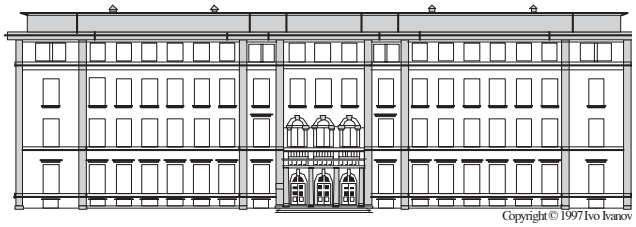

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Investigation Report on Project:

Cytoprotective effects of creatine (conventional or optimised [KreVitalin®) in an *in vitro* model of Lovastatin induced-myotoxicity

Experimental design

Lovastatin and the other HMG-CoA reductase inhibitors (statins) cause clinical myotoxicity manifested as myositis or rhabdomyolysis. Thus they are considered as a suitable model for the in vitro evaluation of toxicity against muscle cells and its management. On this ground the efficacy of conventional and optimised creatine formulations as protective agents against lovastatin-induced myotoxicity was tested in a comparative fashion using RD human muscle cells as an in vitro test system.

As evidenced by a preliminary experiment both creatine formulations under evaluation – conventional and optimised were practically devoid of cytotoxicity in RD cells, within a concentration range of 0.1-1 mmol/L.

Experimental protocol

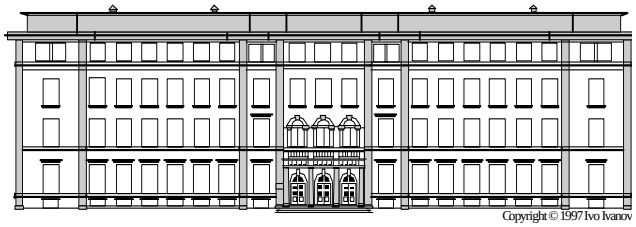
Exponentially growing RD cells were cultured in 96-well microplates and after a 24 h adaptation period they were treated with lovastatin (at 6.25, 12.5, 25 or 100 μmol/L), alone or in combination with 0.2 or 1 mmol/L creatine (conventional or optimised). Following a 72 h continuous exposure the viability of RD cells was assessed using the MTT-dye reduction assay.

Results

In line with the well-established myotoxicity of statins lovastatin caused prominent decrease of the cellular viability of RD cells after 72 h exposure. At the lowest concentration it inhibited the proliferation by %. At concentrations exceeding 25 μmol/L lovastatin caused practically total eradication of viable cells.

The combined treatment of muscle cells with lovastatin and creatine (conventional preparation) ameliorated the myotoxicity of the statin as evident from the data summarized in table and figure. The cytoprotective effects were more pronounced at the higher creatine level (1 mmol/L) (Table 1.; Figure 1.).

The co-administration of RD cells with both lova statin and the optimised creatine resulted in far more prominent protection of the chondrocytes as compared to that registered with the non-processed creatine. In all treatment groups the combination of lovastatin+optimised creatine was associated with significantly higher cell viability as compared to the effects of the myotoxic drug alone (Table 6.; Figure 6).



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Conclusions: Although the precise mechanisms of statin-induced myotoxicity are not elucidated in detail, at the cellular level these drugs caused marked protein synthesis inhibition and ATP-depletion. On this ground the encountered cytoprotective effects of creatine against lovastatin-induced cytotoxicity in RD muscle cells are probably mediated by its anabolic and bio-energetic properties. The superior cytoprotective effects encountered with the processed creatine formulation vs the conventional are most probably an outcome of its superior stability in the course of the experiment.

Project coordinator
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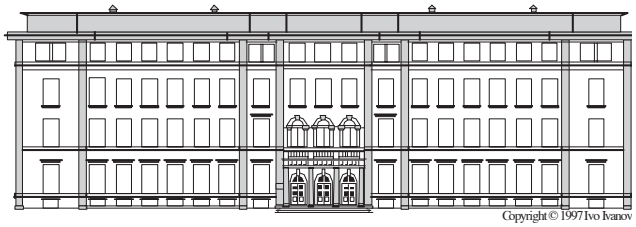
Mrs. Theodora Atanassova, BSc

Sofia August 25th 2007



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Appendix Cytoprotective effects of creatine (conventional or optimised) in an *in vitro* model of Lovastatin induced-myotoxicity. Experimental data.

Table 1. Cytoprotective effects of the conventional creatine formulation against lovastatin-induced myotoxicity against RD cells, as assessed by the MTT-dye reduction assay after 72 h incubation.

Treatment group	% of viable cells		Protection index
	Mean	sd	
Untreated control	100.0	3.8	-
Lovastatin 6.25 μmol/L	40.8*	0.4	-
+ 0.2 mmol/L creatine	42.2*	3.1	1.03
+ 1 mmol/L creatine	44.6*#	1.0	1.09
Lovastatin 12.5 μmol/L	19.1*	1.6	-
+ 0.2 mmol/L creatine	23.2*#	0.4	1.21
+ 1 mmol/L creatine	24.4*#	0.5	1.28
Lovastatin 25 μmol/L	4.1*	3.1	-
+ 0.2 mmol/L creatine	8.5*	7.1	2.07
+ 1 mmol/L creatine	12.6 *#	5.2	3.07
Lovastatin 100 μmol/L	2.2*	2.0	-
+ 0.2 mmol/L creatine	6.7*	2.6	3.05
+ 1 mmol/L creatine	8.9*#	0.9	4.05

* Statistically significant ($p<0.05$) vs. the untreated control; # Statistically significant ($p<0.05$) vs. Lovastatin administered alone (Student's t-test).

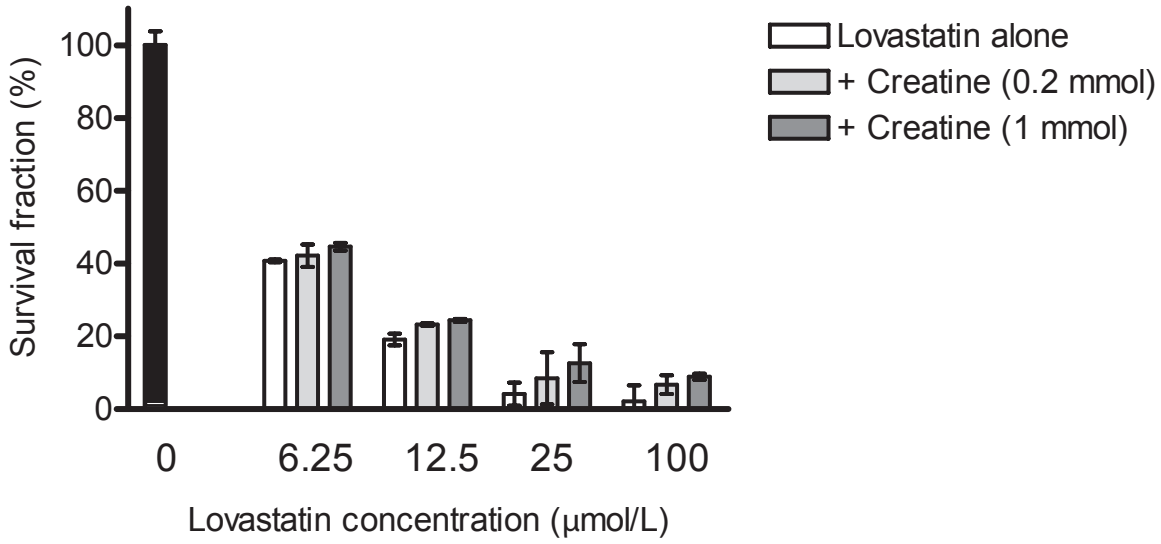

Fig. 1. Cytoprotective effects of the conventional creatine formulation against lovastatin-induced myotoxicity against RD cells, as assessed by the MTT-dye reduction assay after 72 h incubation. Each column represents the arithmetic mean ± sd (n=6).

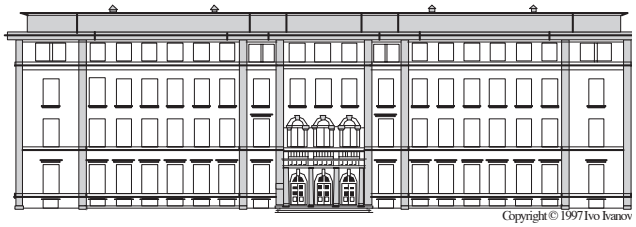

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Table 2. Cytoprotective effects of the optimised creatine formulation against Lovastatin-induced myotoxicity in RD cells, as assessed by the MTT-dye reduction assay after 72 h incubation.

Treatment group	% of viable cells		Protection index
	Mean	sd	
Untreated control	100.0	3.4	-
Lovastatin 6.25 μmol/L	40.8*	0.4	-
+ 0.2 mmol/L opti. creatine	46.6*#	0.6	1.14
+ 1 mmol/L opti. creatine	48.2*#	0.3	1.18
Lovastatin 12.5 μmol/L	19.1*	1.6	-
+ 0.2 mmol/L opti. creatine	24.6*#	0.4	1.29
+ 1 mmol/L opti. creatine	29.8*#	1.2	1.56
Lovastatin 25 μmol/L	4.1*	3.1	-
+ 0.2 mmol/L opti. creatine	11.1*#	5.6	2.7
+ 1 mmol/L opti. creatine	16.0*#	3.5	3.9
Lovastatin 100 μmol/L	2.2*	2.0	-
+ 0.2 mmol/L opti. creatine	10.0*#	1.5	4.54
+ 1 mmol/L opti. creatine	12.5*#	3.3	5.68

* Statistically significant ($p<0.05$) vs. the untreated control; # Statistically significant ($p<0.05$) vs. Lovastatin administered alone (Student's t-test).

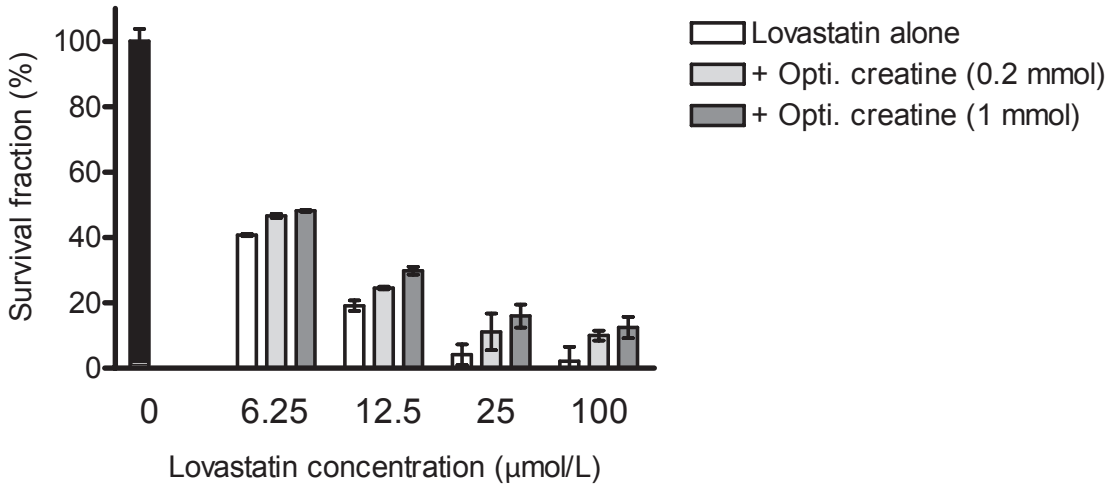

Fig. 2. Cytoprotective effects of the optimised creatine formulation against lovastatin-induced myotoxicity in RD cells, as assessed by the MTT-dye reduction assay after 72 h incubation. Each column represents the arithmetic mean ± sd (n=6).

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