Reviewer's report

Title: Anthracyclins, proteasome activity and multi-drug-resistance

Version: 2  Date: 29 June 2005

Reviewer: Carlos Ciudad

Reviewer's report:

General
In the manuscript Anthracyclines, proteasome activity and multi-drug resistance by Fekete et al., the authors report the ability of several anthracyclines to inhibit proteasome activity. Also the inhibition of P-glycoprotein by MG-132, a well-known inhibitor of the proteasome, is characterized in ECV304 cells. Furthermore, the incubation of MG-132 together with daunorubicin causes the accumulation of daunorubicin in cells overexpressing P-glycoprotein. The authors suggest a possible application of proteasome inhibitors to overcome multidrug resistance in cancer cells.

The observations described in the manuscript are potentially interesting although some of the conclusions are not fully supported by the data.

Some points need to be further elaborated and more data need to be provided to support the conclusions of the present work.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) What is the rationale for a possible activity of anthracyclines as proteasome inhibitors? The background provided in the Introduction section of the manuscript describes the ability of P-glycoprotein inhibitors such as cyclosporin and ritonavir to act also as proteasome inhibitors. However anthracyclines are substrates for P-glycoprotein. The hypothesis for the experimental approach is stated in the Results section but it might be more appropriate to introduce it earlier in the text and to provide references to support such an approach.

2) What is the level of accumulation of daunorubicin in KB8.5 cells incubated with verapamil and daunorubicin? This result would allow a better comparison between the inhibitory effects of the different compounds used to inhibit both the proteasome and P-glycoprotein.

3) The results of the incubations with anthracyclines, verapamil and MG-132 do not support the statement presented in the Abstract and the Discussion sections in which the authors suggest that P-glycoprotein and proteasome have overlapping substrate specificities. Could the inhibition of the proteasome be affecting P-glycoprotein expression at the molecular level?

4) What is the effect on P-glycoprotein activity provoked by MG-132 alone

5) What is the cytotoxicity of MG-132 in ECV304 and KB8.5 cells? The levels of apoptosis are evaluated after 24 hours, but the accumulation of daunorubicin is analyzed after 45 minutes of treatment with MG-132

6) Do all these inhibitors have some structural properties in common that could suggest a general mechanism of action on both proteasome and P-glycoprotein?
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1) The authors state in the Introduction section that vinblastine is able to inhibit P-glycoprotein, this affirmation is not supported by the literature cited in the manuscript.

2) What is the reason to use either doxorubicin or daunorubicin to evaluate the accumulation of the drug in the presence of a proteasome inhibitor?

3) The manuscript needs English revision

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests