Multiple cardiac proteasome subtypes differ in their susceptibility to proteasome inhibitors

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Aims The proteasome is the proteolytically active core of the ubiquitin–proteasome system, which regulates vital processes and which can cause various diseases when it malfunctions. Therefore, the proteasome has become an attractive target for pharmaceutical interventions. Inhibition of the cardiac proteasome by specific proteasome inhibitors has been shown to attenuate cardiac hypertrophy and ischaemia reperfusion injury of the heart. We have resolved the cardiac proteasome into its subtypes and have addressed the key question of how proteasome inhibitors affect single cardiac proteasomal subtypes.

Methods and results The 20S proteasome from rat heart was dissected into three different subpopulations (groups I–III), each comprising 4–7 different subtypes. The major group (group II) comprises standard proteasome subtypes; the two minor subpopulations (groups I and III) contain intermediate proteasome subtypes. All subtypes exhibit chymotrypsin-, trypsin-, and caspase-like activity but to different degrees. We have tested the effect of two common proteasome inhibitors on the chymotrypsin-like activity of all subtypes: 20–30 nmol/L MG132 caused 50% inhibition of all subtypes from groups I and II, whereas 100 nmol/L was necessary to affect group III subtypes to the same extent. However, another inhibitor, bortezomib (VELCADE™), already used clinically, inhibited 50% of the activity of group III proteasome subtypes even below 20 nmol/L, a concentration showing almost no effect on group I and II proteasome subtypes. The caspase-like activity of group II proteasome subtypes was not affected by MG132 and was inhibited by bortezomib only at concentrations above 100 nmol/L.

Conclusion These data show that different inhibitors have differential inhibitory effects on the various cardiac proteasome subtypes. Different cardiac subtypes are inhibited by the same dose of proteasome inhibitor to a different extent.

Keywords Proteasome • Subtype • Activity • Inhibition • Heart

1. Introduction

Beside elimination of proteins, proteolytic processing is an important post-translational mechanism for regulation of vital biological processes. The proteasome is the major pathway for intracellular protein degradation in eukaryotic cells. It is critically involved in cell proliferation by regulation of mitosis and apoptosis, in cell differentiation by regulation of transcriptional gene expression and translation, in cell signalling, metabolic control, immune response, and many other processes.¹ The essential role of the proteasome in these basic cellular functions makes it a key player in numerous pathogenic alterations and thus an attractive target for pharmaceutical interventions.²–⁴ In this regard, the heart is no exception and the proteasome as well as the protein-ubiquitinating machinery have been found to take part in physiological adaptation and pathophysiological alterations.⁵,⁶ The proteasome contributes to removal of oxidized proteins upon cardiac ischaemia and reperfusion injury.⁷ In a pig model of ischaemia/reperfusion damage, inhibition of the proteasome reduced oxidative damage of the heart.⁸ This protective effect involves prevention of the proteasomal inactivation of IkB, the inhibitor of the inflammatory transcription factor NFκB.⁹,¹⁰ Moreover, proteasome inhibition following ischaemia protected from loss of G protein-coupled receptor

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kinase-2 and thus prevented tachyarrhythmias and sudden cardiac death in dogs.11

The proteasome is also involved in the increased protein turnover during the hypertrophic growth response of the heart: induction of cardiac hypertrophy by enhanced work-load or growth factor stimulation was accompanied by increased expression and activity of the cardiac proteasome. Inhibition of the proteasome reversibly suppressed cardiomyocyte hypertrophy in vitro and in vivo.12–14 The underlying molecular mechanism involves reduced activation of NFκB,15 and downregulation of several growth signaling pathways.13 As the proteasome system depends on ATP with regard to its stability and function, reduced proteasomal activity was observed under conditions of decreased levels of ATP such as ischaemia16 or apoptosis of cardiomyocytes.17,18

The proteasome consists of a core complex, designated 20S proteasome, which is the proteolytically active part of an even larger complex, the 26S proteasome that degrades polyubiquitinated substrate proteins. The 20S proteasome has a cylindrical shape that is built-up by four stacked seven-membered rings with a subunit stoichiometry of α1–7, β1–7, β′1–7, α′1–7. Three of the subunits in each β-ring, β1, β2 and β5, contain proteolytically active sites with different specificities, namely caspase-like, trypsin-like, and chymotrypsin-like, respectively (for review see19). For a long time, it has been known that proteasomes are not a homogenous enzyme population but exist as two big subpopulations, designated standard- and immuno-proteasomes,20 the latter of which are characterized by containing immuno-β-subunits β1i, β2i, and β5i, instead of the standard subunits. Additionally, mixed forms of standard and immuno-proteasomes, designated intermediate-type proteasome, have been identified.21,22 Each of the three subpopulations is composed of several proteasome subtypes that are distributed over different cell compartments and show different proteolytic activities.21–23

Partial or differential inhibition of proteasomes can be achieved by the use of diverse inhibitors exhibiting distinct affinities to the three active sites24 or using different doses of the same inhibitor.25 We have recently proposed that the degree of proteasome inhibition determines the cellular effects of proteasome inhibitors as poisons or remedies (for review see26). Although sustained inhibition strongly restricts overall protein turnover and induces apoptotic cell death, partial inhibition of the proteasome mediates protective effects in various tissues including the heart. This differential inhibition of the proteasome may also involve differing susceptibilities of proteasomal subpopulations to proteasome inhibitors. To get further insight into the susceptibilities of cardiac proteasomes to proteasome inhibitors, we have resolved rat heart proteasomes into their spectrum of subtypes and tested the effect of two widely used proteasome inhibitors, MG132 and the clinically tested inhibitor bortezomib, on their specific proteolytic activities.

2. Methods

2.1 Animals

This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). It was also approved by the Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit, Berlin, Germany (T0303/06). Male 8- to 10-week-old Wistar rats were purchased from Harlan Winkelmann GmbH (Borchen, Germany) and sacrificed by carbon dioxide. Their hearts were excised, washed with phosphate-buffered saline, pH 7, for 5 min before being frozen at −20°C.

2.2 Purification of 20S proteasomes

Hearts from 90 rats were pooled and homogenized (1 min at 4°C) in a three-fold volume (v/w) of 20 mmol/L Tris/HCl, 1 mmol/L EDTA, 1 mol/L NaNO3, 1 mol/L DTT, pH 7 (TEAD buffer) by use of a Waring blender. The homogenate was centrifuged at 15000 g for 20 min and the supernatant used for purification of 20S proteasomes as described elsewhere.21,27

2.3 Separation and purification of proteasome subtypes

Purified proteasomes were separated into their subtypes by chromatography on Mini Q (PC 3.2/3) in conjunction with a SMART system (GE Healthcare, Freiburg, Germany) as described previously.21,27 Purified proteasome subtypes were then dialysed against TEAD buffer.

2.4 Determination of proteasome activity

Measurement of proteasome activity was performed using fluorogenic peptide substrates as described elsewhere.21 Chymotrypsin-like activity was measured with Suc-Leu-Leu-Val-Tyr-MCA (200 μmol/L), trypsin-like activity with Bz-Val-Gly-Arg-MCA (400 μmol/L), and the caspase like activity with Z-Leu-Leu-Glu-MCA (400 μmol/L) as substrates. For all assays, 24 ng purified proteasome dissolved in 20 μl TEAD buffer was mixed with 20 μl peptide substrate and incubated at 37°C. After 30–90 min depending on substrate and proteasome subtype the fluorescence of MCA released was measured.21

2.5 Measurement of proteasome inhibition

To measure proteasome inhibition in the presence of proteasome inhibitors, stock solutions of MG132 (10 mmol/L DMSO, Biomol International, Exeter, UK) and Bortezomib (2.6 mmol/L H2O, kindly provided by Millenium Pharmaceuticals, Inc.) were prepared and diluted with TEAD buffer for use. Inhibition tests contained 10 μl purified proteasome (24 ng, corresponding to 3.4 × 10–14 mol) and 10 μl inhibitor solution (final concentrations are given in Results section). The mixture was preincubated for 10 min at 37°C before proteasome activity was measured as described above.

2.6 Electrophoresis

SDS–polyacrylamide gels of 12.5% (w/v) polyacrylamide concentration were run and stained with Coomassie brilliant blue. The first dimension of the two-dimensional gel electrophoresis (2D-PAGE) was performed under non-equilibrium pH-gradient electrophoresis conditions using pH-gradients between 3 and 10.28

3. Results

3.1 Purification of proteasomes

20S proteasomes were purified from pooled rat hearts using a combination of several chromatographic procedures as described elsewhere.27 For further purification of cardiac proteasome subtypes, purified 20S proteasomes were separated by hydrophobic interaction chromatography on a Resource-Phe column into...
three proteasome containing peaks I, II, and III (Figure 1). Analysis of the three proteasome peaks by conventional SDS–PAGE did not reveal any gross difference in the subunit composition that could explain their distinctive surface hydrophobicity (Figure 1). However, detailed analysis by 2D-PAGE revealed clear differences with regard to the subunit composition of the proteasome peaks. As shown in Figure 2, the major proteasome peak II consists exclusively of standard proteasomes, because it contained subunits b1, b2, and b5 but no immuno-subunits. In contrast, proteasomes in peak I contained rather high amounts of immuno-proteasome subunits b1i, b2i, and b5i, and only small amounts of standard-proteasome b-subunits. Proteasomes of peak III contained standard-proteasome b-subunits and only small amounts of immuno b-subunits. Besides the different amounts of immuno-subunits in peak I and III, a splitting up of subunit b1i and a5 into several protein spots can be observed (Figure 2). These differences in the amount and sub-separation of proteasome-subunits may account for the different surface hydrophobicities of the three 20S proteasome peaks.

3.2 Separation of proteasome subtypes

Material from the three 20S proteasome peaks (I–III) was used to resolve the complete subtype spectrum by means of high-resolution chromatography on MiniQ in conjunction with a SMART system. This approach yielded a total of 16 different proteasomal subtypes. The proteasomes of each of the three peaks could be separated into at least three major subtypes, but their overall pattern was clearly different from each other (Figure 3). Whereas the standard proteasome of peak II contains three major (II-2, II-3, and II-4) and one minor (II-1) subtype, the intermediate-type proteasomes of peaks I and III each comprise 5 and 6 subtypes, respectively. In order to eliminate contaminating material from neighbouring subtypes, all 15 different proteasome subtypes isolated from the rat heart were re-chromatographed on MiniQ. We then further analysed the subunit composition of the most abundant intermediate subtypes I-3 and I-4 as well as the standard subtypes II-2, II-3, and II-4 by means of 2D-PAGE. No obvious differences with regard to subunit composition...
could be detected between the subtypes within each group (data not shown).

3.3 Proteolytic activities of proteasome subtypes

Standard- and immuno-proteasomes have been shown to differ significantly in their activities.23,29,30 The caspase-like activity was found to be reduced when subunit β1 was replaced by β1i, sometimes in favour of enhanced chymotrypsin-like and trypsin-like activities. The specific activities of the three standard-proteasome subtypes II-2, II-3, and II-4 from rat heart exhibit high chymotrypsin-like activity (Figure 4). The small amount of subtype II-1 did not allow further characterization of this proteasome subtype. Notably, whereas the trypsin-like activity of these three subtypes accounted for only 1–2% compared with the chymotrypsin-like activity, their caspase-like activities overexceeded the latter by 35–50%. The specific chymotrypsin-like activity of the intermediate-proteasome subtypes I-1 and I-5 ranged in the same order of magnitude compared with the standard-proteasome subtypes. In the intermediate-proteasome subtypes, the caspase-like activity was clearly lower than their chymotrypsin-like activity. When the latter was set as 100%, one can calculate a progressive decrease from 82 to 12% in subtypes I-1 to I-5, respectively. Their specific trypsin-like activity ranged between 1 and 4% when compared with their chymotrypsin-like activity (Figure 4). In contrast to the low trypsin-like activity of the intermediate- and standard proteasomes of peak I and II, proteasome subtypes III-1 to III-6 exhibited a higher trypsin-like activity in absolute as well as in relative terms that accounted for 30 and 50% when normalized to their corresponding chymotrypsin-like activity. Subtypes of peak III also showed strikingly low chymotrypsin-like and caspase-like activities comparably to that of peak I subtypes (Figure 4).

These data show that the well-known difference of activities between standard- and immuno-subunit containing proteasomes is also found in proteasome subtypes from rat heart. This indicates that heart tissue contains a large spectrum of proteasome subtypes, each of which has a distinctive pattern of the three proteolytic activities.

3.4 Inhibition of heart proteasome subtypes by MG132

The identification and characterization of a large set of cardiac proteasomal subtypes with differential proteolytic activities prompted us to investigate whether these subtypes also differ in their susceptibility to different doses of common proteasome inhibitors.

We first tested the widely used peptide aldehyde inhibitor MG132, Z-Leu-Leu-Leu-aldehyde, at concentrations from 3.1 to 100 nmol/L on the chymotrypsin-like activity of all proteasome subtypes (except II-1) purified from rat heart (Figure 5A–C). There was no significant difference in the susceptibility of the proteasome subtypes of peak I and II to MG132 (Figure 5A and B), and 50% inhibition of all subtypes from peak I and II was obtained with about 25 nmol/L MG132. Efficient inhibition by 90% was achieved.
Cardiac proteasome subtypes differ in inhibitor susceptibility

3.5 Inhibition of the heart proteasome subtypes by bortezomib

In a second approach, we tested the FDA-approved inhibitor bortezomib (VELCADE™), which is an N-terminally blocked Phe-Leu dipeptide inhibitor containing a C-terminal boronic acid. We tested the inhibitor at concentrations from 3.1 to 100 nmol/L and again found clear differences with respect to its effect on the chymotrypsin-like activity of the different cardiac 20S proteasome subtypes (Figure 5D–F). However, the inhibitory pattern of

Figure 4 Proteolytic activities of the proteasome subtypes from rat heart. Proteolytic activities of all proteasome subtypes (except II-1) isolated from rat heart tissue were determined by the use of the fluorogenic peptide substrates Z-Leu-Leu-Glu-MCA for caspase-like activity (A), Bz-Val-Gly-Arg-MCA for trypsin-like activity (B), and Suc-Leu-Leu-Val-Tyr-MCA for chymotrypsin-like activity (C). Data are mean ± SD of three separate measurements.
bortezomib was strikingly different from that observed for MG132; 50% inhibition of the chymotrypsin-like activity was obtained at bortezomib concentration of about 30 nmol/L with the intermediate- and standard-proteasomal subtypes of peak I and II. In contrast, much lower doses of bortezomib—only 10–20 nmol/L—were required to obtain 50% inhibition of the chymotrypsin-like activity of the intermediate-proteasomes of peak III with subtype III-6 being the most sensitive proteasome subtype. A concentration of 10 nmol/L bortezomib inhibited the intermediate-subtypes I-1 to I-6 by only 20–30%, whereas almost no inhibition was observed with standard-proteasome subtypes II-2, II-3, and II-4 (Figure 5D and E). The same dose effectively inhibited the chymotrypsin-like activity of subtypes III-1 to III-6 by 30–60% (Figure 5F). This is in clear contrast to the reduced susceptibility of these subtypes to MG132 (Figure 5C). Even at a final concentration of 100 nmol/L bortezomib, all proteasome subtypes were inhibited to a similar extent. In recent investigations

**Figure 5** Effect of proteasome inhibitors on the chymotrypsin-like activity of proteasome subtypes from rat heart. Different concentrations of proteasome inhibitor MG132 (A–C) as well as of bortezomib (D–F) were tested for their effects on the chymotrypsin-like activity of proteasome subtypes from peak I (A and D), peak II (B and E), and peak III (C and F). Subtypes of all three peaks are indicated as follows: subtype 1 (open circle), subtype 2 (filled circle), subtype 3 (triangle), subtype 4 (filled triangle), subtype 5 (square), subtype 6 (filled square). The range of inhibitor concentrations to obtain 50% inhibition (dotted line) of the various subtypes (except of III-2 and III-6 in C) is demarcated by vertical arrows. Data are mean ± SD of three independent measurements, except for subtype III-1.
using active-site directed probes and cell or tissue extracts, it was shown that bortezomib binds not only to subunit β5 that catalyses the chymotrypsin-like activity but also to subunit β1, which harbours the caspase-like activity, as well as to their immuno-counterparts β5i and β1i. The concentrations of bortezomib and MG132 used here had no significant effect on the caspase-like activity of the 20S proteasome subtypes from rat heart (data not shown). Since the caspase-like activity is the predominant activity of the major subpopulation of cardiac proteasomes, the standard proteasomes, we tested whether this activity can be inhibited by higher concentrations of the inhibitors. As shown in Figure 6, a concentration of 800 nmol/L MG132 (and even of 1.6 μmol/L; data not shown) did not affect the caspase-like activity of subtypes II-2, I-3, and I-4 at all. Bortezomib, however, inhibited this activity to 50% at a concentration of 200–350 nmol/L. Thus, when compared with the chymotrypsin-like activity, about 10-fold the amount of bortezomib is required to inhibit the caspase-like activity of these proteasome subtypes to the same extent, which is in line with earlier findings.

4. Discussion

In this study, we were able to resolve 15 different proteasome subtypes from cardiac tissue. This high resolution was achieved by separation of proteasome subpopulations due to their differences in surface hydrophobicity allowing an early separation of standard- and intermediate-type proteasomes. Our approach represents a clear improvement compared with earlier studies on proteasome subtypes from various human blood cells and from several rat tissues. Similar to the results obtained with rat skeletal muscle, standard proteasomes (peak II) constitute the majority of 20S proteasomes in rat heart muscle tissue, whereas the two other peaks (peak I and III) contain intermediate-type proteasomes and amount to about 10% of the total 20S proteasome population. Additional separation of the proteasomes due to their different surface charges allowed further resolution of each of the three peaks I, II, and III into 5, 4, and 6 subtypes, respectively. Recently, Drews et al. used the technique of free flow electrophoresis, the separation principle of which is also based on differences of surface charge. While Drews et al. were able to separate seven groups of proteasomes from murine heart, the effectiveness of our ‘two-dimensional’ (hydrophobicity and surface charge) separation procedure is higher. The subunit composition of these various subtypes differs predominantly with regard to their content of standard- and immuno-proteasome subunits. Resolution of single-proteasome populations by 2D-PAGE did not reveal obvious differences in the subunit composition of non-catalytic subunits except for the different sub-separation of the variants of subunit α5. Therefore, we cannot exclude that post-translational modifications such as phosphorylation of proteasome subunits may contribute to the differing biochemical characteristics of the various cardiac subtypes as suggested recently.

The importance of improved resolution of proteasome subtypes in cells and tissues is emphasized by the observation that each proteasome subtype from heart tissue exhibits a characteristic pattern of proteolytic activities. Standard proteasomes (II-1 to II-4) exhibit high caspase- and chymotrypsin-like activities. Partial replacement of standard- by immuno-β-subunits in intermediate-proteasome subtypes I-1 to I-5 significantly lowers the caspase-like activity. The trypsin-like activity is low in both standard- and intermediate-proteasomes of peak II and I, respectively. Notably, the ratio of all three activities is completely different in all subtypes. Subtypes of peak III display a significantly different pattern of proteolytic activities. Similar to the intermediate-type proteasomes of peak I, they contain also a mixture of standard- and immuno-β-subtypes according to 2D-PAGE analysis, but they exhibit the highest trypsin-like activity and at the same time the lowest chymotrypsin-like activity of all heart proteasome subtypes. As this pattern of activities of peak III subtypes is similar to the one we have observed with proteasome subtypes isolated from the microsomal fraction of HeLa cells, our data suggest that the different proteasome subtypes might differ in their subcellular localization. So far, we have not studied the intracellular distribution of the multiple proteasome subtypes in rat heart. Additionally, one has to consider that each cell type generates its own pattern of proteasome subtypes as we have already shown for different blood cells and HeLa cells. The heart tissue is composed of a variety of cell types, the main populations of which, myocytes, smooth muscle cells, and endothelial cells, are subdivided into many specialized

Figure 6 Effect of proteasome inhibitors on the caspase-like activity of peak II proteasome subtypes from rat heart. Different concentrations of proteasome inhibitor MG132 (A) as well as of bortezomib (B) were tested for their effects on the caspase-like activity of proteasome subtype II-2 (filled circle), II-3 (triangle), and II-4 (filled triangle). The range of bortezomib concentrations to obtain 50% inhibition (dotted line) of the various subtypes is demarcated by vertical arrows. Data are mean ± SD of three independent measurements.
forms. This all adds to an enormous complexity regarding the cellular and subcellular distribution of various proteasome subtypes.

The most important finding of our study is that the proteasome subtypes have different susceptibilities to common proteasome inhibitors. The chymotrypsin-like activity of all subtypes of the two major proteasome subpopulations from rat heart, namely I-1 to I-5 and II-2 to II-4, is inhibited to 90% by MG132 at a concentration of 100 nmol/L. The chymotrypsin-like activity of proteasome subtypes III-1 to III-6, however, is affected to only about 50% by 100 nmol MG132/L. This result could explain the partial inhibition (80%) of the chymotrypsin activity of proteasomes in primary cardiomyocytes after their treatment with 100 nmol MG132/L, which allows survival but prevents agonist-induced hypertrophy of these heart cells. The very same dose of MG132 also inhibited the proteasome by 82% in human endothelial cells and increased eNOS protein and activity thereby contributing to improved endothelial function.

Notably, the differing sensitivities of proteasome subtypes to the aldehyde inhibitor MG132 appear to be inhibitor-specific, as we observed an altered inhibitory pattern with the boronic acid inhibitor bortezomib. Low doses of bortezomib inhibited the chymotrypsin-like activity of heart proteasome subtypes to significantly different extents. Standard-proteasome subtypes (II-2 to II-4) from rat heart are basically not inhibited as long as the concentration of bortezomib does not exceed 12.5 nmol/L. On the other hand, intermediate-proteasome subtypes I-1 to I-5 are inhibited to ~30% and subtypes III-1 to III-6 are even inhibited by 50–70%. These results uncover two important facts: first, they indicate that bortezomib affects the various heart proteasome subtypes to remarkably different extents; second, intermediate-proteasome subtypes III-1 to III-6 have very different susceptibilities to the two proteasome inhibitors, MG132 and bortezomib. On the background of differing reaction mechanisms of proteasome inhibitors, it is not surprising that the multiple proteasome subtypes do not react equally with different inhibitors. On the basis of these data, the local concentration of a proteasome inhibitor as well as the type of proteasome inhibitors seems to be crucial for estimating the effect of treatment with proteasome inhibitors and to prevent unwanted side effects as observed recently.

By using model substrate proteins and site-specific inhibitors Kisselov et al. have shown that the contribution of the three active sites of a proteasome to protein degradation depends on the number of susceptible peptide bonds, i.e. amino acid composition, of a protein substrate. Accordingly, proteasomes with different activity profiles—as shown here for the cardiac proteasome subtypes—will have different substrate specificities and/or may process proteins in a different way or at an altered rate. In fact, this was previously observed with proteasome subtypes from skeletal muscle. Supposed that certain proteasome subtypes are crucially involved in mediating myocyte hypertrophy, specific and targeted inhibition of these proteasomes may suppress this pathological process. On the other hand, use of higher and thus unspecific concentrations of the same inhibitor or application of a less specific inhibitor affecting the activity of the whole spectrum of proteasome subtypes may have toxic side effects. Exact knowledge of the specific effects of proteasome inhibitors is essential to understand when and why reduced proteasome activity has beneficial or detrimental effects on heart function and to achieve successful treatment of patients with heart failure. Cardioprotective effects were observed for instance with the lactacystin analogous proteasome inhibitor PS519 following ischaemia and reperfusion in the isolated rat heart. The same inhibitor was shown to prevent left ventricular hypertrophy in a murine model. This observation is in agreement with the suppression of cardiomyocyte hypertrophy after treatment with non-toxic doses of MG132 and its boronate analogue MG262. MG132 was also found to protect rat cardiomyocytes against hyperthermic and oxidative injury. Similar to MG132, bortezomib attenuates cardiac hypertrophy in Dahl salt sensitive rats and prevents tachyarrhythmias as well as sudden cardiac death in an animal model. Recently, it has also been reported that proteasome inhibition prevents mice from coxackievirus-induced myocardial damage. On the other hand, unexpected harmful cardiac side effects, such as cardiac failure, bradycardia and others, occurred in bortezomib treated patients suffering from lung cancer. multiple myeloma or non-Hodgkin lymphoma. With regard to cardiac proteasomes, it is important to note that a concentration up to 12.5 nmol bortezomib/L, which corresponds to a plasma concentration measured in patients treated with bortezomib to cure their solid tumours, does neither affect the chymotrypsin-like activity nor the caspase-like activity of the major population of standard proteasomes in heart tissue.

Treatment of cancer patients with proteasome inhibitors is working at the balance between cell proliferation and cell death and has been proven to be successful in many cases. Many biological mechanisms have been proposed for the beneficial anticancer effects of proteasome inhibitors but to date it is not known, which proteasome subpopulation or subtypes are involved in these mechanisms. Therefore, separation and characterization of proteasome subtypes is a prerequisite for understanding the biological efficacy of proteasomes and to judge the usefulness of inhibitory compounds that modulate the activity of this group of enzymes.

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Cardiac proteasome subtypes differ in inhibitor susceptibility


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