ment from the wrist like a normal wrist watch. Stored measurements (50) can be downloaded to the PC software via infrared transmission and software allows the student to see changes of blood pressure in time. This software was in-
stalled into a special PC for medical students. Digital tono-
meter OMRON served for comparison with classical methods of blood pressure measurement. Cardiovascular and respiratory systems adaptation are demonstrated using a new bicycle ergometer device with an ear pulse rate oscil-
лометric probe. The ergometer allows us to use program-
mes with various degrees of load. Our program of practical exercises for medical students is ready for use.

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Ultrasonographic criteria determining transjugular intrahepatic portosystemic shunt malfunction

Jan Žižka1, Pavel Eliáš1, Antonín Krajina1, Antonín Michl1, Petr Hůlek2, Pavel Ryška2

Charles Univ. Prague, Fac. Med. Hr. Králové: Dept. Radiol1, 1st Dept. Intern. Med.2

Purpose: To evaluate the efficacy of Doppler ultrasono-
graphy (US) in the long-term follow-up of patients treated with transjugular intrahepatic portosystemic shunts (TIPS). Materials and methods: We performed a retrospective re-
view of 1192 Doppler examinations of TIPS carried out at our institution between 1994 and 1999. No. regular shunt
venograms were performed. Sonographic parameters assess-
ed included shunt velocities together with diameter, velo-
city, 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 ste-
noses on Doppler US (positive predictive value 96%).
Within the course of the study, Doppler US missed a signif-
ificant shunt stenosis leading to an episode of gastrointesti-
nal 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. Refine-
ment of scientific technology in recent decades made detec-
tion 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 spec-
trum 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 ear-
ly alterations take place (46). Apoptosis, a morphologically
defined cellular death, is implicated in removal of cells
in keratinocytes and keratinizing tissue. 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: neurodegeneration, apoptosis, and lysosomal cell death. Mitochondria, certain oncoproteins such as Bcl-2 family, and specific catalytic enzyme-
nes participating in cellular demise might serve as targets for pharmacological manipulation. Upregulation or downregu-
lation of programmed cell death has been implicated in ischemic, neurodegenerative, and autoimmune disorders, as well as in oncology and chronic inflammation. The 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 hy-
pothesis on possible implication in medicine and surgery.

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

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 apo-
ptosis 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 microscopical appearance is less marked than that of
necrosis (46). It includes distinctive chromatin condensa-
tion, formation of cytoplasmic „finger-like“ projections,
which detach from the cell body and form apoptotic bodi-
es, and heterophagocytosis of cellular remnants by sur-
rounding 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 popula-
tion (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 lys-
osomes along with their redistribution, development of large
autophagic vacuoles, and specific sequence of organellar re-
moval. Very late in this process nuclear alterations similar
to apoptosis take place (46). Energy resources probably re-
main generous until the very late stage.
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 quantify 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 diphenyllamine assay of DNA fragmentation. Apoptosis can be in most instances identified by characteristic breakdown of DNA into oligo-nucleosomal fragments, which give so called ladderling appearanace on electrophoresis (2). Pioneering observation of this feature should be probably granted to Czech researchers (55). Chemical asymmetry of plasma membrane is a characteristic feature of normal cells. However, early during apoptotic cells express phosphatidylserine residues normal-ly 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 immunomarking 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 these 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 (67). Nuclear pyknosis and karyorrhexis are near-to-definite morphological manifestation of apoptosis. Factors responsible for chromatin condensation and pyknosis include DNases, Acinus and AIF (Apoptosis Inducing Factor). Caspase-activat-ed DNase (CAD) is a cytosolic protein inactivated by heterodimerization with its inhibitor ICAD. This hetero-dimer 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 appearanace 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 conden-sation (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 Becl-2 inactivation via hyperphospho-rylation and induction of apoptosis (57). This means that upstream intracellular mediators of apoptosis initiate cyto-skeletal rearrangements, which in turn potentiate apoptotic caspase via inhibition of anti-apoptotic function of Becl-2 oncoprotein. Moreover, initiation of apoptotic cascade at any point may cause self-amplification and inevitable of death.

Interesting association between inhibition of apoptosis and a gain of metastatic capability was found in cells lac-king expression of cytokine-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 apopto-sis, 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/CcLD5, bac, bak and bax), while the others (e.g. bcl-2, bcl-X) 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-triggering anti-viral defense originally deve-
Apoptosis seems to be an old and conserved reaction in which depolarization of inner mitochondrial membrane occurred. 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 quantify 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 diaphenylamine 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). Pioneering observation of this feature should be probably granted to Czech researchers (55). Chemical asymmetry of plasma membrane is a characteristic feature of normal cells. However, during apoptosis, cells export phosphatidylserine residues normally 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).

Fig. 1: Step model of apoptosis (58): 1. Premitochondrial phase - activation of apoptotic signaling pathways, including so-called upstream caspases (Fig. 1), 2. Mitochondrial phase - loss of mitochondrial inner membrane potential $\Delta \Psi_m$, and subsequent intramitochondrial production of reactive oxygen species are triggers of apoptosis. 3. Postmitochondrial phase - activation and action of apoptotic effectors (catabolic proteases - downstream caspases, nucleases) leading to morphological appearance of apoptosis (Fig. 1).

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. Genetic control of apoptosis is mediated through several gene products. Some of them promote apoptosis (p53, TNF, Fas/CD95, bad, bak and bax), while the others (e.g. bcl-2, bcl-X$_L$) 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-scarring 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 degrada-

### Table: Principal differences between necrosis and apoptosis.

| Feature | Necrosis | Apoptosis |
|---------|----------|-----------|
| Histopathology | Edema | Cellular shrinkage |
| | Damage to organs | Chromatin condensation |
| | Membrane discontinuity | Formation of apoptotic bodies |
| DNA cleavage | Random, diffuse | Interchromosomal cleavage |
| Reaction of surrounding tissue | Inflammation | Phagocytosis, no inflammation |

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).

Data published by Zamzami and associates (31,72) do document, that lowering of mitochondrial transmembrane potential $\Delta \Psi_m$ 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 intra-cellular calcium homeostasis preceded apoptotic changes of nucleus (37,42). Mitochondrial calcium overload initiates opening of a special mitochondrial megachannel and is prone to all further steps of apoptosis (37,42,44).

Loss of electrochemical gradient on inner mitochon- 
drial membrane is mediated by a sudden increase in its permeability. This permeability transition creates a shunt for protons, lowers protonmotive force ∆p, and results in a cessation of mitochondrial ATP synthesis. In all likely- 
hood, permeability transition is caused by opening of spe- 
cific proteinaceous multiple conductance channel, or “megapore” or “megachannel”. This nonselective 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 mo- 
mement 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 (16,56,74).

Moreover, caspases induce liberation of inter-
membrainal phase of apoptosis – reduction of cellular volume (73), but not cellular death itself. 

Immunosuppressant actions of cyclosporine A, tacroli-
mus and rapamycin are mediated by the drug binding to intermembrane target - immunophilin. Drug-immunophilin complex binds to and inhibits the phosphatase calcineurin, thus resulting in modulation of specific cellular responses. 

Immunosuppressant ligands, including nonimmunosuppressants that do not inhibit calcineurin, stimulate regrowth of damaged neuronal perikarya (48). Furthermore, cyclosporin A and tacrolimus inhibits the activity of nitric oxide synthase. Nitric oxide is capable of inducing apoptosis via direct open-
ning of mitochondrial megachannel (49).

Cell death activators, both apoptotic and necrotic, may be identified (54). Cell’s fate is likely to be defined by the in-
sensity and duration of exposure to initiating steps. This is supported by reported sequence of necrosis and apoptosis in glutathione-induced model of ecotocidal neontological death. Early survival of necrotic phase was determined by necro-
sis of mitochondrial procession machinery, and other necro-

References
1. Ankarcrona M, Deheyl P, Bonfoco E et al. Glutamate-induced neuronal death: a nucleus of necrosis or apoptosis depending on mitochondrial function. Neurosci 1995;111:602-6.
2. Arendt M, Morris R, Wyllie A. Apoptosis. The role of the endonuclease. Am J Pathol 1995;146:593-601.
3. Arends M, Morris R, Wyllie A. Apoptosis 1991:319-401.
4. Arends M, Morris R, Wyllie A. Apoptosis induced by p53-dependent and independent pathways. Nat Cell Biol 1993;6:32-52.
5. Arends M, Morris R, Wyllie A. A caspase-activated DNase that degra-

Fig. 3: Electronomicrograph of ruptured mitochondrion after ce-

bral ischemia-reperfusion injury in canine neocortical neuron. Original magnification x 1000.

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Loss of electrical gradient on internal mitochondrion membrane is mediated by a sudden increase in its permeability. This permeability transition creates a shunt for protons, lowers proton motive force ΔPM, and results in cessation of a mitochondrial ATP synthesis. In all likelihood, permeability transition is caused by opening of special proteinaceous multiple conductance channel, or “megapores” or “megachannel”. This nonselective 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. Intracellular proteins capable of inducing caspases (cytochrome c and apoptosis inducing factor AIF) are thus released to cytosol (16,56,74). Moreover, caspases induce liberation of intermembrane space proteins from other mitochondria (36), hence binding in self perpetuating cycle leading to coordination of proapoptotic behavior among all mitochondria in a given cell in a way that precondition permeability transition of inner mitochondrial membrane takes on a chain reaction profile and spreads as widespread affecting entire mitochondrial population (45). Ionization of ATP is a common trait of necrosis and apoptosis (72). End result of the mitochondrial dysfunction leads to biological catastrophe culminating in disintegration of plasma membranes (necrosis), or to activation of apoptotic proteases with subsequent activation of caspases and manifestation of apoptosis. Cell’s decision on which morphological presevation will be preferred depends on intensity of initiating factor and energetic charge of the cell (7,27,38). 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 dependent on anaerobic glycolysis (leukocytes) undergo apoptosis, while cells completely dependent on aerobic glycolysis tend to suffer from necrosis. This is consistent with our findings of necrotic neuronal death in our model of transient seven global cerebral ischemia in dogs, in which we replacedly found to morphologically identify apoptosis (15,47). However, mitochonodrial damage consistent with apoptosis has been observed (Fig.3).

Either preventive or postinsult application of immunomodulant agent cyclosporine A or tacrolimus (FK-506) in settings of disrupted blood-brain barrier protects neurons against neuronal death in the chick embryo in vivo requires protein and RNA synthesis: evidence for the role of cell death genes. Dev Biol 1990;138:104-13.

Conclusion

The current immediate clinical applications of apoptosis-related research constitute estimation of anticancer chemotherapeutic and biologic tumor necrosis factor (TNF) treatment 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 conceived by basic research. Thereafter, applied research should be conducted in setting of experimental animal model with new approaches for therapy of ischemic disorders, malignant diseases, chronic inflammation and many others. Sufficient understanding of apoptosis and cell growth regulation yet requires more years of investigation.

Nevertheless, new millennium may bring a significant breakthrough in treatment of many incurable and incapacitating diseases.

Fig. 3: Electronomogram of ruptured mitochondria after cere- bral ischemia-reperfusion injury in canine neocortical neuron. Original magnification 12 000x. 
References
1. Ankarcrona M, DiPietro J, Boutros P et al. Clonometric studies in cerebrovascu- lar cell death. J Neurochem 1989;53:630-41.
2. Arends M, Morris R, Wyllie A. Apoptosis. The role of the endonuclease. Am J Pathol 1990;136:589-93.
3. Brancolini C, Benedetti M, Schneider C. Microfilament reorganization during apoptosis of chicken erythroblasts. J Cell Biol 1993;122:1075-86.
4. Clarke A, Purdie C, Harrison D et al. Thrombocytopathy in juvenile diabetes mellitus. Lancet 1995;346:939-42.
5. Clarke A, Purdie C, Harrison D et al. Thymocyte apoptosis induced by p53-de- fective cell lines. J Cell Biol 1995;131:247-68.
6. Dietz J, Bergers M, DeCorte M, Morton D. Oncology. In: Schwartz S, Stein G, Spencer P et al., editors. Principles of Surgery. 7th ed. New York: McGraw-Hill; 2000.
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8. Hirsch T, Susin S, Marzo I et al. Mitochondrial permeability transition in apo- ptosis. Oncogene 1997;15:1573-81.
9. Hortelano S, Dallaporta B, Zamzami N et al. Nitric oxide induces apoptosis via opening of mitochondrial megapore. FEBS Lett 1998;427:198-202.
10. Kajstura J, Cheng W, Reiss K et al. Caspase-dependent fragmentation of adeno- sinergic and dopaminergic nerves in the heart: a model for the regulation of myocardial function by neuropeptide. J Biol Chem 1993;268:27375-81.
11. Kolesnikov M, Zholob F. Evidence supporting a role for programmed cell death in local control of ischemia. Stroke 1995;26:2042-8.
12. Enari M, Sakahira H, Yokoyama H et al. A caspase-activated DNase that degra- des nuclear DNA during apoptosis. EMBO J 1995;14:5660-69.
13. Estrov Z, Thall P, Talpaz M et al. Caspase 2 and caspase 3 protein levels as pre- dicators of clinical outcome in gastric cancer. Cancer Res 1996;56:520-5.
14. Farber J, Kyle M, Coleman J. Mechanisms of cell injury by activated oxygen spe- cies. Folia Biol (Praha) 1976;22:335-42.
15. Skalka M, Cejková M, Matyáová J. The sensitivity of chromatin from thymuses of irradiated mice to alkaline solutions. Folia Biol (Praha) 1976;22:335-42.
16. Green D, Kroemer G. The central executioners of apoptosis: caspases or mito- chondrial permeability transition. FEBS Lett 1998;427:198-202.
17. Hirsch T, Marchetti P, Susin S et al. The apoptosis-necrosis paradox. J Exp Med 1996;183:1299-303.
18. Hirsch T, Susin S, Marzo I et al. Mitochondrial permeability transition de-termine the mode of cell death. Oncogene 1997;15:1573-81.
19. Kajstura J, Cheng W, Reiss K et al. Apoptotic and necrotic myocyte cell deaths occur by apoptosis after ischemia-reperfusion injury in the rat liver. Transplantation 1998;65:1099-105.
20. Kothakota S, Azuma T, Reinhard C et al. Caspase-3-generated fragment of gelso- num procollagen is a terminal effector in glomerular injury. J Exp Med 1998;183:891-9.
21. Kojima H, Takenaka T, Nakao K et al. Mucosal injury by apoptosis and cell cycle regulation. Biochem Biophys Res Commun 1987;139:238-46.
22. Kerr J, Wyllie A, Currie A. Apoptosis: a basic biological phenomenon with wide- ranging implications in tissue kinetics. Br J Cancer 1972;26:39-75.
Introduction

Acute myeloid leukaemia (AML) accounts for over 80% of all adult acute leukaeimias (7) and is a characterized by a clonal expansion of immature myeloid cells in all haematopoietic tissues. Many patients progress to AML from preleukaemia myelodysplastic syndrome (MDS) or from chronic myelogenous leukaemia (CML). AMLs show varied morphologic, cytotoxic, 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 mlr1 gene product, Pglycoprotein. 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 indicated 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 radiosensitivity. The result 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.

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 observed in different phases of cell cycle. 2. Delayed interphase apoptosis, where death occurred after 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 have studied the dose-dependent effects of idarubicin on HL-60 cells. Idarubicin is a DNA intercalating agent, which interacts with topoisomerase II and has an inhibitory effect on nucleic acid synthesis.

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

ORIGINAL ARTICLE

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

Martina Macekova1, Jitína Vávrová2, Doris Yokarková1

Charles University in Prague, Faculty of Medicine in Hradec Králové: Department of Medical Biochemistry1; Purkyně Military Medical Academy, Hradec Králové: Institute of Radiobiology and Immunology2; University Teaching Hospital in Hradec Králové: Institute of Clinical Immunology and Allergology2

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MUDr. Jan Franko,
Safářík University Hospital,
Department of Surgery,
třída SNP 1, 040 66 Kotice,
Slovak Republic,
e-mail: franko@kotice.upjs.sk

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56. Skalicky V. Cytochrome c in the apoptotic and antitumour cascades. FEBS Lett 1999;421:27-30.
57. Skulachev V. Reappraisal of the role of cytochrome c in the regulation of cell death in living organisms. FEBS Lett 1999;441:271-7.
58. Skulachev V. Mitochondrial defence against the apoptotic cellular damage. FEBS Lett 1999;441:278-81.
59. Srivastava R, Srivastava A, Korsmeyer S et al. Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. Mol Cell Bioi 1998;18:3705-13.
60. Suri R, Sharma G, Kumar H. Leukocyte specific expression of apoptotic genes in human leukaemia. Mol Cell Bioi 1998;18:6349-56.
61. Tata J. Requirement for RNA and protein synthesis for induced regression of the tadpole tail in early culture. Development 1990;114:17-56.
62. Trumpp B, Brenzer S. The role of cytochrome C2-in cell injury, necrosis and apoptosis. Cell Opin Cell Biol 1992;4:227-32.
63. Trumpp B, Brenzer S. The role of cytochrome C2-in cell injury and cell death. FASEB J 1995;9:278-84.
64. Terzisaki M, Szigeti J, Zhang L et al. Mechanism of action and persistence of necrostatins I and II. Cell death by necrosis and apoptosis. J Cell Bioi 1992;119:3479-86.
65. van Lookeren Campagne M, Gill R. Ultrastructural morphological changes are not characteristic of apoptotic cell death following focal cerebral ischemia in the rat. Neurosci Lett 1998;233:134-7.
66. Walton M, Srinivasan E, Bradlmaier B et al. Annexin V labels apoptotic neutrons following hypoxia-ischemia. Neuroreport 1997;8:1715-7.
67. White R, Sullivan J. Apoptosis. Acid Emerg Med 1999;20:1039-20.
68. Wylie A. Apoptosis: cell death under homeostatic control. Arch Toxicol Suppl 1997;12:1-10.
69. Wylie A. Apoptosis: Death gets a brake. Nature 1998;391:272-3.
70. Wylie A, Kerr J, Currie A. Cell death: the significance of apoptosis. Int Rev Cytol 1998;186:201-306.
71. Yamamoto N, Brandt C, Marzo I, Sunic S, Kosemeyer G. Subcellular and subnuclear routes of action of Bcl2-like antiapoptotics. Oncogene 1998;16:2265-72.
72. Yamamoto N, Hensch T, Danishevsky B, Petri P, Kosemeyer G. Mitochondrial implication in accelerated and programmed cell death: apoptosis and necrosis. J Blood Med Biol 1997;20:35-71.
73. Yamamoto N, Marcketti P, Canesi T et al. Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. J Exp Med 1995;182:385-77.
74. Zhangmy N, Marzetti L, Bradlmaier B, Dorkofsky S. Injured cytochrome c in donor leukocytes. Nature 1998;391:649-55.

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