Growth and Trophic Factors, pH and the Na⁺/H⁺ Exchanger in Alzheimer´s Disease, Other Neurodegenerative Diseases and Cancer: New Therapeutic Possibilities and Potential Dangers

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Abstract: Abnormalities in the intricate intracellular signalling pathways play a key role in the deregulation of either spontaneous (normal or pathological) or induced (therapeutic) cell death mechanisms. Some of these pathways are increasingly becoming molecular therapeutic targets in different processes, ranging from neurodegenerative diseases to cancer. Recent discoveries in research and treatment have shown that failure to induce selective cell apoptosis in hyperproliferative processes, like neoplastic diseases, and the failure to prevent spontaneous cell death in neurodegenerative diseases (HNDDs) such as Alzheimer's disease (AD), multiple sclerosis (MS), amyothrophic lateral sclerosis (ALS), Huntington’s disease (HD), and retinitis pigmentosa (RP), can be interpreted as problems stemming from the same basic mechanisms but moving in diametrically opposed directions. The integrated approach advanced here represents an interdisciplinary attempt to stimulate an integrated vision of two otherwise widely separated areas of research, experimental neurology and oncology. This kind of approach to the prevention of apoptosis (therapeutic antiapoptosis) and/or other forms of cell death in HNNDs, as well as to resistance to therapeutic apoptosis in cancer (pathological antiapoptosis), has the scope to improve the understanding of the dualistic nature of the basic abnormalities underlying the pathological deregulation of cell death. In this context, an intracellular pH (pHi)-related approach to these opposed situations is advanced to provide a unified theory of the apoptosis-antiapoptosis machinery. Some potential therapeutic possibilities opened by these lines of research, regarding the utilization of human growth factors and/or cellular anti-acidification measures directed to sustain cellular acid-base homeostasis in different HNNDs are considered because of their potential therapeutic benefit. Finally, we advance some possible dangers and side-effects raised by these very same treatment efforts.

Keywords: Apoptosis and antiapoptosis, cancer and Alzheimer’s disease, neurodegenerative diseases, pH and Na⁺/H⁺ exchanger, p53, growth factors and tissue repair, trophic factor withdrawal.

"I recently was diagnosed with cancer and Alzheimer’s disease. Thank God I got cancer!"

A patient

"Wisdom is to walk in two opposite directions at the same time"

Lao Tse

INTRODUCTION

Modern medicine faces an almost total lack of success both in the prevention and treatment of human neurodegenerative disease (HNNDs) like Alzheimer's disease (AD), multiple sclerosis (MS), amyothrophic lateral sclerosis (ALS), Huntington’s disease (HD), and retinitis pigmentosa (RP), while good symptomatic treatments are currently available for other processes such as Parkinson’s disease (PD). New ideas and, perhaps, conceptually new lines of research in this area are urgently needed. Recent trends in both basic and clinical biomedical research are leading towards an increasingly in-depth study of apoptosis – programmed cell death (PCD) - as an essential mechanism in cellular homeostasis and tissue kinetics, and thus in the physiological regulation of the whole animal. However, other mechanisms of cell death different from apoptosis have been demonstrated to be important at different stages of neurodegenerative diseases. These different forms of cell death have been well defined before [1-3]. In any case, the pathological deregulation of apoptosis, by either genetic or environmental factors and mechanisms, plays a significant role in a wide variety of different diseases which can be mediated by an over-expressed apoptotic response leading to pathological cell loss (as in autoimmune, degenerative and neurodegenerative diseases) or, conversely, a deficient apoptotic response leading to excessive cell accumulation in the oncogenic process (cancer) [4]. The intricate cellular machinery that maintains a physiological balance of the dynamic
proapoptosis-antiapoptosis (and other pro-death/anti-death mechanisms) cellular programs is currently under a great deal of investigation, both in neurobiological research [1, 5-7] and in oncology research [8-11], with one of the key events of apoptosis at the molecular level being the activation of certain cysteine/aspartate proteases (mainly caspases 3, 8 and 9) [5, 12, 13]. Finally, the general term "apoptosis" has been used during the last few years in attempts to embrace the different programmed biochemical pathways of cell death that accompany tissue injury and neuronal degeneration [10].

From the etiopathogenic point of view, premature and spontaneous cell death in certain degenerative diseases of the central nervous system (CNS) seems to be related to the onset of an irreversible process of progressive cellular loss. Without ruling out other mechanisms of cell death, it is generally accepted that a pathological and excessive degree of spontaneous apoptosis significantly contributes to neuronal loss in the majority of HNNDs [1, 5-9]. The apoptotic process is known to be involved in at least some stages of AD degeneration, leading many investigators to suggest that neuronal death in AD is mediated by an apoptotic process (1, 6-8). Thus, a therapeutic option in processes such as Alzheimer’s disease and other neurogenerative diseases could consist in interrupting the chain of intracellular signals and/or the activity of genes or gene products that link multifactorial-induced neuronal damage with either apoptosis or parallel mechanisms of cell death [1, 5, 14]. For these reasons, the inhibition of certain caspases, mainly the upstream or initiator caspases such as caspases 8 and 9, which are clearly elevated in most neurodegenerative diseases, has been recently considered as a therapeutic target in these degenerative processes of the central and peripheral nervous system [1, 5-7, 15]. However, since the inhibition of caspase 9 suppresses apoptosis in AD and PD but the cells still die, at least one other important mechanism of cell death, termed paraptosis by Bredesen, has been described for HNNDs [2]. This cellular kind of death mechanism does not follow the morphological and functional criteria of apoptosis, is mediated only by caspase 9 activity, is Apaf-1 independent, and blocking the apoptotic arm with caspase inhibitors converts the cell death program to the nonapoptotic arm.

It can be proposed that the application of the knowledge that has been obtained of the systems and cellular mechanisms leading to the suppression of apoptosis - the pathological anti-apoptosis observed in cancer - could be used in preventing or retarding spontaneous cell death in HNNDs [5, 16]. Therefore, searching for common final pathways and key mediators for these two opposite directions: an exaggerated and pathological cell death (in HNNDs) and the resistance to it (in malignant diseases), becomes an important line of research in both areas, either to effectively stimulate cell trophism, growth and proliferation in neurodegenerative diseases [17, 18], or vice versa. Notably, a hierarchical integration of these new and integral conceptual approaches could help us to increase our knowledge of how to inhibit the action of certain oncogenes and growth factors in attempts to selectively kill malignant cells and tumors through metabolic and trophic deprivation. Finally, understanding the mediating and terminal biochemical pathways for both apoptotic and non-apoptotic programmed cell deaths, both caspase-dependent and caspase-independent, offers a real potential for improving our understanding the basics of both neurodegeneration and cancer in order to discover novel therapeutics for their prevention and treatment [2, 3, 19].

D) ONE SIDE OF THE COIN: INHIBITION OF CELL DEATH BY APOPTOSIS IN THE TREATMENT OF ALZHEIMER’S DISEASE AND OTHER HNNDs

Progressive cell apoptosis has been largely considered by many investigators to be a late phenomenon and just one of the causes and mechanisms behind neuronal cell loss in the majority of human neurodegenerative diseases, mainly because of the presence of a wide array of early apoptotic mediators present in vulnerable neurons of AD, including ß-amyloid, oxidative stress, nitric oxide, hydroxynonenal (HNE) oxidants, and other metabolic abnormalities [1, 5-9, 13]. However, other researchers disagree with that hypothesis for different reasons. First, because neuronal cell death in AD takes place over a too lengthy period of time [14]. Second, because the late stages of apoptosis are rarely seen in AD and the phenotypical alterations characteristic of the different stages of apoptosis are not seen in AD [1, 14]. This has led to the interpretation that the wide array of apoptotic markers present in AD may indicate an avoidance of apoptosis (abortosis) rather than completion of such a death program [20]. In any case, a correlation between caspase activation, stimulation of apoptotic pathways and neurofibrillary tangle (NFT) formation has been reported in AD [21]. Also, while initiator-upstream caspases (caspases 8 and 9) are associated with neurofibrillary pathology in AD, downstream-terminal-executioner caspases (caspases 3, 6 and 7) remain unchanged in vulnerable neurons [1]. In consequence, alternative models of programmed cell death, even caspase-independent, have been proposed [22]. It has also been suggested that a certain overlap of sharing of intracellular signals between different cell death mechanisms take place [3]. In paraptosis, for instance, apart from the fact that caspase 9 and Bcl-Xl inhibition suppress apoptosis, the cells still die and the morphological and biochemical criteria are clearly different from apoptosis [2]. These considerations seem to indicate that the most important targets of intervention in neurodegenerative diseases should be early in the loss of synaptic function. Thus, whatever the main initiating factors could be, such as oxidative stress, ß-amyloid accumulation or pH changes, neuroprotective measures should be ideally directed to these early alterations [23, 24]. Lately, the more general concept of PCD (programmed cell death) tries to integrate the different death mechanisms proposed in HNNDs [10, 24].

A feature that is altered in both neurodegeneration and cancer is cell cycle control or, better, lack of control. Pathological hallmarks in AD such as tau, amyloid and ß-protein precursor have been associated to changes in cell cycle control and progression, like re-entry into the cell cycle of terminally differentiated neurons. Both oxidative stress, apparently mediated through a decrease in pH, achieved by inhibiting Na’/H’ exchanger activity [25] (Fig. 1), and abnormal cell cycle events have been considered proximal processes in time to the onset of neurofibrillary tangle (NFT) and the senile plaque [26]. New markers of attempted cell cycle re-entry, like p27 and phosphorylated histone H3 induced by cyclin-dependent kinases [27], have been identified in vulnerable neurons in AD and blamed for the mitotic catastro-
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Apoptosis is known to be induced either by: 1) The active stimulation of mechanisms that induce it (ie. caspases) [2, 11-13], or, 2) by depletion, spontaneous or induced, of certain growth factors (trophic growth factor withdrawal) [16, 30, 31] (Table 1). Thus, there are two therapeutic, anti-apoptotic approaches to these neurodegenerative processes, should attempt: A) To inhibit the mechanisms that induce neuronal loss through a pathological stimulation of intracellular caspases, at least in caspase-dependent apoptosis (3); and B) To try to diminish and/or block a pathologically increased apoptosis mediated by a lack of trophic stimulation through therapeutic utilization of specific human growth factors and tumor-suppressor oncogenes [30, 31]. Both therapeutic possibilities are being considered at the present time by different research groups and in a wide array of clinical and preclinical essays [5-9].

Approach A

Regarding the first therapeutic approach, caspases are intracellular proteases that participate in the apoptotic pathways of human cells, including neurons. The inhibition of such caspases (mainly caspases 3, 4 and 9) has been shown...
useful in preventing programmed neuronal cell death in both in vitro and in vivo models of neurological disease. Such caspase-related apoptotic machinery appear to be involved in early stages of neuronal malfunction and protein aggregation in AD and HD, and also in the pathogenesis of PD and ALS [5]. Regarding AD, it is known that β-amyloid accumulation is a cause of cellular death through apoptosis, a process induced by caspase activation. For this reason, certain pharmacological inhibitors of caspase activity, such as BAF, z-VAD-FMK, etc., are, up to a certain point, able to inhibit neuronal death [11]. However, caspase inhibition achieves only a weak and transitory inhibition of apoptosis and other neurodegenerative phenomena, and even so, not in all the models studied [5-7]. This can be related to the fact that a pathological and excessive spontaneous apoptosis only seems to be, at the most, one of the different mechanisms responsible for neuronal cell death in different HNNDs [2, 5, 6, 20, 23]. Since it is not possible to conduct ‘in vivo’ functional studies in human brains, many of these questions must be answered by using the limited and scarce existing animal models available. Because direct experimental work is so hindered, a theoretical reworking of the known models becomes of paramount importance in order to not only improve existing conceptual approaches but also to create brand new proposals both in analytical work and in therapeutic discoveries.

Systemic or cellular over-expression of pro-apoptotic molecules such as Bax and p53, together with an inhibition of the anti-apoptotic protein Bcl-2, have been considered to be involved in the apoptosis underlying AD, PD and ALS (Table 1 and Fig. 1). Indeed, neuronal cells that over-express Bcl-2 are protected against apoptosis induced by taxol and other drugs that act on the neuronal cytoskeleton [11, 32]. These abnormalities are diametrically opposed to the pathological resistance to apoptosis (pathological anti-apoptosis) that is a hallmark of cancer and that it specifically takes place in both solid tumors and leukaemias [11, 33, 34] (Table 1). Along the same line of anti-apoptotic therapy, the inhibition or an abnormal function of suppressor gene p53 appears to be neuroprotective while at the same time facilitates cancer growth and spread and is also fundamental in tumor resistance to anticancer therapy [4, 35].

The jun-N terminal kinase (JNK), a member of the family of pH-sensitive MAPK kinases (36), has also been implicated in these neurodegenerative processes [6, 16]. For this reason, a series of inhibitors of JNK, like CEP-11347, are being studied because of the preliminary evidence for a therapeutic potential in antagonizing the formation of β-amyloid, a phenomenon induced, in part, by JNK activation [16, 30]. As a consequence, selective inhibitors of JNK3 could very well show a therapeutic effect in the treatment of AD and/or MS, etc., but, unfortunately, at the same time could also stimulate cellular proliferation and even malignant transformation [7, 16] (Table 1).

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<th>Pathological situation</th>
<th>HNDDs (Alzheimer’s disease, etc.)</th>
<th>Cancer</th>
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<td>Trophic factors</td>
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<td>Bcl-2</td>
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Table 1. Trends in Apoptosis-Related Opposed Situations in HNDDs and Cancer. (For More Details, See Text and Refs. Nos. 3-9)
Approach B

The second therapeutic approach to HNDDs considered here consists in counteracting apoptosis driven by deprivation of trophic and/or growth factors such as neuronal growth factor (NGF), a deficit that stimulates neuronal cell apoptosis through up-regulation of the intracellular caspase pathway [11, 31] (Table 1). Massive apoptosis induced by trophic hormone withdrawal was first reported in hormone-dependent tumors [36]. Microenvironment depletion or functional inability of different cell survival growth factors, such as IL-2 and IL-3, etc., also seems sufficient to induce apoptosis [37]. It has been recently suggested that an inadequate production of trophic molecules in tissues of neural origin or a decrease of certain growth factors in the cellular microenvironment of the CNS - a defect that could be secondary to a systemic failure of growth factor production - might have a significant role in abnormal nerve cell death in different HNDDs [31]. Thus, different antiapoptotic therapies for the degenerative diseases of the nervous system based upon the application of drugs that stimulate cell trophism, apart from certain growth factors, such as propargylamines, alpha-2-adrenergic receptor activators, are at the present time in preclinical stages [11, 31, 38].

Neurons, glial cells and the pigmented epithelial cells of the retina all contain platelet-derived growth factors receptors (PDGFR) making them classic therapeutic targets for PDGF, a feature of potential therapeutic significance that opens new possibilities to the treatment of certain HNDDs like retinitis pigmentosa (Table 1). At the same time, the fact that glioblastomas, as well as other different malignant tumors of different genetic backgrounds and origins express PDGF, PDGFR-α and TGF α, underlines a potential prooncogenic danger that such therapeutic protocols may present. In these cases, the autocrine stimulation by PDGF, etc., might contribute to both the onset as well as to the progression of either these highly malignant brain tumors or other malignancies [39, 40] (see below).

From the point of HNDDs therapy, these new therapeutic approaches are of fundamental importance, since the necessary technology for the clinical utilization of autologous human growth factors is now available. Different human growth factors (GFs) can be isolated from human blood in man growth factors is now available. Different human growth factors is an increase in the rate of exchange of Na\(^+\) and H\(^+\) ions and subsequent intracellular alkalinization, a phenomenon mediated mainly through the activation of the membrane-bound Na\(^+\)/H\(^+\) exchanger (NHE) [30, 42-46]. The NHE plays an essential pivotal role in the signal transduction of a multiplicity of intracellular signals induced by different hormones and trophic and growth factors by inducing an elevation of pH\(_i\) [4, 47, 48]. Further, its overexpression or excessive activity is crucial to the functional activation of different oncogenes such as ras and v-mos, in the suppression of the normal antimitumoral activity of suppressor gene p53 [35, 40], and in mediating cell transformation, proliferation and DNA synthesis [35, 43, 45]. The inhibition of this electroneutral membrane exchanger produces intracellular acidification by retaining hydrogen ions within the cell, [33, 37, 50]. It has been repeatedly demonstrated that inducing a severe intracellular acidosis below a certain threshold seems to be an essential factor in setting up a cascade-like chain reaction ending up in apoptosis [19, 37, 43, 51-60]. Indeed, trophic factors with clear-cut antiapoptotic activity stimulate the NHE, which is the main mechanism protecting the cell against a pathological fall in pH\(_i\), and thus, against apoptosis [4, 30, 44]. Among these antiapoptotic-antiacidification factors are the Bcl-2 family and a dysfunctional p53 suppressor gene [35, 42, 43, 51].

In an attempt to extend this knowledge in the opposite direction towards the study and treatment of HNDDs, we can consider that in certain abnormalities of trophic under-stimulation the signals for intracellular acidification could be the same as for spontaneous apoptosis [19, 37, 42, 51-59]. For instance, the activation of caspase-8 is preceded by intracellular acidification in cells induced to apoptosis by somatostatin [13], while the universal inhibitor of caspases, z-VAD-fmk, suppresses apoptosis by preventing a decrease in pH\(_i\) [5, 13]. In sympathetic and cortical neurons, inhibitors for certain caspases involved in the activating machinery of apoptosis, such as IDN-5370, exert a protecting effect on apoptosis [5-7]. On the contrary, intracellular acidification ‘per se’ activates caspases sensitive to z-VAD-fmk which precedes the onset of apoptosis [57]. Similarly, the up-regulation or dimerization of apoptotic Bax proteins, are activated by intracellular acidification, which is similar to what happens with caspase activation [42] (Fig. 1). (For a more complete review of the pathways and maps of intracellular signalling in caspase-mediated apoptosis, see refs. 5 and 38. For an in-depth study of the molecular aspects of caspase inhibition and antiapoptosis as a therapeutic strategy in HNDDs, see refs. 5-9).

The main bulk of available literature leads us to consider that cellular neuroprotection is most likely to be finally mediated by a pH\(_i\)-sustaining effect of intracellular acid-base homeostasis as described for cancer antiapoptosis and/or resistance to chemotherapy [4, 24]. This hypothesis is supported by data that either treatment with z-VAD-fmk or overexpressing of Bcl-2 are known to prevent apoptosis [42] and that both mechanisms prevent the intracellular acidification that systematically precedes apoptosis in different types of cells, a process that can also be set in motion by Fas or somatostatin receptors [3, 19, 33, 52]. This would serve as a warning on the generalized stimulation of protein kinases (PKs) in the treatment of HNDDs since it has also been published that at least three serine/threonine kinases: protein kinase C, calmodulin and MAP kinases can stimulate the NHE, thus increasing pH\(_i\) and inducing intracellular alkalinization.
Since the inhibition of the NHE produces intracellular acidification [33, 37, 50] and, below a certain threshold, becomes an essential factor in stimulating apoptosis [4, 19, 37, 51-56] (Fig. 1), the question is: where does all this lead to in HNDDs? It becomes evident that any “cell acidifying” pro-apoptotic agent would most likely lead to accentuate pathological processes whose main defect lies in a tendency towards spontaneous apoptosis (and/or related programmed cell death mechanisms) and progressive cell loss in any of the neurodegenerative diseases under study. However, a new paradox seems to arise here, since the potential benefit of increasing intracellular Na⁺ induced by the activity of the NHE in neurons leads to a secondary activation of the Na⁺/Ca⁺ exchanger, which in turn may stimulate the process of neuronal cell death via intracellular Ca⁺ overload. In this situation, NHE inhibition would protect neural cells from apoptosis-mediated death [60].

In a recent review on an integral model for cancer pathogenesis and treatment we have, literally, advanced the following conclusion: “What it is still missing, and is urgently needed at this point, is an integrated time-space intracellular pH-related map leading to the stimulation of acid-induced selective apoptosis (ref. 4, pp.16-17). This is partly because many of the mechanisms targeting proton extrusion pathways that allow cells to retain protons in order to induce a low pH-mediated apoptosis remain still to be elucidated [61]. However, there are an increasing number of research groups studying the cause-effect relationship among pH and programmed cell death (PCD) in a number of different systems (summarized in Fig. 1). In this figure we have tried to consider the evidence so far available on the low pH-mediated apoptosis relationships. Most significantly, the seminal work of Vincent el al., in neurons, have shown that just lowering intracellular (i. c.) pH from 7.36 to 7.09/7.00 through different methods activates three low pH-dependent endonucleases which set in motion a PCD increasing DNA fragmentation and decreasing at the same time neuronal survival [24]. This group have demonstrated that nitric oxide (NO), which has also been linked to the development of several neurodegenerative disorders, from PD to AD, induces neuronal PCD and that this is mediated by the rapid induction of an acidic i. c. pH environment rather than through caspase activity. They concluded that counteracting a drop in pH, by peptide growth factors, for instance, can be a possible therapeutic target against neuronal degeneration [24]. Endonuclease activation during pH-related apoptosis has also been shown by other groups after observing that i. c. acidification correlates with DNA digestion through a low pH$_i$ stimulation of DNase II [62, 63] (Fig. 1). ICE-protease activation and DNA fragmentation are also induced by a decrease of pH$_i$ during apoptosis, which places acidification as an early mediating step in the apoptotic cascade (Fig. 1). Further, inhibiting the NHE also facilitates apoptosis mediated by CD95 (Fas/Apo-1) [64]. Acidification-mediated apoptosis also takes place by SHP-1 mediated somatostatin (SST) and fas ligation through cleavage of the NHE1 and a decrease in pH$_i$, in a process that precedes caspase 8 activation and apoptosis [13, 51] (see also Fig 1). Lastly, neuronal death has been observed in different neuronal tissues of animals genetically lacking NHE [65, 66].

While Na⁺/H⁺ exchanger activation increases resistance to cell death, hydrogen peroxide suppresses Na⁺/H⁺ exchanger activity and gene expression thereby increasing sensitivity to cell death triggers [67]. In this line, hydrogen peroxide induces i. c. acidification, which stimulates cytochrome C release and caspase activation prior to apoptotic cell death [68] (Fig 1). This is important since oxidative stress is widely recognized to be one of the earliest changes detected in neuronal degeneration in HNDDs. It has also been suggested that a mitochondria-mediated decrease in pH$_i$ releases cytochrome C by activation of cytosolic caspases, and this is an early event in inducing apoptosis [57]. Acidic stress also up-regulates Bax protein, which through caspase activation is followed by PARP cleavage and DNA fragmentation in G1 tumor cells [69]. Other authors have also considered intracellular acidification to be an essential and early step in apoptosis [57, 59, 70], however there are also reports stating that an acid pH is not always essential for apoptosis to take place [71]. Furthermore, cell acidification mediated through the inhibition of the Na⁺/H⁺ exchanger has been shown to prevent the protein tyrosine kinase-dependent suppression of apoptosis in different cellular systems [72].

On the contrary, and from the point of view of treatment, molecules like Bcl-2, PMA, monensin, chloroquine, imidazole, as well as different trophic factors like G-CSF or IL3, elevate pH$_i$ and thus prevent cells from undergoing apoptosis both in malignant and non malignant cells [19, 43, 56, 73--77] (Table 1). Maintaining pH$_i$ at an alkaline level (pH$_i$=7.60), also the prevents neuronal injury, suggesting both a critical role for i. c. pH in PCD and a possible role for therapeutic cell alkalinization in the prevention and treatment of neuronal degeneration. In the same vein, antiapoptotic Bcl-2 has been shown to directly prevent cellular acidification and thus, cellular injury [51, 52] (Fig. 1). Finally, the utilization of certain growth factors opens new possibilities in the treatment of HNDDs because trophic factor withdrawal may induce acidification-mediated apoptosis, a feature of pathogenetic importance in HNDDs, and also because the program of cell suicide can be initiated by removal of different growth factors [24, 37].

While it can be concluded that inhibition of apoptosis in cancer cells appear to be completely suppressed by either preventing intracellular acidification or directly inducing intracellular alkalinization, the bulk of evidence also suggests that any measures directed to prevent a pH$_i$ fall in Alzheimer’s disease and other HNDDs as a therapeutic target, would be in the right direction in order to contribute to cell survival and prevent spontaneous-pathological cell death, either apoptotic or parapoptotic. To this effect, this perspective opens a brand new line of possibilities in the preventive and/or therapeutic treatment of HNDDs, and introduces in consideration, as a key and pivotal therapeutic target, any drug or biological compound, alone or in combination (imidazole, chloroquine, neurotrophic or other growth factors, oncogenes, cell proteins, gene products, platelet transfusions) that would activate the NHE, sustaining the pH$_i$ within normal limits and so normalize intracellular acid-base homeostasis (Table 1).
II) THE OTHER SIDE OF THE COIN: THERAPEUTIC INDUCTION OF HNNDs-LIKE APOPTOSIS IN THE TREATMENT OF MALIGNANT DISEASES. A DIALECTIC BETWEEN OPPOSITES OR A COINCIDENTIA OPPOSITORUM?

The above approach used to analyze cell death in HNNDs could facilitate the recognition of new targets and mechanisms of possible therapeutic exploitation in both HNNDs and malignant diseases. In the latter case, these considerations help to understand why specific inhibitors of the NHE of the amiloride series or other drugs that block NHE (endostatin, angiostatin, squalamin, 2-metoxiestradiol, anti-VEGF Ab, thalidomide) [56, 58-60], have been increasingly paid attention to as direct antitumoral agents during the last few years. Some of them have also proved effective in increasing either activity and/or specificity (via synergy) of different anticancer drugs [4, 43, 45, 82]. In this "pro-apoptotic" therapeutic line, it has been repeatedly demonstrated that inducing a severe intracellular acidification below a certain threshold is also directly related to the onset of apoptosis after treatment with drugs such as etoposide, camptothecin, cycloheximide, lovastatin or paclitaxel [42, 44, 51]. Finally, a series of pro-apoptotic drugs, all of them sharing the unique characteristic of inducing cell acidification, have also been tested in a multiplicity of studies in different attempts to overcome MDR (multiple drug resistance) to antitumor agents in a variety of cancer cells and tissues. These drugs include verapamil, cyclosporine A, tamoxifen, amiodarone, bafilomycin A and nigericin [33]. Finally, time-activity studies have demonstrated that the intracellular acidification of apoptosis takes place downstream of the activity of the ICE/CED-3 protease, because the inhibition of the protease with z-VAD fluoromethylketone (FMK) completely prevents the lowering of pH, DNA digestion and other events associated with apoptosis [81].

Since apoptosis, at least malignant cell apoptosis, usually takes place under conditions of intracellular acidification (for a review, see Ref. 4), it becomes highly important to stress the recently published observations that the final molecular mechanism responsible for the loss of the protective function of p53, and subsequent carcinogenesis, is directly related to a mechanism responsible for the loss of the protective function of this acid-base factor in both HNDDs and cancer. Thus, intracellular acid-base homeostasis represents a key and specific molecular mechanism that should always be taken into account as a high priority issue in the study and research of any apoptosis-mediated diseases, either neoplastic or neurodegenerative.

Since the deleterious effect of an elevated pH in preventing therapeutic apoptosis in malignant disease represents a key factor in the pathogenesis of pH-related multiple drug resistance (MDR) [4, 53-56, 77], this helps to better explain the role of a high pH in the drug resistance induced by most, if not all, the different growth factors, gene products and cytokines studied in this context [26, 46]. Further evidence in this area arises from the fact that the elevation of pH induces by imidazole or chloroquine prevent Fas-mediated apoptosis, while the induction of intracellular alkalinization plays a fundamental role in the suppression of apoptosis by a wide array of other molecules, such as PMA, IL-3 and platelet stimulating factor [33, 42, 57]. It can be concluded that, independently of the effector molecule, cellular alkalinity, or at least, a non-acidic pHi is a critical factor in cancer cell survival in all conditions and types of malignant processes of whichever genetic origin [4, 35, 42-45, 49, 68, 82]. The inability of a high pHi to induce selective cell apoptosis can also be related to parallel research lines in translational oncology like the attempts of inhibition of mitogen-activated protein kinases (MAPK) through inhibition of such PKs with drugs like imantib mesylate or gefitinib [83], as well as hitting molecular targets and mechanisms leading towards overcoming resistance to inhibitors of protein kinases [8, 84-86] (Table 1). An integration of all these observations underlines the importance of knowing in detail the common and/or pivotal and/or final pathways mediating cell apoptosis in order to selectively induce it for therapeutic purposes in cancer [4, 8, 9, 15, 56, 74, 75], or inhibit cellular death in certain HNNDs [2-4], two opposite pathological processes that also appear to protect from developing the other one, at least to a certain point [87, 88].

III) POTENTIAL THERAPEUTIC APPLICATION OF HUMAN GROWTH FACTORS IN HNNDs

Recent groundbreaking research reported the recent discovery of a decreased secretion and release of VEGF in the supernatants of circulating natural killer (NK) immune cells in patients with AD compared to normal controls and patients with other types of senile dementias [90]. These findings become even more significant if, as these authors suggest, the importance of VEGF in brain angiogenesis, neuroprotection and cerebrovascular exchange of nutrients is taken into account. Recent evidence points to a pivotal role for VEGF in neuronal protection [91, 92]. Also, a lack of activity of VEGF has been shown in neurodegenerative diseases in spite of the fact that no differences in serum VEGF levels were found between AD patients and controls in that study [93]. Other authors have found co-accumulation of VEGF with β-amyloid apart from a strong binding of VEGF to it, suggesting that secondary VEGF deficiency under hypoperfusion may contribute to neurodegeneration and vascular dysfunction in the progression of AD [94].

From a therapeutic point of view, applying neurothrophic factors in certain neurodegenerative diseases, such as ALS, has proven of significant benefit in certain animal models of non-human neurodegenerative diseases (NHNDDS). In this regard, direct application of vascular endothelial growth factor (VEGF), a proposal that could be considered "dangerous" in an oncological setting, delays the onset of paralysis, improves motor function and increases survival in a rat model of this disease [95, 96]. In the same vein, mice lacking epidermal growth factor receptor (EGFR) develop neurodegeneration of unknown etiology through Akt-caspase dependent apoptosis of the frontal cortex [97], offering another example of neuronal degeneration induced by trophic factor depletion. In this system, an activated Ras oncogene can prevent neuronal death, another case of a "dialectic between opposites"
between cancer and HNNDs, which conforms to the subject of the following section of this contribution. Finally, the emerging role of other growth factors, like fibroblast growth factor (FGF) and its interaction with its receptors, has been reported to present a significant role in brain neuronal trophism, repair and protection, and may play a role in both the treatment of certain mental disorders [98].

From a therapeutic perspective, the use of different growth factors in a wide variety of human pathologies can be achieved by its loco-regional application or autologous systemic administration and it is a relative simple procedure in clinical medicine [41]. This new research field allows for important and exciting new perspectives of tissue replacement in any clinical situation where a lack of one or more trophic factors is involved in its pathogenesis, including degenerative pathology where stimulation of new tissue growth or regrowth is necessary. For instance, Fig. (2) shows how human tendon cell cultures proliferate after only six days of treatment with platelet rich clot (PRCR) with a high concentration of platelet-derived growth factors (PRGFs). Clot preparations containing high amounts of certain growth factors and metabolites that stimulate cell trophism, growth and proliferation are being used with increasing frequency in different medical and surgical contexts, especially in inducing new tissue formation and accelerated tissue repair [17, 18, 89].

Preparations of concentrated plasma rich in autologous growth factors (PRGFs) are obtained through the application of previously described methods [17, 41]. These releasates of growth factors contain high concentrations of mitogenic and pro-angiogenic factors such as platelet-derived growth factor (PDGF), β-transforming growth factor (TGF-β1), EGF (epidermal growth factor), vasoendotelial growth factor (VEGF), platelet-derived endothelial growth factor (PDEGF), insulin-like growth factor 1 (IGF-1) and hepatocyte growth factor (HGF), as well as cytokines PF4 and CD40L, apart from lower concentrations of other factors like b-FGF. Some of these factors show a potent stimulating effect on cell growth, proliferation and viability, to a large extent because of their positive effects on angiogenesis. New vessel formation induced in these experiments is of paramount importance in achieving a rapid repair of different healthy tissues [18, 89]. Further preclinical and clinical experimental protocols using cells of neural origin are strongly advised by these preliminary results.

Potential Dangers of Growth Factors (GFs) Therapeutics

Most recently, the question has been raised if the local and/or systemic application of growth factors could present undesirable secondary effects, such as fostering cellular transformation in vivo, thus leading to an increase in the frequency of new malignant tumors as well as worsening cancer growth and dissemination of already existing malignancies, at least in the areas surrounding loco-regional GFs infiltration. Along this line, it is well known that some of these GFs behave as oncogenes in a variety of cell systems and are associated with malignant transformation directly mediated by inducing a high pH [43, 44, 99]. For instance, pseudopod formation, a key feature in stimulating tumor infiltration of surrounding tissues, can be induced by hepatocyte growth factor receptor (HGFR), as well as by other biophysical and energy-related factors such as the low interstitial tumors pH and a highly specific reversed pH/pHe tumor gradient [4, 47, 80]. In the same vein, both local tumor growth and metastatic dissemination are processes known to be highly dependent on tumor angiogenesis [55]. An elevated plasma level of VEGF is considered a marker in the activity of certain malignant processes, like soft tissue sarcomas, reflecting both tumor degree and response to treatment [100]. Similarly, urinary levels of bFGF in patients recently diagnosed with leukaemia are up to seven times higher than in the normal situation, and even higher than in any other malignancy [78]. This also justifies the rationality behind the fact that an
array of therapies in clinical oncology target the inhibition of VEGF or PDGF (p.e.: imantib mesylate) in different attempts of controlling tumor angiogenesis [38, 101, 102]. The fact that platelets themselves are the origin of VEGF, IGF-1, HGF, bFGF and other highly angiogenic factors, leads to the conclusion that platelet-derived molecules can be involved in fostering both tumor growth and metastatic spread of malignant tumors in human beings [34, 40, 103, 104].

The doubt arises that if the exogenous utilization of GFs like PDGF, VEGF and others, while having a potential value in neurogenesis and neural stimulation [105], could present undesirable side-effects because of the possibility of either increasing oncogenesis and/or speeding up the spread and aggressiveness of a clinical or subclinical metastatic process [106]. The same holds true for the suggestion that treatment with anti-apoptotic measures against p53 could be useful in HNDDs, as has been suggested by Waldeimer [7]. Since a normal functioning of p53 has low pHt-related anticancer activity and suppresses oncogenesis, its therapeutic inhibition in diseases like AD or PD could become problematic because of the possibility of initiating neoplastic transformation and further stimulation of malignant growth and spread [35, 49]. All the above considerations suggest that the loco-regional and/or systemic application of GFs as a therapeutic strategy in neurodegenerative disorders could become related to the occasional onset and/or accelerated growth of malignant tumors. For this reason, patients being treated with loco-regional applications of GFs should have no evidence of the presence of a malignant process in the area of treatment. However, on the weight of so far available evidence, the possibility that this could happen can be considered quite remote. First, because the stimulatory period of exogenous and/or autologous human GFs is relatively short [41]; and second, because local extravascular application of GFs contained in concentrated platelet preparations will most likely have no effect regarding increased systemic levels of these GFs.

From all the above considerations, it can be concluded that even the presence of a history of malignancy in areas of the organism far from the treated area (muscles, joints, maxilo-facial surgery, diabetic ulcers) [17, 18, 89, 107] could be excluded as a therapeutic contraindication a priori. A vast clinical experience of several years and hundreds of patients treated with platelet-rich plasma with high concentrations of GFs has not shown a single case of malignant tumors being induced by these procedures [18, 41, 89, and E. Anitua, personal communication]. However, before more clinical data is available, it would be prudent to avoid the loco-regional utilization of plasma-rich platelet preparations. In the case of dental implants, for instance, any patient with a previous history of head and neck cancer or leukaemia should be excluded until further data becomes available [108].

It is necessary to emphasize that since one of the main mechanisms through which most of these growth factors stimulate cell proliferation is through the up-regulation of the NHE and the subsequent increase in pHt, this deleterious growth stimulus could potentially be suppressed by inhibitors of the NHE [44, 45]. Since a pathological and persistent intracellular alkalinization is increasingly recognized to be an specific and fundamental primary cause of both cell transformation and subsequent dissemination [4, 46, 47, 80, 99], it will be necessary to take into account that any single cause or multifactorial mechanism that induces a pathologically high pHt or, similarly, a chronically reduced microenvironmental extracellular pH (pHe), has serious possibilities of inducing the onset and/or spread of a malignant process [43, 45, 48, 80, 89]. Finally, it is necessary to mention that while there is a certain degree of contradiction among different groups regarding the relationships among pHt changes, apoptosis and bFGF and VEGF expression [19, 46, 109], a close and direct relationship between tumor growth, local and distant spread and NHE activity has recently been shown [4, 46, 47].

IV) CONCLUSIONS. TIGHTROPE WALKING ON THE EDGE OF THE COIN. TOWARDS A “BOTH SIDES NOW” INTEGRAL PERSPECTIVE

This contribution, while not trying to be an in-depth revision of the intricate intracellular molecular pathways in normal or pathological cellular homeostasis and apoptosis, may open new conceptual interrelationships not previously considered. One of its main points is to raise the possibility that intracellular acidification may be directly involved in the mediation of at least certain cases of spontaneous and pathological apoptosis and/or any other PCD program in HNDDs, just as happens in successful therapeutic stimulation of apoptosis in cancer treatment [56, 110]. It may be that, beyond the multiple etiological causes and early mediating mechanisms, an intracellular acid-base mechanism could be the pivotal and/or common final event in down-regulating the caspase-mediated, or even caspase-independent, intracellular signalling pathway leading to neural cell death and/or β-amyloid accumulation in Alzheimer’s disease and other HNDDs [37, 42, 44, 48-52, 56-59, 68, 74-76, 105, 111, 112]. This homeostatic acid-base cellular initial approach to both HNNDs and cancer has been a largely disregarded area in modern neurological and oncological research. If such intracellular acid-base abnormalities could be proven to take place regularly in the early or late mediation of cell death in HNNDs, an entirely new line of therapeutic possibilities would open through the use of autologous or heterologous pHt-elevating growth factors, cytokines and gene products in an attempt to improve cell viability and prevent the undue cell death arising in HNNDs, either through pathological “spontaneous” apoptotic cell death and/or parallel, related or not, PCD mediating mechanisms. After an in-depth analysis of the presently available data dealing with both pro-apoptotic and anti-apoptotic situations (Table 1), it can be concluded that the therapeutic failures both in malignant and in neurodegenerative diseases might have the same ultimate nature but deal with opposite underlying mechanisms and run in opposite directions.

From a therapeutic point of view, the main two main conclusions of this integrated review are: 1) We need to look for a selective i. c. acidification to produce apoptosis in cancer treatment and in overcoming MDR, and 2) It is necessary to search for measures able to prevent the same i. c. acidification and cellular death in HNNDs. Finally, regarding the relationships of HNDDs, cancer, and the possible utilization of growth factors and certain anti-apoptotic substances as potential therapeutic agents in human trials in human neu-
rodent degenerative diseases, the following points should be advanced:

a) It is advisable to undertake further clinical studies to detect the presence, decrease or even absence of both systemic and cellular (in platelets, for instance) antiapoptotic GFs and antiapoptotic substances (PDGF, VEGF, NGF, tyrosine kinases, Bcl-2, Bcl-Xl) in HNDDs (Table 1) [5-7, 17, 18, 89, 108]. The necessary technology for these purposes is already available.

b) Any measures leading towards preventing intracellular acidification in HNDDs could become an anti-apoptotic approach that would help to maintain a healthy cellular homeostasis [43, 48, 74]. Any factor that would stimulate and/or cooperate to up-regulate and express the NHE1, and any drug or biological compound, alone or in combination (imidazole, chloroquine, neurotrophic or other growth factors, oncogenes, cell proteins, gene products, plasma-rich growth factors (PRGFs), platelet transfusions) present a significant potential benefit in the prevention and treatment of HNNDs diseases (Table 1). This approach would be mainly aimed towards activating NHE and/or parallel mechanisms that would sustain the pH, within normal-physiological limits in order to regulate intracellular acid-base homeostasis, so preventing PCD programs and spontaneous cellular death programs in HNDDs. In this same line, propargylamines, alpha-2-adrenergic receptor activators and different antiapoptotic drugs are presently in pre-clinical stages [11, 31, 38].

c) On the contrary, methods directed to selectively decrease intracellular pH in cancer therapy would favour selective cancer cell apoptosis and the overcoming of multidrug resistance (MDR). However, the loco-regional or systemic use of platelet-derived GFs and/or certain other cytokines in an oncological setting could speed up the growth and dissemination of already present malignant tumors or leukemias, and even induce them de novo [4, 45, 47, 104-106]. Up to now, however, there has not arisen any evidence in medical practice that the loco-regional application of preparations containing concentrated amounts of plasma-rich platelet-derived growth factors can become a potential danger for humans not previously diagnosed of a malignant process.

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REFERENCES


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