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Biochemical Pharmacology

Biochemical Pharmacology 61 (2001) 769–777

Commentary

Molecular targets for pharmacological cytoprotection

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Abstract

Cell death is common to many pathological conditions. In the past two decades, research into the mechanism of cell death has characterized the cardinal features of apoptosis and necrosis, the two distinct forms of cell death. Studies using *in vivo* disease models have provided evidence that apoptosis is induced by an array of pathological stimuli. Thus, molecular components of the machinery of apoptosis are potential pharmacological targets. The mechanism of apoptosis can be dissected into: (i) the initiation and signaling phase, (ii) the signal amplification phase, and (iii) the execution phase. Reflecting on the diversity of apoptotic stimuli, the initiation and signaling phase utilizes a variety of molecules: free radicals, ions, plasma membrane receptors, members of the signaling kinase cascades, transcription factors, and signaling caspases. In most of the apoptotic scenarios, impairment of mitochondrial function is an early event. Dysfunctioning mitochondria release more free radicals and hydrolytic enzymes (proteases and nucleases), amplifying the primary death signal. In the final phase of apoptosis, executioner caspases are activated. Substrates of the executioner caspases include nucleases, members of the cellular repair apparatus, and cytoskeletal proteins. Partial proteolysis of these substrates leads to distinctive morphological and biochemical changes, the hallmarks of apoptosis. The first steps toward pharmacological utilization of specific modifiers of apoptosis have been promising. However, since the potential molecular targets of cytoprotective therapy play important roles in the maintenance of cellular homeostasis, specificity (diseased versus healthy tissue) of pharmacological modulation is the key to success.

Keywords: Cytoprotection; Apoptosis; NF-κB; NOS; PARP; Caspase

1. Introduction

During the 1980s, painstaking research in different fields of biomedical science revealed a new concept in the regulation of cell destiny. All animal cells are armed with genetic machinery to commit suicide. Cells constantly require receipt of survival signals to prevent activating the suicidal machinery. Only physiologically functional cells survive; damaged, aberrant, infected, nonfunctional, or developmentally redundant cells die through a type of cell death termed apoptosis. Research in histology, genetics, and molecular biology has defined two principal patterns of cell death: apoptosis and necrosis.

2. Necrosis

Necrosis is considered as the pathological form of cell death. The main outcome of biochemical events is a loss of cellular ion homeostasis. The increased intracellular calcium concentration results in activation of calcium-dependent DNAses, phospholipases, and proteases. Morphological studies have reported nonspecific organellar damage. Necrotic cells swell and lyse, emptying their cytoplasmic and nuclear content into the intercellular space, sparking inflammation.

3. Apoptosis

Apoptosis is characterized by distinct, well-defined biochemical changes. The nuclear DNA is digested into oligo-

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Abbreviations: AD, Alzheimer's disease; AIF, apoptosis-inducing factor; ALS, amyotrophic lateral sclerosis; Apaf-1, apoptotic protease activating factor-1; APP, amyloid-β precursor protein; DFO, desferrioxamine; GSH, glutathione; JNK, Jun N-terminal kinase; MAO, monoamine oxidase; MESNA, 2-mercaptoethane sulfonate; MPT, mitochondrial permeability transition; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NAD^+, nicotinic acid amide dinucleotide; NAIP, neuronal apoptosis inhibitory protein; NF-κB, nuclear factor-κB; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; PD, Parkinson's disease; PIG-3, p53-induced gene 3; PARP, poly(ADP-ribose) polymerase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF, tumor necrosis factor; and ZVAD-fmk, benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone.

0006-2952/01/$ – see front matter
PII: S0006-2952(00)00585-2

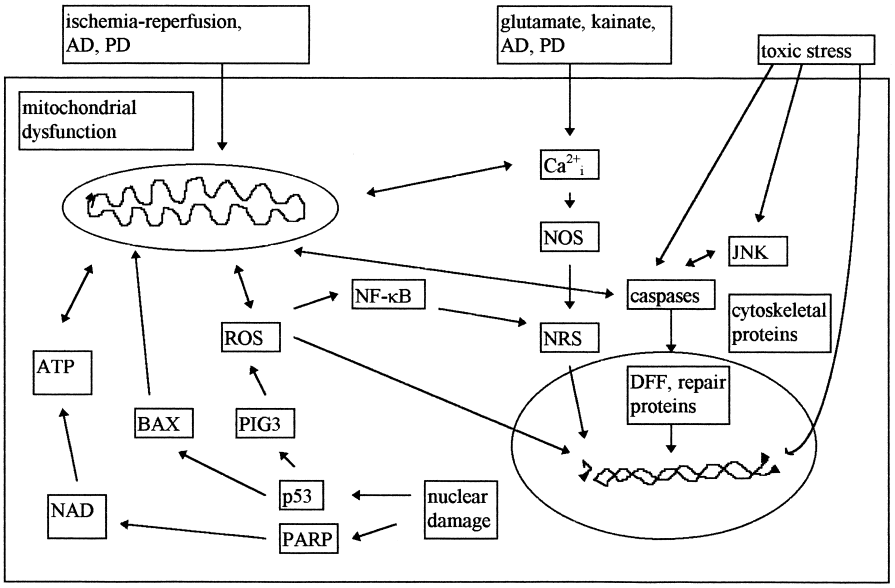

Fig. 1. Mechanism of apoptosis induced by pathological stimuli. Initiator damage, either extracellular (free radicals, excitotoxins, chemicals, radiation) or intracellular (organellar dysfunction), directly targets intracellular organelles or activates apoptotic signal transduction (JNK, calcium, signaling caspases). The primary death signal is amplified by mitochondria (ROS, calcium, caspase activation). The final phase is orchestrated by executioner caspases that cleave a set of proteins involved in cytoskeletal assembly and DNA metabolism.

nucleosomal fragments, and activation of caspases yields partially proteolysed proteins. In some cell types, activation of tissue transglutaminase has been reported, resulting in highly cross-linked membranous structures, termed apoptotic bodies. Apoptosis is an ATP-dependent, active process. In some cell culture models, changes in gene expression are a requirement, hence the term programmed cell death. Morphological changes include nuclear condensation, cell shrinkage, and membrane blebbing. Intracellular organelles remain intact in the early stages of apoptosis. In *in vitro* models, apoptosis is followed by a secondary necrosis. *In vivo*, however, the remnants of apoptotic cells are cleared up by professional phagocytes or nearby cannibal cells. Thus, apoptosis does not induce inflammation. Although apoptosis has been considered the physiological form of cell death, inappropriate activation of apoptosis by pathological or toxicological stimuli (e.g. mild ischemia, virus infection, chemotherapy) may cause or contribute to a variety of diseases, including stroke, AIDS, and neurodegenerative diseases.

There is evidence that apoptosis occurs by a mechanism that has been conserved throughout animal evolution. Therefore, results obtained from studies on more accessible models may be directly relevant to the mechanism of cell death in humans. This has fostered optimism that it may be possible to control apoptosis by the development of drugs that target molecular components of the death machinery.

Apoptotic cell death has been well documented in various ischemic, degenerative diseases, as well as in patients receiving radiation or chemotherapy. This has led to a surge in research into novel therapies to protect the diseased tissue against cell death. On the contrary, induction of apoptosis in rapidly growing tumor cells is the goal of chemotherapy and radiation treatment. This strategy unavoidably harms normal cells and tissues that have a high cell turnover. Rational drug design opens the door to highly specific cytoprotective drugs that protect healthy tissue without reducing the efficacy of the anticancer therapy. Modulation of apoptosis induced by physiological stimuli (e.g. TNF, Fas-ligand, and growth factor deprivation) is outside the scope of this commentary.

The main targets of pharmacological inhibition of apoptosis have been depicted in Fig. 1. The pathway of apoptosis although complex can be dissected into: (i) the initiation and signaling phase, (ii) the signal amplification phase, and (iii) the execution phase. The initiation–signaling phase is the most diverse, both mechanistically and topologically. The initiation of cell death by excitotoxins [1] and some forms of toxic stress (e.g. UV) [2] is a plasma membrane event. The signaling phase includes calcium-activated processes, activation of signaling caspases, and stress-induced protein kinases such as JNK [see Ref. 3 for review]. Chemotherapeutic agents (e.g. cisplatin, camptothecin) target nuclear DNA metabolism. Excessive DNA damage activates p53, which orchestrates the apoptotic response by inducing the expression of Bax (a proapoptotic Bcl-2 homology protein) and PIG 3 that forwards the apoptotic message to mitochondria [see Ref. 4 for review]. PARP is also activated by single- or double-stranded DNA brakes. PARP activation and the consequent depletion of its substrate, NAD^+, contribute significantly to cell death if DNA damage is extensive [5]. The least specific is apoptosis induced by ionizing radiation that generates ROS, which then inflict damage on the nucleus and mitochondria [6]. The primary death signal is transduced to mitochondria by

signaling caspases [7], Bax [8], ROS [9], or elevated calcium [10]. Mitochondrial damage leads to the generation of ROS and the release of calcium, cytochrome *c*, and AIF [reviewed in Ref. 10], thus greatly amplifying the primary death signal. A variety of death signals converge to activate the executioner caspases. Caspase death substrates include cytoplasmic and nuclear proteins involved in DNA repair, replication, RNA splicing, cytoskeletal structure, and cell division. Once caspases are activated, the morphological changes of apoptosis ensue, and the killing process cannot be stopped [reviewed in Ref. 11].

4. Free radicals

Mammalian cells need to protect themselves constantly against oxygen-derived free radicals. Under normal conditions, an equilibrium exists between prooxidant and antioxidant pathways. Upon receiving stress stimuli, the redox imbalance leads to the accumulation of ROS. ROS have long been regarded as the main mediators of apoptosis [12]. ROS play a dual role: (i) as mobile messengers of damage, and (ii) as executioners by damaging membranes, proteins, and DNA.

In the past two decades, the leading strategy was to find molecules that reconstitute cellular GSH, the endogenous antioxidant. The prime candidates were low molecular weight thiols or precursor molecules that, in addition to aiding the maintenance of cellular GSH homeostasis, directly scavange free radicals. Early work has demonstrated that *N*-acetylcysteine was effectively deacylated by hepatocytes and supported GSH synthesis [13]. L-Oxo-thiazolidine-4-carboxylate breaks down to L-cysteine [14] and can be used immediately in the GSH cycle. MESNA, a clinically used uroprotector, has also been shown to reconstitute endogenous GSH levels [15]. Membrane-permeable glutathione mono- and diesters are especially efficient in boosting cellular GSH content [16]; however, there have been reports on toxicity, possibly due to contaminating compounds. Amifostine (WR-2721, Ethyol), a drug that has received much attention recently, is an analog of cysteamine. This phosphorylated prodrug is dephosphorylated by a membrane-associated phosphatase to its active thiol form (WR-1065). Treatment with WR-1065 causes dramatic elevation of cellular glutathione and cysteine levels, accompanied by marked protection against radiation treatment [17]. Direct scavanging of $OH^{\cdot}$, donation of H from its SH function [18], and facilitation of cellular cysteine uptake [17] contribute to this effect. Amifostine is a broad spectrum cytoprotective agent, effective against the most common cytotoxic drug-related toxicities [reviewed in Ref. 19]. These findings highlight a crucial role for ROS in the cell death pathways.

Primary ROS are metabolized extensively through the Fenton and Haber–Weiss reactions [20]. These reactions are mediated by ferrous–ferric and/or cuprous–cupric ions. Lending support to the concept that ion chelators are potential cytoprotective agents, the iron chelator DFO has been shown to improve survival and physiological function in various models of cerebral [21], cardiac ischemia-reperfusion [22], and neurodegenerative [23] diseases. The 21-aminosteroids (Lazaroids) were designed as membrane-specific antioxidants by attaching a membrane-localizing steroid to an antioxidant amine, which can act either as a lipid peroxyl radical scavanger or as an iron chelator [24]. These drugs have subsequently been shown to be effective against CNS trauma and ischemia [25]. Dexrazoxane (ICRF-187), a drug already approved to protect heart tissue against doxorubicin toxicity [26], acting presumably via its hydrolysis product, ADR-925, is able to chelate iron as well as other ions [27]. However, the iron ADR-925 chelate is a good catalyst of the formation of hydroxyl radicals [28]. Nevertheless, ADR-925 efficiently removes iron from the iron–doxorubicin complex and, therefore, could avoid site-specific damage on DNA by orienting damage toward less sensitive targets.

Perhaps the most recent strategy in cytoprotection by utilizing antioxidant pathways is the synthesis of small molecules that mimic antioxidant enzyme activities. EUK-8, a salen-manganese complex, is a combined superoxide dismutase–catalase mimic, a prototype molecule of a new class of synthetic scavangers [29]. They act catalytically, presumably enhancing their efficiency over noncatalytic ROS scavangers, for example, vitamin E. EUK-8 has displayed significant protection against diseases involving severe tissue damage [30]. The glutathione-peroxidase-like activity of the biologically active selenoorganic compound ebselen [31] comes as no surprise, since we know that both GSH peroxidase and phospholipid hydroperoxide GSH peroxidase are selenoenzymes. Ebselen has significantly ameliorated delayed ischemic neurological deficits and subsequent cerebral infarction in patients with severe subarachnoid hemorrhage [32]. A new aspect on potential therapeutic application of ebselen has arisen with the observation of the reactivity of ebselen toward peroxynitrite [33], a particularly damaging radical, believed to be a mediator of many diseases. In keeping with this, it has been shown recently that the superoxide and peroxynitrite scavenger MnTBAP [manganese(III) 5,10,15,20-tetrakis (4-benzoic acid) posphycin] prevents apoptosis of motor neurons induced by Zn-deficient SOD commonly found in ALS patients [34].

5. JNK pathway

JNK can be activated by a variety of cytotoxic stresses, such as ionizing radiation, hydrogen peroxide, UVC light, heat shock [2], γ-radiation [35], and drugs [36], by conditions that mimic neurodegenerative diseases [37,38], and by trophic factor withdrawal [39].

JNK activation is either a very early event, mediated by

a stress-induced kinase cascade [40], or a relatively late event, mediated by caspases [36,37,41]. The downstream members of the pathway remain obscure. c-Jun appears to be a downstream mediator, since mice harboring a mutation in the c-*jun* locus that removes a subset of JNK phosphorylation sites are protected from kainate-induced apoptosis in the hippocampus [42]. Other mediators could be p53 and Bax [43]. A candidate effector is Fas ligand, which is induced in response to JNK activation in PC12 cells [44].

Inhibition of the JNK pathway, by expression of a dominant-negative, kinase-inactive mutant of the JNK-activating SEK-1 kinase, blocked stress-induced JNK activation and cell death [2]. More recently, a pharmacological inhibitor, CEP-1347, has been developed that is specific for the JNK pathway [45]. Taking advantage of the comfort of using a pharmacological agent, it has been quickly shown that inhibition of the JNK pathway attenuates neuronal cell death induced by MPTP [46] and excitotoxic neurotransmitters [47].

6. NF-κB/calcium–NOS–PARP pathway

PARP is expressed at a high level throughout the cell cycle. Upon activation by DNA strand breaks, PARP catalyzes the transfer of poly(ADP-ribose) groups from NAD^+ onto nuclear proteins. A role for PARP in apoptosis [48] and DNA repair [49] has been suggested. However, the first results with PARP-1 −/− mice were ambiguous [50,51]. Ultimately, work using either knockout mice or inhibitors of PARP has shown that disruption of PARP function renders the animals resistant to cerebral ischemia [52], reduces ischemia-reperfusion injury in the heart and skeletal muscle [53], confers resistance to endotoxic shock [54], and reduces streptozotocin toxicity on islet cells [55].

Upstream of PARP are DNA-damaging free radicals. In addition to ROS, RNS significantly contribute to DNA damage. NO, the primary RNS, is made either by the inducible form (iNOS) or by the constitutive, calcium-activated form (cNOS) of NOS. Tissues with highly inducible iNOS are particularly prone to peroxynitrite-induced DNA damage and subsequent PARP-mediated cell death. NF-κB, a redox-sensitive transcription factor, has been shown to mediate cytokine iNOS induction and the consequent destruction of beta cells [56]. The NF-κB–iNOS pathway is also implicated in glucose-induced endothelial cell death [57]. These findings indicate a special role for NF-κB in mediating pathological tissue damage.

In neurons, intracellular calcium, through activation of calcium-dependent nNOS, is generally regarded as the main inducer of pathological NO synthesis [58]. In many cases of neuronal injury, including those associated with stroke, certain neurotoxins induce an excess release of glutamate, which through synaptic NMDA receptors increases intracellular calcium. Supporting this notion is the fact that nNOS knockout mice have significantly smaller cerebral infarcts.

In summary, NF-κB, NOS, and PARP seem to be equally promising targets for pharmacological cytoprotection, and a variety of inhibitors of NF-κB [59], NOS [60], and PARP [61] exist. Nevertheless, given the facts that the pathology of both NOS [62] and NF-κB [63,64] knockout mice is much more severe than the "almost normal" phenotype of PARP-1 knockout mice [50], at present PARP looks to be the most suitable target. Reports on a plethora of new molecules with potent PARP inhibitory activity have been published recently. BGP-15, a nicotinic amidoxime derivative, attenuates ischemia reperfusion injury [65] and reduces cisplatin-induced organ toxicity [66]. INH_2BP, a benzopyrone derivative, has been described as the most specific PARP inhibitor, both *in vitro* and *in vivo* [67]. In addition to preventing peroxynitrite-induced injury [68], it has been shown to reverse the malignant phenotype of E-ras 20 cells [69] in a PARP-dependent manner [67].

7. p53

p53 is a tumor suppressor gene whose loss or inactivation is the most common single lesion in human neoplasia [70]. Lack of p53 is accompanied by high rates of genomic instability, rapid tumor progression, resistance to anticancer therapy, and increased angiogenesis [71]. Inactivation of p53 is viewed as unfavorable, and much effort has been expended to facilitate anticancer treatment by restoring p53 or reversing the cancerous phenotype [72]. However, the role of p53 in cancer treatment is not limited to its involvement in killing tumor cells. p53 is highly expressed in several tissues, and it is these tissues that are damaged by anticancer therapy [73]. Utilizing high throughput screening, Gudkov and colleagues isolated a compound, PFTα (pifithrin-α), that temporarily and reversibly blocks p53-dependent transcriptional activation and apoptosis, thus rescuing normal cells and reducing the side-effects of cancer therapy [74].

8. Caspases

Caspases, the aspartate-specific intracellular cysteine proteases, play an essential role during apoptotic death. Based on their structure and location in the cell death pathways, caspases can be divided into long prodomain, initiator, and short prodomain effector caspases [75]. Analysis of the phenotypes of knockout mice has provided important insights into the functions of the caspases *in vivo* [76]. Caspases contribute to cell death in a cell-type- and death-signal-dependent manner. Accordingly, knocking out caspase function impairs apoptosis, leading to the production of excess oocytes, brain malformation, and abnormal heart development [75]. As inappropriate apoptosis is in-

duced in many diseases, including ischemic vascular disease (heart attack, stroke) and degenerative diseases (AD, motor neuron diseases), there has been a tremendous effort to develop caspase inhibitors for pharmacological use, based on the substrate cleavage sites of caspases [77].

In a myocardial ischemia model, intravenously administered ZVAD-fmk reduced damage to heart muscle and protection correlated with a decrease in cardiomyocyte apoptosis [78]. Studies have shown efficient inhibition of neuronal damage by caspase inhibitors in a middle cerebral artery occlusion/reperfusion model [79] and in bacterial meningitis [80]. Caspase-3 is involved in APP processing, resulting in elevated Aβ formation [81]. Caspases thus appear to play a dual role in the pathogenesis of AD by proteolytically processing APP and mediating neuronal death.

While these effects are promising and legitimize caspases as potential therapeutic targets, issues such as drug delivery, specificity, and permeability should be addressed, particularly if caspase inhibitors are to be used in chronic degenerative diseases.

9. Mitochondria

Inhibitors of mitochondrial ATP conservation induce rapid ROS generation, MPT pore opening, and release of cytochrome *c*, Apaf-1, and AIF. Cytochrome *c* in cooperation with Apaf-1 activates the caspase-9 pathway, while AIF, a nuclease, directly translocates into the nucleus and digests chromatin into ~50 kbp fragments. Most importantly, however, mitochondria are amplifying death signals originating from the nucleus or plasma membrane. Mitochondrial dysfunction is a common theme of cell death induced by a variety of stimuli.

A number of different approaches to stabilize mitochondrial function have been published [see Ref. 82 for review], but only a few mitochondrial targets have been defined at the molecular level.

MAO, which is localized to the outer mitochondrial membrane, clears dopamine from the cytosol of dopamine neurons. Because dopamine turnover is elevated in the parkinsonian brain, surviving dopamine neurons are exposed to an increased flux of hydrogen peroxide, a product of this enzyme reaction [83]. Although a number of *in vitro* and animal model studies of PD have provided evidence of neuroprotection by the MAO inhibitor deprenyl, in clinical trials deprenyl has failed to delay the progression of PD [82].

Soon after the discovery that cyclosporin A was a specific inhibitor of the MPT, reports began to appear showing protection by cyclosporin A against toxicity from oxidative stress, hypoxia-ischemia, and toxic chemicals [84,85]. Since cyclosporine A also interacts with calcineurin, a protein phosphatase known to regulate cell death pathways [86], additional mechanisms have been considered. Unlike cyclosporine A, however, FK 506, another calcineurin inhibitor, failed to provide protection against cortical damage following experimental traumatic brain injury. Therefore, the cytoprotection by cyclosporine A, at least in this model, is most likely due to inhibition of MPT [87].

10. Specificity

Most of the potential molecular targets for pharmacological cytoprotection are proteins that play important roles in cellular homeostasis. Consequently, pharmacological modulation of these functions is a double-edged sword. This is especially true for apoptosis-signaling molecules. NF-κB is a ubiquitous transcription factor that governs the expression of many genes (e.g. chemokines, cytokines, growth factors, and antibodies). Activation of NF-κB correlates with severe traumatic brain injuries as well as neurodegenerative diseases [59]. On the other hand, NF-κB confers resistance to TNF and cancer therapy-induced apoptosis [88]. Moreover, within a single cell type, NF-κB can function as both a proapoptotic and an antiapoptotic regulatory factor [89]. Similarly, nitric oxide [90] and JNK [91] are also Janus-faced molecules. Thus, there is layer upon layer of complexity.

In acute injuries, these adverse effects appear less limiting. In chronic treatment regimens, however, specificity might become a key issue. Drugs potentiating endogenous cytoprotective responses to injury without affecting healthy tissue look most suitable. Bimoclomol, a hydroxamic acid derivative, has been shown to boost stress-induced heat-shock protein synthesis [92,93], an endogenous, almost universal cytoprotective response, without modulating HSP levels in unstressed cells.

Protection of normal tissue from the cytotoxic effects of cancer therapy is the most challenging issue. Blocking cell death pathways can reduce the efficacy of the anticancer treatment. One approach is local or regional detoxification of the chemotherapeutic agent or its toxic metabolite [94]. Local detoxification is only possible when the target of toxicity is easily accessible. In regional detoxification, the antidote is administered systematically, but, due to its pharmacological, pharmacokinetic, and metabolic properties, it reacts with the damaging compound exclusively at the target site of toxicity.

Other strategies are based on the differences in metabolism and/or regulation between cancerous and normal cells. Carnitin, which facilitates entry of long-chain fatty acids into mitochondria for their utilization in energy-generating processes, has been shown to protect normal tissue without decreasing the antitumor effect of adriamycin [95]. This specificity most likely is based upon the lower dependence of cancer cells on mitochondrial ATP synthesis.

Reversible inhibition of p53 activity during anticancer treatment provided significant protection for normal cells in animal tumor models without affecting the efficiency of therapy against p53 $-/-$ cancer cells [74]. The protective

effect was p53 dependent, since no protection was observed in p53 −/− mice. Since local hypoxia is a potent activator of p53, experiments are underway to determine whether PFTα can prevent tissue damage in heart and brain ischemia [74].

Amifostine, a broad range cytoprotective drug, utilizes a surprising strategy to selectively protect healthy tissue: greater uptake by normal cells. This is due, in part, to a higher concentration of alkaline phosphatase in normal cells [96]. This enzyme is responsible for dephosphorylation of amifostine to the free thiol, the transformation competent form of the drug. The uptake mechanism is also different; healthy tissues actively concentrate amifostine, in contrast with tumor cells that passively absorb the drug [97].

11. Challenge from biotechnology

The main components of the cell death machinery are proteins. Consequently, gene therapy, armed with all the tools to specifically modulate gene expression, appears to be the method of choice. However promising, only a few studies have been published to date.

It has been shown recently that adenovirus-mediated *in vivo* expression of the antiapoptotic human *Bcl-2* gene in murine livers significantly attenuates ischemia-reperfusion injury [98].

Another work evaluated the impact of NAIP, a protein implicated in the pathogenesis of neurodegeneration in spinal muscular atrophy [99]. In that study, Xu *et al.* found that in a transient forebrain ischemia model intracerebral injection of an adenovirus vector overexpressing NAIP reduced ischemic damage in the rat hippocampus.

Inhibition of gene expression or replication can be achieved by using antisense probes. Taylor and colleagues [100] used peptide nucleic acids, complementary to a short mitochondrial DNA (mtDNA) sequence harboring a point mutation or a deletion. The peptide nucleic acid probes selectively inhibited the replication of the mutated mtDNA *in vitro*. The difficulties of mitochondrial peptide nucleic acid uptake must be solved before extensive application of this method becomes possible.

Gene replacement therapy would be the ultimate treatment, and this should lead to permanent health. However, it is currently impossible to introduce genes into mitochondria, and this lack of mitochondrial transformation is a major obstacle for gene therapy of mitochondrial disorders. Gene therapy of nuclear genes seems to be more straightforward, offering the luxury of tissue specificity by using tissue-specific promoters and/or by modulating viral capsid proteins that interact with the cellular receptor. Although substantial progress has been made in developing suitable vectors, significant hurdles remain: the efficiency of gene transfer is often low, the duration of gene expression is short, and the antiviral immune response severely limits repetitive treatment. Most importantly, the threat of the spontaneous occurrence of a replication competent virus, although minimal, cannot be ruled out.

12. Conclusions

Several factors could limit the pharmacological utility of cytoprotective therapies. It is unclear whether physiological function would be improved by preventing the death of what may well be irreparably damaged cells. In addition, nonspecific inhibitors of programmed cell death can have deleterious effects in the neonate in whom the risk of apoptosis inhibitors can be amplified if critical developmental events are disrupted.

Although many therapeutic agents prevent ischemic injury in experimental animals, progress has been slow in applying cytoprotective efficacy into clinical practice.

New techniques, such as DNA microarray technology, will enable researchers to determine complete gene expression profiles, giving unprecedented insights into the differences between the regulatory and metabolic pathways of normal and diseased cells. This all may result in the design of more specific treatment regimens and fewer side-effects.

Acknowledgments

The authors would like to thank Katalin Zoldi Kovacs and Mary Hart for their help in the preparation of the manuscript. Research in the laboratory of P. Krajcsi is supported by Grants OTKA T 029622 and ETT-317/2000.

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