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ORIGINAL PAPER

LIPOFUSCIN AND CEROID PIGMENTS: CELLULAR HALLMARK OF CEREBRAL SENESCENCE

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Abstract. Lipopigments (LPs) - lipofuscin and ceroid - are the main marker of brain vulnerability, distress, aging and connected pathology. Lipofuscin is the basic feature of cellular senescence, as ceroid is the cummulation product of aggressive external (environmental) and/or intrinsic (mainly genetic) factors. During ontogenesis, neuronal LPs progressively accumulate, as a time dependent phenomenon. In the aged neurons, LPs are present in all cellular compartiments: massively in every perikaryon areas and dendrites, also in axons, and even in terminal buttons. They constantly coexist and are significantly correlate with important changes in nerve cell biochemistry and morphology, such as neuronal loss, decrease in the surface/volume of neurosoma, dendritic aberrations, simplifications and destructions, axonal enlargements to meganeurites, considerably reduction of cortical myelin, and synapses loss. Moreover, neuronal LP accumulations coexist with glial LP storages, in all types of glia (astrocytes, oligodendrocytes, but especially in microglia). Glial systems play an important role in collecting of neuronal LPs. Owing to their transporting properties, and migration capacity of microglia, glial cells deposit the LP clusters in pericapillary areas. Thus, LP conglomerates appear in the whole nervous tissue, from neurons to perineuronal glia, neuropil, pericapillary glia and endothelial cells, realizing specific patterns of LP architectonics. Direct, causal interrelations, critical LP concentrations, which generate cascades of negative subcellular events, and indirect, associative impairment correlations determine characteristic neuropathological aging profiles. These specific and associate negative neuropathologic consequences of LP accumulations have multiple and detrimental impacts on neuron and glia homeostasis, from neurono-glial function to central nervous system physiology.

Key words: accumulations and storages of neuronal and glial lipopigments, causal and associate damages correlated with lipopigments, neuropathological aging profiles.

BIOLOGY as PROCESSES in CORRELATION

The correlation process of variables and data, introduced for the first time in genetics and heredity by Sir Francisc GALTON (1822-1911) in his book *Natural Inheritance*, is very important in knowledge, science and biomedicine (Galton, 1889).

During ontogenesis, the time-dependent processes and especially aging change the morpho-functional systems from homogeneous and harmonic into heterogeneous and dysharmonic. This transformation is determined by causal relationships, acummulations of unbalances, interdependences and covariates - expressed also by correlations (positive, negative or zero).

Correlative Directions in Neurosciences

In particular, the multitude of studies and researches, ranging from molecular and subcellular levels to comparative neuroanatomy and neuropathology and to cognitive and behavioural sciences, created the large bases and opportunities to correlate the parameters, processes and phenomena.

Moreover, the morphological ultradifferentiation and functional over-specialization of the nervous tissue, comparatively with other tissual types, also impose the use of correlation methodologies in brain research. In addition, the resulted data of correlations and connections show new aspects, links and directions, and contribute to the better understanding of neurobiology and human beings.

Therefore, the ontogenesis, longevity, and normal and pathological brain aging correlated with the factors which control, regulate and disturb central nervous system (CNS) structure and function open new ways in knowledge and intervention (Riga and Riga, 1998).

Links between Rate of Aging and Biological Variables

Systematic researches in comparative zoology (concerning Invertebrata and Vertebrata), in systems morphology and senescence of mammals, and bio-gerontology show interesting connections, such as the following:

- relationship in mammals between the rate of aging (as deduced from maximum life span) and the size of the animal (body weight), metabolic scales/rates; the production of free radicals being an attractive explanation;
- correlation in mammals between longevity and brain size/weight, as an absolute or relative (to body weight) value (Lynch and Bi, 2003; Riga, 2003);
- links between longer-lived species with a slower rate in accumulation of lipofuscin-age pigment and grown resistance of brain autooxidation with increasing live span.

Biochemical and Morphopathological Correlations in Brain Aging and Neurodegenerative Disorders

Brain senescence in humans is a complicated and heterogeneous process with high regional specificity and individuality. Therefore, important CNS aging connections can be pointed out between:

- neuronal density and different types of glia presence and reactivity (Landfield et al., 1981; Morgan et al., 1999);
- brain lipopigments (LPs) in neurons and glia (Riga and Riga, 1994; Riga and Riga, 1995a);
- LPs in relationship with mitochondria pathology: mtDNA mutations and gigant mitochondria (Brunk and Terman, 2002), with anabolic organelles (Riga and Riga, 1995b) and with lysosomal dysfunction (Lynch and Bi, 2003; Riga and Riga, 1994); and
- LPs and Alzheimer pathology: neurofibrillary tangles, amyloid plaques and meganeurites (Abraham, 2000; Abraham, 2001; Bi et al., 1999; Braak, 1984; Braak and Braak, 1988).

BRAIN LIPOPIGMENTS (LPs)

General Characteristics of LPs

LPs are represented by lipofuscin and ceroid. Lipofuscin, progressively accumulated in ontogenesis, is the hallmark of cellular senescence. Ceroid, pathologically formed, is the stamp of external (environmental) aggressions and of internal factors (cellular distresses, including also genetic factors). At some time of their evolution, LPs display almost identical biophysical, biochemical and morphological characteristics, properties and structure (Porta, 1991).

By their implication and negative consequences on neuronal and glial biochemistry and physiology, LPs represent the main marker of brain vulnerability, distress, normal and pathological aging, and associated diseases (Riga and Riga, 1995a).

LPs in the Brain

LP accumulations in the CNS (Figs. 1 - 4) have some important features:

- brain ubiquity: in all regions and zones, from cerebrum to spinal cord (Riga and Riga, 1974);
- presence in all nervous tissue components: in whole cellular types (Cervós-Navarro and Sarkander, 1983), from different kind of neurons - post-mitotic cells, to glia (astrocytes, oligodendrocytes, but especially microglia) - mitotic cells, and to pericytes and endothelial cells;
- specific patterns of LP architectonics (pigmento-architectonics), in close relation with senescence and age-related pathology (Braak and Braak, 1988);
- LPs evolution in two-stages (Riga et al., 2006a): stage I - LPs increase in number, surface, volume, complexity, in both neurons and glial cells; stage II -

LPs become a constancy in inflammatory-degenerative nervous pathologies (Alzheimer's dementia, Parkinson's disease etc.).

LPs in both Neurons and Glia

In the aged neurons, LPs - these biological garbage are present in all cellular compartiments (Cervós-Navarro and Sarkander, 1983; Riga and Riga, 1995a): massively in every perikaryon areas and dendrites (Figs. 1 - 3), but also in axons, and even in presynaptic components.

Moreover, in the neuroglia, from perineuronal glia to neuropil and pericapillary glia, LP storages occur in all cellular partitions (Cervós-Navarro and Sarkander, 1983; Riga and Riga, 1995a; Riga and Riga, 2015): gliosomas (Fig. 4), glial dendrites and arborizations, and capillary end-feet.

NEURONAL CORRELATES of LPs

During ontogenesis, longevity and aging, as time dependent phenomena, neuronal LPs accumulate progressively, in various quantities. They constantly coexist and are significantly correlated with important negative synergistic metabolic and cell-subcellular events: modifications in neuron/glia index; changes of cyto-, pigmento- and myelo-architectonics; alterations in structure and function of subcellular systems.

Neuronal LPs and Neuron/Glia Index (Number)

In aging and aged-related neurodegenerative pathology (Alzheimer's and Parkinson's diseases etc.), constant expansion of neuronal LPs is associated with neuronal loss and simultaneous increase of reactivity and number of neuroglial cells (Braak and Braak, 1988; Finch et al., 1999; Gonzáles-Scarano and Baltuch, 1999; Landfield et al., 1981).

In the brain, glial activations (Finch et al., 1999; Morgan et al., 1999), the canonical features of mammalian aging and mediators of inflammatory and degenerative diseases, basically consist of:

- astrocyte hyperactivity, fibrous phenotype (increased levels of glial fibrillary acidic protein - GFAP); and
- microglia activation (increased expression of major histocompatibility complex (MHC) class II antigens and rised levels of transforming growth factor-beta-1 mRNA - TGFβ-1).

They are attenuated by food and caloric restriction (Finch et al., 1999; Morgan et al., 1999).

Neuronal LPs and Neuron Morphology

Neuronal LP conglomerates realize specific brain patterns of pigmento-architectonics (Braak, 1984; Riga and Riga, 1974), and modify neurono- and myelo-architectonics (Braak, 1984). They are correlated with: decrease in the

surface/volume of neurosoma; dendritic aberrations, simplifications and destructions; axonal enlargements to meganeurites (Bi et al., 1999; Braak and Braak, 1988; Lynch and Bi, 2003); considerably reduction of cortical myelin; and synapses loss.

Neuronal LPs and Apoptotic Processes

Programmed cell death via apoptosis signifies a morphologic pattern of cell death affecting single cells (Dănăilă et al., 2013). Apoptosis is induced by the activation of a family of proteases (cysteine endo-peptidases), called caspases (cysteine aspartate-specific proteases). Caspase activation can be initiated by two molecular pathways (Delhalle et al., 2003):

- extrinsic pathway - the DR (death receptor) pathway, which is induced by ligand binding to TNFR (tumor necrosis factor receptor) superfamily members;

and

- intrinsic pathway - the mitochondrial pathway, which is triggered by mitochondria in response to intracellular injuries, such as DNA damage.

Morphological and biochemical changes are represented by cell shrinkage, condensation of chromatin, formation of cytoplasmic blebs, release of mitochondrial cytochrome c, fragmentation of cell DNA into multiples of 180 bp (base pairs), and cell fragmentation into membrane-bound small apoptotic bodies, that are eliminated (cleared) through phagocytosis by neighboring cells (Anderson, 2003). Being encoding in cell genetic programme, apoptosis becomes the main mechanism for cellular deletion in the regulation of cell population, a key factor of tissual homeostasis, in embrio- and morphogenesis, an arbiter of cellular growing and differentiation.

The other major mechanism of cell death is necrosis, pathological cell death. Necrosis affects groups of cells or parts of an organ structure. Necrosis is caused by external noxious factors, is not genetically controled, and is accompanied by inflammation.

In biological wear and tear, aging and neurodegenerative pathology, the apoptosis can be premature started, activated and/or accelerated. In this way, it is a close correlation between senescent mitochondria, old lysosomes filled with LPs (biological dirt) and the induction of apoptosis. Old mitochondria generate elevated quantities of superoxide and hydrogen peroxide. If large amounts of hydrogen peroxide diffuse into lysosomes (by autophagocytosis of giant and senescent mitochondria), they disturb stability of lysosomal membrane, with subsequent leak into cytosol of lysosomal lytic enzymes. Moderate release of lysosomal enzimes can induce apoptosis (Brunk and Terman, 2002), while marked discarge of these enzymes cause cell death by necrosis.

Neuronal LPs and Anabolic Subcellular Systems

- Neuronal LPs and anabolic subcellular systems are in inverse correlation. Extension of LP clusters is in connection with: decrease of ribosomal RNA, total

RNA and water-soluble proteins, and consecutive diminution in number and surface/volume of polyribosomes and rough endoplasmic reticulum (Fig. 3), (Riga and Riga, 1995b; Riga et al., 2003).

Neuronal LPs and Mitochondrial Pathophysiology

In addition, progressive neuronal LP storages are associated with: increase of the oxidative stress attack (Beckman and Ames, 1998; Fosslien, 2001; Harman, 2003); decrease of the antioxidative defence (Ames et al., 1993); cumulation of mtDNA mutations (Brunk and Terman, 2002; Terman and Brunk, 2004); increase the number of damaged, impaired, defective, and giant mitochondria with a low rate of their degradation (Brunk and Terman, 2002); and decrease the number and area of normal and healthy mitochondria (Fig. 3).

The mitochondrial-lysosomal axis theory of aging (Brunk and Terman, 2002) demonstrates that mitochondria and lysosomes of postmitotic cells (such as neurons and cardiac myocytes) suffer the most remarkable age-related alterations amoung all cellular organelles. Moreover, by continuous oxidative stress and reactive oxygen species (ROS) production, the damaged oxidated mitochondrial components and structures become the main sources in LP accumulations and storages via old, tertiary lysosomes.

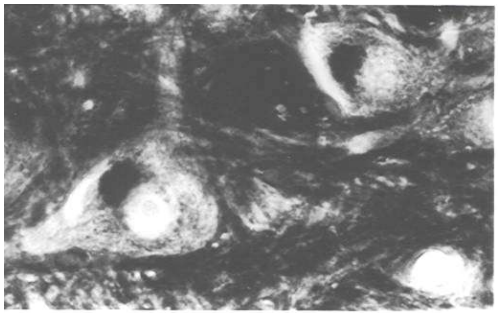

Fig. 1. Old rat (26.6 months). Brain. Pontine reticular formation. Light microscopy (Sudan black B). Large masses of neuronal LPs, gathered into perinuclear-unipolar clusters. X 900.

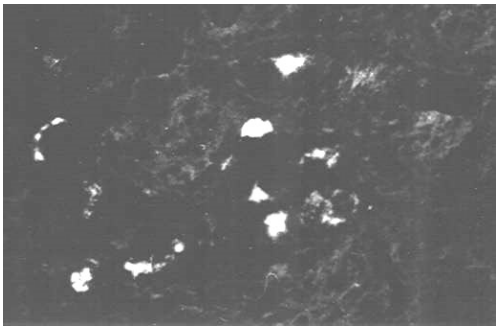

Fig.2. Old rat (26.6 months). Brain. Pontine reticular formation. Fluorescence microscopy (Autofluorescence). Extensive perinuclear, uni- and bipolar accumulations of neuronal and glial LPs. X 650.

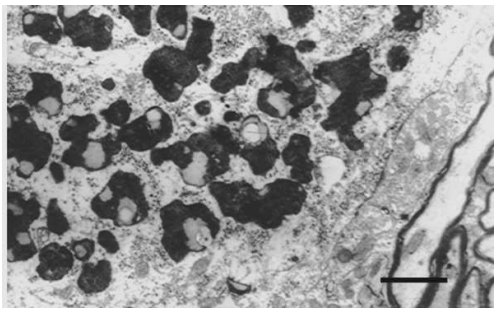

Fig. 3. Old rat (26.6 months). Brain. Cerebral cortex. Pyramidal neuron. Electron microscopy. Numerous polycyclic conglomerates of LPs, tend to cluster and to occupy a wide surface of the neuroplasm. Bar: 0.5 μm.

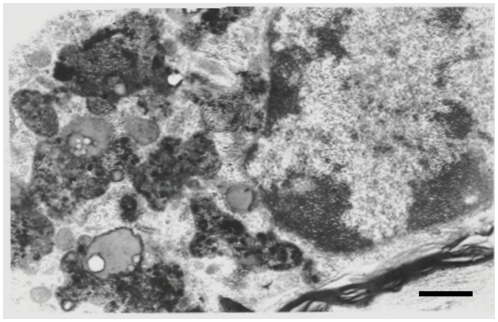

Fig. 4. Old human (80 years). Brain. Cerebral cortex. Vb pyramidal layer. Electron microscopy. Correlation in gliosoma of parenchymal microglia between aggregated LP deposits and reduced representation of free ribosomes and rough endoplasmic reticulum. Bar: 0.5 μm

Neuronal LPs and Proteasome - Catabolic Subcellular Systems

Neuronal LPs and catabolic subcellular systems shown interesting correlations. For examle, progressive LP accumulations and aggregations interact and are associated with:

- proteasome (multicatalytic proteinase complexes) instability and inhibition (Grune et al., 2004; Keck et al., 2003);
- lysosome (center of main hydrolases) dysfunction (Evans, 1993; Lynch and Bi, 2003; Porta, 1991), i.e. decreased activity of cathepsin L [EC3.4.22.15], a thiol proteinase, with advance in age;
- augmented amount of some lysosomal hydrolytic enzymes (Lynch and Bi, 2003), i. e. increased activity and concentration of cathepsin D [EC3.4.23.5], a carboxy proteinase;
- deficient and poor function of cellular recycling systems; and finally with
- accumulations of water-insoluble proteins, oxidized proteins, advanced protein glycation/glycooxidation end products, advanced lipid peroxidation end products, as pluri-metabolic sources and compounds of subcellular garbage (Riga et al., 2004; Terman and Brunk, 1998; Terman and Brunk, 2004).

Therefore, aging can be explained as a catabolic malfunction (Terman and Brunk, 2004). In addition, "garbage" accumulation theory of aging (Terman, 2001) considers the agglomeration of intracellular waste materials, results of imperfect intracellular degradations as fundamental feature of senescence.

Neuronal LPs and Cytoskeleton Abnormalities

LP storages negatively interact with neuron structure, and are constantly presented and correlated with appearance and development of cytoskeleton damages, as well as with amyloid deposits, and amyloid-related pathology (Braak, 1984; Grune et al., 2004; Riga et al., 2011a; Riga et al., 2011b).

Cytoskeleton abnormalities are represented by pathological filaments, which contribute to the formation of three different lesions, referred to as senile (amiloid, neuritic, argyrophilic) plaques, neurofibrillary tangles, and neuropil threads (Braak and Braak, 1988). Together with dystrofic neurites (meganeurites), microgliosis and astrocytosis, they form the neuropathological picture of Alzheimer's disease (Abraham, 2001).

Senile (amyloid) plaques, located within the neuropil, vary in diameter from 15 to 200 μm. They are formed from an intricate feltwork of pathologically changed and often ballooned processes of nerve cells (dendrites, as well as axons), reactive astrocytes, activated microglia, and frequently, a core of extracellularly deposited amyloid. Amyloid represents an extracellular protein storage, composed by the Amyloid β peptide - Aβ, a proteolytic fragment of the amyloid precursor protein - APP. In plaques, Aβ is associated with several other molecules: complement components, serine protease inhibitor α1-anti-chymotrypsin, heparan sulfate proteoglycans and apolipoprotein E (Abraham, 2000). In addition, these Aβ-associated compounds contribute to the aggregation of Aβ and its resistance to proteolysis. Moreover, Aβ induces an inflammatory reaction by stimulating microglia. Activated microglia secrete proinflammatory cytokines and ROS (reactive oxygen species), that are detrimental to the nervous tissue.

There is an interesting relation between neurofibrillary tangles (tightly packed bundles of paired helical filaments located within neurosoma, whence they may extend into proximal portions of the dendrites) and LPs (lipofuscin and ceroid). In general, the central bundes of the tangle form a dense and intricate feltwork around the storage of LP granules within the neurosoma (Riga et al., 2011b).

Neuropil threads, inconspicuous structures loosely scattered through the neuropil, are formed of small bundles of paired helical filaments contained in slender thread-like profiles. They do not cluster or accumulate in patches, columns or other storages.

An important conclusion for human beings appears. The regressive neuro-pathological changes, as seen in the senescent brain, mimic to a certain extent, the morphological characteristics observed in Alzhemer's dementia and in neuronal ceroid-lipofuscinoses (Braak, 1984; Braak and Braak, 1988). The main difference is that the neurodegeneration process progresses slowly or very slowly, and it is far less marked than that seen in the diseased CNS (Riga et al., 2011a).

GLIAL CORRELATES of LPs

Glial Paradox

Moreover, neuronal LP deposits coexist with glial LP storages, in all types of glial cells (astrocytes, oligodendrocytes, but especially in microglia), Fig. 4.

Thus, the glial paradox appears in brain aging and aged-related pathology. Neuroglia, mitotic cells, having moderate-to-high rate of divisions, are overloaded with LP conglomerates, characteristic of neurons, post-mitotic cells - long-life cells, other than phagocytic-degraded neuronal apoptotic bodies (Riga and Riga, 2006b).

Glial Activation and Functions

Microglial cells degrade oxidized extracellular proteins (Grune et al., 1997; Stolzing et al., 2002), and neuronal apoptotic bodies (Stolzing and Grune 2004), subsequent sources of LPs. But the large amounts of glial LPs (often up to 80%-90% of glioplasm) can be explain also by neurono-glial transfer of neuronal LPs. In this way, glia become collectors of neuronal LPs from the neurosoma periphery (Riga and Riga, 2006a; Riga and Riga, 2006b).

Glial Collection, Storage, Processing and Transport of LPs

Glial systems play an important role in collecting of neuronal LPs. Owing to their transporting properties, and migration capacity of microglia, glial cells deposit the LP clusters in pericapillary areas.

These natural recycling and purge mechanisms can be activated and completed by neuro-metabolic, anti-oxidative, neurovascular and nootropic therapy (Riga and Riga, 1995b; Riga et al., 2004).

INTERRELATIONSHIPS between BRAIN and AGING

Direct, causal interrelations, critical LP concentrations, which generate cascades of negative lifelong subcellular events, and indirect, associated impairment correlations determine characteristic neuropathological aging profiles.

Specific and associate negative neuropathologic consequences of LP storages have multiple and detrimental impacts on neuron and glia homeostasis, from neurono-glial function to CNS biochemistry and physiology (Riga and Riga, 1998).

CORRELATIONS betwwen AGING, BRAIN with PRO-LONGEVITY THERAPY

At the beginning of this new 3rd millennium, the innovative and holistic patterns for health systems are directed in the early detection, prevention and treatment of diseases, as well as in reversal of age-related dysfunctions and disorders. In this way, the concepts of "anti-aging medicine" (Goldman and Klatz, 2004; Riga, 2003) and “health-longevity medicine” (Riga et al., 2012) was developed and consolidated.

Anti-homeostatic actions of psychic distress, oxidative stress, decreased anti-oxidative defence, time-dependent LP accumulations and of subcellular dys-functions contribute to the initiation, maintenance, and magnifying of brain stress-aging cascade (distress-impairment-aging-frailty-polypathology), in accelerated and etio-pathogenic directions.

Inversely, in therapeutic conception, the anti-stress, anti-oxidative, anti-impairment, anti-aging, anti-frailty and anti-polypathology therapies represent strong natural ways in rejuvenation and prolongevity medicine, in anti-aging revolution and health-longevity evolution (Klatz and Goldman, 2003; Riga et al., 2012):

In the main, the complementary directions of anti-aging and pro-longevity strategies are represented by:

- metabolic support and activation of general and brain homeostasis, homeo-dynamics, redundance and vitality (Riga et al., 2005b; Riga et al., 2012);
- adapting stimulation in hormesis, including low level of stress (Rattan, 2005); cerebral and psychic activation and training therapies;
- caloric restriction with adequate nutrition - CRAN (Best, 2004; Goto et al., 2002; Riga, 2003), caloric restriction mimetics (Yu, 2015);
- daily physical activity and resistance exercises (Goto et al., 2004; Little, 2003; Yu, 2015);
- reformulate the body-mind connection by the continuous activation of main social determinants regarding human health-vitality-longevity (permanent work-activity, brain rebuilding and body reconstruction).

Concomitant application of these sanogenetic and provitality-longevity strategies will increase and mentain health in the world.

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