Introduction

Loss of cellular populations is a key limiting factor in many medically and socially high-impact diseases. Refinement of scientific technology in recent decades made detection of cell death possible. Identification of different morphological and signaling aspects, as well as variances in requirement for energy enabled us to construct a theory of three main types of cell death: necrosis, apoptosis, and lysosomal cell death. Mitochondria, certain oncoproteins such as Bcl-2 family, and special catalytic enzymes participating in cellular demise might serve as targets for pharmacological manipulation. Upregulation or downregulation of programmed cell death has been implicated in ischemic neurodegenerative, and autoimmune disorders, as well as in oncology and chronic inflammation. This minireview brings a short overview of genesis and development of theories on programmed cell death and apoptosis, summarizes basic relevant facts on apoptotic mechanisms and draws a new hypothesis on possible implication in medicine and surgery.

Pathways of Cellular Demise

The term ‘programmed cell death’ appeared in 1960s in works on cell elimination in metamorphosing insect (30,31,50). This refers to a genetically invoked form of death (6). Morphologically it may assume a picture of either apoptosis or lysosomal cell death. Although morphological descriptions consistent with apoptosis have been present in literature since the 19th century (32), it has not been sooner than in 1970s, when Kerr and associates (22) unequivocally defined its histological appearance. Apoptosis serves as a one of three models of cell death during ontogenesis and its morphological appearance is less marked than that of necrosis (6). It includes distinctive chromatin condensation, formation of cytoplasmic „finger-like“ projections, which detach from the cell body and form apoptotic bodies, and heterophagocytosis of cellular remnants by surrounding tissue. Absence of inflammation, cellular edema and significant subcellular damage is characteristic, but is observed in necrosis. Histological appearance tends to be conserved with respect to type of cell and damage (65). Prompt phagocytosis as well as the absence of inflammato-ry reaction persuades pathologist to greatly underestimate the contribution of apoptosis in cell removal from population (67).

Cytoplasmic cell death may be seen in cells possessing large cytoplasm and is heralded by early and vast changes in cytoplasm. These involve increase in a number of lysosomes, and an accumulation of proteinaceous debris. Early alterations take place in the cytoplasm (46). Energy resources probably remain generous until the very late stage.

Key words: Cell death, Apoptosis, Oncoprotein, Mitochondria, Caspase, Calcium

Ultrasonographic criteria determining transjugular intrahepatic portosystemic shunt malfunction

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Purpose: To evaluate the efficacy of Doppler ultrasonography (US) in the long-term follow-up of patients treated with transjugular intrahepatic portosystemic shunts (TIPS). Materials and methods: We performed a retrospective review of 1192 Doppler examinations of TIPS carried out at our institution between 1994 and 1999. No. regular shunt venograms were performed. Sonographic parameters assessed included shunt velocities together with diameter, velocity, flow volume, and congestion index of the main portal vein (MPV). To the best of our knowledge, the congestion index of the MPV was evaluated for the first time in a large group of patients with TIPS. Results: The sensitivity of Doppler US for detection of shunt occlusion was 96% and for shunt stenosis 94%. We encountered 4 false positive test results on Doppler US (positive predictive value 96%). Within the course of the study, Doppler US missed a significant shunt stenosis leading to an episode of gastrointestinal bleeding or ascites recurrence only in seven cases. Conclusion: Doppler US is an effective primary imaging method for the follow-up of patients with TIPS. Invasive shunt venography should be reserved only for therapeutic purposes.

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Introduction

Loss of cellular populations is a key limiting factor in many medically and socially high-impact diseases. Refinement of scientific technology in recent decades made detection of various forms of physiological cell death possible. Physiological cell death is very distinct from necrosis. It occurs in two distinct models, and perhaps a whole spectrum between these two. Classic apoptosis, in which most of the early morphological changes occur in the nucleus, and a lysosomal or cytoplasmic cell death, in which the early alterations take place (46). Apoptosis, a morphologically defined cell death, is implicated in removal of cells during ontogenesis, physiological cellular turnover in adults, cytokine-induced tumor involution, clonal selection of T lymphocytes, gerontopathology, neurodegenerative disorders, immunopathologic states, and even myocardial or cerebral ischemia (6,7,22-70). However, a loss of cell’s susceptibility to inducers of physiological cell death is likely an important component in development of malignancy and maintenance of drug resistance (46,68,69). Therefore, after years of academic ignorance, processes of programmed cell death have become a hot issue in scientific world.

Pathways of Cellular Demise

The term ‘programmed cell death’ appeared in 1960s in works on cell elimination in metamorphosing insect (30,31,50). This refers to a genetically invoked form of death (6). Morphologically it may assume a picture of either apoptosis or lysosomal cell death. Although morphological descriptions consistent with apoptosis have been present in literature since the 19th century (32), it has not been sooner than in 1970s, when Kerr and associates (22) unequivocally defined its histological appearance. Apoptosis serves as a one of three models of cell death during ontogenesis and its morphological appearance is less marked than that of necrosis (6). It includes distinctive chromatin condensation, formation of cytoplasmic „finger-like“ projections, which detach from the cell body and form apoptotic bodies, and heterophagocytosis of cellular remnants by surrounding tissue. Absence of inflammation, cellular edema and significant subcellular damage is characteristic, but is observed in necrosis. Histological appearance tends to be conserved with respect to type of cell and damage (65). Prompt phagocytosis as well as the absence of inflammatory reaction persuades pathologist to greatly underestimate the contribution of apoptosis in cell removal from population (67).

Cytoplasmic cell death may be seen in cells possessing large cytoplasm and is heralded by early and vast changes in cytoplasm. These involve increase in a number of lysosomes along with their redistribution, development of large autophagic vacuoles, and specific sequence of organellar removal. Very late in this process nuclear alterations similar to apoptosis take place (46). Energy resources probably remain generous until the very late stage.
Apoptosis seems to be an old and conserved reaction. Apoptosis has been observed in those cells only, in which depolarization of inner mitochondrial membrane occurred.

$m, and subsequent intramitochondrial production of $\Delta\Psi_m$ block apoptosis and promote cell survival (41, 71).

The genes $ced-3$ and $ced-4$ are essential for cell death; $ced-9$ antagonizes the activities of $ced-3$ and $ced-4$, thus resembling bcl-2 family in mammals. $ced-3$ is a counterpart of $Bcl-2$, while $ced-4$ resembles that of mammalian $Apaf-1$ (Apoptosis Protease-Activating Factor 1).

Classical studies have been carried out in a worm, *Caenorhabditis elegans* model, which features extensive removal of organelles in a controlled and energy-dependent manner. Susceptibility of cells to suicide varies significantly. Genetic control of apoptosis is mediated through several gene products. Some of them promote apoptosis (p53, TNF, Fas/CD95, bax, bak and bad), while the others (e.g. bcl-2, bcl-XL) block apoptosis and promote cell survival (41, 71).

**Subcellular Mechanisms of Apoptosis**

Apoptosis seems to be an old and conserved reaction based on a self-scavenging anti-viral defense originally developed in primitive eukaryotes. Execution of this process involves inhibition of protein synthesis at the level of translation initiation, proteolysis specifically involving degradation of DNA repair mechanisms, and polymorphonuclear degradation. This complex molecular signaling system includes feedback mechanisms tending toward activation of all elements of the execution platform if only one element is initially engaged (67).

Because morphological alteration of nucleus was first observed in apoptotic cells, it was considered a coordinator of whole process. However, studies on enucleated cells have shown that they can die with apoptosis as well (52). Although cellular suicide program is genetically encoded, its translation immediately before execution of apoptosis is not required. Apoptosis in certain cellular populations can be prevented with transcriptional and/or translational inhibitors (29, 43, 60). However, similar approach in different settings may provoke or accelerate apoptosis (5, 10).

The effector tool of apoptosis is a class of cysteine proteases localized in the mitochondria, also termed ICE-like enzymes (Interleukin-1β-Converting Enzyme) according to the first discovered member of this family. Two pathways of cellular suicide exist. One triggered by signals created within the involved cell (unbalanced oxidative stress, calcium overload) and the other one initiated by signals generated from outside of the cell (TNF, Fas ligand, NO, glucocorticoids, actinomycin D). The intrinsic signals activate caspase-9, while extrinsic engages caspase-8. Both modes converge to sequential activation of other caspases, all process thus resembling limited proteolysis seen with blood clotting. Activated caspases digest structural proteins and degrade chromosomal DNA leading to death of the cell. Cell’s decision to commit suicide is driven by death activators and is counterbalanced by the action of trophic factors, among them nerve growth factor, basic fibroblast growth factor, interleukin 2, insulin, and others.

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Nuclear pyknosis and karyorrhexis are near-to-definite hallmarks of apoptosis. Factors responsible for chromatin condensation and pyknosis include DNases, Acinus and CAD. Membrane continuity preserved on the other hand, studies employing microtubule-damaging drugs such as vinblastine suggest that mitochondrial damage is an important event in Bcl-2 inactivation via hyperphosphorylation and induction of apoptosis (57). This means that upstream intracellular mediators of apoptosis initiate cytoskeletal rearrangements, which in turn potentiate apoptotic cascade via inhibition of anti-apoptotic function of Bcl-2 oncoprotein. Moreover, initiation of apoptotic cascade at any point may cause self-amplification and inevitable cell death.

Interesting association between inhibition of apoptosis and a gain of metastatic capability was found in cells lacking expression of cytoskeleton-bound Death Associated Protein (DAP) kinase (20). Restoration of normal DAP kinase expression in high-metastatic tumor cells suppressed their metastatic ability. Links among suppression of apoptosis, cytoskeleton rearrangements, and neoplastic immortality and metastatic capability need further elucidation.

Susceptibility of cells to suicide varies significantly. Genetic control of apoptosis is mediated through several gene products. Some of them promote apoptosis (p53, TNF, Fas/CD95, bax, bak and bad), while the others (e.g. bcl-2, bcl-XL) block apoptosis and promote cell survival (41, 71).

Mechanisms of apoptosis include caspase-dependent and caspase-independent pathways.
Necrosis is opposed to physiological cell death (Tab. 1). It represents a morphological counterpart of energy resources loss, membrane penetrations, ruined control of ion flow and osmolysis resulting in uncontrolled loss of cellular content (14,61,62).

Using classical staining procedures it is almost impossible to quantitatively apoptosis due to a very short lifetime of morphologically evident apoptosis, which is believed to be at the level of tens of minutes. Classical microscopic analysis was the first method used to define apoptosis, and it should be kept in mind, that none of modern methods has replaced it. However, a myriad of new methods suitable for detection of certain apoptotic features has emerged, among them annexin V immunohistochemistry (66), multiparameter flow cytometry (11), TUNEL (TdT-mediated dUTP-biotin nick end labeling) staining, and diphencyline assay of DNA fragmentation. Apoptosis can be in most instances identified by characteristic breakdown of DNA into oligonucleosomal fragments, which give so called ladder appearing on electrophoresis (2). Pioneer observation of this feature should be probably granted to Czech researchers (35). Chemical asymmetry of plasma membrane is a characteristic feature of normal cells. However, early during apoptotic cells export phosphatidylserine residues normal confined to the inner leaflet of the plasma membrane to the outer leaflet, thus flagging apoptotic cell to phagocytes (35). This feature has been recently employed to identify apoptotic cells using immunolabeling techniques against annexin V, cell membrane confined phospholipid binding protein with a high affinity for phosphatidylserine (66).

Morphological manifestation of apoptosis is linked to its terminal stage. Only those latter stages of the whole process are heralded by cell rounding, cytoplasm blebbing, and nuclear condensation and fragmentation. Acquisition of typical apoptotic morphology is dependent on caspase-mediated and energy-dependent rearrangements of cytoskeleton.

Nuclear pyknosis and karyorrhexis are near-to-definite morphological features of apoptosis. Factors responsible for chromatin condensation and pyknosis include DNases, Acinus and AIF (Apoptosis Inducing Factor). Caspase-activated DNase (CAD) is a cytosolic protein inactivated by heterodimerization with its inhibitor ICAD. This heterodimer splits by action of caspase-3 on ICAD and CAD translocates into the nucleus, where it exerts typical inter-nucleosomal chromatin cleavage (12). Acinus (apoptotic chromatin condensation inducer in the nucleus) is newly described chromatin-condensation factor involved in apoptosis. For full activation it requires double caspase cleavage and features an unique peculiarity as it exerts its chromatin-condensing action without any detectable DNase activity (49). Both Acinus and CAD lead to histological appearance of karyorrhexis. Yet another factor, mitochondrial AIF, participates in nuclear changes. However, it produces large scale DNA fragmentation into pieces around 50 kb in length and gives a picture of peripheral chromatin condensation (58).

Caspases have been found to mediate cleavage of many cytoskeleton-associated proteins, among them Gas2 (3), gelsolin (25), and fodrin (35). Detachment of apoptotic cells from plate or from other tissue cells was found to be a consequence of calpain-mediated cleavage of cytoplasmic domain of integrin β3 subunit (40), which is required to maintain cellular adhesion and cytoskeletal association. On the other hand, studies employing microtubule-damaging drugs such as vincristine suggest that microtubule damage is an important event in Bcl-2 inactivation via hyperphosphor- ylation and induction of apoptosis (57). This means that upstream intracellular mediators of apoptosis initiate cyto- skeletal rearrangements, which in turn potentiates apoptotic cascade via inhibition of anti-apoptotic function of Bcl-2 oncoprotein. Moreover, initiation of apoptotic cascade at any point may cause self-amplification and inevitable cell death.

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Classical studies have been carried out in a worm Caenorhabditis elegans model, which features extensive removal of cell population during ontogenesis. Genes responsible for programmed cell death were identified as cell death genes (ced), and their corresponding proteins were termed CED. Although cellular suicide program is genetically encoded, its translation immediately before execution of apoptosis is not required. Apoptosis in certain cellular populations can be prevented with transcriptional and/or translational inhibitors (29,43,60). However, similar approach in different settings may provoke or accelerate apoptosis (5.10). The effector tool of apoptosis is a class of cysteine proteases localized in apoptotic caspases, also termed ICE-like enzymes (Interleukin-1β-Converting Enzyme) according to the first discovered member of this family. Two pathways of cell suicide exist. One triggered by signals created within the involved cell (unbalanced oxidative stress, calcium overload) and the other one initiated by signals generated from outside of the cell (TNF, Fas ligand, NO, glucocorticoids, actinomycin D). The intrinsic signals activate caspase-9, while extrinsic engages caspase-8. Both modes converge to sequential activation of other caspases, all process thus resembling limited proteolysis seen with blood clotting. Activated caspases digest structural proteins and degrade chromosomal DNA leading to death of the cell. Cell’s decision to commit suicide is driven by death activators and is counterbalanced by the action of trophic factors, among them nerve growth factor, basic fibroblast growth factor, interleukin 2, insulin, and others.

Data published by Zamzaki and associates (31,72) document, that lowering of mitochondrial transmembrane potential ΔΨm and subsequent intramitochondrial production of reactive oxygen species are triggers of apoptosis. Apoptosis has been observed in those cells only, in which depolarization of inner mitochondrial membrane occurred.
It has been reported that disturbances of mitochondrial function and integrity paralleled by disruption of intracellular calcium fluxes preceded apoptotic changes of nuclei (37,42). Mitochondrial calcium overload initiates opening of a special mitochondrial megachannel and is prerequisite of all further steps of apoptosis (31,42,44).

Loss of electrochemical gradient on inner mitochondrial membrane is mediated by a sudden increase in its permeability. This permeability transition creates a shunt for protons, lowers promotive force $A_{\text{P}}$, and results in a cessation of mitochondrial ATP synthesis. In all likelihood, permeability transition is caused by opening of special proteinaceous multiple conductance channel, or "megachannel". This nonspecific channel is probably permeable to any atomic ion as well as water and ions and forms at the junction of inner and outer mitochondrial membranes (45). Its opening gives rise to massive ion movement accompanied by water with resulting edema and rupture of outer mitochondrial membrane. Intermembrane proteins capable of inducing caspases (cytochrome c and apoptosis inducing factor AIF) are thus released to cytosol (35,56,74). Moreover, caspases induce liberation of intermembrane proteins from other mitochondria (36), hence binding in self perpetuating cycle leading to coaggregation of proapoptotic behavior among all mitochondria in a given cell or tissue, so that permeability transition of inner mitochondrial membrane takes on a chain reaction profile and spreads as outbreak affecting entire mitochondrial population (37,42). Loss of a mitochondrial transmembrane potential is a common trait of necrosis and apoptosis (72). End result of the mitochondrial dysfunction leads to biological catastrophe culminating in disintegration of plasma membrane (necrosis), or to activation of apoptotic proteases with subsequent activation of endonucleases and manifestation of apoptosis. Cell's decision on which morphological preservation will be preferred depends on intensity of initiating factor and energetic charge of the cell (27,28,39). Cells low in energy undergoes uncoordinated process of necrosis, yet cells rich in sufficient energy sources experience apoptosis. Other explanation may be that cells mainly depend on anaerobic glycolysis (leukocytes) undergo apoptosis, while cells relying on aerobic glycolysis tend to suffer from necrosis. This is consistent with our findings of necrotic neuronal death in our model of transient seven minute global cerebral ischemia in dogs, where we repeatedly failed to morphologically identify apoptosis (15,47). However, mitochondrial damage consistent with apoptosis has been observed (Fig. 3).


Current immediate clinical applications of apoptosis-related research constitute estimation of anticancer chemotherapeutic efficacy in drug-resistant tumor cells, prediction of treatment failure in acute leukemia (13), or reduction of allotropic reperfusion injury after transplantation (24). Basic research has also recently questioned use of lactated Ringer solution for acute shock therapy (9).

New knowledge of cellular death control is to be con-

ceived by basic research. Thereafter, applied research should extend our pharmacological armamentarium with new approaches for therapy of ischemic disorders, malig-
nant diseases, chronic inflammation and many others. Sufficient understanding of apoptosis and cell growth regu-
lation yet requires more years of investigation. Never-
theless, new millennium may bring a significant break-

through in treatment of many incurable and incapacitating diseases.

References


It has been reported that disturbances of mitochondrial function and integrity paralleled by disruption of intracellular calcium stores and consequent apoptotic changes of nucleus (37,42). Mitochondrial calcium overload initiates opening of a special mitochondrial channel and is proposed as a further steps of apoptosis (3,42,44).

Loss of electrochemical gradient on inner mitochondrial membrane is mediated by a sudden increase in its permeability (37,42). This permeability transition creates a shunt for protons, lowers proton motive force ∆Ψm, and results in a cessation of mitochondrial ATP synthesis (37,42,43). In all likelihood, permeability transition is caused by opening of special proteinaceous multiple conduit channel, or “megapore” or “megachannel”. This nonspecific channel is probably permeable to any atomic ion as well as water and forms at the junction of inner and outer mitochondrial membranes (45). Its opening gives rise to massive ion movement accompanied by water with resulting edema and rupture of outer mitochondrial membrane. Intermembrane proteins capable of inducing caspases (cytochrome c and apoptosis inducing factor AIF) are thus released to cytosol (36,56,74).

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Either preventive or postinjury application of immunomodulator agent cyclosporine A or tacrolimus (FK-506) in settings of disrupted blood-brain barrier protects neurons against apoptosis induced by ischemia/reperfusion injury (18,58,72). These compounds inhibit formation of mitochondrial permeability transition, and performance of mitochondrial function (41,58,72). Mitochondrial DNA mutations (mitosporidial DNA mutations) and others (8) Mutations in the p53 tumor suppressor gene are among the most frequent genetic abnormalities identified in human solid malignancies. In species with an exceptionally high frequency of malignant transformation to performance of mitochondrial DNA, cellular calcium chelators (63,64) and natural inhibitors of apoptosis - some of protein products of bcl-2 gene family. Employment of free radical scavengers can only retard terminal phase of apoptosis - reduction of cellular volume (73), but not cellular death itself.

Immunosuppressors actions of cyclosporine A, tacrolimus and rapamycin are mediated by the drug binding to mitogen-activated protein kinases (MAPKs). MAPKs complex binds to and inhibits the phosphatase calcineurin, thus resulting in modulation of special protein phophorylation. Immunosuppression ligands, including immunosuppressors that do not inhibit calcineurin, stimulate regrowh of damaged central neurons (48). Furthermore, it is tacrolimus which inhibits the activity of nitric oxide synthase. Nitric oxide is capable of inducing apoptosis directly opening of mitochondrial megapore (19).

Cell death activators, both apoptotic and necrotic, may be identical (54). Cell’s fate is likely to be defined by the intensity and duration of exposure to initiating steps of apoptosis. This is supported by reported sequence of necrosis and apoptosis in glial stimulating induced model of excitotoxic neuronal death. Early survival of necrotic phase was determined by inability of mitochondrial generating machinery, and cells surviving the necrotic phase underwent apoptotic transfiguration (1).

Conclusion

Known associations of apoptosis controlling and cellu- lary growth gene mutations include familial adenomatous polyposis (APC gene), hereditary malignant melanoma (re- sults of cyclin-dependent kinases), Lynch syndromes (microsatellite DNA mutations) and others (8). Mutations in the p53 tumor suppressor gene are among the most frequent genetic abnormalities identified in human solid malignancies. Besides malignancy, downregulation of apoptosis controlling mechanism is seen with inability to handle some forms of chronic inflammations (26,51), e.g. ulcerative colitis and rheumatid arthritis. Upregulation of apoptosis has been implicated in many autoimmune, neurodegenerative, and ischemic disorders (21,63,77).

Current immediate clinical applications of apoptosis-related research constitute estimation of anticancer chemother- apy effectiveness (33), survival prediction in acute leukemia (13), or reduction of allotropic reperfusion injury after transplationation (24). Basic research has also recently questioned use of lactated Ringer solution for acute shock therapy (9).

New knowledge of cellular death control is to be con- ceived by basic research. Thereafter, applied research should be extended to clinical field by means of new therapeutic agents. New combinations of agents will be developed with new approaches for therapy of ischemic disorders, malig- nant diseases, chronic inflammation and many others. Sufficient understanding of apoptosis and cell growth regu- lation yet requires more years of investigation. Never- theless, new millennium may bring a significant break- through in treatment of many incurable and incapacitating diseases.

References

Acute myeloid leukaemia (AML) accounts for over 80% of all adult acute leukaemias and is characterized by a clonal expansion of immature myeloid cells in all haematopoietic tissues. Many patients progress to AML from preleukaemic myelodysplastic syndrome (MDS) or from chronic myelogenous leukaemia (CML). AMLs show varied morphologic, cytometric, immunologic and cytogenetic characteristics and varied sensitivity to conventional chemotherapeutic regimes. Sixty percent to 70% of patients with de novo AML initially achieve complete remission. However, the majority of these patients relapse and eventually die of the disease. The first described and best characterized mechanism of resistance is mitr1 gene product, P-glycoprotein. This molecule spans the cell membrane and act as an efflux pump for toxins, including chemotherapy drugs such as anthracyclines, vinca alkaloids and topoisomerase II inhibitors. The biological bases of drug resistance and relapse in AML are not understood and prognoses are still largely based on descriptive parameters. Several lines of evidence indicate that apoptosis plays roles in responses of AML patients to chemotherapy. Aldridge and Radford (1) showed that differences between human haematopoietic cell lines, in the rate of induction of apoptosis after irradiation were generally related to the functioning of cell cycle checkpoints. Whereas the rapidly dying and radiosensitive HSB-2 cell line underwent apoptosis at different points in the cell cycle, the more slowly dying cell lines showed a variety of cell cycle arrest profiles and initiated apoptosis after accumulation of cells in the G2 phase. AML cells showed a markedly longer G2 arrest that correlated with their greater radioresistance. The results suggest that the total length of time available for DNA damage repair (regardless of whether this time occurs as arrest in G1, S or G2), prior to potential activation of apoptosis, is a critical determinant of radiosensitivity in human haematopoietic cell lines.

Introduction

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The mode of induction of apoptosis is dependent upon the cell type and the type and concentration of cytostatic drug used. Three different routes to the induction of apoptosis are described (6): 1. Interphase apoptosis, where death occurred in different phases of cell cycle. 2. Delayed interphase apoptosis, where death occurred following arrest in G2 phase. 3. Mitotic/delayed mitotic death, where death occurred after one or more cell division. (6). To investigate whether the sensitivity of leukaemias to chemotherapeutic agents depends on the abilities of leukaemia cells to respond to therapeutic insult by induction of apoptosis, we used the DNA intercalating agent, which interacts with topoisomerase II and has an inhibitory effect on nucleic acid synthesis.

ORIGINAL ARTICLE

DOSE DEPENDENT BIOLOGICAL EFFECTS OF IDARUBICIN IN HL-60 CELLS: ALTERATIONS OF THE CELL-CYCLE AND APOPTOSIS

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Summary: TP-53 deficient cells of human leukaemia HL-60 die by massive apoptosis after treatment by high (50-100 nmol/l) doses of DNA damaging agent Idarubicin, regardless of the cell-cycle phase, in which they are affected. In contrary, after relatively low dose 10 nmol/l the cells die after cell-cycle arrest in G2 phase. The results show, that apoptosis induced by idarubicin could appear independently of the cell-cycle phase and that period in which apoptosis is observed is related to the dose of Idarubicin.

Key words: HL-60; Idarubicin; Apoptosis; G2 cell-cycle arrest