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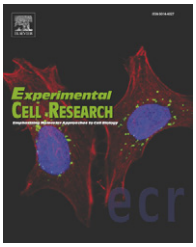

Research Article

Inhibition of ErbB2 by receptor tyrosine kinase inhibitors causes myofibrillar structural damage without cell death in adult rat cardiomyocytes

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ABSTRACT

Inhibition of ErbB2 (HER2) with monoclonal antibodies, an effective therapy in some forms of breast cancer, is associated with cardiotoxicity, the pathophysiology of which is poorly understood. Recent data suggest, that dual inhibition of ErbB1 (EGFR) and ErbB2 signaling is more efficient in cancer therapy, however, cardiac safety of this therapeutic approach is unknown. We therefore tested an ErbB1-(CGP059326) and an ErbB1/ErbB2-(PKI166) tyrosine kinase inhibitor in an in-vitro system of adult rat ventricular cardiomyocytes and assessed their effects on 1. cell viability, 2. myofibrillar structure, 3. contractile function, and 4. MAPK- and Akt-signaling alone or in combination with Doxorubicin. Neither CGP nor PKI induced cardiomyocyte necrosis or apoptosis. PKI but not CGP caused myofibrillar structural damage that was additive to that induced by Doxorubicin at clinically relevant doses. These changes were associated with an inhibition of excitation–contraction coupling. PKI but not CGP decreased p-Erk1/2, suggesting a role for this MAP-kinase signaling pathway in the maintenance of myofibrils. These data indicate that the ErbB2 signaling pathway is critical for the maintenance of myofibrillar structure and function. Clinical studies using ErbB2-targeted inhibitors for the treatment of cancer should be designed to include careful monitoring for cardiac dysfunction.

Introduction

The epidermal growth factor receptor (EGFR) family plays a critical role in the development and maintenance of the heart. ErbB2 and ErbB4 null mutant mice display impaired development of trabeculae and the embryos die before embryonic day 11, whereas the conditional deletion of ErbB2 in the adult heart leads to dilated cardiomyopathy and an increased sensitivity to anthracyclines [1–3]. Clinical trials with the immunotherapeutic agent trastuzumab (Herceptin®) support the hypothesis of a critical role of the ErbB2 receptor in the heart. Inhibition of human ErbB2 (HER2) with trastuzumab, given either in combination with or after

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chemotherapy, prolongs survival in women with HER2 positive metastasizing breast cancer [4] and improves disease free survival in the adjuvant setting [5,6]. However, alone and in combination with chemotherapeutic agents like doxorubicin (Doxo), trastuzumab therapy was associated with cardiac dysfunction [4]. We showed, that in adult cardiomyocytes treatment with anti-ErbB2 antibodies changes MAPK-signaling and increases Doxo- or paclitaxel-induced myofibrillar disarray, which may explain contractile dysfunction seen in patients [7].

Based on these previous observations, we hypothesized that ErbB2-inhibition with tyrosine kinase inhibitors would induce myofibrillar disarray and contractile dysfunction in cardiomyocytes. We found, that a ERBB1/ErbB2 tyrosine kinase inhibitor induces similar changes as antibodies to ErbB2. Since the inhibition of ErbB2-receptors in cancer cells promotes cell death, we expected to find similar effects in myocytes. We did not find induction of cell death in cardiomyocytes either with a single EGFR- or with the combined EGFR/ErbB2 tyrosine kinase inhibitor.

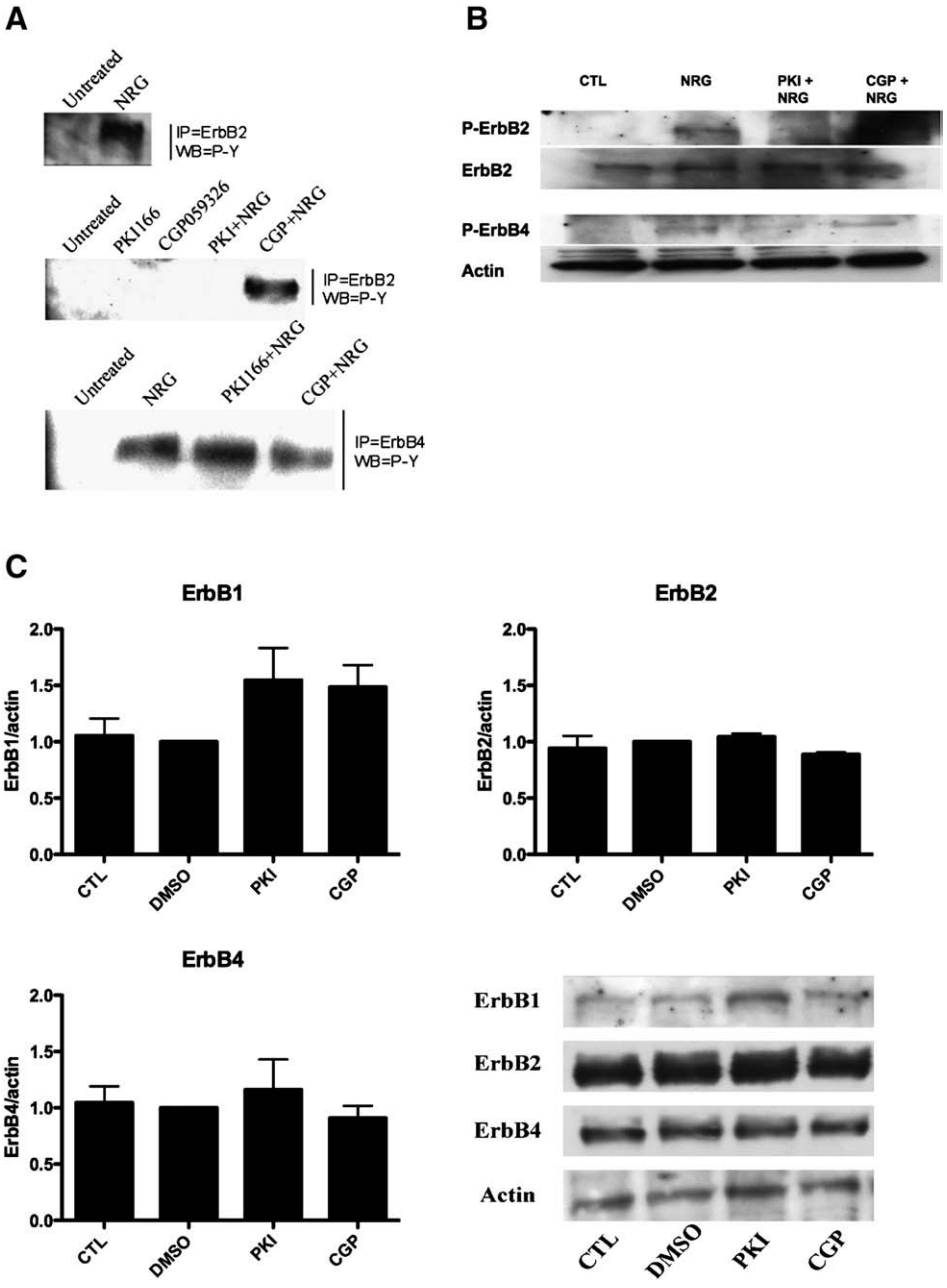

Fig. 1 – ErbB receptor phosphorylation and protein amount. (A) ARVM cultured for 10 days were treated for 3 h with tyrosine kinase inhibitors (PKI 1 μM and CGP 1 μM) followed by NRG 10 ng/ml for 10 min and immunoprecipitation/Western blot was performed as indicated. NRG activated both ErbB2 and ErbB4 receptors; PKI blocked NRG-induced activation of ErbB2 whereas CGP did not. Neither PKI nor CGP inhibited NRG action on the ErbB4 receptor. (B) ARVM were treated as in A and Western blot was performed as indicated including phospho-specific antibodies for ErbB2 and ErbB4. (C) ARVM cultured for 10 days were treated for 48 h with DMSO 1 μl/ml, PKI 1 μM, CGP 1 μM and Western blot was performed for ErbB1/2/4 and actin as loading control. Neither PKI nor CGP reduced the receptor proteins.