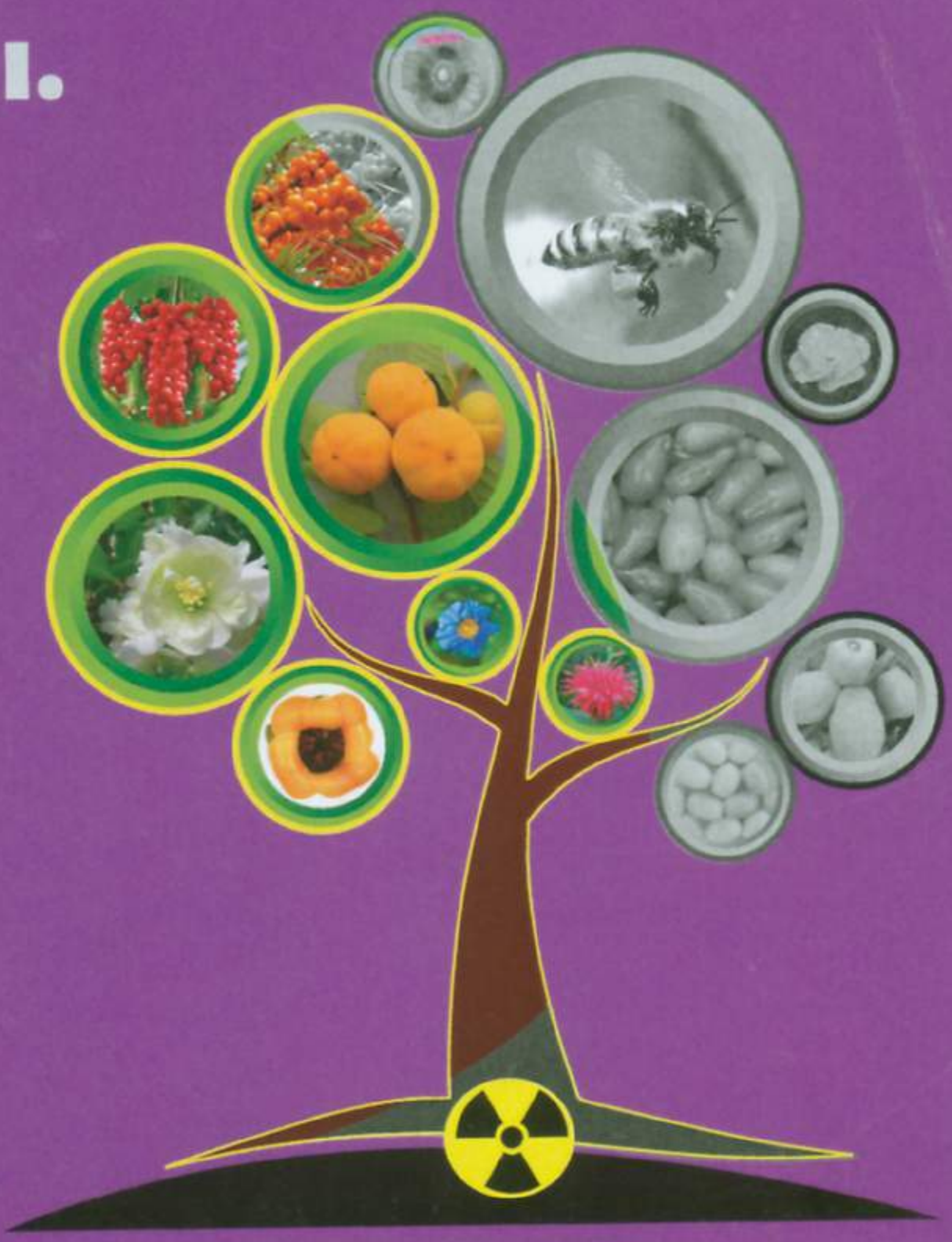
BIODIVERSITY AFTER THE CHERNOBYL ACCIDENT

PART I.



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EFFECT OF RADIATION ON LYSOZYME AMYLOID FIBRILS

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Ionizing radiation has long been recognized as an inducer of several cancers but several studies suggests an additional role in increasing the risk of noncancerous diseases, including neurodegeneration. Our observed results are coincident with observation that various type of radiation can lead to structural changes of matter.

Keywords: radiation, neurodegenerative diseases, amyloid fibrils

Introduction

The aggregation of proteins into amyloid fibrils is associated with a wide variety of diseases, ranging from neurodegenerative Alzheimer's and Parkinson's diseases to systemic amyloidoses. A common feature associated with most of these neurodegenerative diseases is the formation of extended, β-sheet rich, proteinaceous fibril aggregates, known as amyloids. Despite the differences in function, structure, and amino acid sequences, the disease-related proteins shared defining characteristics such as a β-sheet structure motif, fibrillar morphology, birefringence to polarized light upon staining with Congo red, insolubility in most solvents, and protease resistance. Compelling evidence indicates that amyloid aggregation is critical for neurodegeneration and therefore the preventing of amyloid formation and/or the reduction of amyloid assemblies is potentially the most effective therapeutic approach for the treatment of amyloid-related diseases (De Felice, 2002).

Daily we are confronted with low doses of ionizing radiation from natural and man-made sources. However, also medical radiation is increasing and this leads to a worldwide increase in the population exposure. There is no clear evidence proving or disproving that ionizing radiation is causally linked with neurodegenerative diseases such as Parkinson's and Alzheimer's. However, it is known that high doses of ionizing radiation to the head (20–50 Gy) lead to severe learning and memory impairment which is characteristical for Alzheimer's. It is known that low doses of ionizing radiation may cause immediate and persistent impairment of cardiac mitochondria, Complexes I and III being the main targets (Azimzadeh, 2011). The mechanism of radiation-induced mitochondrial dysfunction both in the heart and brain is not completely understood. Radiation-induced damage is probably caused by mutations and deletions or indirectly through the formation of reactive hydroxyl radicals. High doses of ionizing radiation are also known to affect learning and memory processes but even considerably lower doses may have an effect. Increased oxidative stress and a generalised depression of the mitochondrial electron transport chain activity have been observed in AD patients (Parker, 1994). Kempf et al. (2013) suggest that mitochondria play a central role in the radiation response followed by neuroinflammation and oxidative stress. Subsequent cellular effects lie in the reduction in neurogenesis and cerebrovasculature followed by neurodegeneration.

Nanoparticles (NPs), due to their size-dependent physical and chemical properties, have shown remarkable potential for a wide range of applications over the past decades. Because the magnetic fields are not harmful to a human organism (although this question is still opened for high strength magnetic fields) magnetic nanoparticles can be used for biomedical *in vivo* and, *in vitro* applications such as MRI contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery, and cell separation (Gupta, 2005). Recent study shows that nanoparticles can significantly influence the kinetics of protein amyloid fibrillation, associated with a wide variety of diseases, ranging from neurodegenerative Alzheimer's and Parkinson's diseases to systemic amyloidoses. Compelling evidence indicates that amyloid aggregation is critical for neurodegeneration and therefore the preventing of amyloid formation and/or the reduction of amyloid assemblies is potentially the most effective therapeutic approach for the treatment of amyloid-related diseases (De Felice, 2002). Thus, nanoparticles are being explored for their role in diagnosing, preventing, treating or even causing amyloid diseases.

In addition to amyloid pathology, in present work we have investigated effect of radiation on amyloid fibrils and fibrils in presence of nanoparticles, and we concern our study to investigate the ability radiation to enhance effect of magnetic nanoparticles (NPs) stabilized by perchloric acid on preformed lysozyme fibrils. Previously we have demonstrated that magnetic nanoparticles are capable to inhibit amyloidogenesis and depolymerize amyloid aggregates (Bellova, 2010; Siposova 2012). Therefore, combination of magnetic nanoparticles with radiation will give us a possible more effective system with both, effective anti-amyloid properties and targeted capability.

Materials and methods

In vitro formation of lysozyme amyloid fibrils (LAF) was achieved under conditions leading to formation of partially unfolded conformers, which allow intermolecular interactions required for triggering process of amyloid fibrillization. To these preformed fibrils, we add nanoparticles at final concentration 183.75 μg/mL (to 147 μg/mL of fibrils), ratio 1.25 : 1 (NPs : LAF). After 1 hour incubation we apply radiation at four different times of exposure: 3, 7, 15 and 30 minutes.

Bremsstrahlung gamma rays of betatron B-25 (Uzhgorod National University) were used for irradiation of prepared samples. Inner accelerated electron beam of 15 MeV produce a continuous gamma spectrum from zero up to 15 MeV. At the distance of 1 meter from bremsstrahlung target, the doserate of exposure was near 7–9 R/m (röntgen/minute). Exact measurements gave such values of exposure doses 20.8±0.5 R, 76.6±1.8 R, 122±3 R and 244±6 R respectively. It should be emphasized that we are using the exposure dose on the spot of the irradiated sample location and the absorbed dose depends on the sample matter.

After irradiation, using atomic force microscopy we examined effect of radiation. Samples were deposited by drop casting on the freshly cleaved mica surface and after adsorption were washed with ultrapure water and left to dry. AFM images were taken by a scanning probe microscope (Veeco di Innova, Bruker AXS Inc., Madison, USA) in a tapping mode using uncoated silicon cantilever NCHV un-mounted with force constant 42 N/m and nominal resonance frequency ~320 kHz.

Results and discussion

In the absence of NPs and radiation, lysozyme amyloid fibrils (Fig. 1) shows typical amyloid morphology of aggregates displaying long fibrillar structure and protofibrillar twisting. The thicker species appeared to arise by a lateral association of the fibrils. Short dosing intervals (dose 21

and 77 R) caused no significant changes; the fibrils have similar morphology to that detected for lysozyme fibrils (control). 30 min. radiation (dose 244 R) caused significant changes in amount as well as morphology of fibrils. Fibrils are thinner, very short parts of fibrils and amorphous aggregates were observed in comparison to fibrils irradiated for 15 min. which exhibit only slightly changes.

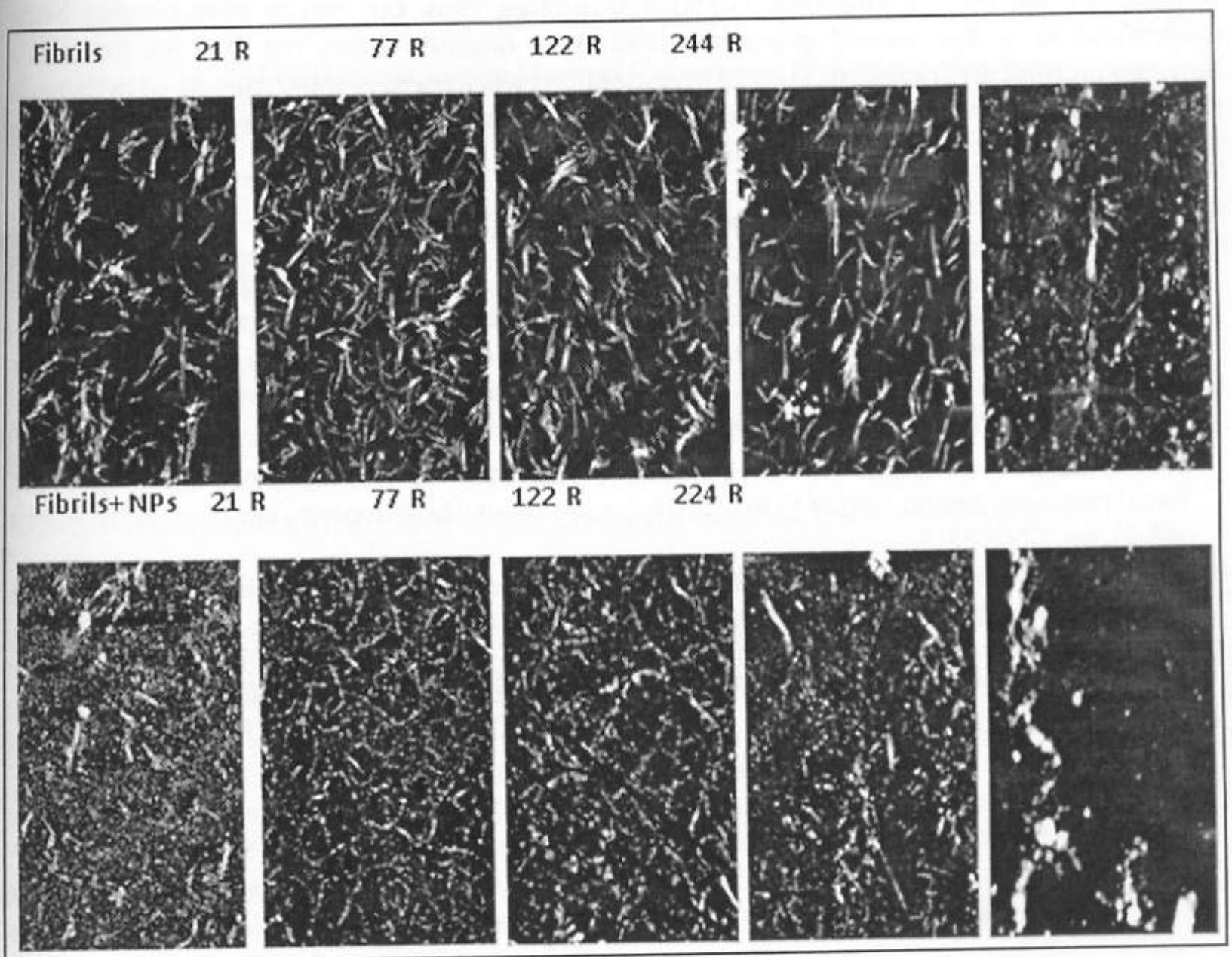

Figure 1 AFM of fibrils irradiated alone and presence of nanoparticles
The xy scale is 10 × 10 μn. The resolution of image was 512 pixels per line (512 × 512 pixels/image) and scan rate 0.5 kHz. No smoothing or noise reduction was applied. The data analyses were performed using NanoScope Analysis 1.2 software

The irradiation effect on fibrils was enhanced in presence of nanoparticles, as documented by AFM scans presented in lower row. The extensive decrease of the amount of amyloid aggregates and very short parts of fibrils were observed (especially after 15 and 30 minutes of radiation). Moreover, after 30 min. of radiation only amorphous structures were observed.

AFM images confirmed that changes in the shape and amount of amyloid aggregates depend on the presence of nanoparticles and exposure to radiation, and clearly proved that the ThT fluorescence decrease is caused by the reduction of amyloid aggregates (data not showed). In our previous work we proved destruction of fibrils after 24 h incubation (Bellova, 2010; Siposova, 2012). Application of radiation enhanced the destruction of fibrils in presence of NPs and similar extent of destruction was observed already after 1 h (incubation of fibrils + NPs) and dosing interval 15 min. (corresponding to dose of 122 R). Longer radiation doses lead to total destruction of fibrillar morphology.

Conclusions

Ionizing radiation has long been recognized as an inducer of several cancers but several studies suggests an additional role in increasing the risk of noncancerous diseases, including neurodegeneration. Our observed results are coincident with observation that various type of radiation can lead to structural changes of matter. Thus, our results may provide better understanding of the general effect of radiation on organisms and the possible interaction between proteins and radiation. Further investigations will help to identify the role of radiation in the etiology of neurodegenerative diseases such as Parkinson's and Alzheimer's disease, because they are considered to be late effects of radiation.

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