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New insights in the treatment of chondrosarcomas; role of the hydroxy-methyl-glutaryl-CoA reductase inhibitors "*in vitro*"

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Chondrosarcoma is a very aggressive chemo- and radio-resistant tumor of the adulthood. The search of an effective treatment for this muscle-skeletal tumor has not been successfully achieved. Consequently, the investigation of new drugs for chondrosarcomas is still a priority in bone tumors. Statins are lipid-lowering agents, with pleiotropic effects on the mevalonate pathway, the products of which are vital for a variety of key cellular functions. These drugs have been tested as chemotherapeutic agents in some types of tumors, however there is no evidence of its use in chondrosarcomas. The aim of this work was to evaluate the usefulness of statins treatment *in vitro* in chondrosarcoma cell lines.
Rat chondrosarcoma cell lines (LTC and 422) were grown under conventional conditions. The following parameters were studied after administration of simvastatin at different doses (vehicle, 0.1, 0.3, 1.0, 3.0 and 10 μM) and during different times (24, 48 and 72 hours): cell growth rate by cell counting; cell viability by Trypan blue cell exclusion assay and by MTT; morphologic changes; apoptotic response studied by DAPI, flow cytometry with PI/annexine V and by DNA laddering; cell cycle alterations were analyzed by flow cytometry with PI; and cell motility was studied by cell wound assay. Data was presented as mean ± SEM of at least three different experiments. A rejection level of $p < 0.05$ will be considered significant. It was observed that statins induced: 1. cell rounding and decreased ability of cell adhesion to the substrate; 2. decreased cell viability, assessed by MTT assay, with IC_{50} between 1–2 μM; 3. induction of time- and dose-dependent cell apoptosis; 4. cell growth arrest in G1 and G2/M phases with decreased S phase; 5. alteration of cell motility.

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Dual tyrosine kinase inhibitor PKI 166 alters the contractile structure and function of rat cardiomyocytes

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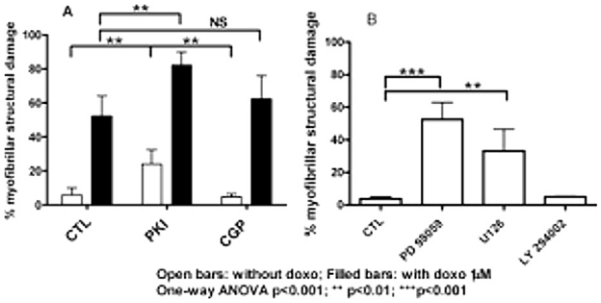
Background: Treatment of selected breast cancer patients with the anti-ErbB2-antibody Herceptin® improves survival but can cause significant cardiac dysfunction, particularly in combination with doxorubicin (doxo). Newer data suggest that combined blockage of ErbB1/EGF- and ErbB2-receptors with dual tyrosine kinase inhibitors (TKI) might further improve anti-cancer efficacy but cardiac safety is unknown. We therefore investigated the effect of two selective, reversible TKI on cardiomyocyte survival and contractile function.
Material and methods: We tested the effect of an ErbB1-(CGP059326-CGP;1 μM/48h) and a dual ErbB1/ErbB2-(PKI166-PKI;1 μM/48h) tyrosine kinase inhibitor in an in-vitro system of cultured (10 days) adult rat ventricular cardiomyocytes and assessed the effect on myocyte 1. LDH release and MTT assay, 2. TUNEL, 3. integrity of the contractile apparatus (immunofluorescence) 4. MAPK- and PKI3-signaling and 5. contractile function (video-edge detection; electrically paced at 2 Hz).



Results: *Myocyte survival*: Neither CGP nor PKI induced necrosis or apoptosis. In contrast, doxo led to a dose-dependent increase of both necrosis and apoptosis.
Myofibrillar Structural Damage (MSD): Since ErbB2-antibodies, especially in combination with anthracyclines, induce MSD to the cardiac contractile apparatus, we tested if CGP or PKI alone or in combination with doxo (1 μM) had a similar effect. PKI but not CGP alone caused MSD (figure A), which was significantly worsened by the addition of doxo (1 μM). Inhibition of MAPK/ERK1/2- (PD98059 50 uM and U126 5 μM), but not of PI3/AKT-signaling (LY294002 10 μM), led to a similar MSD (Figure B).
Signaling: CGP and PKI reduced phosphorylation of AKT (CGP by 40% (NS) and PKI by 30%, $p < 0.05$ vs. CTL, $N = 3$), whereas only PKI reduced ERK 1/2 phosphorylation (30% vs. CTL, $p < 0.05$, $N = 3$).
Contractility: PKI but not CGP attenuated fractional shortening (FS) by 25% (NS). PKI and doxo combined further reduced FS by 70% ($p < 0.0001$; $n = 400$ cells)
CONCLUSIONS: The dual ErbB1/ErbB2-TKI PKI166 can cause MAPK-dependent structural damage to the myocyte contractile apparatus which leads to contractile dysfunction. However, it does not induce myocyte death, suggesting a different pathophysiological mechanism than doxorubicin-induced cardiotoxicity. Only combination of PKI 166 with doxorubicin leads to an additive cardiotoxic effect. Clinical studies with ErbB2-blocking TKI should carefully monitor for cardiac dysfunction.

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Nutritional calcium regulates synthesis of the tumor-preventing steroid hormone 1, 25-dihydroxyvitamin D3 in the mouse colon

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Epidemiological data suggest a protective role of calcium and vitamin D against colorectal tumor pathogenesis. It has been demonstrated previously that colonic epithelial cell hyperproliferation and hyperplasia induced in mice fed a Western-style diet could be reversed by the addition of calcium to the diet. We propose that nutritional calcium in the intestinal lumen can act as a growth-regulator and may prevent cancer by direct reduction of colonocyte proliferation. This could occur by modulating colonic vitamin D synthesis. We investigated a possible interaction between dietary calcium and the colonic 1, 25-D_3-synthesizing machinery. Synthesis of 1.25-D_3 from its precursor 25-hydroxyvitamin D_3 is catalyzed by the mitochondrial cytochrome P450 enzyme, 25-hydroxyvitamin D_3-1alpha-hydroxylase (CYP27B1), which is present also in human colon carcinoma cells. 25-hydroxyvitamin D_3-24-hydroxylase (CYP24) is the enzyme responsible for the first step in 1.25-D_3 catabolism.
We quantified mRNA levels by real time RT-PCR using the comparative $\Delta\Delta C_T$ method. CYP24, p21, and the proliferating cell nuclear antigen (PCNA) protein levels were measured by revealed by immunohisto-chemistry.
We investigated the concentration-dependent action of dietary calcium on colonic expression of vitamin D receptor (VDR), CYP27B1, CYP24, and on proliferation as shown by PCNA and p21 expression in a mouse model. Mice were fed a modified AIN-76 diet containing 20% lactose and 0.9%, 0.1% or 0.04% calcium. We measured calcium content of the feces and found a significant reduction of fecal calcium content in animals fed low calcium diet. Low fecal calcium concentration had a promitotic effect on crypt cells, increasing PCNA protein expression. Quantitative evaluation of the cyclin dependent kinase inhibitor p21 showed less sensitivity to lowered fecal calcium: only the diet containing 0.04% calcium was able to reduce p21 expression. While low dietary calcium did not affect expression of VDR, we observed a moderate but insignificant increase in CYP27B1 mRNA expression. Low calcium diets significantly augmented CYP24 mRNA expression in the ascending colon, without affecting it at all in the descending colon. We confirmed our claim that low fecal calcium content is the regulator of colonic CYP24 expression by showing *in vitro*, in the Caco-2 colon cancer cell line, that 0.0 mM calcium in a medium without any 1, 25-D_3 indeed increased CYP24 mRNA levels.
We conclude that low dietary and thus luminal calcium concentrations increase CYP24 levels, which may result in reduced colonic accumulation of 1, 25-D_3, decreased p21 expression leading to hyperproliferation and unhindered tumor progression.
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Publication
Basic science

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Nitric oxide reverse arginine deiminase (ADI) induced anti-proliferative activity through NFKB and BCL-XL

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Arginine deiminase (ADI) is an inducer of apoptosis *in vitro* and has an anti-tumor effect *in vivo*. And, nitric oxide (NO) has been reported to be an