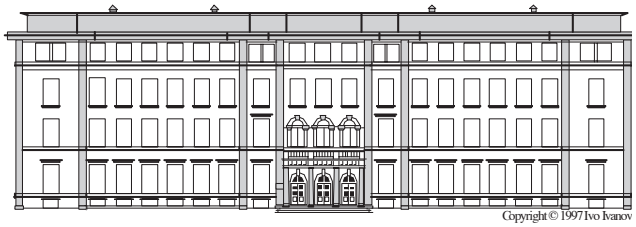

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Investigation Report on Project:

Cytoprotective effects of creatine (conventional or optimised) in an *in vitro* model of ciprofloxacin induced-joint toxicity

Experimental design/Experimental protocol

Fluoroquinolones e.g. ciprofloxacin cause toxic injury to the weight bearing joints in juvenile animals. Their effects have been well demonstrated in human chondrocytes as well, which makes these antibiotics useful model drugs for the assessment of joint toxicity and its treatment. The chondroprotective effects of conventional and optimised creatine formulations were tested in a comparative fashion using ciprofloxacin-induced cytotoxicity in SW1353 human chondrocytes as an in vitro model of joint-injury. A preliminary experiment showed that both creatine formulations – conventional and optimised were practically non-toxic against this cell line, within a concentration range of 0.1-1 mmol/L. Exponentially growing SW1353 cells were plated in 96-well microplates and after a 24 h adaptation period they were exposed to ciprofloxacin (at 7.5, 15, 30 or 60 µg/mL), alone or in combination with 0.2 or 1 mmol/L creatine (conventional or optimised). Following a 72 h continuous exposure the cellular viability was assessed using the MTT-dye reduction assay.

Results/ Conclusions:

The sole application of ciprofloxacin caused prominent cytotoxicity in SW1353 chondrocytes, in a concentration-dependent manner. Thus at the lowest level of 7.5 µg/mL the antibiotic lowered the cellular viability by ca. 20%, while at 60 µg/mL only 36 % of the cells were viable. The co-administration of ciprofloxacin and (as conventional preparation) ameliorated the cytotoxicity of the antibiotic as evident from the data summarized in table 1. and figure 1. The chondroprotective effects were more pronounced at the higher creatine level (1 mmol/L). The combined treatment of SW1353 cells with ciprofloxacin and the optimised creatine was associated with far more pronounced protection of the chondrocytes. In all treatment groups the combination of ciprofloxacin+optimised creatine was associated with significantly higher cell viability as compared to the effects of the drug alone (Table 2., Figure 2.).

The superior cytoprotective effects encountered with the processed creatine formulation vs the conventional are most probably an outcome of its superior stability under the conditions of the experiment.

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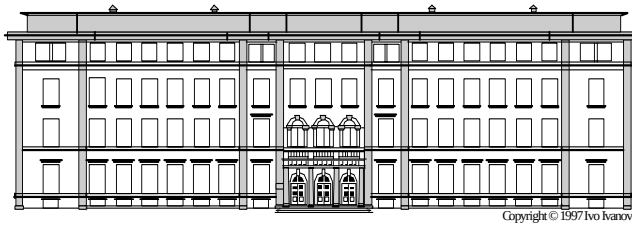
Sofia August 25th 2007



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Appendix . Cytoprotective effects of creatine (conventional or buffered) in an *in vitro* model of ciprofloxacin induced-joint toxicity. Experimental data.

Table 1. Cytoprotective effects of the conventional creatine formulation against ciprofloxacin-induced cytotoxicity in SW1353 human chondrocytes, 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**	**-**
Ciprofloxacin 7.5 μg/ml	**81.6***	**3.0**	**-**
+ 0.2 mmol/L creatine	**86.5***	**3.6**	**1.06**
+ 1 mmol/L creatine	**89.7*#**	**1.7**	**1.10**
Ciprofloxacin 15 μg/ml	**78.2**	**1.4**	**-**
+ 0.2 mmol/L creatine	**82.0***	**3.0**	**1.05**
+ 1 mmol/L creatine	**84.7*#**	**1.9**	**1.08**
Ciprofloxacin 30 μg/ml	**58.8***	**4.8**	**-**
+ 0.2 mmol/L creatine	**67.5*#**	**1.9**	**1.15**
+ 1 mmol/L creatine	**74.2*#**	**2.1**	**1.26**
Ciprofloxacin 60 μg/ml	**36.1***	**3.8**	**-**
+ 0.2 mmol/L creatine	**43.1*#**	**1.3**	**1.19**
+ 1 mmol/L creatine	**46.9*#**	**1.7**	**1.30**

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

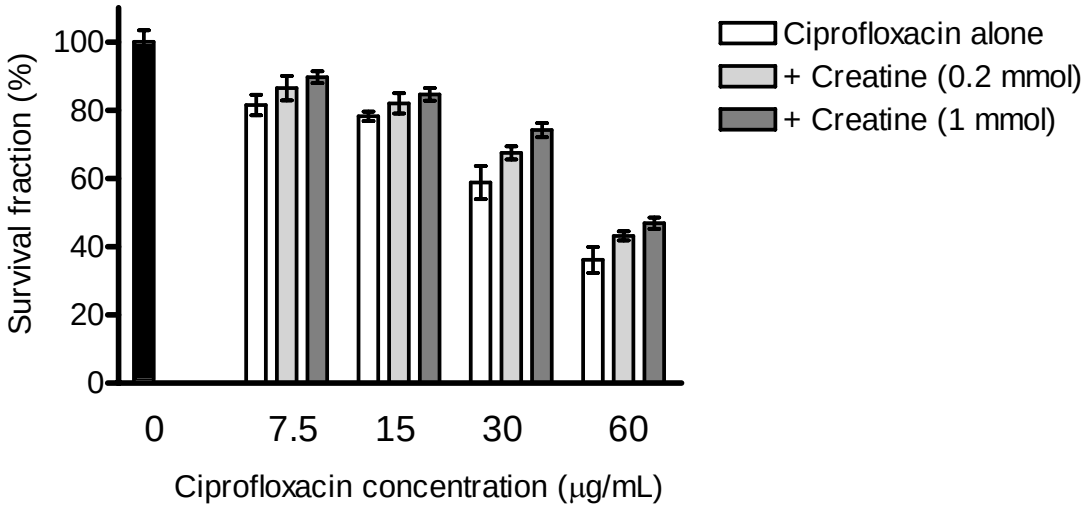

Fig. 1. Cytoprotective effects of the conventional creatine formulation against ciprofloxacin-induced cytotoxicity in SW1353 chondrocytes, as assessed by the MTT-dye reduction assay after 72 h incubation. Each column represents the arithmetic mean ± sd (n=6).

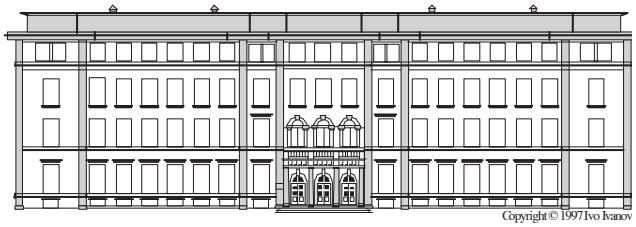

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Table 2. Cytoprotective effects of the conventional creatine formulation against ciprofloxacin-induced cytotoxicity in SW1353 human chondrocytes, as assessed by the MTT-dye reduction assay after 72 h incubation.

Treatment group	% of viable cells		Protection index
	Mean	sd	
Untreated control	100.0	3.4	-
Ciprofloxacin 7.5 μg/ml	81.6*	3.0	-
+ 0.2 mmol/L opti. creatine	94.1	1.3	1.15
+ 1 mmol/L opti. creatine	95.2	2.4	1.17
Ciprofloxacin 15 μg/ml	78.2*	1.4	-
+ 0.2 mmol/L opti. creatine	89.1*#	4.3	1.14
+ 1 mmol/L opti. creatine	93.9*#	3.2	1.20
Ciprofloxacin 30 μg/ml	58.8*	4.8	-
+ 0.2 mmol/L opti. creatine	72.5*#	1.1	1.23
+ 1 mmol/L opti. creatine	78.3*#	3.7	1.33
Ciprofloxacin 60 μg/ml	36.1*	3.8	-
+ 0.2 mmol/L opti. creatine	50.8*#	5.7	1.41
+ 1 mmol/L opti. creatine	58.2*#	3.6	1.61

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

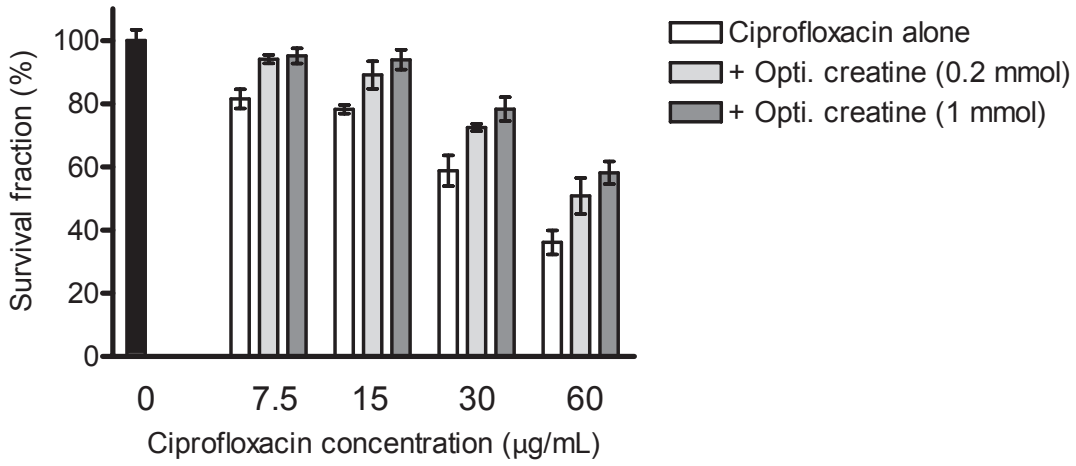

Fig. 2. Cytoprotective effects of the optimised creatine formulation against ciprofloxacin-induced cytotoxicity in SW1353 chondrocytes, as assessed by the MTT-dye reduction assay after 72 h incubation. Each column represents the arithmetic mean ± sd (n=6

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