A critical look at cardiovascular translational research

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Every decade can be characterized on the basis of fashionable crazes, trends, and vogue words. Science is no different from fashion in this respect, and one of the newly created words at the end of the millennium is the term “translational research.” This term, which is in danger of becoming an empty phrase because of overuse, refers to the process of transferring, from bench to bedside, findings in basic science into clinical practice, e.g., diagnostic procedures and therapeutic concepts. Translational research is not a one-way process because observations made in the clinic are often a stimulus to return to the bench to further investigate additional specific aspects of a treatment in cell biology. However, whereas a close collaboration between basic scientists and clinicians is in general a good thing, there are intense pressures placed on this approach by institutions that have a financial interest in developing new therapies. In such cases, there is a danger of proceeding too rashly with a novel therapeutic approach with the consequence that clear data from animal studies may be ignored and clinical trials performed that have little likelihood of providing a long-term significant benefit for more than a small group of patients.

GENE REPLACEMENT THERAPY

Relatively few attempts have been made to date to perform gene transfer in the human cardiovascular system. Thus the majority of the information currently available has been obtained from animal models. Such models are only of limited use because a cardiovascular disorder manifest in an animal may not truly reflect the chronic and complex pathophysiological situation in humans. Nevertheless, many of the problems associated with the transfer of genetic material into the cardiovascular system (targeting, kinetics of expression, and eventual side effects) can be addressed in such a manner.

Probably the best examples of successful translational research relate to cancer and especially to the elucidation of the role of the p53 tumor suppressor gene in the regulation of programmed cell death or apoptosis. Approximately 50% of cancer patients carry a mutation in the p53 gene and exhibit decreased expression of the wild-type gene. Whereas the demonstration of this absolute difference between the healthy and the diseased state was the result of bench science, the translational approach to this research required basic scientists and clinicians to exploit this difference to treat cancer patients. As the expression of the wild-type gene seemed to be decreased in cancer patients, it was decided to transfet patients with the wild-type p53. The results of the first phase I clinical trial of p53 gene therapy in which a retroviral wild-type p53 construct was directly injected into tumor sites in lung cancer patients were exceedingly impressive (51). Although it would be ideal to have a systemically administrable treatment/cure for cancer, which would also target unrecognized metastases, the direct application of the p53 gene circumvented one of the main difficulties associated with gene replacement therapy, i.e., targeting. Major obstacles in gene therapy are (that is after the target gene has actually been identified) getting the administered gene to the tissue of interest without being concentrated in a secondary, irrelevant location or removed from the body entirely. Once there, the gene product should be rapidly and selectively expressed, but the time frame of expression is difficult if not impossible to predict, and it is the timing of the appearance of the gene product that is a major determinant of the clinical outcome. One example is the recent demonstration of the adenovirus-mediated local expression of human tissue factor pathway inhibitor (TFPI) in the rabbit carotid artery following balloon angioplasty. This intervention eliminated shear stress-induced recurrent thrombosis without affecting the systemic coagulation status (42). Because this effect was observed 6 days after transfer, TFPI transfer would seem like an attractive clinical approach to deal with recurrent thrombosis. However, although gene transfer was performed directly after arterial injury, there would have been a necessary delay in the expression of TFPI, because the expression of a gene product usually requires a time lag of between 12 and 24 h. The timing of transgene expression may therefore be too late to be of real clinical benefit because tissue factor expression increases within a few hours of injury.

The duration of gene product expression is another important factor determining clinical outcome. One example is the gene transfer of the endothelial nitric oxide (NO) synthase (eNOS) into the lung of mice (10). The reason for attempting such an approach is that the production of NO by eNOS appears to be crucial to...
countercurrent pulmonary vasoconstriction caused by chronic hypoxic stress (53). Whereas eNOS gene transfer resulted in a clear rightward shift of the pressure-flow relationship, the expression of eNOS was most probably transient because the half-life of the expression of a marker gene was 5–7 days (10). It is questionable whether or not such a transient expression of a gene product can bring about a significant therapeutic improvement.

Experimental attempts to overexpress eNOS in various arteries have somewhat surprisingly revealed that the adventitial, rather than endothelial, transfection with eNOS appears to produce some of the most positive results (11, 22, 46, 47, 58). It is, however, unclear to what extent the function of eNOS is altered when expressed in nonendothelial cells, given that enzyme activity is modulated by alterations in its phosphorylation as well as by changes in intracellular Ca²⁺ (15). Moreover, unlike endothelial cells, fibroblasts and vascular smooth muscle cells are not directly exposed to fluid shear stress, which is the most important physiological stimulus for the generation of NO.

The topic of eNOS gene transfer can be used to highlight another potential problem associated with gene therapy, i.e., whether or not the prolonged expression of a given gene product is actually desirable. Enhancing the expression of eNOS has received a lot of attention in the treatment of atherosclerosis since the bioavailability of NO, a fundamental vasodilator and antiatherogenic principle, appears to be decreased. However, unlike the case of the p53 tumor suppressor gene, there is no consistent evidence that a lack, functional defect, mutation, or polymorphism of eNOS underlies cardiovascular diseases (8, 21, 23, 30, 38, 39, 48, 52, 55, 59). Moreover, in preeclampsia (40, 43) and several animal models of hypertension (4, 9, 44, 61), the development of endothelial dysfunction is associated with an increase rather than a decrease in eNOS expression. In some experimental models such a long-term overexpression of eNOS is associated with a decrease rather than an increase in endothelium-dependent relaxation as a consequence of a concomitant increase in the formation of superoxide anions (O₂⁻) and the downregulation of NO effector pathways, most notably that of the soluble guanylyl cyclase expression in smooth muscle cells (4, 45). An increase in NO production fits well with the suggestion that peroxynitrite, the reaction product of NO and O₂⁻, is formed within the vasculature of patients with preeclampsia or atherosclerosis and is associated with increased nitrotyrosine immunostaining (5, 50). In addition, despite reports that the transfer of eNOS enhances endothelium-dependent relaxation in hypercholesterolemic rabbits (47) and induces a relatively maintained decrease in the blood pressure of spontaneously hypertensive rats (26), it should be emphasized that the functioning of eNOS is dependent on the expression of several cofactors, especially tetrahydrobiopterin (H₄B). A lack of cofactor availability, which is a possible consequence of transfecting vascular smooth muscle cells and fibroblasts with eNOS, may have the opposite effect to that intended by transforming eNOS into an O₂⁻-generating enzyme (14, 49, 60, 62, 63) and thus further impairing vascular reactivity.

**ENDOTHELIAL DYSFUNCTION, ANGIOTENSIN-CONVERTING ENZYME INHIBITORS, AND STATINS**

One of the first positive examples of translational research in the cardiovascular area is the development of a relatively simple technique to assess endothelium-dependent dilator responses, in either the forearm or the coronary system of patients. The starting point in this case was the fundamental observation by Furchgott and Zawadzki (17) that a factor, later identified as NO, that is released from acetylcholine-stimulated endothelial cells can relax arterial preparations. The subsequent finding that agonist- and flow-induced vasodilator responses, which basically reflect the amount of bioavailable NO generated by endothelial cells, were depressed in animal models and subjects with hypertension and atherosclerosis led to the concept that this procedure could be used as a convenient diagnostic test for endothelial dysfunction.

As mentioned above, endothelial dysfunction is currently believed to be related to an imbalance in the endothelial production of NO and O₂⁻, resulting in the shut down or inactivation of certain intrinsic anti-hypertensive and antiatherogenic mechanisms. Thus redressing the balance in vascular NO/O₂⁻ production is an attractive therapeutic goal in the treatment of cardiovascular disease. A vast amount of experimental data provides evidence that angiotensin-converting enzyme (ACE/kininase II) inhibitors are able to do just that, i.e., increase NO and decrease vascular O₂⁻ production, partly by inhibiting the generation of angiotensin II and partly by prolonging the half-life of bradykinin. Moreover, reports of an impressive protective effect of ACE inhibitors on cardiovascular complications in patients (20) and in animal models (13) without decreasing blood pressure suggests that ACE inhibitors possess additional effects. Such observations prompted a reinvestigation of the effects of ACE inhibitors on endothelial cells, with the outcome being the demonstration of a crosstalk between ACE and the B₂ kinin receptor, such that the ACE inhibitor group of compounds, independent of ACE inhibition, appears to be able to modulate B₂ kinin receptor signalling (6, 27, 29).

ACE inhibitors have also recently been reported to promote angiogenesis in a rabbit model of hindlimb ischemia (16). Although the exact mechanism underlying this effect is unknown, it may involve the increased expression of hepatocyte growth factor (31, 64), which is currently characterized as one of the most potent growth factors specific to the endothelium (35) and which may protect against the development of endothelial dysfunction (34, 36, 37).

Another example where clinical observations have sent scientists back to the bench relates to observations made using 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors or “statins.” The cardiovascular benefits of this group of compounds extends beyond
their lipid-lowering effects, and although not reported to significantly affect the regression of atherosclerotic plaques, it stabilizes them, restores endothelial function, and dramatically decreases the incidence of cardiovascular events (stroke and myocardial infarction). At a cellular level, statins inhibit the synthesis not just of cholesterol but also of isoprenoids. The upregulation of eNOS expression by statins occurs via a mechanism independent of cholesterol but mediated by the inhibition of the isoprenoid geranylgeraniol and the subsequent geranylgeranylation of the GTPase Rho (25). It was also hypothesized that inhibition of isoprenylation of p21ras proteins could account for the statin-induced, but eNOS-independent, suppression of vascular smooth muscle cell proliferation. Indeed, lovastatin has been reported to inhibit cyclin-dependent kinases resulting in a cell cycle arrest in vascular smooth muscle cells (32). A similar effect has been demonstrated in cultured mesangial cells and is related to the translational upregulation or impairment of p27kip1 (a cyclin-dependent kinase inhibitor) protein degradation (56).

As a result of the current obsession in the cardiovascular field with NO, there has been a tendency to ignore the fact that there are many other endothelium-derived vasoactive factors that exhibit vasodilating or constricting properties. The production of endothelium-derived contracting factors, for example, may increase, and a cyclooxygenase-derived product is known to increase in essential hypertension (54). In addition, the increased production of the ω-hydroxylation product of arachidonic acid 20-hydroxyeicosatetraenoic acid, which is thought to be involved in the development of myogenic tone in the kidney, may contribute to the development of hypertension by elevating renal vascular resistance (2, 19). Moreover, there are vessels and vascular beds in which NO plays little or no role in mediating vasodilatation, and although this function is still controlled by the endothelium, the autacoids mediating this response are prostacyclin and the endothelium-derived hyperpolarizing factor. Indeed, the magnitude of endothelium-dependent dilations elicited by agonists or an increase in flow are barely different in wild-type versus eNOS knockout mice.

THE DARK SIDE

Taking information gleaned from exhaustive investigations in cell culture, in isolated organs, and in animal models of disease and applying it to a clinical situation that is currently untreatable with classical therapy is, in principle, a thorough and (almost) ideal way of treating disease. The approach of elucidating an obvious and causal defect associated with a given pathophysiological situation and using modern molecular medicine to correct this defect has an enormous potential for clinical application. Thus it is understandable that there is a tendency to idealize this approach and to believe in its infallibility. There is however a dark side to translational research. In what way can translational research be negative? Perhaps the translation of a basic finding to the clinic is too fast or fails to take into account basic considerations related to the sequence of a clinical condition. The other problems relate to the interference in a given project by external pressure groups to force the development of treatments for potentially fatal and expensive conditions.

Classical cases of unsuccessful treatment approaches are the use of tumor necrosis factor-α (TNF-α) antibodies and NO inhibitors in sepsis. It should be noted that, following a septic insult, there is a clear sequence of cytokine synthesis, which may be critical to the cascade of events leading to septic shock. The first cytokine to peak in plasma is TNF-α (~1 h), followed by interleukin (IL)-6 (3 h) and IL-1β (3–6 h), this sequential cytokine production likely reflects carefully orchestrated gene expression and regulation and is of importance to the subsequent cascade of metabolic events. An overlap also exists in the actions of certain cytokines, such that conditions exist in which IL-1β, TNF-α, and other cytokines can elicit the same end effect. Monotherapy, rendering only one cytokine ineffective, is therefore likely to be only of limited benefit. Effective treatment with neutralizing antibodies also depends on the time of administration, and whereas anti-TNF-α antibodies decrease lethality and the circulating concentrations of IL-1 in experimental animals if given before an endotoxic challenge, little or no benefit is seen if antibodies are administered after endotoxin (7, 28, 33, 57). Despite such knowledge gained from experimental investigations, a huge investment has been made in the development of TNF-α antibodies for the treatment of human sepsis. Not surprisingly, these clinical studies have provided negative results with antibodies being unable to alter the overall pattern of cytokine activation or the profound disarrangements in physiological function that accompany severe sepsis (1, 12).

Back to the topic of NOS, but in this case the inducible NOS (iNOS), there is overwhelming evidence that this enzyme plays a role in the hyporeactivity to vasoconstrictor agents in sepsis. Given this information, the development of selective iNOS inhibitors as therapy for septic shock became a priority of a number of pharmaceutical concerns. The first clinical reports have proven to be disappointing and have provided conflicting results, demonstrating both beneficial and detrimental effects (18). Moreover, a high mortality rate in NOS inhibitor-treated patients indicates that iNOS inhibition has only limited effects on outcome (3). On hindsight, this was to be expected because prevention of iNOS induction does not prevent the fatal outcome of shock. Indeed, the induction of septic shock is just as lethal to iNOS knockout mice as it is to wild-type mice (24). One point that has only recently achieved attention is that in many cases the induction of iNOS is not an annoying side effect but an essential cytoprotective step, e.g., in the liver. In addition, NO is a potent weapon used by numerous cells in the fight against infection, and inhibition of iNOS or the genetic deficiency of iNOS can profoundly affect the time course of infection and inflammation (41).
WHAT IS THE TAKE HOME MESSAGE?

For translational research in the cardiovascular area to be effective, it is necessary (as in the case of p53) to exploit a real difference between the healthy and diseased state. Such instances are unfortunately rare. In the majority of cases, gene polymorphisms or absolute alterations in enzyme or protein expression and function do not seem to correlate with the manifestation of a given disease. Thus, whereas the goals of translational research represent a form of utopia to which one has to strive, it is unlikely that an effective therapy can be rapidly developed using such an approach. The simple truth is that we do not fully understand the complex pathophysiology of the diseases that exert the most significant drain on health care resources, and thus, from a financial point of view, have the highest priority for the development of an alternative therapy. We can go back to eNOS and endothelial dysfunction for a last example. A few years ago, just as it became clear that a defect in the generation of bioactive NO was implicated in hypertension, atherosclerosis, etc., programs were started to find substances that enhance the expression of eNOS and restore NO production to “normal” levels. It took quite some time to develop screening systems and synthesize lead compounds that effectively enhance the expression of eNOS. The only problem is that we now know that eNOS expression is, more often than not, increased in situations associated with endothelial dysfunction. From currently available data it would be perhaps a better idea to concentrate on maintaining eNOS in a fully functional capacity by enhancing the expression of enzymes such as GTP cyclohydrolase, which catalyze the formation of H4B, the lack of which transforms eNOS from an NO-generating enzyme into one that generates O2⁻.

Whereas such an example cannot detract from the fact that translational research is one of the potentially most powerful approaches for the next millennium, we have to accept the fact that there is an inevitable amount of trial and error involved and that programs will have to be flexible enough to reorientate in the light of the findings continuously coming from basic science. The financial pressures associated with translational research are not to be ignored and can only be expected to increase, but perhaps it is worthwhile to stress the need for a well-balanced approach to translational research. Preventive medicine should, for example, receive much more attention because, at the end of the day, prevention is always better than cure.

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