JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 3, NO. 2, 2018

EDITORIAL COMMENT

Exaggerated Cardiotoxicity of Sunitinib in Stressed 3-Dimensional Heart Muscles*

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Twenty years ago, oncology was revolutionized by the introduction of the anti-Her2 antibody trastuzumab (Herceptin, Genentech, South San Francisco, California; 1998) for the treatment of patients with Her2-positive breast cancer. It represents a direct application of the proto-oncogen concept that had been developed by Bishop and Varmus in the early 1980s (1,2), for which they were awarded with the Nobel Prize in Physiology or Medicine in 1989. Other examples, such as the approval of imatinib (Glivec, Novartis, Basel, Switzerland) in 2001 for the treatment of Philadelphia chromosome-positive chronic myeloid leukemia, followed and raised hopes that cancer can one day be eradicated by an individualized therapy. In this concept of personalized medicine, malignant tumors are understood as the consequence of individual (activating) somatic mutations of a proto-oncogen that drives cellular growth and makes the tumor cell "depending" on the respective signaling pathways. The right choice of drug blocking such pathways should kill the tumor, but not the normal tissue.

However, not all hopes have been fulfilled. Most solid tumors appear to quickly develop resistance against the growth-suppressing anticancer drugs and appear to be much more heterogeneous than anticipated. Disappointingly, even the combination of several "targeted" drugs, whose choice was based on the molecular analysis of various somatic mutations of tumor cells, did not yet yield convincing clinical success (3).

Furthermore, most "targeted" drugs are not so specific as the term may suggest. In fact, most of the popular tyrosine kinase inhibitors (TKIs) block numerous kinases with similar potency, some like sunitinib >50 kinases (4). But, blocking more and more cellular growth pathways comes at a price, because the growth pathways used by tumor cells for their pathological growth are the same that are necessary for other cells, including cardiomyocytes, to grow in response to increased demand and protect against stress (5). An early example of the importance of such growth-promoting cascades required under stress was the discovery that cardiac-specific deletion of gp130, the receptor of the cytokine cardiotrophin, is without apparent phenotype under basal conditions, but leads to severe dilated cardiomyopathy in mice subjected to pressure overload (6). Other pathways include epidermal growth factor receptor signaling (ErbB2, or Her2), the PI3 kinase pathway, AMP-activated protein kinase (AMPK, a nodal point of metabolic control), the ubiquitin proteasomal system, the autophagy lysomal pathway, the control of (histone) acetylation (addressed by HDAC inhibitors), and, mostly indirectly, vascular endothelial growth factor-mediated maintenance of adequate perfusion (7). Many of these pathways are addressed by TKIs, explaining their cardiotoxic side effects, particularly under stress. The main stressors in this respect are parallel treatment with the prototypic cardiotoxin

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

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The author attests he is in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2018.01.011

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 3, NO. 2, 2018
APRIL 2018:277–9

doxorubicin and the presence of arterial hypertension. In the first studies with trastuzumab in patients with breast cancer, left ventricular dysfunction was seen in only 3% to 7% of patients when trastuzumab was given alone, but in 28% when given in combination with doxorubicin and cyclophosphamide (8).

The principles of the cardiotoxicity of antitumor drugs, as described in the previous text, have been formulated through experience with the ErbB2 (Her2) antibody trastuzumab (9). In the meantime, however, hundreds of small-molecule TKIs have been developed that target multiple pathways, raising the risk of adverse effects (10). The problem has been widely recognized, and has led not only to clinical guidelines and consensus papers (11), but also to activities by the U.S. Food and Drug Administration to improve the preclinical discovery of cardiotoxic side effects of anticancer drugs (12,13). Preclinical risk assessment traditionally focuses on proarrhythmic effects mediated by inhibition of the repolarizing I_{Kr} current (carried by the hERG channel), and numerous drugs failed to reach clinical application for such side effects. However, cardiotoxicity by TKIs and other novel anticancer drugs encompasses not only proarrhythmic effects, but also reduced cardiomyocyte survival and contractile function. Here, traditional cell culture assays fall short. Freshly isolated adult cardiomyocytes quickly degenerate in culture, and isolated hearts or heart muscles (e.g., Langendorff-perfused hearts, trabeculae from human atrial appendages, papillary muscles, and Purkinje fibers from animals) are dying preparations that offer a maximum experimental window of a few hours. Neonatal rat cardiomyocyte cultures are more stable, but they are of rodent origin, immature, and not well suited for the determination of contractile function. It was on this background that we developed 3-dimensional heart muscle constructs 20 years ago (14). These hydrogel-based engineered heart tissues (EHTs) are stable over weeks and allow the determination of contractile force of contraction under controlled conditions.

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In this issue of *JACC: Basic to Translational Science*, Truitt et al. (15) report the effects of the multi-TKI sunitinib on contractile force, apoptosis, and mitochondrial potential of neonatal rat- and human-induced pluripotent stem cell (hiPSC)-derived EHTs (called cardiac microtissues [CMTs]). They used an adaptation of the original micropost EHT technique (16) developed by Boudou et al. (17) and showed that sunitinib at a clinically relevant concentration of 1 μmol/l induces a time-dependent activation of caspase 3/7 (indicator of increased apoptosis rate). At higher concentrations (10 μmol/l), sunitinib also decreased beating rate and diminished the increase of contractile function that was observed in control CMTs. Sunitinib slightly decreased the mitochondrial transmembrane potential. The latter was not rescued by activation of AMPK; this result was unexpected, as others had previously shown sunitinib toxicity to be at least partially related to its inhibitory effect on AMPK (18). Finally, activation by sunitinib of caspase 3/7 was confirmed in CMTs from hiPSCs, indicating that it occurred across species. Data on contractile function of hiPSC-CMTs were not reported. The data by Truitt et al. (15) nicely confirm earlier work on cardiotoxic effects of sunitinib and other TKI on cultured human cardiomyocytes (19–21), rat 3-dimensional (3D) EHTs (22), or cardiac function of murine hearts in vivo (23). The studies consistently indicate that the multi-TKI sunitinib has a particularly high cardiotoxic potential when compared with more specific compounds such as erlotinib. The likely reason is sunitinib's parallel inhibition of several signaling pathways that are essential for the maintenance of normal cellular function, including the EGFR, VEGFR, platelet-derived growth factor receptor (PDGFR), AMPK, and others, interfering with mitochondrial energy metabolism, apoptosis, and autophagy.

The novel and most interesting aspect of the present study occurred when the authors repeated their experiments in CMT cultured on stiffer-than-normal microposts. This increase in afterload exaggerated the toxicity of sunitinib, providing an experimental proof that the cardiotoxicity by this (and likely other) TKI is augmented in situations of cardiac stress. This result is consistent with earlier data on rat EHT (24) and a genetic model of cardiomyopathy (25), in which stiffer posts induced and exaggerated the phenotype. A recent meta-analysis demonstrated that sunitinib increases the risk of arterial hypertension 7- to 10-fold (26). Thus, the high incidence of left ventricular dysfunction seen with this compound (risk ratio: 4.3 [26]) is very likely due to a direct cardiotoxic effect combined with the parallel drug induction of arterial hypertension.

The possibility to study such combined effects in vitro is a welcome step forward and shows the power of the advanced 3D cell culture models for preclinical drug testing. The consistency of the effects of sunitinib in several in vitro and in vivo studies and the good correlation with clinical data indicate that the in vitro assays are valid to predict the clinical profile of TKI and other anticancer drugs. Yet, more work is needed to

compare the cardiotoxicity of several compounds under defined conditions and in blinded, interlaboratory-comparisons, preferentially on hiPSC-derived cardiomyocytes and their 3D derivatives. Such experiments have the chance to better predict the specific cardiotoxic potential of novel drugs before approval. At this point, oncologists and cardiologists can only be advised to choose the drug that is likely to be the least cardiotoxic, and be alert to any effect not only on the heart, but also on blood pressure.

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KEY WORDS afterload, cardiac tissue engineering, cardiotoxicity, induced pluripotent stem cells, tyrosine kinase inhibitors