Myocardial ischemic injury and protection

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The European Commission organized the workshop "Cardiovascular research in the next ten years" in Brussels, Belgium in May 2004. The aim was to prepare the concrete basis for the European conference "The Future of Cardiovascular Research in Europe" which took place two weeks later and dealt with perspectives of theoretical and clinical cardiovascular research in Europe. Fifteen experimental cardiologists from all over Europe were invited, each of whom was entrusted to elaborate on the prognosis for a particular topic of cardiovascular research. I was entrusted with the design of the future of experimental approaches for the study of the mechanisms that participate in the development of myocardial ischemic injury and protection. The present paper was prepared for this workshop.

GENERAL BACKGROUND

At present, cardiovascular diseases represent the most important health risk factors because they are responsible for more than 50% of total mortality (1). Among them, acute coronary occlusion is the leading cause of morbidity and mortality, and according to the World Health Organization, it will be the major global cause of death by the year 2020 (1).

The history of ischemic heart disease is relatively brief and represents a very convincing example of the rapid development of cardiology as a scientific discipline (2,3). Myocardial infarction was first described clinically in 1910, but the precise diagnosis was possible only after the introduction of the electrocardiogram into clinical practice in the 1920s. Before 1961, patients with acute myocardial infarction who were fortunate enough to reach the hospital were treated largely by benign neglect. They were sedated and placed on bed rest for five to six weeks. In 1961, Julian (4) articulated the concept of the coronary care unit, which should include the treatment of arrhythmias, cardiopulmonary resuscitation with external ventricular defibrillation and well-trained nurses. The introduction of the coronary care unit caused an immediate 50% reduction in in-hospital mortality. Since 1963, the in-hospital mortality has decreased stepwise by almost 70% with the introduction of thrombolysis, acetylsalicylic acid, invasive cardiology and cardiac surgery. Modern therapy, together with effective secondary prevention, has significantly increased the two-year survival of patients after myocardial infarction by 75% during the past 30 years (3). The impressive progress in the prognosis, diagnosis and therapy of ischemic heart disease would have been impossible without the notable achievements of the 20th century which were critical for the further development of cardiology (5), eg, electrocardiogram, the Framingham Heart Study, lipid hypothesis of atherosclerosis, coronary care units, echocardiography, thrombolytic therapy, heart catheterization and percutaneous coronary intervention, open heart surgery and implantable defibrillators. It should be noted that the described achievements are the result of very close collaboration of theoretical and clinical cardiologists, and in almost every instance, these advances came from interdisciplinary and international collaborations (2). This suggests that cardiology belongs to medical disciplines in which the cooperation of basic and clinical cardiologists has a long-lasting tradition of acting as an engine, driving scientific progress forward.

Although the management of ischemic heart disease will centre on the development of effective primary prevention, the impact of these strategies may be limited. Therefore, there is an urgent need for effective forms of secondary prevention and, in particular, treatment that will limit the extent of evolving myocardial infarction during the acute phase of coronary occlusion. Based on these presumptions, future cardiovascular research should concentrate on four consecutive periods of the development of myocardial injury:

- pathogenetic mechanisms involved in **cardiac protection** against ischemia;
- pathological and molecular factors responsible for **myocardial cell death**;
- positive and negative consequences of **myocardial reperfusion**; and
- possibilities of **myocardial regeneration** or the attenuation of adverse remodelling.

Preserving the viability of ischemic myocardium should be the major therapeutic target.

CARDIAC PROTECTION

The present

The degree of ischemic injury depends not only on the intensity and duration of the ischemic stimulus but also on the level of cardiac tolerance to O_2 deprivation and other components of ischemia. Therefore, it is not surprising that the interest of many experimental and clinical cardiologists during the past 40 years has been focused on the question of how cardiac tolerance to ischemia might be increased.

Already in the late 1950s, the first observations appeared showing that the incidence of myocardial infarction was lower in people living at high altitude (6). These epidemiological observations were later repeatedly confirmed in experimental

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studies using simulated hypoxia (7). In the early 1970s, interest concentrated on the possibilities of limiting infarct size pharmacologically (8); however, this effort was not successful because it became increasingly obvious that the clinical observations did not correspond with the optimism of experimental results. After the period of skepticism, the discovery of a short-term adaptation of the myocardium, the so-called 'preconditioning' by Murry et al (9), opened the door of a new era of cardiac protection. Several years after the description of acute cardiac protection by ischemic preconditioning (IP), a second delayed window of protection was observed (10). At present, the long-term adaptation to chronic hypoxia (CH) and short-term IP are examples of the potent cardioprotective phenomena. Both restrict infarct size, improve postischemic contractile dysfunction and reduce arrhythmias. The intensity of protection is stronger in IP; however, the duration of protection is significantly longer after adaptation to CH (hours versus weeks, respectively). Unfortunately, the molecular mechanisms of cardiac protection have not yet been satisfactorily explained. Signalling for IP involves triggers (eg, adenosine, several G-protein-coupled cell surface receptors and second messengers) and mediators (eg, specific protein kinase isoforms, tyrosine kinases and free radicals), and finally results in the activation of the ATP-dependent potassium channels (K_{ATP}) at the sarcolemma and in the mitochondria. Whether K_{ATP} merely plays a role in signal transduction or whether it is the end effector still remains to be established. Although a number of other end effectors have been proposed, such as the permeability transition pore and the Na^+/H^+ exchanger, there are insufficient data available to support any one end effector to the exclusion of the others (11). The molecular mechanisms of the cardioprotective effect from adaptation to CH have been much less studied and the understanding of its signalling is still far behind that of IP (12).

Future directions

Perhaps the most pressing task for future research is to find the end effectors of IP, including better characterization of the mitochrondrial K_{ATP} channel, the definition of its molecular identity and its direct (physical) or indirect relation to the permeability transition pore. Furthermore, precise information about the protein kinase isoforms and their structural requirements is needed, as is the explanation of the dual roles of free radicals and nitric oxide. For this purpose, novel techniques are necessary that would allow researchers to work with areas such as the permeability transition pore, mitochrondrial K_{ATP} channel or gap junctions (11,13). Because most of these pathways (eg, protein kinases, mitochondrial K_{ATP} channels and free radicals) are also involved in the mechanisms of adaptation to CH, it seems that they belong to general endogenous protective pathways (12). This suggests that future research should examine both the redundancy and specificity of signalling.

As far as the possible clinical relevance of IP is concerned, it should be mentioned that despite the enormous effort during the past 20 years, the results have not fulfilled the expectations because of its short-term protective effect and the lack of convincing results from clinical studies. Future research should concentrate on the pharmacological agents that are capable of mimicking the protective effects of preconditioning, such as K_{ATP} channel openers, and on possibly prolonging the cardioprotective effect of IP to increase its use in clinical situations. It may be worth mentioning the Impact Of Nicorandil in Angina (IONA) study (14) which showed that the K_{ATP} opener nicorandil was effective in reducing the rates of the combined end points of coronary artery disease mortality, myocardial infarction and admission for chest pain.

In discussing the clinical relevance of adaptation to CH, it is necessary to stress that CH can be found in common cardiopulmonary diseases, such as chronic ischemic heart disease and chronic obstructive lung disease. Clinical profit from this phenomenon depends on the balance between the benefit (cardiac protection) and potential risks (pulmonary hypertension, right ventricular hypertrophy) (15). An interesting aspect that should be analyzed to understand human susceptibility to myocardial infarction may be the sex and age differences in cardioprotection. A particularly rewarding aspect of basic research in the field of cardioprotection should be the possibility of the immediate translation of new discoveries obtained from in vitro experiments into the clinical setting, particularly those of coronary bypass surgery and percutaneous coronary intervention (16).

MYOCARDIAL CELL DEATH

The present

Reduced blood flow to the myocardium causes metabolic, functional and morphological changes. At the level of the myocyte, both dysfunction due to impaired excitation-contraction coupling, electrical instability, altered ionic homeostasis and a shift from aerobic to anaerobic metabolism, on the one hand, and irreversible myocyte loss on the other, are believed to contribute to disease progression. Cardiomyocytes can undergo cell death by two different mechanisms: necrosis and apoptosis (17). The increased interest into the research of apoptosis in cardiology (in contrast to necrosis) mainly stems from the hope that understanding the mechanism of apoptosis in cardiac myocytes may provide new strategies to prevent myocyte loss. A major determinant for the success of this novel approach is the degree to which apoptosis contributes to total myocyte loss and to what extent this loss of contractile mass can be prevented to reduce functional deterioration and mortality. However, only a few reports (18) provide evidence for the potential of antiapoptotic therapy to improve the outcome in cardiac disease.

Future directions

There are still several unresolved issues in apoptosis that need to be addressed by future research (18,19). At the molecular level, it still remains uncertain which mechanisms initiate the apoptotic process in cardiac myocytes. Although several interventions (eg, catecholamines, atrial natriuretic peptide and angiotensin II) were shown to induce apoptosis in cultured myocytes, their role in human disease must be established. It will be of major interest to discern the apoptotic pathways that lead to DNA fragmentation and positive terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) staining in clinically relevant experimental models. These pathways include mitochondrial-dependent versus receptor-mediated pathways, caspase-dependent versus apoptosis inducing factor-induced apoptosis and proapoptotic versus antiapoptotic signalling pathways. Furthermore, there are increasing doubts that evidence for apoptosis that is solely based on TUNEL staining and even DNA laddering may be sufficient to prove apoptotic cell death; morphological alterations of myocytes in situ will also be required. Of great importance is the definition of the

implications of myocyte apoptosis and its extent for the progression of disease and thus, to delineate the potential of an antiapoptotic therapy in human disease. More studies are required to provide evidence for the pathophysiological importance and clinical outcomes to support the current hopes that the apoptosis of myocytes will become a new target for future therapeutic intervention.

REPERFUSION

The present

Early coronary reperfusion has proved to be the only way to limit infarct size in the clinical treatment of evolving acute myocardial infarction; however, there is also evidence from animal studies (20-22) that reperfusion may contribute to a further increase tissue damage, a phenomenon known as 'reperfusion injury' (including arrhythmias, enzyme release or severe intramyocardial hemorrhage). O_2 free radicals have been clearly shown to be generated on the restoration of blood flow and to be potentially harmful, and their role as main mediators of reperfusion injury were soon widely accepted (20). Piper et al (21) suggested three initial causes of immediate reperfusion injury (apart from O_2 radicals): re-energization, the rapid normalization of tissue pH and rapid normalization of tissue osmolality. Taken together, during ischemia-reperfusion, cardiomyocytes are exposed to a sequence of adaptive and injurious events. In principle, two components can be discerned, one that develops during ischemia (ischemic injury) and a second that develops during reperfusion (reperfusion injury). The term 'reperfusion injury' is in fact a misnomer, because it is the severity of ischemia that sets the stage for the development of reperfusion injury, and the appropriate term should be 'ischemia-reperfusion injury' (22).

However, concerns about the potential clinical significance of reperfusion injury were soon put aside by results from clinical studies. The experience obtained from millions of patients with evolving acute myocardial infarction has demonstrated that reperfusion therapy may be more or less beneficial, depending on the circumstances, particularly on how early it is applied (eg, The PRAGUE Study [23,24]). Reperfusion injury is therefore considered by many cardiologists to be either nonexistent (reperfusion-associated phenomena as accelerated expression of pre-existent injury) or clinically irrelevant (in relation to the importance of ischemic injury [25]). It must be mentioned that in contrast to the skeptical view of cardiologists, many cardiovascular surgeons are convinced of the existence of the potentially adverse effects associated with the restoration of normal myocardial perfusion (26). However, both experimental and clinical cardiologists agree that the main target in reperfusion is the restoration of microcirculation, and the most striking example of postischemic microvascular incompetence is the so-called no-reflow phenomenon.

Future directions

Future research should, therefore, bring together the opinions of both basic and clinical cardiologists. From the experimental point of view, it would be desirable to better understand the molecular mechanisms involved in the initial minutes of reflow, to determine whether the mechanisms originate in cardiomyocytes and/or in vascular or bloodborne cells, and whether reperfusion induces the death of noninjured cells or accelerates the death of cells that were injured during the period of ischemia. Of great importance would be the clarification of the role of apoptosis in ischemia/reperfusion injury (19) and the function of mitochondria in this process, particularly through the opening of the permeability transition pore (27). It may be interesting to explore the new approach of the reperfusion injury salvage kinase (RISK) pathway that was suggested by Hausenloy and Yellon (28). Therapeutic relevance involves the reduction of reperfusion injury and restoration of microcirculation based on treatments targeting the previously discussed mechanisms to minimize the impact of acute coronary syndromes on survival and the quality of life. Therapeutic strategies that are able to attenuate reperfusion, particularly during cardiac surgery, are very much needed.

MYOCARDIAL REGENERATION

The present

Myocardial regeneration consists of the repopulation of irreversibly damaged muscle with new contractile cells to restore function in the necrotic areas, thereby improving global heart function (29). Until recently, cardiomyocytes were thought to be terminally differentiated cells, implying that hypertrophy was the only available form of growth. This concept was recently revised on the basis of both experimental and clinical studies (30,31) which showed that some adult cells can re-enter a mitotic cycle. However, the magnitude of this self-repair mechanism is far too limited to compensate for the massive loss of cardiomyocytes resulting from a large infarction. Therefore, the only practicable perspective is an exogenous supply of cells for effective remodelling of injured areas. According to Menasché (29), exogenous cells should satisfy the following criteria:

- be easy to collect and expand;
- form stable intramyocardial grafts;
- be able to electromechanically couple with host cardiomyocytes and beat in synchrony with them; and
- be devoid of arrhythmogenic and oncogenic effects.

Unfortunately, ideal cells fitting this description are not yet available for clinical use. The first attempts began with the transplantation of immature cardiomyocytes in infarcted myocardium and the results demonstrated a beneficial effect on cardiac function (32). From a clinical perspective, however, the use of fetal tissue is not feasible because immature cardiomyocytes are not available for therapeutic measures in humans (32). Therefore, alternative sources of cells, which might be more readily available, such as skeletal myoblasts and stem cells, are under investigation. Cell replacement therapeutic strategies based on either the replacement of scarred tissue by precursor cells (skeletal myoblasts) or attempts at regenerating the scarred tissue by bone marrow-derived stem cells, embryonic stem cells and endothelial progenitor cells might be regarded as the scientific model of what can be achieved.

Future directions

Several key problems remain for the future (29,32):

- the determination of the optimal cell type in relation to the specific patient situation;
- improvement of tissue processing and culturing techniques;

- the development of means to enhance cell survival, possibly by the concurrent induction of gene- or cell-based angiogenesis;
- the selection of the most appropriate method of cell delivery; and
- the development of objective methods for controlling the efficacy of transplantation therapy.

It may be of interest to mention that the cardiac myocytes of cold-blooded animals proliferate during their entire lifespan and are, thus, able to replace lost tissue; in contrast, the mammalian heart loses this proliferative capability soon after birth. An understanding of these phylogenetic differences might be the basis for cardiac regenerative therapy in the future (33).

CONCLUSIONS AND PERSPECTIVES

We are living in an era in which the influence of molecular medicine and basic research on clinical practice has never been more pronounced. The recent achievements in molecular biology and genetics have opened the door for substantial progress in many medical disciplines, including cardiology. This, however, requires a new strategy for future cardiovascular research. The huge amount of available genetic information should now be exploited for integrative cardiovascular pathophysiology. Such an approach must, first of all, include developmental and sex differences, which are particularly important for the field of ischemic heart disease; therefore, experimental cardiovascular research can no longer be restricted to males of uncertain age. Clinical epidemiological studies have shown that ischemic heart disease is no longer the disease of the fifth and higher decades of life, but their origin and consequences may be essentially influenced by risk factors that are already acting during development. Moreover, it is necessary to mention that healthy, immature myocardium is more tolerant to ischemia than the myocardium of adults, and that the cardioprotective effects of adaptation to CH and IP are significantly less expressed during early ontogeny (34). Therefore, it follows that experimental studies of the pathogenetic mechanisms of myocardial injury and protection must shift to the early ontogenetic period (33). Sex differences have a pronounced influence on the type and severity of cardiovascular diseases, including the susceptibility to ischemic heart disease. Recent results indicate that some sex differences may exist even on a molecular level (eg, a different number of K_{ATP} channels and the microvascular generation of free radicals [35]). There is increasing evidence that the field of cardiology is not exempt from the basic biological fact that men and women are different, and that this may need to be reflected in the therapies they receive (36). Basic research should follow this imperative.

Future cardiovascular research should include the following general aspects:

- evaluate the role and proportion of already described molecular pathways; the descriptive approaches will gradually disappear;
- distinguish between the acute, chronic and pleiotropic effects of different drugs under in vitro and in vivo conditions with respect to possible clinical use;
- use clinically relevant genetic models;
- study the possible alteration in intracellular signalling to find the decisive steps that are responsible for the abnormal control of cell growth, contractile function, lipid metabolism, cardiac ischemic tolerance, etc; and
- study the molecular mechanisms of cardiovascular diseases not only in healthy individuals but also under different pathological conditions.

It is hoped that patients in future decades will profit from the progress of basic cardiovascular research, allowing many useful treatments to become available. Simultaneously, there should be a greater focus on prevention, using different early markers of myocardial injury. Moreover, the development of suitable animal models of human cardiovascular diseases is very much needed and remains as important as ever.

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