Recent studies show that the late delayed radiation-induced brain injuries are the main cause of complications caused by radiation therapy. At the histological level, vascular anomalies, demyelination, and irreversible necrosis of the white matter have been described [5]. It is now generally accepted that the brain is the major dose-limiting organ in the clinical radiation therapy [Wong, van der Kogel, 2004]. According to the conventional view, the late radiation-induced brain injuries result from reduction in the proliferative activity of glial cells or endotheliocytes. According to different studies, the incidence of radiation-induced necrosis ranges from 3 to 24% [4]. The lowest incidence was noted in the conventional fractionated radiation therapy (with 60 Gy irradiation dose) [52]; the highest incidence occurred when stereotactic radiosurgery was used [Marks et al., 2010]. Cognitive impairments are typically considered separately as side effects of radiation therapy. Six months after fractionated radiation therapy, cognitive impairments are described in 50—90% of neurooncological patients [41]. According to the results of clinical trials [36], cognitive deficit is the second most significant factor (after survival) affecting the quality of life after radiation therapy. It is particularly remarkable that cognitive deficit can be detected in the absence of any structural changes in the brain tissue [Shai et al., 2006].

Acute radiation syndrome (from several days to several weeks) and early delayed (up to 6 months) injuries are transient and respond to corticosteroid treatment. In contrast, late radiation-induced injuries are irreversible and aggravate over time, which is one of the major problems of clinical radiation therapy. The issue becomes more complicated due to the fact that it is presently quite impossible to predict individual sensitivity of a patient to irradiation, which can vary from hypersensitivity to resistance, and to correct the dose or exposure mode accordingly. Furthermore, radiation-induced necrosis can be clinically indistinguishable from continued tumor growth. Therefore, there is a need for developing differential diagnosis methods.

No noninvasive biochemical markers of the radiation-induced brain injuries currently exist. At the same time, experimental works over the past 20 years have revealed new cellular and molecular mechanisms that are induced in the brain by ionizing radiation exposure. The key molecules of these mechanisms are likely to be detected in the peripheral blood and, thus, become biological markers of ionizing radiation exposure affecting the brain tissue.

2. Molecular mechanisms induced by ionizing radiation

As well as any other medium, the brain absorbs the energy induced by ionizing radiation. As a result of absorption processes, ionization and atomic excitation occur. The effect of ionizing radiation is estimated to be about 105 electrons per Gray per cell [67]. Ionization causes the formation of free radicals — high-reactivity atoms with unpaired electrons. Free radicals react with brain molecules resulting in their damage or structural changes [61]. Signaling pathways initiated by ionizing radiation occur in two separated cell compartments: the nucleus and cytoplasm. Nuclear processes are initiated by DNA lesion that leads to the cell cycle arrest in order to promote the repair of strands; cytoplasmic processes include activation of receptor tyrosine kinases without ligand binding, which is mediated by inhibition of activ-
ity of protein phosphatases under the influence of reactive oxygen species.

2.1. Nuclear processes: repair of DNA double-strand breaks

Events in the cell nucleus induced by ionizing radiation include detection of DNA double-strand breaks, involvement of repair proteins in the area of lesion, and subsequent cell cycle arrest [62]. If DNA lesions are not to be repaired, apoptosis occurs. A key role in this process is played by two members of the phosphoinositol-3-kinase (PI3K) family: ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR) protein kinases [37]. ATM and ATR are activated by the involvement in the area of lesion; they phosphorylate many substrates including cell cycle proteins and DNA repair enzymes [22]. Inherited ATM defect is observed in autosomal recessive ataxia telangiectasia disorder associated with hypersensitivity to ionizing radiation due to inefficient DNA repair. On the other hand, increased activation of phosphorylated ATM can be observed in glioma stem cells, making these cells resistant to ionizing radiation [73, 74].

In the case of glioblastomas, according to some authors [52], the combined use of the alkylating agent temozolomide (TMZ) increases the risk of radiation-induced necrosis development over five times, which increases tissue sensitivity to radiation exposure. The repair enzyme O6-methylguanine DNA methyltransferase (MGMT) in its turn protects DNA against alkylating agents such as TMZ. Promoter methylation inhibits enzyme transcription, which increases tissue sensitivity to the radiation therapy effects [25]. Thus, the determination of the MGMT methylation status may become a method for evaluating radiosensitivity and the risk of radiation-induced necrosis development.

The success of repair of double-strand breaks eventually determines whether a cell will survive or apoptosis will occur. Initiation of apoptosis or necrosis starts not immediately, only after 3 or 4 cycles of cell division during attempts of DNA repair [66]. Pharmacological inhibition of DNA repair is currently regarded as a strategy of tumor cell radiosensitization [62].

2.2. Cytoplasmic processes: activation of receptor tyrosine kinases

Cytoplasmic events caused by radiation are more diverse as compared with processes in the cell nucleus. Formation of oxygen and nitrogen free radicals with the participation of mitochondrion is enhanced at early stages [8]. Free radicals inhibit the protein tyrosine phosphatase activity, resulting in activation of receptor tyrosine kinases (RTKs) regardless of ligand presence [15]. RTKs phosphorylate a number of proteins initiating the great number of intracellular cascades; the best described one is the epidermal growth factor receptor (EGFR) signaling pathway. The activation of this pathway causes the proliferative and anti-inflammatory cell response. It is known that the extracellular part of the receptor can be detected in blood when using available immunoenzym method [27]. Thus, one can assume that the receptor levels in blood may correlate with radiosensitivity. Little is currently known about the level of EGFR biological variation in the healthy brain tissue. However, it has been shown that the glioma molecular subtypes significantly differ in the expression level of this receptor [30].

2.3. Convergence of nuclear and cytoplasmic signaling pathways

DNA lesion as well as RTK activation result in activation of several transcription factors (TFs), thus initiating gene transcription [11]. NFkB and STAT3 are the best-studied ones among all TFs activated by ionizing radiation [2, 44]. Experimental studies revealed that activation of these TFs is a key factor of neuroinflammation initiation that to a significant extent determines cellular radiosensitivity [1]. Activated TFs are transferred into the nucleus and bind to promoter regions of DNA. This enhances the expression of the cell cycle progression regulatory genes (cyclin D1), the genes of angiogenesis (VEGF) [44], the genes of structural changes in the extracellular matrix and invasion (MMPs), and the genes of enormous number of neuroinflammatory response regulators (TNF alpha, IL-1, IL-6, IL-8, COX2, CXCR4) [6, 40]. Many authors agree that it is neuroinflammation that plays the main role in the mechanisms of ionizing radiation damaging effect on the brain tissue [1].

3. Cellular mechanisms of radiation-induced brain injuries

As described above, the effects of radiation exposure on the brain are mediated by both the formation of reactive oxygen species and apoptosis initiation when double-strand breaks cannot be repaired during several cell cycles. Thus, actively dividing cells and oxygen-rich cells are the most radiosensitive ones. In the CNS they are presented by oligodendrocytes, vascular endothelium, and various precursor cells. Consequently, according to the classical point of view, brain radiation-induced injury is caused by endothelial cell lesions and mitotic cell death of oligodendrocytes. Certain confrontation between advocates of the glial and the vascular theories of the development of delayed radiation-induced brain injuries existed for a long time. Experimental studies revealed that the death of both oligodendrocytes and endothelial cells may lead to the development of radiation-induced necrosis. At present, however, it becomes clear that the development of radiation-induced brain injuries in the actual pathogenetic situation can completely be related neither to mitotic death nor to destruction of certain cell types. It results from complex dynamic interactions between various brain cell subpopulations: microglia, neurons, astrocytes, oligodendrocytes, and endothelial cells. Furthermore, the development of cognitive impairments due to radiation exposure is among the most researched aspects of radiation-induced brain injury.
may occur irrespective of cytological and morphological damages [61].

**3.1. Endothelium**

As mentioned above, many authors consider the endothelium damages to be a key factor in the development of radiation-induced brain injuries. The advocates of the vascular theory refer to a great number of experimental studies showing that radiation causes vascular wall thinning of brain blood vessels, structural changes in the capillaries, and reduction in the amount of endothelial cells [4, 50]. It has been shown that clinically relevant doses of ionizing radiation cause changes in the permeability of the blood—brain barrier due to the imbalance between the expression of metalloproteinases and their inhibitors, type IV collagen degradation; changes in VEGF, angiotensin I, and angiotensin II expression [34; Won Hee Lee et al., 2012]. At the same time it is known that radionecrosis may be developed in the brain in the absence of microvascular changes [54]. Furthermore, it has been shown that certain substances (agonists of peroxisome proliferator-activated receptor-gamma, inhibitors of angiotensin-converting enzyme) prevent the development of cognitive impairments in rats after the radiation exposure. However, they do not affect the radiation-induced vascular changes [72]. Thus, the delayed radiation-induced brain injuries cannot only be explained by damage to endothelial cells.

**3.2. Oligodendrocytes**

It is assumed that damage to oligodendrocyte precursor cells results in inability of the brain tissue to substitute dead oligodendrocytes. Eventually, it causes demyelination and white matter necrosis. Actually, it was shown that single doses of more than 3 Gy or fractionated doses exceeding 4.5 Gy cause a progressive decrease in the number of oligodendrocytes in the rat’s brain within 24 h after exposure [28]. These data correspond to early transient demyelination and acute radiation syndrome that are observed in clinical practice. However, these results do not explain the late development of radiation-induced necrosis occurring some months after the treatment. Moreover, it has been shown that progressive cognitive impairment in old rats caused by the same dose is not accompanied by reduction in the number of oligodendrocytes, myelin fibers thinning, or decrease in the number of fibers [58]. Thus, the correlation between oligodendrocyte damages and the development of radiation-induced brain injuries is not as clear as it was previously thought.

**3.3. Astrocytes**

About a half of all the glial brain cells are astrocytess, which is four times higher than the number of neurons in the human brain [Hansson, 1988]. It was previously thought that astrocytes perform mainly structural and supporting functions, but it is becoming evident now that these cells are represented by a heterogeneous population that plays an important role in modulation of synaptic transmission, regulation of neurogenesis, and differentiation of various precursor cells [55]. In addition, astrocytes and endothelial cells maintain the brain—blood barrier integrity [21]. Ionizing radiation causes astrocyte activation, which is accompanied by increased secretion of glial fibrillary acidic protein (GFAP), cyclooxygenase, and intercellular adhesion molecule-1 (ICAM-1) providing invasion of foreign immunocompetent cells, such as leukocytes [68, 75]. GFAP secretion is increased both in the acute and chronic phases of radiation-induced brain injury [55]. GFAP is known to be detected in the peripheral blood in a number of CNS pathological states [14], this protein can be considered as one of the potential biomarkers of the effects of ionizing radiation on brain tissue.

**3.4. Microglia**

About 12% of all the brain cells are microglia [17]. Being a homologue of macrophages, inactivated microglia cells play a role of a monitoring agent of homeostasis [60]. Like any other damaging factor, radiation induces microglia activation, which is followed by significant cell shape changes, proliferation, and becoming of secretory phenotype. Activated microglia is one of the main regulators of neuroinflammatory response when secreting a wide variety of mediators: TNF alpha, IL-1, IL-6, MCP1, and ICAM-1 [20, 33]. Enhanced expression of these mediators occurs at the gene and protein levels [29]. It can be expected that the aforementioned mediators may be detected in blood when permeability of the blood—brain barrier changes, which reflects that neuroinflammation and microglia activation processes occur in response to radiation exposure.

**3.5. Neurons**

Neurons were previously thought to be resistant to radiation exposure due to their inability to divide. At present, however, it is shown that significant changes in expression of the NMDA-receptor subunits, impairment in glutamatergic transmission and long-term potentiation occur in response to ionizing radiation in neurons [38, 65]. These mechanisms are significant for implementation of the processes of synaptic plasticity and thinking; the disorders occurring in these processes are among the most severe side effects of the influence of radiation exposure on the brain. It is interesting that these neuron activity disorders seem to be related neither to the changes in the number of neurons (including a decrease in their amount) nor to the level of myelination processes in the extensions of nerve cells [58].

**3.6. Interaction between cell subpopulations**

A very delayed manifestation of the worst side effects of ionizing radiation on the brain (radiation-induced necrosis and cognitive impairment) is one of the most intriguing issues in the field of studies of the radiation effect on the brain. In an effort to settle these questions, series
of long-term studies were performed by different researchers. They assessed the expression of a number of genes and proteins in different periods ranging up to several months after the exposure to ionizing radiation [43, 63]. Moravan et al. [43] estimated the expression of a number of neuroinflammation molecules: ICAM-1, TNF alpha, GFAP, and SSL2. A similar pattern of expression was revealed for most compounds: early enhancement (from several hours to several days), subsequent decline and rise again at the later stages (from 6 months to 1 year). The morphology of cells expressing these molecules in this case significantly changed. In the first few hours after exposure, expression was observed mainly in the minute vessels; at the later stages, it was detected in the cells with a secretory phenotype. GFAP showed the earliest secondary enhancement of gene expression. At the same time, enhanced expression of MHC-II (marker of microglia activation) was first observed 30 days after exposure [43].

Thus, one can assume that the neuroinflammation becoming chronic results from changes in cell populations involved in inflammatory response: from vascular endothelium to astrocytes and microglia at the latest stages. Proteins reflecting activity of these cells may be transferred into the blood during neuroinflammation. Thus, they can be regarded as potential markers of various stages of radiation-induced brain injuries.

4. Complex pathophysiological mechanisms

4.1. Neuroinflammation

A growing number of data points to the fact that it is precisely the neuroinflammation that plays a leading role in the development of radiation-induced damage. As evidenced a few years ago, inflammatory reactions play an important role in the development of radiation-induced necrosis; the studies revealed the efficacy of glucocorticoids [Martins et al., 1979] and COX-2 inhibitors in treatment of radiation-induced brain injuries [Khan et al., 2004]. Later, numerous studies revealed that astrocytes, microglia, endothelium, and immune cells express mediators of inflammation both in acute and in delayed stages of the development of radiation-induced injury. Enhanced expression of cytokine proteins (IL1B, TNF alpha, and IL-6), intercellular adhesion molecules (ICAM-1), and transcription factors contributing in anti-inflammatory processes (NFKB) was detected in the brain exposed to ionizing radiation [43]. Sustained activation of microglia was maintained in the rodent brains 6 and 9 months after exposure [10]. Recent studies show that inhibition of neuroinflammation prevents the development of delayed radiation-induced cognitive impairment in animals [Jenrow et al., 2013]. The hippocampus is known to be the most sensitive area in the whole-brain radiotherapy [Jenrow et al., 2012] and the area most sensitive to neuroinflammation processes [47]. Many authors directly relate the development of somnolence syndrome to activity of inflammatory mediators [Ballesteros-Zebadúa, 2012]. Moderate doses of radiation can decrease the subsequent cell death of neurons mediated by neuroinflammation in rats [Titova et al., 2010]. Curiously, it was found that the decline in expression of anti-inflammatory mediators in response to radiation exposure occurs with age in the rat brain [34]. Meanwhile, there are data on risk reduction of radiation-induced necrosis in adults compared to children [48]. Thus, a great variety of multi-aspect information points up the prominent role of neuroinflammatory processes in the pathogenesis of various radiation-induced brain injuries, including radiation-induced necrosis and cognitive impairments.

4.2. Neurogenesis

Progenitor cells are hypersensitive to ionizing radiation. Dose-dependent cell death of neural precursor cells, reduced proliferation, and inhibition of cell differentiation into neurons are observed in the hippocampus of rodents exposed to radiation [3, 72]. A dose of 10 Gy does not cause demyelination and development of radiation-induced necrosis in young rats. However, this dose results in only 3% of new neurons being formed in the hippocampus of animals when compared to intact rats [42]. Recent clinical data have confirmed the reduction in the number of progenitor cells, resulting from radiation therapy in neurooncological patients [46].

In young rats, the reduction of neurogenesis induced by clinically relevant radiation doses correlates with the development of delayed cognitive impairments [32, 51]. Meanwhile, it should be noted that no radiation-induced reduction of neurogenesis is observed in old rats in spite of the functional disorders [Greene-Schloesser et al., 2012].

4.3. Endocrine mechanisms

Endocrine mediators play a significant role in maintaining the healthy brain function and in adaptation to damaging factors. Specifically, thyroid hormones and sex steroids play a vital role in neurogenesis; estradiol and progesterone may protect neurons against cell death [53]; glucocorticoids are endogenous key regulators of neuroinflammation [19]. The role of endocrine mechanisms in the development of radiation-induced injuries has not yet been surveyed in detail. However, it has been found recently that radiation therapy results in long-term changes in the function of the endocrine system in 80% of patients [13]. The observed changes appear to be linked to pituitary gland damage and are not associated with the damage to the hypothalamus. Experimental studies performed in the acute phase after radiation exposure revealed coordinated induction of inflammatory responses in the hypothalamus (enhanced expression of IL1B and NFKB) and an increased blood level of corticosterone, which was followed by enhanced transfer of the glucocorticoid receptor in the nuclear fraction of hypothalamic cells [64]. These responses most likely reflect com-
pensatory activation of anti-inflammatory endocrine response mechanisms to radiation-induced neuroinflammation. One may assume that the changes induced by radiation therapy in endocrine reactions in patients can play a significant role in controlling the loss of the brain inflammatory response and in the subsequent development of radiation-induced injuries. Thus, the variation in glucocorticoid synthesis assessed by the level of these hormones in the peripheral blood may apparently reflect the risk of developing radiation-induced brain injury.

5. Potential biomarkers of radiation-induced brain injuries

Little is known about biomarkers of radiation-induced brain injuries. However, based on the above noted clinical and experimental studies, one can find appropriate approaches to the risk assessment and radiation-induced brain injury monitoring.

5.1. Inflammatory mediators

Neuroinflammation is apparently a key factor of radiation-induced brain injury development. One can expect that inflammatory mediators are likely to be detected in blood as the brain—blood barrier permeability changes in development of radiation-induced brain injury. Meanwhile, the use of inflammation mediators as diagnostic or prognostic biomarkers of radiation-induced brain injuries may be linked to overcoming certain limitations. Primarily, it comes to lack of specificity and to the dual role of inflammatory responses in nervous tissue injury.

Lack of specificity. Neuroinflammation is a common process in all cerebral pathologies. Therefore, inflammatory mediators are less likely to be used as specific biomarkers of radiation-induced injuries and, for example, in the differential diagnosis of radiation-induced necrosis or gliomas with continued growth. The diagnosis specificity may be improved in this case by simultaneously determining the tumor growth biomarkers. Meanwhile, endothelial cells (some of the major cell types involved in inflammatory response) are damaged as a result of brain exposure to radiation. This leads not only to changes in the brain—blood barrier permeability and causes hypoxia, but also to changes in the contribution of these cells to the inflammatory response regulation. Thus, one can expect that the inflammatory response as a result of radiation exposure possesses specific qualitative or quantitative characteristics.

The dual role of neuroinflammation in brain injury. The inflammatory response of the brain is initially a defensive reaction of the nervous tissue dedicated to the removal of damaged cells and stimulation of regenerative processes. Most likely the cell death is mediated not by inflammation alone, but also by dysregulation of its processes or the inflammatory response becoming chronic. The level of mediators of inflammation would thus be expected to reflect to a greater extent the severity of ionizing radiation exposure on the brain tissue than the likelihood of radiation-induced injury development. Using intracranial injury and other cerebral pathologies with evident component of the neuroinflammation as an example, it is well known that the level of inflammatory mediators may be correlated both with the favorable and unfavorable disease outcomes at several points in time. Apparently, the parallel determination of other markers that might point to adaptive or non-adaptive direction of the inflammatory process, the following brain cell activation or cell death could be the solution to this issue.

5.2. The markers of brain cell activation and damage

Experimental studies suggest that the development of radiation-induced brain injuries and the inflammatory response becoming chronic occurs during sequential activation of various brain cell subpopulations. It is clear from studies of other cerebral pathologies that a number of markers in peripheral blood may reflect the processes of nervous tissue cell activation or cell damage. Specifically, GFAP is regarded as biomarker of astrocyte activation; VEGF, VCAM, and ICAM-1 may reflect the endothelium activation [14] [Patrick et al., 2013].

Determination of molecules that are fragments of radiation-damaged cells in blood may become an approach to molecular diagnosis of radiation-induced injuries as well. Specifically, the test for anti-aquaporin-4 antibodies has already shown to be a reliable diagnostic technique for determining demyelinating diseases of the CNS [16]. The level of these antibodies in blood serum would be expected to correlate with the severity of radiation-induced necrosis.

Ubiquitin C-terminal hydrolase (a neuron-specific enzyme) [6, 35], enzyme-specific products of alpha II-spectrin degradation (reflecting the calpain or caspase-3 activity) [Mondello et al., 2010], the fragment of protelytic cleavage of NMDA receptor or NMDA receptor antibodies, matrix metalloproteinases and their inhibitors (involved in extracellular matrix remodeling) [68], EMAP II cytokine (involved in microgliosis) [70], nitrotyrosine (involved in oxidative stress) [Ryan et al., 2010] etc. can be regarded as potential markers.

5.3. Markers of processes in the nucleus

Examination of lymphocytes as the only blood cells containing full DNA is used to quantify the effects of radiation in clinical radiology [9]. Varied chromosome aberrations (terminal deletions, translocations, ring chromosomes, and dicentric chromosomes) may result from radiation exposure [Granzotto et al., 2011]. Severity of these changes may reflect the "success" of repair mechanisms at equal doses of radiation exposure. Meanwhile, only a small number of lymphocytes are located in the brain, and it is extremely difficult to discover them in blood. At the same time, as noted above, a great number of repair proteins is concentrated and structurally modified in the area of DNA double-strand breaks. Identifying
Radiation-induced necrosis and cognitive impairments are the most severe complications of radiation therapy. Their development is characterized by asymptomatic disease course, late manifestation, irreversibility, and distinct interindividual variability. This determines an acute need to study the mechanisms regulating these processes and to search for relevant objective diagnostic and prognostic biomarkers. Literature data reveal a great variety of cellular and molecular mechanisms of pathogenesis of the radiation-induced brain injuries. The key processes most likely are not confined to mitotic death of oligodendrocytes and endothelial damage; they also include the initiation of neuroinflammatory response resulting in chronic form and the subsequent activation of different brain cell subpopulations. The mechanisms of systemic physiological control involving the endocrine system are likely to play a significant role. One can hope that the key molecules of these processes may become the first noninvasive biomarkers of the radiation-induced brain injuries.

**Conclusion**

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