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Role of Cell Death in Toxicology: Does It Matter How Cells Die?

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Keywords

drug metabolism, toxicity, hepatocytes, calcium, ROS, cell death

Abstract

My research activity started with studies on drug metabolism in rat liver microsomes in the early 1960s. The CO-binding pigment (cytochrome P450) had been discovered a few years earlier and was subsequently found to be involved in steroid hydroxylation in adrenal cortex microsomes. Our early studies suggested that it also participated in the oxidative demethylation of drugs catalyzed by liver microsomes, and that prior treatment of the animals with phenobarbital caused increased levels of the hemoprotein in the liver, and similarly enhanced rates of drug metabolism. Subsequent studies of cytochrome P450-mediated metabolism of toxic drugs in freshly isolated rat hepatocytes characterized critical cellular defense systems and identified mechanisms by which accumulating toxic metabolites could damage and kill the cells. These studies revealed that multiple types of cell death could result from the toxic injury, and that it is important to know which type of cell death results from the toxic injury.

INTRODUCTION

I spent my early childhood living with my family on a farm in southern Sweden. This was during World War II, and Sweden was rather isolated from the rest of the world. Since we were living on a farm, we did not suffer from lack of food or other locally produced daily necessities. Purchase of imported items was restricted by national regulations (e.g., coffee, tea), or they were not available at all (e.g., tropical fruits).

I started school when I was six years old and spent the next four years going to a local rural school with about 50 other pupils and three teachers. The school was located 3 km from home, and I either walked or cycled to school; this was quite safe since there was almost no traffic due to gasoline rationing. My second school was located in Vadstena, a small town some 20 km away from home. Since there was no adequate daily transportation available, my parents paid for me to live with a local family. Hence, I could only visit home during weekends. Since we had long school days and lectures until 3 PM on Saturdays, and I had to return to school on Sunday afternoon, weekends were rather short.

At the age of 15, I finished secondary school and continued my education at a *gymnasium* in Motala, another town some 15 km further away from home. After three years I graduated with *studentexamen* and was ready to start my university studies. There had been much discussion in the family whether I should become a farmer like my father or choose another occupation requiring further studies. However, in the 1950s male students had to do approximately one year of military training between finishing *gymnasium* and starting university studies. Unfortunately, my own military training ended with a car accident after two months, which hospitalized me for almost a year. This accident also made me change my original plans to study law at Stockholm University. Instead, I decided to apply for medical school, and I started as a medical student at the Karolinska Institute in Stockholm in 1958 (**Figure 1**).

Figure 1

First class of medical students in anatomy at the Karolinska Institute in 1958. The author can be seen in the middle of the fourth row from below (*red arrow*).

Medical school lasted for six years—three years of preclinical studies and three years of clinical courses. A major advantage for me was that medical students with an interest in science could engage in research between the courses; some of them completed their graduate studies and got their PhD degree before finalizing their MD program. In my case, I started on a research project in pathology after three years of medical studies. During the following few years, I was either engaged in laboratory work or attended clinical courses until I defended my PhD thesis in 1965 and concluded my medical studies in 1967. This was possible thanks to an assistant position in the pathology department at the Karolinska Institute, which also helped to cover the living costs for me and my family during my remaining studies.

EARLY STUDIES ON DRUG METABOLISM

My research activity started with my PhD studies (1961–1965), which took place at the pathology department at Sabbatsberg Hospital (Karolinska Institute) and at the Wenner-Gren Institute for Experimental Biology at the University of Stockholm. The latter had started as a private research institution funded by the Swedish industrialist Axel Wenner-Gren, but was later incorporated into the University of Stockholm. My thesis supervisor, Professor Lars Ernster, PhD, was Head of the Division of Physiological Chemistry at the Wenner-Gren Institute. He and his wife, Edit, had arrived in Sweden in 1945 together with the last group of refugees from Nazi-occupied Budapest, an escape organized by the Swedish diplomat Raoul Wallenberg. The main research focus of Lars's unit at the Wenner-Gren Institute was on mitochondria and their role in cellular energy metabolism. He was a world-leading scientist in this field, and his unit was visited regularly by many of his famous international colleagues. However, Lars had also developed an interest in the structure and functions of the endoplasmic reticulum (ER) during a sabbatical stay with Philip Siekevitz and George Palade at the Rockefeller Institute in New York, and my work started with attempts to subfractionate rough- (i.e., ribosome-coated) and smooth-surfaced liver microsomes and to investigate the enzyme composition of the subfractions. To achieve this, we subjected the postmitochondrial supernatant of a rat liver homogenate to density gradient centrifugation in the presence of Cs^+ or Mg^{2+} ions. The fractions obtained were analyzed, but the results did not display any significant differences in enzyme activity between the rough and smooth vesicles, although there were previous suggestions that the smooth-surfaced ER would be particularly active in drug metabolism.

In 1958, the German biochemist Martin Klingenberg discovered the CO-binding pigment (later termed cytochrome P450) of liver microsomes during a sabbatical stay with Britton Chance at the Johnson Foundation in Philadelphia (1), and a few years later Ronald Estabrook and his colleagues demonstrated its role as the terminal oxidase in steroid hydroxylation catalyzed by adrenal cortex microsomes (2). Thanks to previous work by Axelrod, Brodie, and colleagues at the NIH, it was known that liver microsomes could catalyze the nicotinamide adenine dinucleotide phosphate (NADPH)-linked oxidative metabolism of various drugs (3). These activities were originally believed to be attributed to separate enzymes present in the ER membranes. However, our early studies suggested that cytochrome P450 might serve as a common monooxygenase also in oxidative drug metabolism in liver microsomes, and a detailed characterization of the drug-metabolizing enzyme system was part of my thesis work (4). The role of cytochrome P450 in drug metabolism was further supported by the discovery that treatment of experimental animals with certain drugs or carcinogens, notably phenobarbital and methylcholanthrene, led to induction of several-fold increased levels of cytochrome P450 in the liver microsomes and similarly enhanced rates of oxidative drug metabolism (5). A detailed characterization of the enzyme induction process and its relationship to the concomitant proliferation of the liver ER membranes formed the second part of my thesis work (6).

The studies on enzyme induction and liver ER membranes were subsequently extended to investigate possible effects of phenobarbital pretreatment on other drug-metabolizing activities in the liver, specifically glucuronic acid conjugation, which was also found to increase in activity in treated animals (7); in contrast, some other microsomal enzymes (e.g., glucose-6-phosphatase) showed a decrease in specific activity. Further experiments made it increasingly clear that the effects on the liver ER of the two inducers used in our experiments (i.e., phenobarbital and methylcholanthrene) were different, and that separate isoforms of the P450 monooxygenase were affected. We also spent time looking for endogenous substrates of liver microsomal cytochrome P450 (e.g., saturated fatty acids, steroids, hydrocarbons) and obtained further evidence for the heterogeneity of the hemoprotein. Numerous studies were devoted to the spectral interaction between cytochrome P450 and its substrates and to the characterization of cytochrome P450 systems in tissues other than the liver, notably the kidney, lung, and small intestine. The ω-hydroxylation of saturated fatty acids turned out to be a major activity catalyzed by rat kidney microsomes, which was also found to be inducible by adding such fatty acids to the animals' diet (8). The substrate specificities of both hepatic and kidney cytochromes P450 were also subjects of my postdoctoral research at the Department of Pharmacology at Yale University (perhaps the most impressive pharmacology department in the world at this time) and the Department of Biochemistry at the University of Texas Southwestern Medical School at Dallas. A few years before my visit, Ron Estabrook had left the Johnson Foundation in Philadelphia to become chairman of the latter department, which he had soon made into the mecca of cytochrome P450 research, where much of the progress in the field was made during the coming decades. Hence, the P450 group in Dallas attracted visitors from P450 research centers all over the world for many years to come.

STUDIES WITH FRESHLY ISOLATED HEPATOCYTES

In 1973 I attended an Alfred Benzon symposium on the regulation of hepatic metabolism in Copenhagen and was much impressed by some of the studies presented at the meeting, which were using freshly isolated hepatocytes. The hepatocytes were obtained by collagenase perfusion of the rat liver and could be kept viable in suspension for many hours. It seemed obvious to me that this technology could be used for more integrated studies of hepatic drug metabolism, since the cells would catalyze both phase I and phase II [i.e., conjugation with sulfate, glucuronic acid, and glutathione (GSH)] reactions. Hence, it appeared that this experimental model could be used to study several of the drug metabolic reactions that occur normally in the liver, as well as the supply of required cofactors and other potentially rate-limiting steps. The fact that the isolated hepatocytes could function both as a metabolic system and as a sensitive indicator of cytotoxic effects was a further advantage of this technology. In collaboration with Peter Moldéus in our department, we perfected the isolation procedure and characterized the obtained cell suspensions in detail (9). They were then used to investigate the cellular uptake and metabolism of various drugs and other xenobiotics (10). By consecutive incubations of rat hepatocytes and similarly isolated cells from the small intestine and kidney, we could reconstitute the overall metabolism of several drugs and xenobiotics (11). Since the intracellular pool of GSH functions not only as a regulator of important metabolic reactions, but also as the prime protective mechanism against the accumulation and toxic effects of reactive and electrophilic drug metabolites, we spent extra time investigating the intracellular distribution, synthesis, and turnover of GSH in the isolated cells (12). We also studied the fate of extracellular glutathione (both GSH and glutathione disulfide) and characterized a novel GSH-oxidase activity localized in the plasma membrane at the cell surface.

After moving to the Department of Forensic Medicine at the Karolinska Institute in 1971, my research became more toxicologically oriented. Following the pioneering studies of Brodie,

Gillette, and colleagues, bromobenzene and acetaminophen had become model agents in studies of drug-induced hepatotoxicity (13). Accordingly, we incubated these drugs with isolated hepatocytes to characterize their cytotoxic effects (14). This experimental approach was also used for studies of the biosynthesis and metabolism of GSH conjugates, and together with Dean Jones, who was a postdoc in our group at this time, we could reconstitute the entire sequence of reactions known to be involved in the metabolism of acetaminophen to mercapturic acid derivatives, using combinations of cells isolated from the rat liver, small intestine, and kidney. Our early studies with acetaminophen and bromobenzene not only revealed the critical importance of GSH conjugation in cell defense, but also indicated a role for the Ca^{2+} ion as a mediator of cytotoxicity (15). This observation was further substantiated in studies with hepatocytes exposed to redox-cycling quinones [i.e., menadione (2-methyl-1,4-naphtho-quinone)], which were performed together with Giorgio Bellomo and Pierluigi Nicotera, both of whom joined our group at the Karolinska Institute at this time (16, 17). The results demonstrated that menadione cytotoxicity was due to a combination of thiol oxidation and disruption of intracellular Ca^{2+} homeostasis triggered by the generation of reactive oxygen species (ROS) during redox cycling of the quinone; inhibitor studies showed that the two electron reduction of menadione to the more stable hydroquinone (menadiol) was protective. The cytoskeleton was found to be particularly sensitive to menadione toxicity, and the formation of characteristic blebs on the surface of the hepatocytes was ascribed to a perturbation of the normal organization of the microfilament system caused by the oxidation of thiol groups in actin and the activation of Ca^{2+}-dependent proteolysis (**Figure 2**). A subsequent study showed that incubation of the hepatocytes with exogenous ATP, but not with succinate, resulted in intracellular Ca^{2+} accumulation (18). Although the bulk of the accumulated Ca^{2+} was sequestered by the mitochondria, formation of surface blebs on the hepatocytes exposed to extracellular ATP suggested that this treatment also led to an increase in cytosolic free Ca^{2+} concentration. When hepatocytes loaded with Ca^{2+} by preincubation with ATP were exposed to either menadione or *tert*-butyl hydroperoxide, the toxicity of both compounds was markedly potentiated.

FURTHER INVESTIGATION OF THE ROLE OF THE CALCIUM ION IN CYTOTOXICITY

The hypothesis that the Ca^{2+} ion might serve as a potential death trigger dates back to Fleckenstein's early observation that an influx of Ca^{2+} into cardiomyocytes might be the mechanism

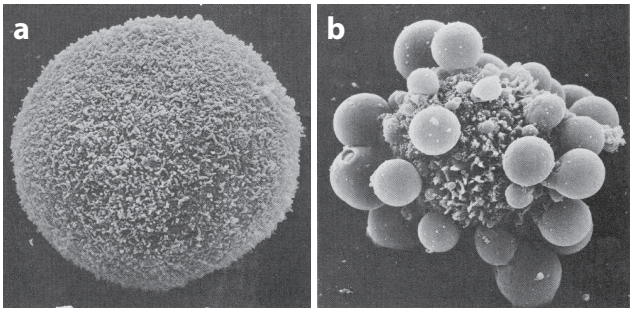

Figure 2

Scanning electron micrographs of hepatocytes isolated from a rat liver and incubated in the (*a*) absence or (*b*) presence of menadione. The surface blebs were ascribed to a perturbation of the cytoskeleton triggered by the generation of reactive oxygen species during redox cycling of the quinone. Figure adapted from Thor et al. (16).

underlying cardiac pathology after ischemia (19). The idea that Ca^{2+}, which is present at millimolar concentrations extracellularly, might accumulate in damaged cells that have lost their ability to maintain the normal Ca^{2+} concentration gradient seemed reasonable. More recently, however, it became clear that nondisruptive alterations in Ca^{2+} signaling could also have adverse effects. The question we addressed was whether such perturbations might be a common, early factor in the development of toxic cell injury. For this purpose, we developed a methodology to measure the releasable amount of Ca^{2+} in the ER and mitochondria of isolated hepatocytes. Subsequent experiments revealed that multiple hepatotoxins caused decreased Ca^{2+} content in both the ER and the mitochondria of the hepatocytes. The effect on mitochondrial Ca^{2+} seemed to be due to a loss of membrane potential, whereas the loss of ER Ca^{2+} was associated with a decreased cytosolic GSH level and was probably due to the inhibitory effects of electrophilic species on the ER Ca^{2+} pump (20). In experiments with isolated mitochondria, we could show that the oxidation of nicotinamide adenine dinucleotide (NADH)/NADPH during the metabolism of menadione or *tert*-butyl hydroperoxide resulted in a release of intramitochondrial Ca^{2+}. Recycling of the released Ca^{2+} caused damage to the mitochondria with loss of membrane potential and increased inner membrane permeability. Thus, our studies with organelle fractions supported the findings with isolated hepatocytes that effects on ER- and mitochondrial Ca^{2+}-sequestering mechanisms resulted in a disruption of intracellular Ca^{2+} homeostasis, important to the early development of toxic cell injury. The resulting increase in cytosolic Ca^{2+} concentration could explain the typical alterations in cell surface structure seen during the early phase of toxic injury (21). A schematic representation of the cytotoxic effects caused by oxidative stress and disruption of calcium homeostasis is shown in **Figure 3**.

Our early calcium work was not restricted to the potential role of the Ca^{2+} ion in cytotoxicity, but also included studies of intracellular Ca^{2+} compartmentalization and signaling, notably nuclear

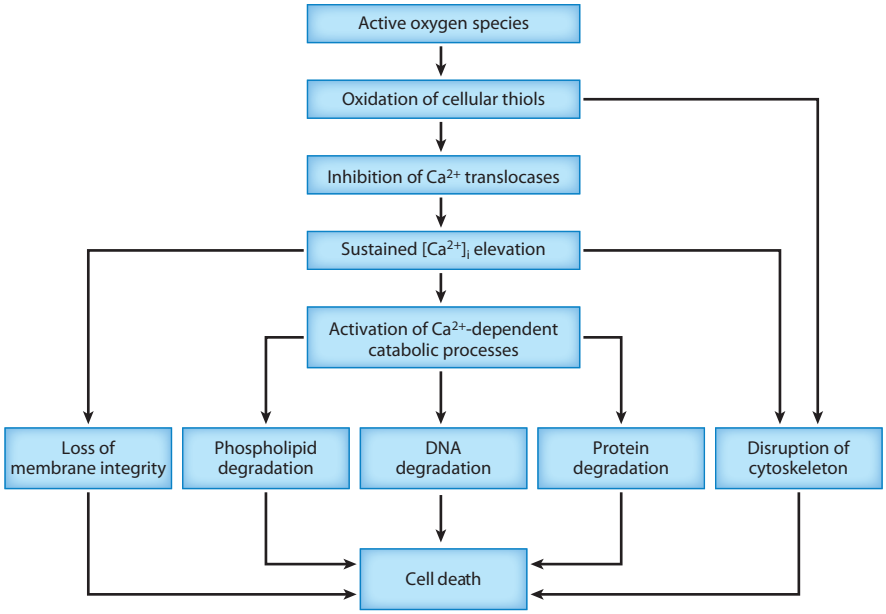

Figure 3

Schematic illustration of the effects of oxidative stress on intracellular calcium compartmentalization in hepatocytes.

Ca^{2+} fluxes (22). A study of Ca^{2+}-activated DNA fragmentation in liver nuclei can be seen as an early link to our future work on apoptotic cell death (23).

EARLY WORK ON APOPTOTIC CELL DEATH

Being trained as a pathologist, I had been confronted with the characteristic morphology of apoptotic cell death in medical school. However, the apoptosis concept developed by Kerr, Wyllie, and Currie was not published until 1972 (24), and it caught my research interest more than a decade later. The trigger was a presentation by Andrew Wyllie at a Society of Toxicology meeting in New Orleans in 1986, where both of us were speakers at an American-European debate on mechanisms of cell injury. I was fascinated by his talk, and back in Stockholm I discussed it with my group and suggested that we should perhaps get involved in this exciting research. David McConkey—a newly arrived graduate student in the laboratory—volunteered to travel to Edinburgh to learn how to measure apoptosis in Wyllie's laboratory. This was our entry into the apoptosis field.

Our initial approach concerned glucocorticoid-induced apoptosis in human thymocytes and can be seen as an extension of our previous work on Ca^{2+}-mediated DNA fragmentation in hepatic nuclei, assuming that a similar process was involved in apoptotic cell death in thymocytes (25). At this time, apoptosis research was largely focused on the nuclear changes (nuclear apoptosis), and on a search for endonuclease(s) that might be responsible for the DNA ladder formation seen in the dying cells. It was further anticipated that DNA ladder formation would be calcium dependent, since a previous study had concluded that this was true for thymocyte nuclei (26). This work resulted in a growing list of Ca^{2+}-dependent endonucleases, which we thought could possibly be responsible for apoptotic DNA fragmentation. Only several years later did we learn that such DNA fragmentation was the result of caspase-activated nuclease activity. However, the observation that incubation of an isolated rat liver nuclear fraction, devoid of caspase activity, in the presence of ATP and Ca^{2+} resulted in a chromatin fragmentation pattern indistinguishable from that seen in apoptotic cells (23) raised questions about the possible contribution of other nucleases to apoptotic DNA fragmentation.

Our studies of the apoptotic process were subsequently extended to include additional cell types (e.g., lymphocytes, leukocytes, aortic smooth muscle cells, pancreatic β-cells, cerebellar granule cells, and a variety of cancer and noncancer cell lines) and triggers of apoptosis other than glucocorticoids (e.g., the CD95 antibody, oxidative stress, and multiple toxic agents) (27). In many of these studies, disruption of Ca^{2+} signaling (e.g., by ER stress) was found to be an important mediator of apoptosis. Further, in collaboration with Nancy Thornberry and Don Nicholson at Merck, we performed an early study on CPP32/Apopain (later termed caspase-3) in CD95-mediated apoptosis (28). The study was followed by detailed investigations of the involvement of the growing caspase family of proteases in apoptosis together with Boris Zhivotovsky, who had joined our group in Stockholm and had previous experience with cell death research from a successful career at the Leningrad Research Institute of Radiobiology.

Much of our work on caspases concerned caspase-2, which is activated early in response to DNA-damaging antineoplastic agents and appears to be important for the engagement of the mitochondrial apoptotic pathway. Hence, fully processed caspase-2 was found to stimulate the release of cytochrome *c* and Smac/DIABLO from mitochondria (29). This occurred independently of the Bcl-2 family proteins, and inactivation experiments revealed that the proteolytic activity of caspase-2 was not required for the effect on the mitochondria. Exactly how antineoplastic agents activate caspase-2 is not known, but it seems that a functional interaction between caspase-2 and p53 is essential for the initiation of the apoptotic process by chemotherapeutic drugs (e.g., 5-fluorouracil) (30).

Our early experiments indicated that the apoptotic process was influenced by the intracellular redox state. Hence, when Andrew Slater moved to our group at the Karolinska Institute from a postdoctoral position at Rockefeller University, we initiated a series of studies on the modulation of the apoptotic process by oxidants and antioxidants (31). These studies supported an important role for the mitochondria in the regulation of apoptosis, which has been a major research topic in our laboratory ever since.

MITOCHONDRIAL REGULATION OF APOPTOSIS

Our work on the role of mitochondria in cell death started with studies on mitochondrial calcium metabolism and the protective effect of mitochondrial Ca^{2+} buffering against oxidative cell damage. After Wang and associates (32) had discovered the mitochondrial pathway of caspase activation, this work was extended and intensified. In collaboration with colleagues at the University of Bergen in Norway, Boris Zhivotovsky and colleagues (33, 34) characterized the apoptotic process induced by microinjection of cytochrome *c* into a variety of cell types in detail. These experiments demonstrated that the presence of the intact hemoprotein in the cytosol was enough to trigger caspase activation and apoptotic cell death. Moreover, the lag phase seen in noninvasive models of apoptosis induction was markedly shortened. Following this study, much of our work has focused on potential mechanisms of cytochrome *c* release preceding caspase activation in intact cells. Early studies had suggested that the proapoptotic Bcl-2 family proteins Bax and Bak would be responsible for the membrane permeabilization preceding cytochrome *c* release and caspase activation. Our studies demonstrated, however, that Ca^{2+}-mediated permeability transition pore (PTP) formation could play a similar role for cytochrome *c* release, particularly when apoptosis was the result of exposure to toxic agents. Together with Vladimir Gogvadze, our prime expert on mitochondria, we characterized the PTP-mediated cytochrome *c* release process in detail and showed the protective effect of inhibitors of either mitochondrial Ca^{2+} uptake (ruthenium red) or PTP formation in the inner mitochondrial membrane (IMM) (cyclosporin A) (35).

Cytochrome *c* is normally attached to the outer surface of the IMM by an association with the anionic phospholipid cardiolipin. In fact, we and others had previously shown that the interaction of cytochrome *c* with cardiolipin determines the amount of the hemoprotein that can be released during apoptosis induction. The molecular interaction between cardiolipin and cytochrome *c* involves electrostatic as well as hydrophobic interactions and hydrogen bonding. In fact, it has been suggested that one of the acyl chains of cardiolipin may be inserted into a hydrophobic pore in cytochrome *c*, whereas the others extend into the phospholipid bilayer. Because of its highly unsaturated acyl chains, cardiolipin is prone to oxidative damage by mitochondrial ROS generation. This, in turn, decreases its binding affinity for cytochrome *c* and facilitates the formation of a soluble pool of the hemoprotein in the intermembrane space. Hence, based on experiments by Martin Ott, a master's degree student in our group, several years ago we suggested that the release of cytochrome *c* during apoptosis signaling might be a two-step process, involving detachment of the hemoprotein from oxidized cardiolipin followed by its release into the cytoplasm through Bax/Bak-induced pores in the outer mitochondrial membrane (OMM) (36). Strong support for this hypothesis came from findings by Valerian Kagan and associates that the cytochrome *c*–cardiolipin complex catalyzes H_2O_2-dependent peroxidation of the phospholipid and subsequent release of the hemoprotein into the cytosol through pores in the OMM (37). The two-step concept of cytochrome *c* release has since been supported by a host of studies.

Mitochondria can also trigger caspase-independent apoptosis. In fact, apoptosis inducing factor (AIF) was the first mitochondrial protein discovered to mediate nuclear DNA fragmentation and cell death (38). AIF is N-terminally anchored to the IMM, where it exerts NADH oxidase activity.

Upon treatment with some apoptotic inducers, AIF can be cleaved from its membrane anchor, generating a soluble 57-kDa AIF fragment, which is subsequently released into the cytosol together with several other mitochondrial proteins, including cytochrome *c* and Smac/DIABLO. AIF is then translocated into the nucleus, where it contributes to large-scale DNA fragmentation and chromatin condensation resulting in cell death. As shown in collaboration with Erik Norberg, a graduate student in our group, and Boris Zhivotovsky, the release of truncated AIF from the IMM was preceded by calpain 1 cleavage of the bound AIF, which was triggered by a prolonged increase of cytosolic/intramitochondrial Ca^{2+} (39). Subsequent work demonstrated that this increase was the result of import of extracellular Ca^{2+} via hyperpolarization-activated cyclic nucleotide-gated channel 2 (HCN2) (40). We further found that, in addition to activating calpain, the imported Ca^{2+} stimulated mitochondrial ROS production leading to oxidative modification of AIF, making it more susceptible to calpain cleavage (41). However, it is important to note that AIF-mediated apoptosis is an important death mechanism only in some cell types when treated with certain stimuli. Our experiments were performed with primary cultures of cerebral cortical neurons or non-small-cell lung carcinoma cells that were exposed to protein kinase C inhibitors, notably staurosporin and PKC412.

CROSS TALK BETWEEN DIFFERENT MODES OF TOXIC CELL DEATH

As mentioned above, toxic cell death was originally regarded to be of the necrotic type and due to damage-induced loss of vital cell functions, or to the inhibition of signaling pathways required for cell survival. Other modes of cell death were either not yet known, or considered not to be of toxicological significance. This view has changed dramatically during recent years. Multiple other modes of cell death have been identified, characterized, and found to sometimes result from toxicity (e.g., apoptosis, autophagic cell death, necroptosis, ferroptosis, pyroptosis, mitotic catastrophe), all of which result from the activation of cellular death programs (**Figure 4**). Moreover, it has also been shown that necrotic cell death might be genetically programmed (necroptosis) and a result of the activation of intracellular signaling pathways. In addition to necrosis, all above-mentioned

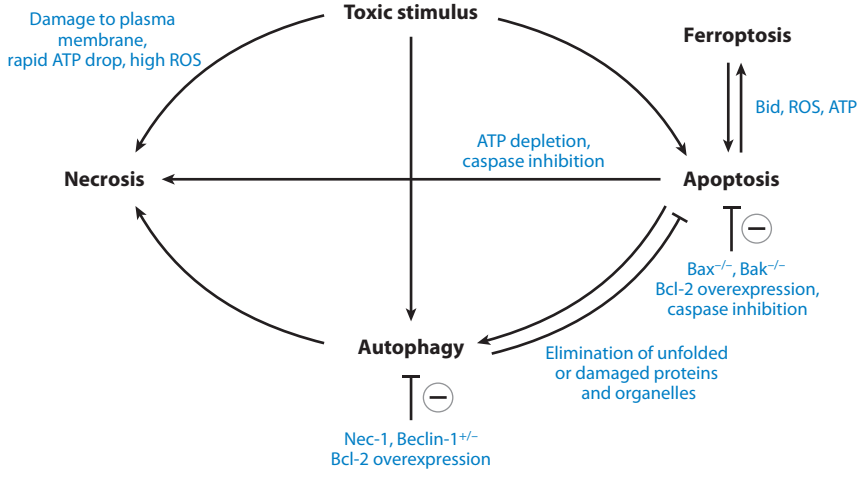

Figure 4

The cross talk between several modes of cell death is influenced by members of the Bcl-2 family of proteins and by the ATP level, reactive oxygen species (ROS) production, and caspase inhibition.

cell death modalities have been shown to sometimes result from the action of toxic agents on the cells, although induction of autophagy might often serve a protective function (42).

Whether toxicity leads to one mode of cell death or another depends on both the cell type and the nature and dose of the death trigger. Furthermore, it is well known that intracellular energy depletion causes necrotic cell death, whereas caspase activation via the apoptosome complex requires dATP/ATP. Andrew Wyllie suggested early on that apoptosis might be induced by injurious agents of lesser amplitude than those causing necrosis in the same cells, and that this might occur more readily in cell populations primed for apoptosis. Such dose dependency is nicely illustrated by one of our old studies, which showed that a pancreatic β-cell line progressively underwent discrete responses of proliferation, apoptosis, and necrosis as the concentration of a pro-oxidant quinone in the medium was gradually increased (43).

Studies in our and other laboratories have since shown that a variety of chemicals can exert their toxicity via induction of apoptosis. Glucocorticoid-induced apoptosis in thymocytes and lymphocytes was an early example thereof, and subsequently it was found that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) could also trigger apoptosis in thymocytes, although at concentrations much higher than those shown to cause thymic atrophy in vivo (44). Additional examples of apoptosis-inducing chemicals include heavy metals (e.g., Cu, Cd, MeHg, Pb), organotin compounds, and dithiocarbamates. Chemical toxicants that can induce autophagic cell death include cytostatics, ionophores, and oxidants, whereas ER stress is known to be able to cause apoptosis as well as autophagic cell death (42). TCDD and cadmium are among the environmental contaminants that can induce cell death by either apoptosis or autophagy. It is of interest to note that cells are not always subject to only one death program and that there are molecular switches within the programs. Hence there are reported examples of the target cell displaying morphological features of both apoptosis and necrosis, and it is known that caspase inactivation during apoptosis might redirect the cell to die by either necrosis/necroptosis or autophagic cell death (**Figure 4**). The latter observation illustrates the frequent finding in vivo that distinct cell death modalities may coexist within the same lesion, and that interference with certain signaling pathways or critical cell functions might result in cell killing by a different mechanism. This is illustrated by our study of the interplay between apoptosis and necrosis in glutamate-induced neuronal cell death, in which we found that glutamate can induce either early necrosis or delayed apoptosis in cultures of cerebellar granule cells (45). During or shortly after exposure to glutamate, a subpopulation of neurons died by necrosis. In these cells, the mitochondrial membrane potential collapsed, the nuclei swelled, and intracellular debris was scattered in the incubation medium. Neurons surviving the early necrotic phase recovered mitochondrial potential and normal energy levels, but later underwent apoptosis. These observations suggest that mitochondrial function is a critical factor that determines the mode of neuronal death in excitotoxicity. Conversely, postapoptotic necrosis due to intracellular Ca^{2+} overload has been demonstrated in cells whose vital Ca^{2+}-extruding proteins in the plasma membrane had been inactivated by caspase cleavage during the apoptotic phase. Taken together, these findings suggest that massive intracellular calcium accumulation triggers necrotic cell death, but also that surviving cells can subsequently die from apoptosis.

PERSPECTIVES

The discovery of multiple genetically programmed modes of cell death, and their signaling networks, has opened new avenues for studies of the mechanisms by which toxic chemicals might cause tissue damage. Of particular importance is the observed role of the calcium ion and ROS in the activation and modulation of multiple cell death pathways, since a wide variety of toxicants (including the rapidly expanding group of engineered nanomaterials) are known to affect

intracellular Ca^{2+} homeostasis and/or redox balance (46, 47). The same is true for many of the traditional environmental pollutants, which can trigger apoptotic, necroptotic, or autophagic cell death in various in vitro models, although it remains questionable whether the same mechanisms are responsible for their toxicity in vivo.

So, does it matter how cells die? Yes, for our understanding of the potential hazards of exposure to foreign chemicals, it certainly does. Cell death is the ultimate outcome of toxicity, and new knowledge about the cellular targets and mechanisms activated by toxic compounds is important for risk assessment and for our attempts to design less toxic analogs. So, is better understanding of the various cell death programs also important for the future of toxicology? Again, the answer is yes! Cell death research is a typical example of an area of potential cross-fertilization between toxicology and basic science. There are many examples of mechanistic studies with toxic compounds, which have contributed to our understanding of fundamental biological processes in the past. Similar studies might continue to reveal important contacts between the disciplines and thereby promote the future development of toxicology.

OTHER ACADEMIC ACTIVITIES AND MERITS

When I officially retired as Professor of Toxicology at the Karolinska Institute in 2004, I had been employed in various positions at this institution since 1967. In addition to my activities in research and teaching, I served on various faculty committees and for seven years as Dean of the Karolinska Medical School (1983–1990). I was also Chair of the Toxicology Department (1984–1990) and thereafter Director of the Institute of Environmental Medicine, which was established at the Karolinska Institute in 1990.

For more than 30 years I was a member of the Nobel Assembly at the Karolinska Institute, which awards the Nobel Prize in Physiology or Medicine. During most of this time, I also served on its Nobel Committee. The Nobel work provides you with a broad overview of research in the field of biomedical sciences and is therefore also a stimulus for your own scientific activities.

My academic merits further include membership in the Royal Swedish Academy of Sciences and Academia Europeae, and I am also a Foreign Associate Member of the Academy of Medicine of the National Academy of Sciences (United States). I have received honorary degrees from several universities in Europe and South America, and I have been elected an Honorary Member of the American Society for Pharmacology and Experimental Therapeutics, the American Society for Biochemistry and Molecular Biology, the Society of Toxicology (United States), and the Italian and Swedish Societies of Toxicology.

I have also been honored with the Distinguished Lifetime Toxicology Scholar Award by the Society of Toxicology (United States) and the first European Cell Death Organization (ECDO) Career Award for Excellence in Cell Death Research. I was the first elected President of ECDO.

I am certainly most grateful for these signs of appreciation by my colleagues.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding or financial holdings that might be perceived as affecting the objectivity of this review.

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