Author's response to reviews

Title: Metabolic and morphological alterations induced by proteolysis-inducing factor from Walker tumour in C2C12 myotubes.

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Author's response to reviews: see over
We thank to Kent Lundholm for the considerations and comments, and we have now added to the main text.

With regards to whether the Walker cells contribute themselves to production of the WF, we believe that; Professor Tisdale and colleagues have shown PIF was produced by tumour cells in vitro e.g. MAC16 (Todorov et al, J Biol Chem, 1997; 272: 12279-12288) and human melanoma G361 (Todorov et al, Br J Cancer, 1999; 80: 1734-1737). Although there have been no direct measurements of WF in tumour tissue, in analogy with other studies (Todorov et al. Br J Cancer 1999; 80: 1734-1737. Cabal-Manzano et al Br J Cancer 2001; 84: 1599-1601) it is most likely to be produced by the Walker-256 tumour than host tissue secondary to inflammation as we have previously published the effect of Walker ascitic fluid on placenta tissue compared to pregnant rats injected with serum (from other rats) or ascitic fluid provided from a rat’s peritoneal transudate and we have found damage effects on placenta tissue and fetal reabsorption only in pregnant injected Walker ascitic fluid which were similar to pregnant tumour-bearing rats (Gomes-Marcondes et al., Cancer Res Ther Cont 5:277-283, 1998; Toledo & Gomes-Marcondes, Oncol Res 11(8): 359-366, 1999). Another fact is that we have tested the Ehrlich tumor and the western blot of the ascitic fluid from Ehrlich tumour did not show any band similar to PIF. Therefore, since the Walker-256 tumour has been extensively used as an experimental model to induce cachexia in rats, its ascitic fluid causes placental and fetal damages, and there have been no studies on whether it produces a factor similar to PIF, some experiments are needed to show the relationship between PIF and Walker Factor.

With regards to the increased nucleic acid, although it is not statistically significant but tend to be increased, probably it may due to DNA synthesis without cell division so that the total DNA content per cell increases, besides it is only speculative indicating cellular activity, but the WF can not stimulate cell replication, we have already tested.

As the same methodology to measure the protein synthesis using 2,6[3H] phenylalanine utilized by MJ Tisdale and colleagues, we have used extensively. We believe that even the 3H is transferred to solvent, the radioactivity left in the solute form (as pellet) is the labeled protein as the same methodology realized in muscle tissue.

It was a mistake in Methods about the “Walker factor” purification. It is not the precipitate but the supernatant, and it is now corrected in the main text. The Walker factor was purified following these steps:

1) The ascitic fluid obtained from Walker 256 tumour-bearing rats was centrifuged to remove the tumour cells
2) The supernatant was added to ammonium sulphate, so the high molecular weight proteins of the supernatant were precipitated and then centrifuged (3000 xg).
3) This supernatant was dialysed with PBS to remove the ammonium sulphate
4) Then it was filtered and concentrated by filtration through on Amicon Ultra centrifugal filter, 10,000 MW cut off (Millipore Corporation, USA) prior to concentration by affinity chromatography

The WF has both immunological and molecular weight characteristics identical to PIF, although it appears to be less potent in inducing a biological response. PIF shows activity in the nM range, whereas the WF requires concentrations up to 1µM for maximal biological effect. PIF is a complex sulphated glycoprotein with a short core peptide chain to which is attached both N- and O-linked oligosaccharide chains. Over 80% of the molecule is carbohydrate, and the biological activity of PIF is due to the complex oligosaccharide chains, maybe the reason for this difference (PIF and WF) could be due to small differences in the composition of the oligosaccharide chains, which are essential for the biological activity of PIF (Todorov et al, J Biol Chem, 1997; 272: 12279-12288).
Reviewer's report

Title: Metabolic and morphological alterations induced by proteolysis-inducing factor from Walker tumour in C2C12 myotubes.

Version: 1 Date: 18 July 2007
Reviewer: Luiz Claudio C Fernandes

Reviewer's report:
General

We thank Dr LC Fernandes for the comments and considerations about the manuscript. With concern to MDA measurement, we understand it underestimates the total lipid peroxidation, but as many other researchers (Lykkesfeldt J Clin Chim Acta. 380(1-2):50-8, 2007 (an interesting review); Bachur et al. Appl Physiol Nutr Metab. 32(2):190-6, 2007; Celik & Suzek. Chem Biol Interact. 167(2):145-52, 2007; Hoffman et al. J Strength Cond Res. 21(1):118-22, 2007; Karanth & Jeevaratnam Int J Vitam Nutr Res. 75(5):333-9, 2005; Gumieniczek, et al. Clin Chim Acta. 314(1-2):39-45, 2001) indicate that the MDA content can estimate the oxidative stress occurrence, we opted to infer the oxidative stress using the MDA assay, and we have already published results of MDA indicating the increase on oxidative stress in myotubes cell culture (Yano & Marcondes, Free Radic Biol Med. 39(10):1378-84, 2005.). We agree with the methodology using the ferrous oxidation in xylenol orange, although this assay is not standardized in our lab and we suggested the confirmed oxidative stress not only by the MDA content but also the decrease in cell answer to WF decreasing the GST activity.

We agree with the points written below and they are now corrected in the main text.

Minor Essential Revisions.
Page 2, first line first word. Background? It is now corrected in the main text.
Page 2 last line. The cachexia syndrome occurs in up to 80% of cancer patients... What is the source of this information? Cachexia researchers love to say this sort of thing but what sort of information supports this point? Add reference. We have now corrected in the main text.
Page 3, paragraph 2, line 10, seen in animals bearing cachexia-inducing tumors.8. What is that? is it a reference? It is the reference 8 – Lorite et al.,1998 . It is now corrected.
Results. Page 9 paragraph 2, line 3-5. I think the information concerning to refs 31,32 should be add to discussion section. I did not see any reason to be in here. Do you agree with that? There are many stiles to write. I use to include some references in the “Results” item as I think it could direct the reader to the focused point.
Page 9. I did not see ... a concentration- and time-dependent manner. In fact it was clear to me that the concentration of 25 was the most efficient at any time for MTT, NRU and NAC. I did not see a curve-response shape. I suggest to change the sentence. It is now changed. To show a time-curve it should be presented or elaborated as a different manner.
Page 11. The authors report the findings in the Figure 6 (B-E), but do not say anything about F-H. Why? It is now mentioned in the main text that in these concentrations the effects on cells morphology is more evidenced.
Discussion. Page 13, Line 11. The way you wrote the sentence I understand that GST and MDA have the same results mainly after 48 and 72 h. But in the figure 3, MDA changes only at 24 hs. Rewrite the sentence because was true only for GST, please. It is now properly wrote.
Page 23, figure 6 legend ... to-cell contact,..... It is now corrected.