrunken mitochondria are seen. × 8 500

Ryc. 5. Niedokrwienie, 4 godz. przeżycia. Róg Amona. Widoczne okołonaczyniowe wypustki astrocytów (As) są obrzmiałe i zawierają nieliczne struktury śródplazmatyczne. Zwraca uwagę obrzmienie aksoplazmy zakończeń synaptycznych (gwiazdki) z nierównomiernym rozkładem pęcherzyków i obecnością drobnych, obkurczonych mitochondriów. Pow. 8 500 ×

Fig. 6. Ischemia, 6 h survival. Hypothalamus. Fragment of neuron and neuropil elements exhibit severe ultrastructural abnormalities indicating their complete disintegration. × 12 700

Ryc. 6. Niedokrwienie, 6 godz. przeżycia. Podwzgórze. Fragment komórki nerwowej i elementy neuropilu wykazują ciężkie uszkodzenia ultrastrukturalne wskazujące na ich dezintegrację. Pow. 12 700 ×



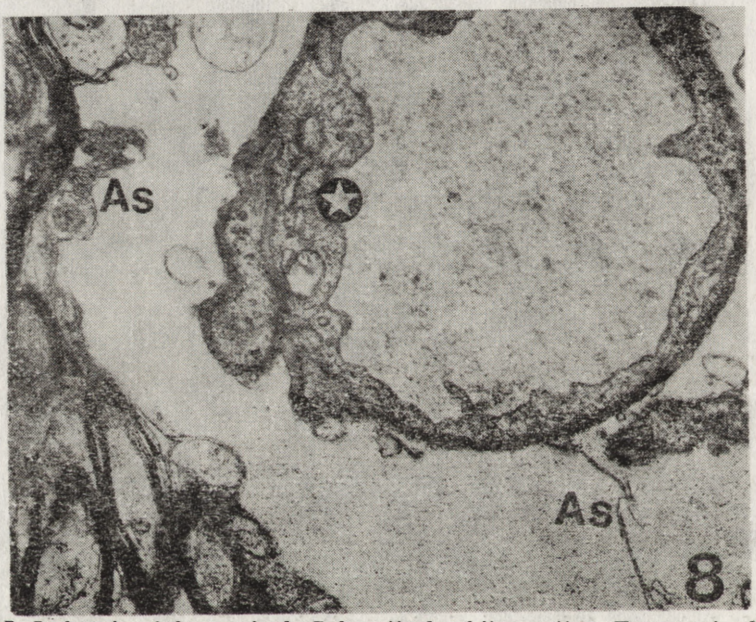


Fig. 7. Ischemia, 4 h survival. Subcortical white matter. Fragment of severely swollen astrocyte (As). Neighbouring nerve fibers with unchanged myelin sheaths reveal swollen mitochondria (M) and small vesicular structures in the axoplasm. × 12 700

Ryc. 7. Niedokrwienie, 4 godz. przeżycia. Podkorowa istota biała. Widoczny fragment znacznie obrzmiałego astrocyta (As). Położone w sąsiedztwie aksony, z nieuszkodzonymi osłonkami mielinowymi zawierają w aksoplazmie obrzmiałe mitochondria (M) i drobne struktury pęcherzykowate. Pow. 12 700 ×

Fig. 8. Ischemia, 6 h survival. Subcortical white matter. Severely swollen perivascular astrocytic processes (As), almost totally deprived of cytoplasmic organelles with disrupted cellular membranes. Note swelling of the capillary endothelial cells (asterisk). × 12 700

Ryc. 8. Niedokrwienie, 6 godz. przeżycia. Podkorowa istota biała. Znacznie obrzmiałe okołonaczyniowe wypustki astrocytów (As), niemal całkowicie pozbawione struktur cytoplazmatycznych z uszkodzonymi błonami komórkowymi. Zwraca uwagę obrzmienie komórek śródbłonka naczyniowego (gwiazdka). Pow. 12 700 ×

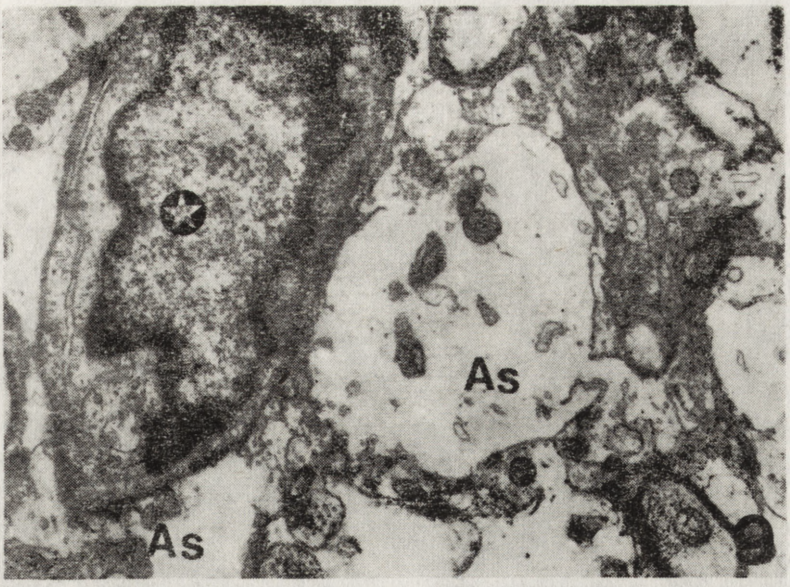
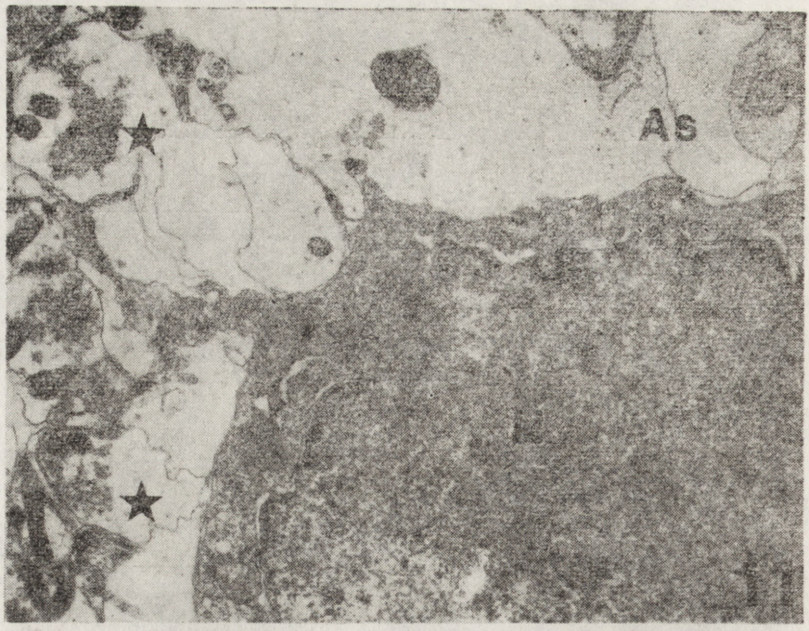


Fig. 9. Ischemia with indomethacin, 6 h survival. Thalamus. Remarkably swollen perivascular astrocytic processes (As) with reduced number of cytoplasmic organelles. Swollen endothelial cells (asterisk) narrowing the capillary lumen. × 7 400

Ryc. 9. Niedokrwienie z indometacyną, 6 godz. przeżycia. Wzgórze. Bardzo znaczne obrzmienie okołonaczyniowych wypustek astrocytów (As) zawierających szczątkowe struktury cytoplazmatyczne. Widoczne jest obrzmienie komórek śródbłonka (gwiazdka) zwężające światło naczynia. Pow. 7 400 ×

Fig. 10. Ischemia with indomethacin, 6 h survival. Thalamus. Note few shrunken mitochondria and vacuoles (v) in slightly swollen perivascular astrocytic processes (As) and varying in number vesicles in synaptic buttons (asterisks) × 9 000

Ryc. 10. Niedokrwienie z indometacyną, 6 godz. przeżycia. Wzgórze. Zwracają uwagę skąpe obkurczone mitochondria i wakuole (v) w cytoplazmie lekko obrzmiałych okołonaczyniowych wypustek astrocytów (As) i zmienna ilość pęcherzyków w zakończeniach synaptycznych (gwiazdki). Pow. 9 000 ×



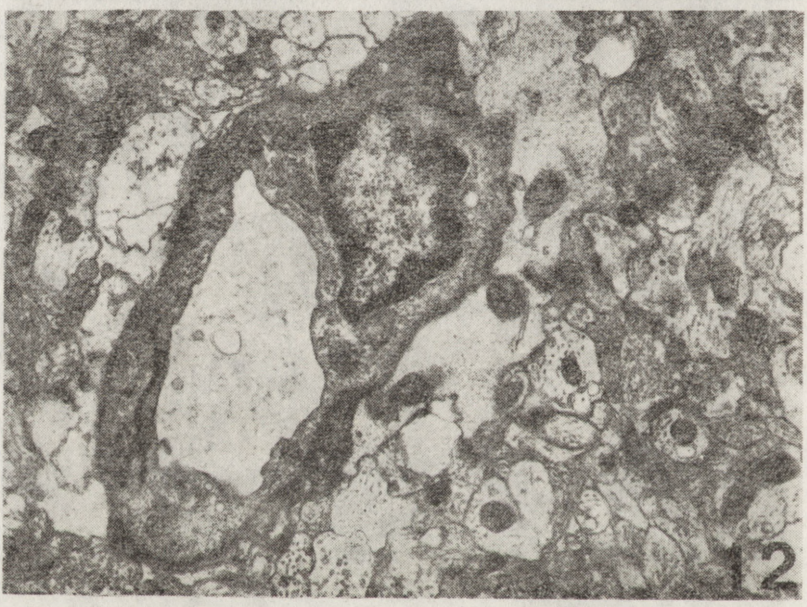


Fig. 11. Ischemia with indomethacin, 6 h survival. Hypothalamus. Fragment of ischemic neuron (N). Synaptic endings (asterisks) exhibit swollen axoplasm with great number of vesicles. Note greatly swollen astrocytic processes (As) with reduced number of cytoplasmic organelles. × 12 700

Ryc. 11. Niedokrwienie z indometacyną, 6 godz. przeżycia. Podwzgórze. Fragment neuronu (N) z typowymi cechami uszkodzenia niedokrwiennego. Zakończenia synaptyczne (gwiazdki) o obrzmiałej cytoplazmie zawierają znaczną ilość pęcherzyków; skupionych w nieregularnych ugrupowaniach. Obrzmiałe wypustki astrocytów (As) pozbawione struktur cytoplazmatycznych. Pow. 12 700 ×

Fig. 12. Ischemia with indomethacin, 2 h survival. Cerebral cortex. Note only slight swelling of some astrocytic processes around unchanged blood vessels. × 12 700

Ryc. 12. Niedokrwienie z indometacyną, 2 godz. przeżycia. Kora mózgu. Wokół naczynia o prawidłowej strukturze widoczne są tylko pojedyncze wypustki astrocytów z nieznacznym obrzmieniem. Pow. 12 700 ×

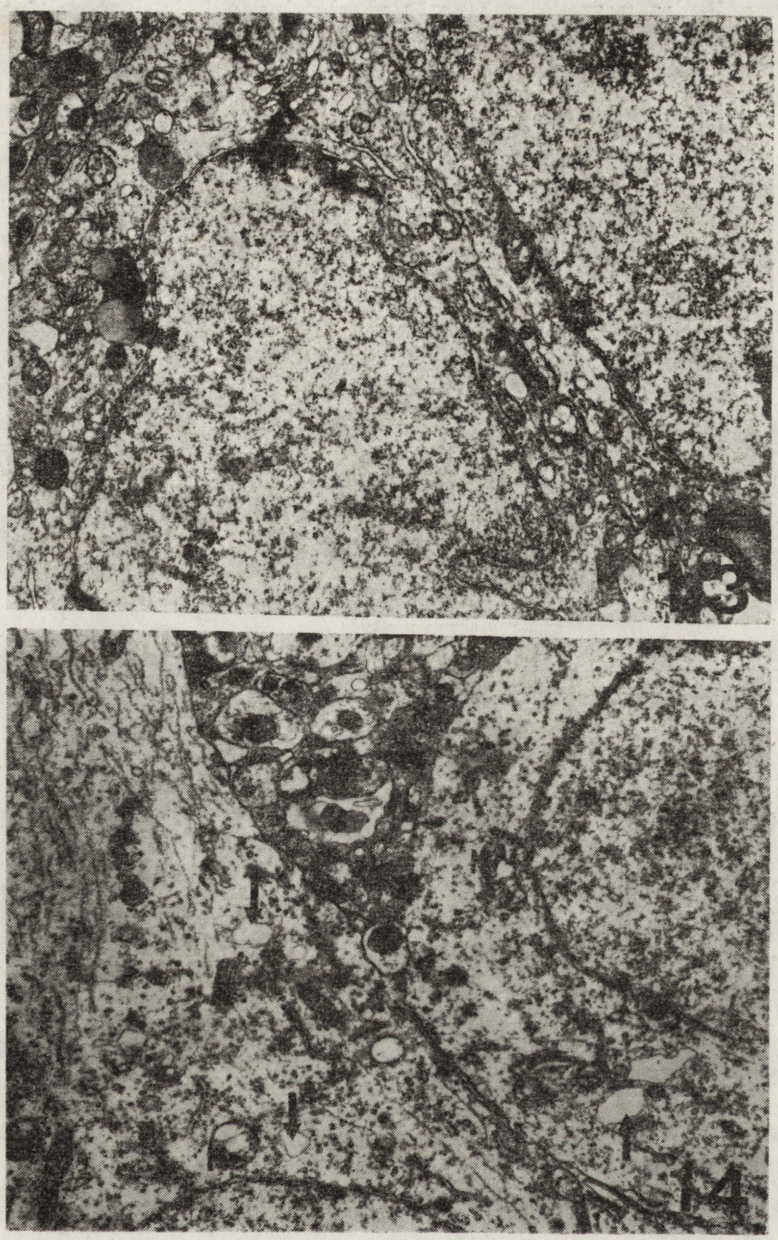


Fig. 13. Ischemia with indomethacin, 4 h survival. Ammon's horn. Fragments of two unchanged pyramidal neurons. × 8 500

Ryc. 13. Niedokrwienie z indometacyną, 4 godz. przeżycia. Widoczne fragmenty dwóch niezmienionych piramidowych komórek nerwowych. Pow. 8 500 ×

Fig. 14. Ischemia with indomethacin, 6 h survival. Cerebral cortex. In a fragment of apparently normal neuron, some swollen mitochondria (M), dilated Golgi profiles and channels of rough endoplasmic reticulum are visible (arrows). × 6 400

Ryc. 14 Niedokrwienie z indometacyną, 6 godz. przeżycia. Kora mózgu. We fragmencie skądinąd niezmienionej komórki nerwowej widoczne są pojedyncze obrzmiałe mitochondria (M) oraz poszerzone kanały siatki śródplazmatycznej i zbiorniki aparatu Golgiego (strzałki). Pow. 6 400 ×

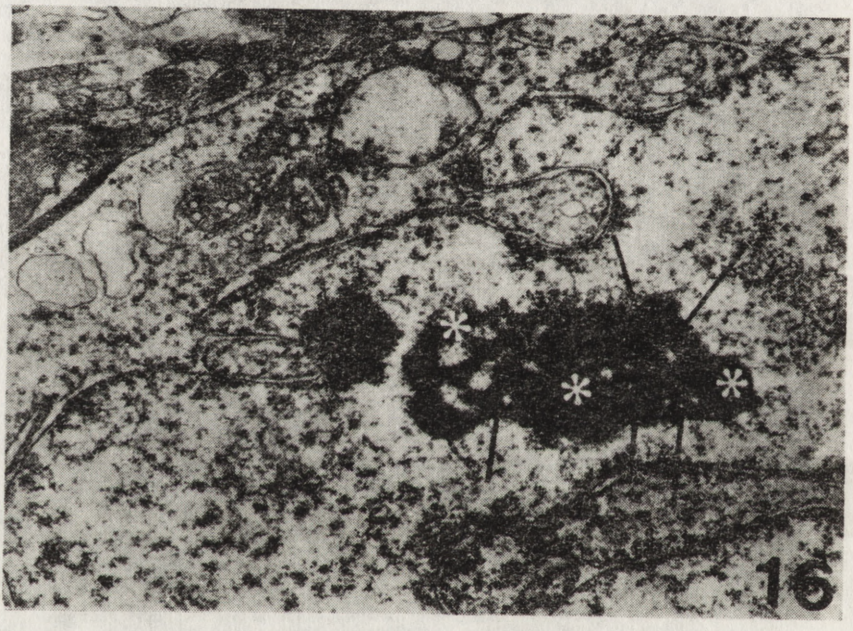


Fig. 15. Ischemia with indomethacin, 4 h survival. Subcortical white matter. Apparently normal ultrastructure of most of the axons, myelin sheaths and glial processes. × 12 700

Ryc. 15. Niedokrwienie z indometacyną, 4 godz. przeżycia. Podkorowa istota biała. Prawidłowy obraz mikroskopowo-elektronowy większości włókien osiowych, osłonek mielinowych i wypustek glejowych. Pow. 12 700 ×

Fig. 16. Ischemia with indomethacin, 6 h survival. Thalamus. Neuron with features of nucleolar microsegregation. Note partial segregation of filamentous (arrows) and granular (asterisks) parts of nucleolus. × 12 700

Ryc. 16. Niedokrwienie z indometacyną, 6 godz. przeżycia. Wzgórze. Neuron z cechami mikrosegregacji jąderka. Zwraca uwagę częściowy rozdział włókienkowej (strzałki) i ziarnistej (gwiazdki) części jąderka. Pow. 12 700 ×

observed after 4 h of recovery (Fig. 13). However, on the 6th h of the postischemic period some ultrastructural abnormalities appeared in the cerebral cortex. They consisted in a slight perivascular astrocytic swelling, dilatation of cysterns and channels of endoplasmic reticulum and Golgi system and mitochondrial swelling in some nerve cells (Fig. 14). On the contrary, the white matter structures were apparently normal in all observation stages (Fig. 15).

In all experimental and control animals treated with indomethacin, great number of neurons and glial cells revealed nucleolar abnormalities taking the form of microsegregation and accumulation of perichromatin granules (Fig. 16). Except that, no other ultrastructural pathological changes were found in the control groups throughout.

DISCUSSION

In general, the present electron microscope study confirmed our previous light microscopic observation, concerning influence of cyclo-oxygenase inhibition by indomethacin on the tissue sequela of temporary brain ischemia. Indomethacin administered 45 min before carotid arteries ligation for 30 min did not prevent the appearance or influenced further evolution of focal tissue lesions localized in posterior hypothalamic and septal regions as well as in dorso-lateral thalamic nuclei. Pathological changes in these brain regions in indomethacin-treated animals did not differ essentially from those in untreated ones. In the course of 6 h observation they evolved to full tissue necrosis, the subsequent stages of which were similar to those described by Brierley et al. (1973), Garcia et al. (1978) and Jenkins et al. (1979). The only slight difference between the treated and untreated animals consisted in less intense astrocytic swelling in the former ones. Our previous supposition, concerning the possible appearance of thrombocyte aggregations in the blood vessels on the necrosis periphery was not confirmed. The aggregations were totally absent from both treated and untreated animals. This agrees well with previous observations of Hossmann et al. (1980).

In the selectively damaged brain areas, in the previous studies on the influence of indomethacin on the cerebral microcirculation following brain ischemia, focal ischemic foci were found in the recovery period both in untreated and treated animals (Mossakowski, Kwiatkowska-Patzer 1982). Although the exact brain angioarchitectonics in Mongolian gerbils is not known, a detailed inspection of the material presented by Levy and Brierley (1974) and Tamura et al. (1981) allows to consider the above mentioned regions as the borderline zones between watersheds of larger branches of the internal carotid arteries and the carotid and vertebral-basilar systems. Since the classical work of Zülch (1955), the

vascular borderline zones are known to be the areas of severe hemodynamic disturbances, depending on the systemic hemodynamics. The presence of the latter in the Mongolian gerbils after both uni- and bilateral carotid artery ligation was clearly shown by Ito et al. (1975) and Kapuściński and Mossakowski (1983).

Indomethacin pretreatment markedly influenced the electron microscope picture of the remaining brain structures. In untreated animals ultrastructural tissue pathology, the same as in the above described areas, was present in such brain structures as neocortex, Ammon's horn and subcortical white matter. The most striking abnormality consisted in glial swelling, involving mostly astrocytic perikarya and perivascular processes, and ischemic lesions of neurons including their extensions and synaptic endings. In indomethacin-treated animals pathological changes of this nature were not present at all or they appeared in much less intense form solely on the 6th hour of postischemic period.

The mechanism of the preventive action of indomethacin remains open. As an inhibitor of cyclo-oxygenase activity it remarkably reduces the synthesis of endogenous prostaglandins in the brain. Abdel-Halim et al. (1978) showed that single application of indomethacin in a dose of 3 mg/kg b.w. decreases cerebral synthesis of prostaglandins to 20 percent for a period of 8 h. This led to the supposition of its protective effect against the action of vasoactive prostaglandins, accumulation of which in the brain after ischemia of different type and duration was found by Ruszczewski (1977), Gaudet et al. (1979, 1980), Iannotti et al. (1981), Moscovitz, Coughlin (1981a) and Bhakoo et al. (1982). Improvement of the cerebral blood flow after ischemia, resulting from indomethacin pretreatment was described by Furlow and Hallenbeck (1978), Moscovitz and Coughlin (1981b), Boulu et al. (1982) and Kägström et al. (1983). However, Hallenbeck and Furlow (1979) and Hallenbeck et al. (1982) demonstrated the positive effect of the drug only when indomethacin was applied in combination with prostacycline PGI_2 and heparin. On the other hand, Harris et al. (1982) noticed even a harmful effect of indomethacin on the ischemic brain. Our own observations on the influence of the drug on the postischemic cerebral microcirculation indicated its partial efficacy, consisting in prevention of patchy secondary ischemic foci, spread over most of the grey brain structures with no effect on postischemic venous hyperemia and focal secondary ischemia in vascular borderline zones (Mossakowski, Kwiatkowska-Patzer 1982).

Even more controversial are the results concerning the influence of indomethacin on the blood flow in undamaged brain. Most of the authors have demonstrated that indomethacin administration results in reduction of the cerebral blood flow and decrease of vascular reactivity, to the increased CO_2 level (Pickard, MacKenzie 1973; Pickard et al. 1977; Hedquist 1977; Bill 1979; Sakabe, Siesjö 1979; Dahlgren et al. 1981).

On the contrary Busija and Heistadt (1983) found that indomethacin influenced neither cerebral blood flow nor vascular reactivity to hypercapnia. According to Hierton's observation (1981) indomethacin does not reduce cortical blood flow, but diminishes it in the brain stem structures and cerebellum. Similar regional differences were observed by Pickard et al. (1976) and Vlachov (1976), who stated that indomethacin at the same time reduced the total cerebral blood flow but dilates pial vessels.

In the light of the above presented data and the results of own electron microscope observations, the solely vasogenic mechanism of the protective action of indomethacin seems questionable. Its cytoprotective action, preventing alterations of cellular membrane permeability and their metabolic consequences, is more probable. The fact that early ultrastructural pathology of the ischemic brain is dominated by severe astrocytic swelling, which is lacking in indomethacin-treated animals, appears to support such a view. This concept was also brought up by Crockard et al. (1980), Iannotti et al. (1981), Bhakoo et al. (1982) who related the preventive effect of indomethacin on postischemic brain edema to its cytoprotective action. Ischemic brain edema, at least in its early stages, is of cytotoxic nature, resulting from altered permeability of cellular membranes (Klatzo 1975). Astrocytic swelling is one of its fundamental ultrastructural evidences. As swollen astrocytic processes are known to compress cerebral capillaries, it seems possible that the preventive influence of indomethacin on the disturbances of regional cerebral flow can also be connected with its effect on membrane permeability. This would also explain lack of patchy ischemic foci spread over grey structures of the brain in indomethacin-treated animals.

The molecular mechanism of cytoprotective action of indomethacin is unknown. Perhaps it can be related to its inhibitory influence on the activity of phospholipase A_2 (Jesse, Franson 1979). Volpi et al. (1980) demonstrated that indomethacin reduced the permeability of the cellular plasma membrane for calcium. Its antagonistic action to calcium ions was also shown by Northover (1977). Pappius and Wolf (1977) although denying the influence of indomethacin on the vasogenic brain edema, demonstrated its protective effect on numerous metabolic parameters in the brain damaged by various factors.

Two other questions concerning indomethacin action require short discussion. The first is its time-dependence shown in our electron microscope study. Preventive effect of indomethacin against generalized astrocytic swelling and ischemic damage to the nerve cells was clearly shown to occur during the early stages of postischemic period. It was obviously less at the 6th h of recovery period, as shown by the appearance of slight perivascular astrocytic swelling and some features of nerve cells injury. It seems plausible to connect this with the relatively short half-

-life of the drug. In our previous study, when keeping ischemic animals for longer survival periods, indomethacin was administered every subsequent 6 h.

The second question concerns the presence of a rather unexpected finding in the brains of indomethacin-treated animals. In all of them ultrastructural features of nucleolar microsegregation were seen in both nerve and glial cells. This may indicate a noxious side-effect of the drug on nuclear RNA metabolism. This problem, forms subject of an accompanying paper (Gajkowska et al. 1984).

WPŁYW INDOMETACYNY NA OBRAZ ULTRASTRUKTURALNY WCZESNYCH NIEDOKRWIENNYCH USZKODZEŃ MÓZGU U CHOMIKA MONGOLSKIEGO (*Meriones unguiculatus*)

Streszczenie

Oceniono obraz mikroskopowo-elektronowy mózgu chomików mongolskich, u których 30-minutowe obustronne podwiązanie tętnic szyjnych poprzedzono dootrzewnowym podaniem indometacyny w dawce 10 mg/1 kg masy ciała. Wyniki porównano z materiałem zwierząt, u których w tych samych warunkach doświadczalnych nie stosowano indometacyny.

Stwierdzono, że indometacyna pozostawała bez wpływu na pojawienie się i rozwój ognisk uszkodzeń tkankowych w obszarach pogranicza unaczynienia przez duże pnie pochodzące od tętnic szyjnych wewnętrznych oraz pogranicza między układem szyjno-wewnętrznym i kręgowo-podstawnym. Były to ogniska w okolicy tylnego podwzgórza, przegrody i grzbietowo-bocznych jąder wzgórza. Występujące tu zmiany w okresie 6 godzin obserwacji ewoluowały do stadium martwicy tkanki zarówno u zwierząt leczonych, jak i nie leczonych. Podanie indometacyny zapobiegało natomiast uogólnionym uszkodzeniom tkanki występującym w korze mózgu, jądrach podstawy oraz w podkorowej istocie białej. W szczególności chroniło ono przed pojawieniem się tu obrzmienia astrocytów oraz niedokrwiennych zmian komórek nerwowych, nawet w obszarach charakteryzujących się wybiórczą wrażliwością neuronów na niedostatek tlenu. Osłaniające działanie indometacyny wiązano z jej wpływem cytoprotekcyjnym. U wszystkich zwierząt, którym podawano indometacynę stwierdzono nieprawidłowości w strukturze jąderka, występujące zarówno w komórkach nerwowych, jak i glejowych, w postaci mikrosegregacji i pojawienia się w nadmiarze ziaren interchromatynowych. Zmiany tego typu uznane są za morfologiczny wykładnik zaburzeń w syntezie i/lub transporcie kwasów nukleinowych. Biochemiczne potwierdzenie tego faktu ograniczy niewątpliwie ze względu na następstwa metaboliczne użyteczność indometacyny jako środka ograniczającego skutki tkankowe niedokrwienia mózgu.

ВЛИЯНИЕ ИНДОМЕТАЦИНА НА УЛЬТРАСТРУКТУРНУЮ КАРТИНУ РАННИХ ИШЕМИЧЕСКИХ ПОВРЕЖДЕНИЙ МОЗГА МОНГОЛЬСКОГО ХОМЯКА (*MERIONES UNGUICULATUS*)

Резюме

Оценивалась электронно-микроскопическая картина мозга монгольских хомяков, у которых внутрибрюшинное введение индометацина в дозе 10 мг/кг веса тела, предшествовало двусторонней перевязке шейных артерий. Результаты сравнивались с материалом животных, у которых в таких же экспериментальных условиях не применялся индометацин.

Было обнаружено, что индометацин не влиял на появление и развитие очагов тканевых повреждений в пограничных областях больших сосудов, происходящих от внутренних шейных артерий, а также на границе между внутришейной и позвоночно-базальной системами. Это были очаги в области заднего гипоталамуса, перегородки и спинно-боковых ядер зрительного бугра. Изменения, наблюдаемые в течение 6 часов наблюдений, развивались до стадии некроза ткани, как у лечимых, так и у нелечимых животных. Однако, введение индометацина предотвращало общее повреждение ткани, выступающее в мозговой коре, базальных ядрах и подкорковом белом веществе. В частности, оно предохраняло от появления отека астроцитов и ишемических изменений нервных клеток, даже в областях характеризующихся избирательной чувствительностью нейронов к недостатку кислорода. Охраняющее действие индометацина объясняется его цитопротекционным влиянием. У всех животных, которым вводился индометацин были обнаружены нарушения в структуре ядрышка, выступающие как в нервных, так и глияльных клетках, в виде микросегрегации и появления в избытке интерхроматиновых зерен. Нарушения этого типа рассматриваются как морфологический показатель нарушений в синтезе и/или транспорте нуклеиновых кислот. Биохимическое подтверждение этого факта, ввиду метаболических последствий, несомненно ограничит пригодность индометацина как средства, ограничевающего тканевые последствия ишемии мозга.

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