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BIOCHEMISTRY AND CYTOLOGY OF TOXIC ACTIONS: A REVIEW ON APOPTOSIS ROLE

R. Stoika

Department of Regulation of Cell Proliferation and Apoptosis
Institute of Cell Biology, NAS of Ukraine
14/16, Hrushevskyi St., Lviv 79005, Ukraine
e-mail: stoika@cellbiol.lviv.ua

The aim of the review is to present principal approaches in studying cellular and biochemical mechanisms of the action of highly toxic agents towards the mammalian cells. The USA Environmental Protection Agency recognizes the existence of >4 million toxic compounds. While the "exotoxins", including the pharmaceutical drugs, originate mostly from exposure to the environment, the "endotoxins" are produced as a response in the body-to various stressesand appear as radical and non-radical reactive oxygen species, specific hormones (ex. Estradiol), and toxins of the emotional stress and negative memories.Toxins accumulate mostly in the adipose tissue of the body, however, they alsoare found in the umbilical cord and breast milk. In liver which is the main detoxification organ, toxins (usually lipid-soluble) are converted by Cytochrom P450 enzymes to the intermediary metabolites that are more water-soluble (Phase 1 of detoxification), and then conjugated in Phase II detoxification pathways with different chemical groups (sulfation, acetylation, methylation) or natural compounds (glucuronidation or conjugation with gluthatione or specific amino acids, such as glycine, taurine, glutamine, ornithine, arginine). At the final stage of detoxification, water-soluble derivatives of toxins are complexed with bile acids and excreted with faeces, or transferred to blood serum and excreted via kidney in the form of urine. The main methods used for evaluation of cytotoxic action of specific toxic agents, alkaloids, and anticancer drugsare briefly described in the review. Changes in plasma membrane of the apoptotic cells (membrane budding, externalization of phosphatidylserine, and redistribution of membrane glycoconjugates) caused by toxic actions are characterized. These changes recognized as "find me", "eat me" and "don't eat me" signals,are exposed by the apoptotic cells for the phagocytes (ex. Macrophages). Biochemical mechanisms of the intracellular pro-apoptotic signaling (caspase cascade, mitochondria-dependent processes, DNA splitting in cell nucleus) are also described in regard of the cytotoxic actions. Thus, if not neutralized in liver, toxic agents cause complicated and multi-level changes in cells of tissues and organs, involving apoptosis pathways switched on cell surface and including mitochondria, cytosol and nucleus.

Keywords: review, toxic agents, liver detoxification, mammalian cells, apoptosis, biochemical mechanisms.

The USA Environmental Protection Agency (https://www.epa.gov) recognizes the existence of >4 million toxic compounds [12].They include two main types of toxins: 1) "exotoxins" that originate mostly from exposure to the environment (industrial chemicals, pesticides, plasticisers, life style toxins (e.g. cigarettes, alcohol, recreational drugs, caffeine, sugar), heavy metals, artificial food additives, pharmaceutical drugs, bacterial endotoxins); 2) "endotoxins" that are produced as a response of the body to various stresses (radical and non-radical reactive oxygen species, specific hormones (ex. Estradiol), and toxins of the emotional stress and negative memories).

Toxins accumulate mostly in the adipose tissue of the body, however, they are alsofound in the umbilical cord and breast milk. Liver is the main detoxification organ, and toxins (usually

lipid-soluble) are converted there by Cytochrom P450 enzymes (CYP enzymes) to the intermediary metabolites that are more water-soluble (Phase 1 of detoxification). There is a big family of genes coding for CYP enzymes that are involved in metabolism of xenobiotics, including drugs and exogenous toxins [13]. In a result of the oxidation, reduction, hydrolysis, hydration, dehalogenation reactions in Phase 1, superoxide and other reactive oxygen species are produced. Besides specific enzymes, several co-factors (riboflavin (Vitamin B2), niacin (Vitamin B3), pyridoxine (Vitamin B6), folic acid, Vitamin B12) and other nutrients (glutathione, branched-chain amino acids, flavonoids, phospholipids) take part in the transformation of toxin molecules.

The intermediary metabolites appearing in Phase 1 are conjugated in Phase II of the detoxification pathways with different chemical groups (sulfation, acetylation, methylation) or natural compounds (glucuronidation or conjugation with gluthatione or specific amino acids, such as glycine, taurine, glutamine, ornithine, arginine). In order to avoid primary and secondary tissue damage caused by the reactive oxygen species produced in Phase 1, several antioxidants and other protective nutrients including plant derivatives are involved. These are: carotenes (Vitamin A), ascorbic acid (Vitamin C), tocopherol (Vitamin E), Selenium, Copper, Zink, Manganese, Coenzyme Q10, thiols found in garlic, onions and cruciferous vegetables), bio-flavonoids, solymarin, picnogenol). While the activity of CYP 1A1 and CYP 1A2 enzymes is increased in Phase 1, no substantial induction of these enzymes is observed in Phase 2 [13].

At the final stage of detoxification, water-soluble derivatives of toxins are complexed with bile acids and excreted with faeces, or these derivatives are transferred to blood serum and excreted via kidney in the form of urine.

Below, we willaddress negative consequences of the action of toxic agents that have not been neutralized in liver by such detoxification mechanisms as cytochrome P450 oxidases, UDP-glucuronosyltransferases, or glutathione *S*-transferases. These agents induce cell death mainly by apoptosis or secondary necrosis (in case of long lasting action or high dose of toxicant). The main methods used for evaluation of cytotoxic action of varioustoxic agents, including the anticancer drugs,are: light and fluorescent microscopy, Western-blot analysis of the pro-apoptotic (ex. active caspases, cytochrome C) and anti-apoptotic proteins (ex. Bcl-2, PARP-1), DNA comet or TUNEL analyses, DNA fragmentation (laddering) analysis, others.

Plasma membrane of the apoptotic cells stays morphologically intact, however, membrane budding, externalization of phosphatidylserine from the inner to outer layer of plasma membrane, and redistribution of membrane glycoconjugates take place during apoptosis [9]. Membrane budding can be an ultra-rapid change at apoptosis.It was found that the microinjection of apoptosis inducernodularin (protein phosphatase inhibitor) into the target cells (293 cells, Swiss-3T3 fibroblasts, promyelocytic IPC-81, NRK cells) caused budding of plasma membrane as soon as in 45 sec [10]. An exposure of phosphatidylserine on the outer layer of plasma membrane at apoptosis was firstly detected in 1992 [8], and the developed method based on Annexin V assay stays one of the most frequently used for apoptosis detection. Potential biological role of plasma membrane glycoproteins in the apoptotic cells has been cleared up by[3, 11]. All listed changes in plasma membrane of the apoptotic cells arerecognized by the phagocytes (ex. Macrophages) as “find me”, “eat me” and “don’t eat me” signals [14]. The sialic acid of plasma membrane glyco-conjugates can be bound by the siglecs (sialic acid binding Ig-like lectin) [5]. It was demonstrated that siglec-11 prevents of binding “eat me signals” by the complement factors C1q and C3b [7],and, thus, stops clearance of the apoptotic cells.

We found that the 1st type vesiclesreleased by the apoptoticcellsand exposing “eatme” signals are derived from the endoplasmic reticulcum and contain on their surface un-mature glycoepitopes, while the 2nd type apoptotic vesicles are derived by the budding of plasma membrane

of the apoptotic cells [2]. The 1st type vesicles are preferentially recognized and engulfed by the macrophages that provides clearance (elimination) of the apoptotic cells and vesicles. It was demonstrated that the appearance of such "immunogenic" vesicles with changed glyco-epitopes is caused by a complicated biochemical transformation that takes place on the surface of the apoptotic cells under the action of either receptor-dependent (CD95) or receptor-independent (UV-B) inducers of apoptosis [2].

It was found that sialidase activity is involved in changing glycosylation pattern of the apoptotic cells, since inhibitor of neuraminidases – DANA (2-deoxy-2,3-dehydro-N-acetyl-neuraminicacid) – stops a decrease in the amount of sialyl-containing glycoconjugates of plasma membrane of the apoptotic cells [4, 16]. These changes on the surface of the apoptotic cells can be detected as a decrease in binding of the sialo-specific lectins – MAL I ***ma*** SNA[1]. Such characteristics of the apoptotic cells was applied in the developed „ApoLect" Kit that was proposed Bilyy et al. (personal communication) for conducting rapid diagnistics of the presence ofapoptotic cells in whole blood samples („ApoLect" Kit is successfully used at the Department of Immunology and Allergology of Danylo Halytsky National Medical University of Lviv, Ukraine).

At the apoptotic death,surface of dying cells and apoptotic bodies is modified due to production of the reactive oxygen species leading to an appearance of immunologically new antigens [5]. Non-effective clearance of dying cells can cause accumulation of remnants of the apoptotic cells. This can be a serious defect inducing constant presence of cell remnants that are responsible for initiation of systemic autoimmunity at such pathologies as the systemic lupus erymatosus [14]. Below, a sequence of events that accompany clearance of the apoptotic cells is presented: 1) under the effect of apoptosis inducer, intact cells transform into the early apoptotic cells; 2) due to next changes in plasma membrane glyco-conjugates, these cells are changed to late apoptotic cells; 3) normally, both early and late apoptotic cells are subjected to clearance by the macrophages; 4) if, for some reason, such clearance does not take place, the late apoptotic cells are converted to secondary necrotic cells; 5) these necrotic cells and their remnants are recognized by the dendritic cells, and such complexes are presented to the immune lymphocytes which under such action are transformed into plasma cells; 6) the activated plasma cells produce autoantibodies whose action leads to development of the autoimmune disease and inflammation process.

Thus, when viable cells are subjected to the action of some physiological stressing agent (ex. hormone), reversible changes in cell morphology and metabolism take place. However, when the apoptosis inducer (ex. anticancer drug, immune suppressor) affects target cells, the non-reversible early/late apoptotic changes develop. When the action of the apoptosis inducer is rather durable, the non-reversible apoptotic/secondary necrotic changes are observed in the treated cells. Finally, when the necrosis inducer (ex. hyperthermia) is applied, the non-reversible primary necrotic changes take places in the treated cells. For example, when the toxic heavy metal ($HgCl_2$, 10 microM) was applied30 mintowards human T-leukemia cells of Jurkat line, there were no changes in cell morphology and functions, however, when this toxicant was used in <100 microMdose for 20 min, apoptosis was induced, and if the action of >100 microM lasted for 60-120 min,secondary necrosis has developed. Heat (<60 °C, 5 min) treatment of these cells led to their primary necrosis [15]. Another example for time-dependency of Jurkat T-cell death can be the consequences of their treatment with Etoposide, an antineoplastic agent that inhibits DNA topoisomerase II, thereby ultimately blocking DNA synthesis. When it was acting towards T-cells of Jurkat linefor 8 h in 5 microM concentration, 87 % of cells stayed viable. In 12 h of such treatment, the amount of viable cells decreased to 81 %, while in 24 h, 65 % of cells were viable and 16% - late apoptotic. In 32 h, 46 % of cells were viable, 22% – late apoptotic, and 11% – primary necrotic, while in 48 h, only 20% of cells stayed viable, 39 % were late apoptotic, 16% – secondary necrotic, and 18% – primary necrotic [15].

Thus, “Poison is in everything, and no thing is without poison. The dosage makes it either a poison or a remedy” (Paracelsus, 1493-1541). Here we presented a brief description of a long chain of biochemical and physiological processes that leads to detoxification of poisonous agentsin the organism. If this detoxification system does not function properly, then complicated biochemical and cytomorphological processes are induced in the toxicant-targeted cells that leads to their degradation (apoptosis/necrosis) and clearance by phagocytosis without inflammation. An impairment of such elimination of dying cells can lead to development of the autoimmune disease(s) accompanied by inflammationor totumor growth.

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БІОХІМІЯ І ЦИТОЛОГІЯ ТОКСИЧНИХ ДІЙ: ОГЛЯД РОЛІ АПОПТОЗУ

Р. Стойка

Відділ регуляції проліферації клітин та апоптозу
Інститут біології клітини НАН України
вул. Грушевського, 14/16, Львів 79005, Україна
e-mail: stoika@cellbiol.lviv.ua

Метою цього огляду є представити головні підходи у вивченні клітинних і біохімічних механізмів дії високотоксичних чинників на клітини ссавців. За даними Агенції з охорони середовища США у світі є понад 4 мільйони токсичних сполук. Якщо "екзотоксини", включно із фармацевтичними препаратами, діють переважно внаслідок експозиції до середовища, то "ендотоксини" продукуються як відповідь тіла на дію різних стресових чинників і виявляються як радикальні чи нерадикальні активні сполуки кисню, окремі гормони (наприклад, естрадіол) і токсини, які виникають унаслідок емоційного стресу й негативних переживань. Токсини акумулюються переважно у жировій тканині тіла, проте їх також виявляють у пуповинній крові та грудному молоці. У печінці, яка є головним детоксифікаційним органом, токсини (зазвичай жиророзчинні) перетворюються ензимами цитохрому Р450 до проміжних метаболітів, які є більш водорозчинними (Фаза 1 детоксифікації). Опісля останні кон'югуються під час детоксифікаційних шляхів Фази 2 з різними хімічними групами (сульфатація, ацетилюваннння, метилюваннння) чи з природними сполуками (глюкуронідування чи кон'югування з глутатіоном або окремими амінокислотами, наприклад, гліцином, таурином, глутаміном, орнітином, аргініном). На завершальній стадії детоксифікації водорозчинні похідні токсинів утворюють комплекси із жовчними кислотами і видаляються з калом або потрапляють до сироватки крові й видаляються через нирки у вигляді сечі. В огляді коротко описані головні методи оцінки цитотоксичної дії окремих токсичних чинників, алкалоїдів і протипухлинних ліків. Також охарактеризовані зміни в плазматичній мембрані апоптичних клітин (утворення мембранних везикул, екстерналізація фосфатидилсерину і перерозподіл мембранних глікокон'югатів), зумовлені дією токсичних чинників. Ці зміни розпізнаються як сигнали «знайди мене», «з'їж мене» і «не їж мене», які експонуються апоптичними клітинами для фагоцитів (наприклад, макрофагів). Біохімічні механізми внутрішньоклітинного проапоптичного сигналювання (каспазний каскад, процеси, залежні від мітохондрій, розщеплення ДНК в ядрі клітини) також описані з огляду на цитотоксичні дії. Отже, якщо токсичні чинники не нейтралізуються в печінці, то вони викликають складні та багаторівневі зміни в клітинах тканин і органів, у яких задіяні апоптичні процеси на поверхні клітини, а також у мітохондріях, цитозолі та ядрі.

Ключові слова: огляд, токсичні чинники, детоксифікація в печінці, клітини ссавців, апоптоз, біохімічні механізми.

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БИОХИМИЯ И ЦИТОЛОГИЯ ТОКСИЧЕСКИХ ВОЗДЕЙСТВИЙ: ОБЗОР РОЛИ АПОПТОЗА

Р. Стойка

Отдел регуляции пролиферации клеток и апоптоза
Институт биологии клетки, НАН Украины
ул. Грушевского, 14/16, Львов 79005, Украина
e-mail: stoika@cellbiol.lviv.ua

Цель этого обзора – представить основные подходы в изучении клеточных и биохимических механизмов действия высокотоксичных агентов на клетки млекопитающих. По данным Агенции по охране среды США, в мире насчитывают свыше 4 миллионов токсичных соединений. Если “экзотоксины”, включительно с фармацевтическими препаратами, действуют преимущественно вследствие экспозиции к среде, то “эндотоксины” продуцируются как ответ организма на действие различных стрессовых агентов и выявляются как радикальные или нерадикальные активные соединения кислорода, отдельные гормоны (например, эстрадиол) и токсины, которые возникают вследствие эмоционального стресса и негативных переживаний. Токсины аккумулируются преимущественно в жировой ткани организма, однако их также выявляют в пуповинной крови и грудном молоке. В печени, которая является главным детоксификационным органом, токсины (обычно жирорастворимые) превращаются энзимами цитохрома Р450 до промежуточных метаболитов, которые являются более водорастворимыми (Фаза 1 детоксификации). Позже последние коньюгируются во время детоксификационных путей Фазы 2 с различными химическими группами (сульфатация, ацетилирование, метилирование) или с природными соединениями (глюкуронидирование или коньюгирование с глутатионом или отдельными аминокислотами, например, глицином, таурином, глутамином, орнитином, аргинином). На завершающей стадии детоксификации водорастворимые производные токсинов образуют комплексы с желчными кислотами и выделяются с калом или попадают в сыворотку крови и удаляются через почки в виде мочи. В обзоре кратко описаны основные методы оценки цитотоксичного действия отдельных токсичных агентов, алкалоидов и противоопухолевых лекарственных препаратов. Также охарактеризованы изменения в плазматической мембране апоптичных клеток (образование мембранных везикул, экстернализация фосфатидилсерина и перераспределение мембранных гликоконьюгатов), обусловленные действием токсичных агентов. Эти изменения распознаются как сигналы «найди меня», «съешь меня» и «не ешь меня», которые экспонируются апоптичными клетками для фагоцитов (например, макрофагов). Биохимические механизмы внутриклеточной проапоптичной сигнализации (каспазный каскад, процессы, зависящие от митохондрий, расщепление ДНК в ядре клетки) также описаны с точки зрения их цитотоксического действия. Следовательно, если токсичные агенты не нейтрализуются в печени, то они вызывают сложные и многоуровневые изменения в клетках тканей и органов, в которых задействованы апоптические процессы на поверхности клеток, а также в митохондриях, цитозоле и ядре.

Ключевые слова: обзор, токсичные агенты, детоксификация в печени, клетки млекопитающих, апоптоз, биохимические механизмы.