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Inhibitors of the Ubiquitin-Proteasome System as
Potential Therapeutic Agents for Cardiovascular Diseases

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1. Introduction: The Ubiquitin-Proteasome System

The ubiquitin-proteasome system is the major proteolytic system for non-lysosomal degradation of cellular proteins. In 2004, Aaron Ciechanover, Avram Hershko, and Irvine Rose were awarded the Nobel Prize in Chemistry for their original description of ubiquitin-mediated degradation of proteins. This recognition emphasizes the exceptional biological significance of ubiquitin-mediated degradation of proteins in numerous cellular processes such as turnover and quality control of proteins, cell cycle and apoptosis, transcription and cell signaling, immune response and antigen presentation, and inflammation and development.

There are two major steps in the ubiquitin-proteasome degradation pathway: proteins are first covalently tagged with poly-ubiquitin chains and are then degraded by the 26S proteasome. The initial step of the degradation pathway, ubiquitination of proteins, involves covalent binding of the ubiquitin molecule to a lysine residue (Lys) of the substrate. Ubiquitination proceeds along a cascade of enzymatic reactions, in which ubiquitin is first activated by the ubiquitin-activating enzyme E1. With the aid of an E2 ubiquitin-conjugating enzyme, ubiquitin is then covalently linked to the substrate by a specific ubiquitin ligase, E3. There is only one E1 enzyme known, several E2s, and multiple classes of E3s.

For polyubiquitination, activated ubiquitin moieties are processively transferred to the Lys 48 residue of the previously conjugated ubiquitin molecule. This process may be facilitated by a polyubiquitination factor, E4.

The second step in the degradation pathway involves proteolysis of ubiquitinated proteins by the 26 proteasome, a multicatalytic protease that consists of a 20S catalytic core and two 19S regulatory complexes. The 19S complex is composed of at least 19 different subunits that form a lid- and base-like structure. The lid component provides binding sites for polyubiquitinated substrates, and also contains a deubiquitinating activity, which allows recycling of ubiquitin moieties upon substrate degradation. The base component consists of six ATPases that form a ring-like structure and interact with the 20S proteolytic core. These ATPases have chaperone function and are required for the ATP-dependent unfolding of substrates and the opening of the narrow entry pore of the 20S proteasome. The unfolded polypeptide chain is then inserted into the catalytic chamber of the 20S core complex, where it is degraded into peptides of 3-25 amino-acids length. Additionally, several components of the ubiquitin system such as polyubiquitin-binding proteins - which presumably serve as substrate shuttles, several deubiquitinating enzymes - which are required
for the removal and recycling of ubiquitin moieties, and also several E3 ligases are associated
with the 26S proteasome \(^{18}\), which suggests that the two steps, ubiquitination and degradation,
are closely coupled and controlled within the cell.

**Figure 1:** Overview on the ubiquitin proteasome system

### 1.1. Proteolysis by the 20S Proteasome

The 20S proteasome is a barrel-like structure with two-fold symmetry that is composed of
four stacked rings of seven subunits each \(^{19}\). Seven different but related \(\alpha\) subunits (\(\alpha_1-\alpha_7\))
form the two outer rings, whereas the two inner rings contain seven different \(\beta\) subunits (\(\beta_1-\beta_7\)) enclosing a central chamber that contains the proteolytic sites \(^{19}\). A small gated pore in the
centre of the \(\alpha\) rings provides access to the catalytic chambers and enables substrate entry and
product release. This gate is usually obstructed by the N-termini of the \(\alpha\) subunits\(^{19}\), and is
opened by a conformational shift in the N-termini of the \(\alpha\) subunits upon docking of an
activator complex: such as the 19S and 11S regulator complexes \(^{20,21}\). The proteolytically
active sites reside in three of the seven \(\beta\) subunits: \(\beta_1\), \(\beta_2\), and \(\beta_5\). The catalytically active \(\beta\)
subunits contain an N-terminal threonine (Thr) as active site nucleophile to efficiently
hydrolyze peptide bonds. The three proteolytic sites differ in their specificity (Figure 1): the active site of the β1 subunit cleaves peptide bonds preferentially C-terminal of acidic residues and has been termed “caspase-like.” The site located on the β2 subunits is trypsin-like in cleaving mainly after basic residues. The chymotrypsin-like activity is located on the β5 subunits. However, the specificities of the active sites are broader than is implicated by their names. Structural and mutational analysis has revealed that the substrate binding pocket is formed by residues of the catalytic subunit and by neighboring β-subunits. Substrates of one proteolytic site may also regulate the activity of another active site, presumably via binding to non-catalytic modifier sites. The 20S proteasome is therefore not a complex of individual peptidases, but a multicatalytic enzyme whose proteolytic activity is an integrated function of multiple active sites.

1.2. Inhibitors of the 20S Proteasome

One mode of proteasome inhibition is provided by molecules that interact with the α subunits of the proteasome such as PR-39, PI31, and some viral proteins, thereby interfering with the opening of the gated pore. Most proteasome inhibitors, however, act as active-site inhibitors that bind to the substrate pocket of the active sites and directly modify the catalytic N-terminal Thr residues of the β subunits. Specificity and reversibility of this reaction strongly depend on the pharmacophore group of the inhibitor: aldehydes such as MG132 are generally very reactive and form an unstable hemiacetal bond with the hydroxyl group of the Thr. Peptide boronates such as bortezomib and MG262 are more potent and selective inhibitors than are peptide aldehydes. Binding is virtually irreversible due to a very slow off rate. Vinyl sulfones (e.g. NLVS), lactacystin, MLN519 (a modified β-lactone derivative), salinosporamide A (NPI-0052) and B, as well as epoxyketones (e.g., epoxomycin) form irreversible bonds with the active site Thr. Less commonly used inhibitors are the TMC-95s from Apiospora montagnei. These inhibitors bind to the active sites non-covalently and are stabilized by forming a tight network of hydrogen bonds with several residues of the substrate pocket. Structural data indicate that most of the inhibitors are able to block all three active sites at high inhibitor concentrations. However, differential inhibition of the active sites is observed both in vitro and in vivo, due to more or less favorable fitting of the inhibitor into the specific substrate pockets of the active sites.
1.3. Proteasome Inhibitors as Poisons and Remedies

Due to the essential role of ubiquitin-mediated degradation of proteins for a plethora of cellular processes such as cell cycle control, transcriptional regulation, and cell signaling, proteasome inhibition has been established as a powerful and promising strategy for therapeutic intervention in various human diseases. Proteasome inhibitors have been applied to numerous different cell types, in various animal models, and in clinical therapy of malignant diseases.

The proteasome inhibitor bortezomib has been approved as a cytostatic drug for the therapy of multiple myeloma in 2003 and is currently being tested in clinical trials for a variety of other malignancies. At the same time, a growing number of animal studies suggest that proteasome inhibitors may also prove to be valuable remedies for the treatment of non-tumorous diseases. The diverse beneficial effects range from inhibition of inflammation and acute transplant rejection, pharmacological pre- or postconditioning in ischemia-reperfusion injuries to enhanced bone formation, suppression of cardiac fibrosis, and reduced cachectic protein turnover. These diverse applications of proteasome inhibitors in clinical research can be grouped according to the cellular effects as poisons, that induce cell death, or as remedies, which modulate cellular function.

2. Results: Proteasome Inhibitors as Poisons and Remedies in Cardiovascular Disease

In full accordance with the concept of proteasome inhibitors acting either as poisons or as remedies, we observed differential cellular effects of proteasome inhibition when we evaluated proteasome inhibitors as potential therapeutic agents for various cardiovascular diseases. The effects of proteasome inhibition ranged from induction of apoptosis of smooth muscle and endothelial cells to reduced matrix remodeling of cardiac fibroblasts, suppressed hypertrophic growth of cardiomyocytes, and protection of endothelial cells from cell death.

2.1. Inhibition of Restenosis

We have exploited the pro-apoptotic features of proteasome inhibitors for treatment of restenosis, a hyperproliferative wound healing response of balloon-dilated vessels. In a rat model of restenosis, proliferation of vascular smooth muscle cells (VSMCs) is induced by local damage of carotid arteries after balloon dilation, which in turn leads to obstruction of these vessels within 14 days (cooperation with Peter Muschick, Schering AG). Local administration of high doses of the proteasome inhibitor MG132 for 5 minutes immediately after vessel damage markedly reduced development of restenosis in the rat carotid arteries. Reduced neointima formation correlated with diminished immunohistochemical staining for the proliferation markers Ki67 and PCNA indicating reduced proliferation of VSMCs within the lesion. We also observed prolonged apoptosis in balloon-dilated vessels upon treatment with proteasome inhibitor: TUNEL positive staining of rat carotid arteries was still evident 6 hours after tissue damage in inhibitor-treated animals but absent in control vessels. Staining with the macrophage-specific marker ED-1 revealed reduced infiltration of macrophages in proteasome inhibitor-treated animals. Our in vivo results were further corroborated by in vitro studies with primary VSMCs. Short-term treatment of VSMCs with MG132 for only 5 minutes showed dose dependent inhibition of proliferation and induction of apoptosis with 1 mM MG132 after 24 hours. Activation of the pro-inflammatory transcription factor nuclear factor κ B (NFκB) was blocked by proteasome inhibition. These data demonstrate that inhibition of the ubiquitin-proteasome system effectively reduces neointima formation in vivo, which corresponds to strong anti-proliferative, anti-inflammatory, and pro-apoptotic effects in vitro and in vivo. Our data suggest the ubiquitin-proteasome system as a new target in the prevention of vascular restenosis.


While we were working with primary VSMCs, we observed induction of several proteasomal genes in response to proteasome inhibition. Concerted upregulation of all mammalian 26S proteasome subunit mRNAs was confirmed by comprehensive real-time RT-PCR analysis. Induction is mediated by coordinated transcriptional activation of proteasomal genes as it could be blocked by inhibition of RNA-polymerase II activity. Transcriptional activation of
proteasomal genes was also observed for several immortal cell lines. In cooperation with Elke Krüger from the Institute of Biochemistry of the Charité, we were able to demonstrate that proteasome inhibitor-mediated transcriptional activation of proteasomal genes results in increased de novo protein synthesis of all subunits and in enhanced biogenesis of proteasomes. These experiments strongly indicate that the homeostasis of mammalian proteasomes is regulated by a positive autoregulatory feedback mechanism that allows compensation of severely reduced proteasomal activity. This newly expressed proteasome might not only compensate inhibited protein breakdown but might even transiently enhance proteasomal degradation beyond a compensatory response. Thus, these results point toward a more complex understanding of the cellular effects of proteasome inhibition.

2.2. Suppression of Cardiac Fibrosis


The essential role of the proteasome in transcriptional regulation prompted us to analyze the effects of proteasome inhibitors on gene expression of cardiac fibroblasts. Inhibition of the proteasome in rat cardiac fibroblasts suppressed expression of matrix metalloproteinases (MMP) 2 and 9 in a dose dependent manner as determined by in-gel zymography and real-time RT-PCR. Interestingly, expression of several collagen isoforms such as collagen Iα1, Iα2, and IIIα1 was also reduced. Proteasome inhibition significantly reduced activation of NFκB in cardiac fibroblasts. As these effects were observed with low and non-toxic doses of proteasome inhibitors, we tested the potential therapeutic use of proteasome inhibitors in an animal model of myocardial remodeling. Systemic treatment of spontaneously hypertensive rats (SHR) over 12 weeks with the proteasome inhibitor MG132 resulted in pronounced reduction of cardiac fibrosis in SHRs compared to control animals (as assessed by quantitative morphometry of sirius-red-stained sections of left ventricles in the lab of Bernd Hocher). These findings correlated well with significantly reduced RNA levels of collagen Iα2 and IIIα1 and reduced expression of MMP2 in the left ventricles. Importantly, systemic long-term treatment with MG132 was well tolerated, indicating that the observed reduction in cardiac
fibrosis is not due to cytotoxic side effects. We conclude that inhibition of the ubiquitin-proteasome system may provide a new and attractive tool to interfere with collagen and matrix metalloproteinase expression, and might therefore represent a promising remedy for the therapy of myocardial remodeling (see also our patent application PCT/EP 03/08205).

2.3. Suppression of Cardiomyocyte Hypertrophy


Inhibitors of the proteasome interfere with transcriptional regulation of growth signaling pathways and block cell cycle progression of mitotic cells. As growth signaling pathways are highly conserved between mitotic and post-mitotic cells, we hypothesized that proteasome inhibition might also be a valuable approach to interfere with hypertrophic growth of post-mitotic cardiomyocytes. To test this hypothesis, we analyzed the effects of proteasome inhibition on hypertrophic growth of neonatal rat cardiomyocytes. Partial inhibition of the proteasome effectively suppressed cardiomyocyte hypertrophy as determined by reduced cell size, inhibition of hypertrophy-mediated induction of RNA- and protein synthesis, reduced expression of several hypertrophic marker genes, and diminished activation of brain natriuretic peptide-mediated luciferase activity. Importantly, suppression of hypertrophic growth was independent of the hypertrophic agonist used. Expressional profiling, subsequent western blot analysis, and luciferase assays revealed that proteasome inhibition induced a cellular stress response with differential regulation of transcriptional activators such as NFκB and AP-1, reduced expression of conserved growth signal mediators, upregulation of heat shock proteins, and impaired G1/S phase transition of cardiomyocytes.

We propose that this stress response makes the cardiomyocytes “numb” for incoming growth signals thereby preventing hypertrophic cell growth. Importantly, this “numbness” does not affect cell viability but is reversible and can be overcome by strong growth stimulatory signals. These data provide important mechanistic insight into the recently observed suppressive effects of proteasome inhibitors in left ventricular hypertrophy and establish partial proteasome inhibition as a new and broad-spectrum approach to interfere with the hypertrophic growth of cardiomyocytes (see also our patent application DE 100 407 42.0-41).
2.4. Improvement of Endothelial Function


Endothelial nitric oxide synthase (eNOS) is the key enzyme of vascular homeostasis since the product of eNOS, nitric oxide (NO), mediates endothelial-dependent vasodilation and has been shown to exert protective effects in atherosclerosis. The aim of our initial study was to investigate whether eNOS expression and activity are regulated by the proteasome. When we treated bovine pulmonary artery endothelial cells with increasing doses of proteasome inhibitors, we observed a biphasic response on RNA and protein expression of eNOS: while low and non-toxic dose induced upregulation of eNOS with increased eNOS activity, higher doses downregulated eNOS on the RNA and protein level. Inhibition of protein synthesis by cycloheximide cotreatment prevented upregulation of eNOS protein, indicating that the increase in eNOS protein is due to enhanced protein synthesis and not due to post-translational stabilization of eNOS upon proteasome inhibition. When we analyzed the functional consequence of proteasome inhibitor-mediated upregulation of eNOS in rat aortic rings, we observed improved endothelial function as evidenced by enhanced endothelial-dependent vasorelaxation upon treatment with proteasome inhibitors. Improved endothelial function was also observed in an aortic ring model of TNFα-induced endothelial dysfunction (Lorenz et al., in revision). Proteasome inhibition has also been shown to downregulate endothelial expression of the vasoconstricting peptide endothelin-1 (ET-1). These data fit into the concept that proteasome inhibitor-mediated improved endothelial function is due to a shift in the balance of vasoconstricting and vasodilating factors in favor of vasodilation. Accordingly, reduced aortic ET-1 expression was associated with diminished blood pressure in DOCA salt sensitive rats, and in hypertensive Dahl salt-sensitive rats (Meiners et al., unpublished observation).

When we performed comprehensive Affymetrix microarray analysis of proteasome inhibitor treated human umbilical cord vein cells (HUVEC), we observed transcriptional regulation of
several other genes involved in endothelial function: whereas pro-atherogenic markers, such as ET-1, HMG-CoA reductase, and monocyte chemoattractant protein-1 were predominantly downregulated, anti-atherogenic markers (e.g. eNOS, plasminogen activator, thrombomodulin), and several genes of the anti-oxidant defense system (see below) were upregulated. This adaptive gene pattern adds another favorable aspect to the beneficial effects of low dose proteasome inhibition on endothelial dysfunction.

2.5. Protection from Stress


In an early study, we analyzed the protective effects of proteasome inhibition in cardiomyocytes with particular regard to the proteasome inhibitor-mediated induction of heat shock proteins (hsp) 60. Short-term incubation of neonatal rat cardiomyocytes with the proteasome inhibitor MG132 resulted in pronounced induction of hsp70 on the RNA and protein level as determined by real-time PCR and Western blotting. Hsp70 induction correlated with enhanced survival of neonatal cardiomyocytes after sublethal heat stress in XTT assays. In a functional assay, pretreatment with proteasome inhibitors was associated with enhanced recovery of the contractile parameters of papillary muscles after 40 minutes of hypoxia. These data suggest that proteasome inhibition mediates cardioprotection by induction of an endogenous cellular stress response as exemplified by upregulation of hsp70.


In endothelial cells, proteasome inhibition induces an anti-oxidative stress response. In our Affymetrix gene chip analysis, we observed differential regulation of a number of enzymes of the endothelial anti-oxidant defence system. In particular, glutathione peroxidase-3 (GPx-3) was markedly upregulated by proteasome inhibition. Expression of several glutathione-S-
transferases was induced moderately. Superoxide dismutase-1 (SOD-1) and heme oxygenase-1 (HO-1) were both upregulated as confirmed by real-time RT-PCR and Powerblot analysis. In contrast, RNA expression of the NADPH oxidase-4 - whose activity is the major source of reactive oxygen species in endothelial cells - was downregulated by proteasome inhibition. Reporter gene assays using the human GPx-3 or SOD-1 promoter coupled to a luciferase reporter gene demonstrated that upregulation of anti-oxidative enzymes is due to transcriptional activation upon proteasome inhibition. We are currently investigating the proteasome inhibitor-mediated induction of GPx-3 in further detail. Preliminary results indicate that the more than 50 fold upregulation of GPx-3 in HUVECs involves additional post-transcriptional mechanisms beside transcriptional activation (Westphal et al., unpublished results). Importantly, the observed transcriptional response of endothelial cells to non-toxic proteasome inhibition is translated into protection against oxidative stress: Pretreatment of HUVECs with proteasome inhibitors for 24 hours markedly inhibited the hydrogen peroxide-induced increase in intracellular reactive oxygen species (ROS), as evidenced by reduced generation of fluorescent dichlorofluorescein.


In a recent study, we have extended our analysis of the protective effects of proteasome inhibition in cardiomyocytes and addressed the question whether the induction of anti-oxidative enzymes contributes to cardioprotection by proteasome inhibition (Dreger et al.). Long-term treatment (48 hours) with low doses of proteasome inhibitors proved to be nontoxic and protected cardiac myocytes from subsequent hydrogen peroxide-induced oxidative stress. This correlated with suppressed formation of intracellular ROS. Immunoblots showed a significant increase of protein levels of SOD-1, GPx-3, HO-1, and catalase. Transient transfection with luciferase reporters driven by the promoters of SOD-1 or GPx-3 revealed proteasome inhibitor-mediated induction of luciferase activity. Comprehensive deletion and mutation analyses identified putative antioxidant response elements (ARE) in both, the GPx-3 and SOD-1, promoters to be not only essential but also sufficient for transcriptional upregulation by proteasome inhibition. Our data suggest that non-toxic proteasome inhibition upregulates anti-oxidative enzymes via an Nrf2-dependent
transcriptional activation of ARE sites. Transcriptional activation of anti-oxidative enzymes might thus contribute to proteasome inhibitor-mediated cardioprotection.

3. Discussion: Right Dose and Cell Type Differentiate a Poison and a Remedy

The above data confirm the concept that proteasome inhibitors are able to act as poisons which induce cell death, but also as remedies which modulate cellular function and protect from cell death. How can we resolve this apparent paradox? Extending the old observation of Paracelsus that “the right dose differentiates a poison and a remedy”, we postulate that for proteasome inhibitors the right dose and cell type differentiate a poison and a remedy: While substantial and prolonged proteasome inhibition results in cell death of tumor and highly proliferative cells, partial and short term impairment of proteasomal function induces a protective stress response in normal and quiescent cells.

3.1. Differential Cellular Responsiveness to Proteasome Inhibition

It has early been noted that one identical dose of proteasome inhibitor has divergent effects on different cell types. Whereas proteasome inhibition induces apoptosis in rapidly proliferating tumor cells, the opposite response – protection from apoptosis – has been observed in differentiated and quiescent cells \(^6^1\). Different tumor cell lines also vary markedly in their sensitivity to proteasome inhibition, although proteasome inhibitors equally effective block the chymotrypsin-like activity of the 20S proteasome \(^6^2\). Even within the same cell type, it is possible to observe differential responsiveness to proteasome inhibition. Systematic analysis by Drexler revealed that a defined dose of proteasome inhibitors is capable of inducing apoptosis in proliferating but not in quiescent human leukemic HL60 cells \(^6^3\). Similar differential responsiveness to induction of apoptosis was observed in rat-1 fibro-blasts \(^6^4\), primary endothelial cells \(^6^5\), and in tumor-derived lymphocytes and hepatocytes (in contrast to normal lymphocytes and hepatocytes) \(^6^6;^6^7\).

3.2. Dose-dependency of Proteasome Inhibition

Another aspect that accounts for the divergent effects of proteasome inhibitors is the dose-dependency of proteasome inhibition. Meriin et al. were the first to analyze the effects of different inhibitor doses within the same cell type \(^6^8\). Whereas high inhibitor doses induced apoptosis of human lymphoid tumor cells, low doses of proteasome inhibitors protected these
cells from apoptosis. Apoptosis was associated with activation of c-jun N-terminal kinase (JNK), whereas protection was attributed to proteasome-inhibitor-mediated upregulation of hsp72 and suppressed JNK activation. Similarly, Lin et al. observed suppression of Sindbis virus-induced apoptosis in a prostate carcinoma cell line by low inhibitor doses, whereas high doses induced apoptosis. A biphasic dose effect of proteasome inhibitors on LPS-stimulated NO production was observed in primary astrocytes: NO production was enhanced by low doses but inhibited by higher doses. Low-dose proteasome inhibition induced basal NO production and expression of eNOS in primary endothelial cells. In contrast, high doses diminished expression of eNOS and concomitant NO production.

In a systematic experiment, we investigated the dose-dependent response of two different proteasome inhibitors – the peptide aldehyde MG132 and the boronate inhibitor MG262 – in HUVEC after 24 hours of treatment. Initially, we defined equipotent doses of these two inhibitors that affected cell viability and apoptosis to a similar extent. High-dose proteasome inhibition clearly committed endothelial cells to apoptosis, as evidenced by 40% reduced cell viability and by pronounced activation of caspases 2, 3, 8, and 9. Low doses of MG132 and MG262, however, did not affect cell viability. Nevertheless, transcriptional profiling revealed pronounced alterations of the gene expression pattern, with upregulation of several enzymes of the endothelial antioxidant defense system and proteins involved in stress response – including, for example, hsp family members hsp70 and hsp22. Factors related to cell cycle progression, at the same time, were primarily downregulated. Notably, induction of this subset of protective genes by nontoxic doses protected endothelial cells from oxidative stress induced by hydrogen peroxide (see above).

When we increased the dose of proteasome inhibitors, we observed a dose-response profile with a clear threshold separating the non-apoptotic (i.e., protective) from the apoptotic state (Figure 2). Remarkably, the differential cellular effects of proteasome inhibition by protective versus apoptotic doses were reflected by a distinct pattern of inhibition of the three proteolytic sites (Figure 2): apoptotic doses induced sustained inhibition of chymotrypsin-like activity by more than 90% and of caspase-like activity by more than 50%. In contrast, protective doses only partially inhibited the proteasome, with 80% reduced chymotrypsin-like activity, but with little effect on the two remaining activities. This observation concurs with a recent study by Kisselev et al., who observed that efficient inhibition of protein breakdown can be achieved only by sustained and simultaneous inhibition of the chymotrypsin-like activity and of a second active site. Taken together, these data indicate that sustained inhibition of
multiple sites is cytotoxic, whereas partial inhibition of only the chymotrypsin-like site is not only well tolerated but results in a protective response of untransformed cells (Figure 2).

At this point the question arises of how the different active sites contribute to substrate breakdown. Site-specific inhibition of the three proteolytic activities in purified proteasomes revealed that the contribution of single active sites to total protein breakdown varies strongly with the amino acid composition of the protein substrate. For example, site-specific inhibition of the trypsin-like activity of purified 26S proteasomes by leupeptin predominantly reduced degradation of substrates that are rich in basic amino acids. These in vitro data indicate that site-specific inhibition of single proteolytic sites of the proteasome may result in preferential stabilization of specific proteins in cells. Unfortunately, there are no cell-permeable active site specific inhibitors available until now. Differential inhibition of the three catalytic sites of the proteasome within the cell can be achieved only by different doses of inhibitors. It is tempting to speculate that dose-dependent differential inhibition of the proteasome might affect specific sets of substrates. Thereby, a defined degree of proteasome inhibition can confer substrate specificity. This idea remains to be proven but the available data are in full agreement with this hypothesis.
Figure 2: The degree of proteasome inhibition determines the cellular effects. HUVECs were treated for 24 hours with different doses of the proteasome inhibitor MG262. Cell viability was assessed by XTT assays. Caspase activation was determined using the ApoAlert Caspase Profiling Plate (BD Transduction Laboratories). Proteasomal activities were assayed in cell extracts using fluorogenic substrates.
4. **Summary and Perspectives**

The therapeutic potential of proteasome inhibitors as cytostatic drugs was early realized. However, it is the cytotoxic potential of proteasome inhibitors that has long hampered their therapeutic application as nontoxic remedies. Here, we provide evidence that proteasome inhibitors are potential remedies for the therapy of various cardiovascular diseases. While local and pronounced inhibition of the proteasome with high doses of proteasome inhibitors have anti-proliferative, anti-inflammatory and pro-apoptotic effects on vascular smooth muscle cells and suppress development of restenosis in rat carotid arteries, nontoxic and partial inhibition of the proteasome with low inhibitor doses induces a variety of beneficial effects in several different cardiovascular cell types:

- reduced expression of collagen isoforms and matrixmetalloproteinases in cardiac fibroblasts, which is associated with suppression of cardiac fibrosis in spontaneous hypertensive rats;
- suppressed hypertrophic growth of cardiomyocytes;
- enhanced expression of eNOS versus downregulation of ET-1 in endothelial cells leading to improved endothelial function of rat aortic rings;
- induction of a conserved stress response with upregulation of hsp70 and several anti-oxidative enzymes in cardiomyocytes and endothelial cells which is associated with protection from thermal and oxidative stress.

Our data confirm the concept that proteasome inhibitors are able to act as poisons which induce cell death but also as remedies which modulate cellular function and protect from cell death. To reconcile these divergent effects of proteasome inhibitors we would like to extend the old observation of Paracelsus that “the right dose differentiates a poison and a remedy”, and postulate that for proteasome inhibitors the right dose and cell type differentiate a poison and a remedy: For a given cell type, the degree of proteasome inhibition determines whether the cell dies or is protected from death. While sustained inhibition of multiple sites strongly restricts overall protein turnover and ultimately results in cell death, partial inhibition of only the chymotrypsin-like site may affect degradation of specific sets of substrates and induces a protective stress response.

This hypothesis might provide a conceptual framework to pave the way for the clinical application of proteasome inhibitors in other diseases beyond cancer, and in cardiovascular diseases in particular. However, application of proteasome inhibitors is clearly limited for
diseases that require chronic treatment, since there is increasing evidence that long-term proteasome impairment may contribute to neurodegenerative disease, cardiac and aortic dysfunction, and aging. For this reason, short-term and/or local treatment with proteasome inhibitors may represent a more feasible approach. In particular, treatment of ischemia-reperfusion injuries and restenosis offer promising clinical applications, as proteasome inhibitors combine acute and local anti-proliferative and anti-inflammatory actions with beneficial systemic effects on endothelial function.

5. Zusammenfassung

Das therapeutische Potential von Proteasominhibitoren als Zytostatika wurde bereits früh erkannt, und verhinderte lange den Einsatz von Proteasominhibitoren als nicht-toxische „Heilmittel“. Die hier dargestellten Daten belegen durch eine Reihe von proof of concept Experimenten, dass Inhibitoren des Proteasoms neue und viel versprechende „Heilmittel“ für die Therapie unterschiedlicher kardiovaskulärer Erkrankungen darstellen. Während die ausgeprägte Hemmung des Proteasoms durch hoch-dosierte Proteasominhibitoren deutlich anti-proliferative, anti-inflammatorische und pro-apoptotische Effekte auf glatte Muskelzellen zeigt, die zu einer verminderten Restenose-Ausbildung in Rattenkarotiden beitragen, vermittelt die nicht-toxische und partielle Hemmung des Proteasoms durch niedrig-dosierte Proteasominhibitoren eine Reihe benefizierter Effekte in unterschiedlichen kardiovaskulären Zelltypen:

- Die reduzierte Expression mehrerer Kollagen Isoformen und Matrixmetalloproteinasen in kardialen Fibroblasten ist assoziiert mit einem deutlich verminderten Grad der kardialen Fibrose in spontan hypertensiven Ratten;
- das hypertrophe Wachstum von Kardiomyozyten wird supprimiert;
- eine erhöhte Expression von eNOS zusammen mit einer vermindertenExpression von ET-1 in Endothelzellen korreliert mit einer verbesserten Endothelfunktion in Rattenaortenringen;
- das Auslösen einer konservierten Stress-Antwort mit Hochregulation von hsp70 und der konzertierte Induktion mehrerer anti-oxidativer Enzyme schützt sowohl Endothelzellen wie auch Kardiomyozyten vor thermalen und oxidativem Stress.

Unsere Daten sind somit in vollem Einvernehmen mit dem Konzept, dass Proteasominhibitoren als „Gift“ – also als zytotoxische Substanzen - oder „Heilmittel“,

6. Danksagung


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Der Dank, den ich meiner Familie und insbesondere meinem Mann schulde, lässt sich nicht in Worte fassen.
7. **Eidesstattliche Erklärung**

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde bzw. welchen Ausgang ein durchgeführtes Habilitationsverfahren hatte;

- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen wurden, sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlerinnen oder Wissenschaftlern und technischen Hilfskräften und die Literatur vollständig angegeben sind,

- der Bewerberin oder dem Bewerber die geltende Habilitationsordnung bekannt ist.

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Datum                                    Unterschrift
8. **Literatur**


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9. Abkürzungsverzeichnis

lysine residue (Lys)
thaone (Thr)
vascular smooth muscle cells (VSMCs)
spontaneously hypertensive rats (SHR)
nuclear factor κ B (NFκB)
matrix metalloproteinase (MMP)
endothelial nitric oxide synthase (eNOS)
jun N-terminal kinase (JNK)
nitric oxide (NO)
derothelin-1 (ET-1)
human umbilical cord vein cells (HUVEC)
heat shock proteins (hsp)
glutathione peroxidase-3 (GPx-3)
superoxide dismutase-1 (SOD-1)
heme oxygenase-1 (HO-1)
reactive oxygen species (ROS)
antioxidant response elements (ARE)