Cytoprotective effects of creatine (conventional or buffered) in an in vitro model of cisplatin induced-nephrotoxicity

Owing to the complex functions of the kidney and its anatomical peculiarities the kidney and its adjacent tissues are often exposed to higher levels of xenobiotics relative to other tissues and organs. Thus the kidney often suffers from the deteriorating effects of both drugs and other xenobiotics, giving rise to toxic effects [1, 2].

The cytoprotective potential of both conventional and buffered creatine formulations was tested in a comparative fashion using a model of cisplatin-induced cytotoxicity. Cisplatin is nephrotoxic drug and the mechanisms underlying this effect are complex and involve free radical generation, oxidative and nitrosative stress, disruption of calcium homeostasis, adduct formation and bioactivation upon binding to glutathione giving rise to toxic reactive thiols [3].

Despite the diverse mechanisms implicated some important secondary events include ATP-depletion with consequent disruption of cellular bioenergetics and homeostatsis and induction of cellular death through apoptosis or necrosis [3] . On this ground we sought to determine the cytoprotective effects of creatine, since it is implicated in the macroergic phosphate cross-over and cellular bioenergetics maintenance [4, 5].

Among the diverse models for in vitro evaluation of kidney toxicity the transformed cell lines appear to be a promising one, as they are easier for manipulation than primary cells and retain some of the characteristic biochemical fetures of the tubulaqr epithelium [1]. The human kidney cell line 293T has been previously described as suitable model for in vitro assessment of renal toxicity and hence we used in this study as a test system.

. A preceding experiment, carried out in order to evaluate the creatine cytotoxicity in 293T cells and hence non-toxic levels, revealed that both conventional and buffered formulation are practically devoid of cytotoxic effects in this cell line, within a concentration range of 0.1-1 mmol/L.

Exponentially growing 293T cells were plated in 96-well microplates and after a 24 h adaptation period they were exposed to cisplatin (at 5, 25 or 50 μmol/L), alone or in combination with 0.2 or 1 mmol/L creatine (conventional or buffered). Following a 72

h continuous exposure the cellular viability was assessed using the MTT-dye reduction assay, as described by Mossmann [6], with minor modifications [7, 8].

Evident from the results obtained cisplatin induced a strong, concentration-dependent decrease of the cellular survival, whereby at 5 µM the viable cells where reduced by 25%, at 25 µM they were reduced by 40%, whereas at the highest concentration tested (50 µM) the cell survival fraction was reduced to 41 % of the untreated control.

The co- administration of a non-stabilized creatine solution was consistent with marginal protective effects, which where generally more pronounced at the higher level of creatine (1 mmol/L). Statistically significant decrease of cisplatin cytotoxicity was encountered only when cisplatin (at 5 µM or 50 µM) was co-administered with 1 mmol/L creatine.

In a dissimilar fashion the buffered creatine led to a prominent, statistically significant and dose-dependent amelioration of cisplatin cytotoxicity in 293T cells. In all treatment groups the combination of cisplatin+buffered creatine was associated with significantly higher cell viability as compared to the effects of cisplatin alone.

The stability of a given compound under the chosen experimental conditions is a crucial prerequisite for optimal activity in vitro. We encountered significant discrepancies between the cytoprotective potential of creatine against cisplatin toxicity, depending on the nature of the creatine formulation evaluated. Thus while conventional creatine displayed only marginal activity, the stabilized buffered creatine formulation proved to afford effective protection of 293T cells against the deteriorating effects of cisplatin, a finding which could be ascribed to the superior stability of the buffered formulation under the conditions of our experimental setup.

Conclusions:

- Creatine, both conventional and buffered is non cytotoxic against the human kidney cell line 293T in a wide range of concentrations.

- When applied concomitantly with the nephrotoxic agents cisplatin the conventional creatine formulation displayed only marginal cytoprotective effects, only when applied at 1 mmol/L.

- The buffered creatine formulation is endowed by a significant, dose dependent cytoprotective potential against cisplatin-induced cytotoxicity in 293T cells.

References:

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Appendix

Table 1. Cytoprotective effects of conventional creatine against cisplatin-induced cytotoxicity in 293T human kidney cells, as assessed by the MTT-dye reduction assay after 72 h incubation.

Treatment group	MTT-formazan absorption		% of viable cells		Protection index
	Mean	sd	Mean	sd	
Untreated control	0.954	0.026	100.0	3.1	-
Cisplatin 5 µmol/L	0.718*	0.019	75.2	2.0	-
+ 0.2 mmol/L Creatine	0.741*	0.017	77.7	1.8	1.03
+ 1 mmol/L Creatine	0.773*#	0.010	81.0	1.0	1.08
Cisplatin 25 µmol/L	0.581*	0.056	60.9	5.9	-
+ 0.2 mmol/L Creatine	0.550*	0.027	57.7	2.9	0.95
+ 1 mmol/L Creatine	0.554*	0.020	58.1	2.1	0.95
Cisplatin 50 µmol/L	0.400*	0.011	41.9	1.2	-
+ 0.2 mmol/L Creatine	0.421*	0.003	44.1	0.3	1.05
+ 1 mmol/L Creatine	0.436*#	0.016	45.7	1.7	1.09

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

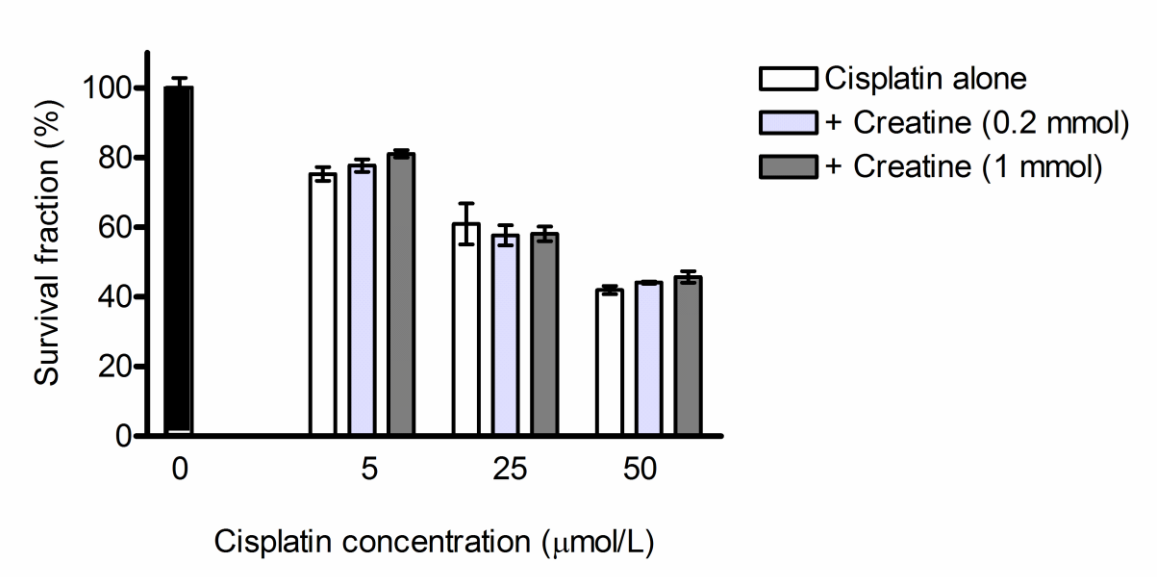

Fig. 1. Cytoprotective effects of conventional creatine against cisplatin-induced cytotoxicity in 293T human kidney cells, as assessed by the MTT-dye reduction assay after 72 h incubation. Each column represents the arithmetic mean ± sd (n=6).

Table 2. Cytoprotective effects of the buffered creatine formulation against cisplatin-induced cytotoxicity in 293T human kidney cells, as assessed by the MTT-dye reduction assay after 72 h incubation.

Treatment group	MTT-formazan absorption		% of viable cells		Protection index
	Mean	sd	Mean	sd	
Untreated control	0.954	0.026	100.0	3.1	-
Cisplatin 5 µmol/L	0.718*	0.019	75.2	2.0	-
+ 0.2 mmol/L Buff. creatine	0.788*#	0.018	82.6	1.9	1.10
+ 1 mmol/L Buff. creatine	0.849*#	0.069	89.0	7.3	1.18
Cisplatin 25 µmol/L	0.581*	0.056	60.9	5.9	-
+ 0.2 mmol/L Buff. creatine	0.646*#	0.016	67.7	1.6	1.11
+ 1 mmol/L Buff. creatine	0.738*#	0.029	77.4	3.0	1.27
Cisplatin 50 µmol/L	0.400*	0.011	41.9	1.2	-
+ 0.2 mmol/L Buff. creatine	0.486*#	0.057	51.0	6.0	1.22
+ 1 mmol/L Buff. creatine	0.494*#	0.040	51.8	4.2	1.24

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

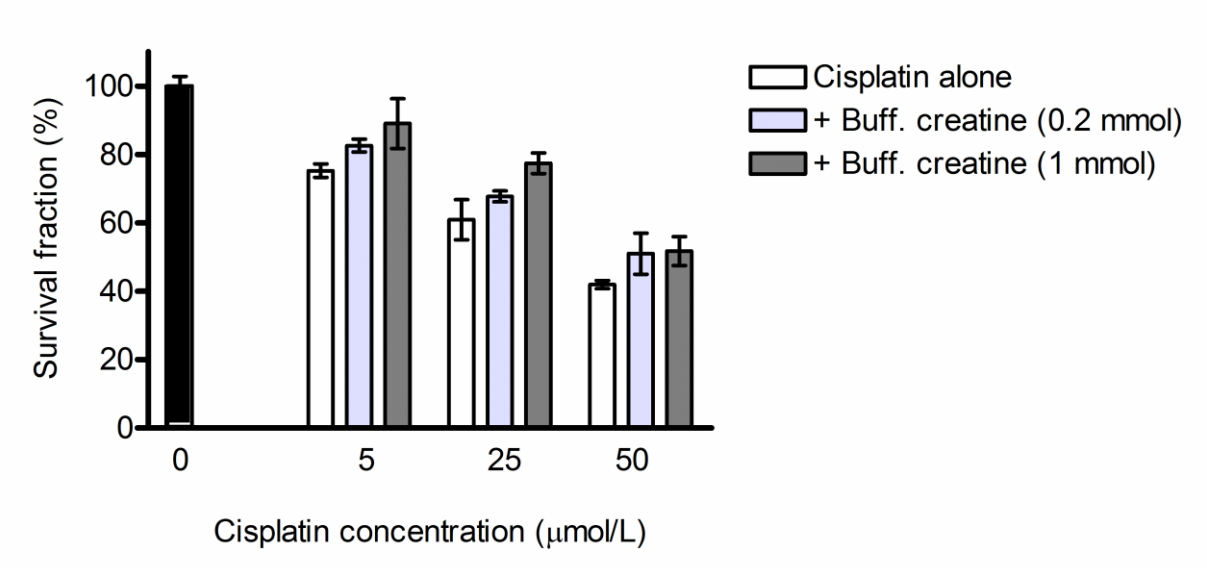

Fig. 2. Cytoprotective effects of the buffered creatine formulation against cisplatin-induced cytotoxicity in 293T human kidney cells, as assessed by the MTT-dye reduction assay after 72 h incubation. Each column represents the arithmetic mean ± sd (n=6).