KRE-CELAZINE™

Break-through chondroprotective joint care product

Kre-Celazine® is a break-through nutrient complex (patents pending) designed to effectively aid the body in its natural role of protecting joint connective tissues. To understand how and why it works, it is first necessary to understand in part the causes of inflammation and the complex interrelated workings in the human body.

Inflammation is a complex stereotypical reaction, our body's response to damage of its cells and vascularized tissues. Inflammatory processes are implicated in mechanisms of a wide array of pathological conditions ranging from common cold, athlete muscle pain to asthma and rheumatoid arthritis.

On a daily basis human cells and tissues must defend themselves against the multitude of different pathogens including different germs (viruses, bacteria, fungi, and protozoan parasites) as well as tumors and a number of various harmful agents or capable of deranging its homeostasis. In order to meet these needs, during the course of evolution, different effector mechanisms capable of defending the body against such antigens and agents have developed. These defense type reactions can be mediated by soluble factors or by cells. If infection occurs as a consequence of the tissue damage, the innate, and later, the adaptive immune systems are triggered to neutralize or destroy the infectious agent.

The discovery of the detailed processes of inflammation has revealed a close relationship between inflammation and the immune response.

The five basic symptoms of inflammation have been] defined first by Ancient Roman and Greek physicians; these include - redness (rubor), swelling (tumor), heat (calor), pain (dolor) and deranged function (functiolaesa). These signs arise due to an increase of vascular permeability, leakage of plasma and certain white blood cells (leukocytes) into the site of inflammation. Early researchers considered inflammation a primary

host defense system. From this point of view inflammation is the key reaction of the innate immune response. In fact inflammatory hyper-reaction to parasites or to allergens can lead to death or to substantial shortening of life, for example anaphylactic shock, or debilitating diseases such as arthritis and gout.

According to different criteria, inflammatory responses can be divided into several categories. The criteria include:

1. **Time** - hyperacute (peracute), acute, subacute, and chronic inflammation;
2. **The Main Inflammatory Manifestation** - alteration, exudation, proliferation;
3. **The degree of tissue damage** - superficial, profound (bordered, not bordered);
4. **Characteristic picture** - nonspecific, specific;
5. **Immunopathological Mechanisms**
   - allergic (reaginic) inflammation,
   - inflammation mediated by cytotoxic antibodies,
   - inflammation mediated by immune complexes

Nevertheless, inflammation is often not good for the host organism and in case of so called auto-immunity, is triggered by virtually non-noxious stimuli. Moreover, while acute inflammatory conditions are typically curable and tissue alterations are reversible, in case of chronic infection, there could be significant loss of function associated with disabilities and devastating impact on quality of life.

An issue of particular interest is the spectrum of inflammatory conditions affecting the musculo-sceletal system, which are often initiated by persistent trauma and over-activity i.e. in athletes.

As far as arthritis is concerned, at present, drug treatment is generally palliative and symptomatic and is incapable of altering the time-course and progression of the disease. These current treatments targeting solely the inflammatory aspects of the disease are expensive and none has any appreciable effects on cartilage destruction. Moreover the pharmacotherapy for arthritis and chronic inflammation is associated with significant side effects. Anti-inflammatory drugs act by inhibiting the production of the chemical mediators of inflammation (e.g. aspirin and related drugs, the so called non-steroidal anti-inflammatory drugs NSAIDs) or by down-regulation of immune responses. The second group is generally referred to as immune suppressants (corticosteroids, cylcosporin, azathioprin). NSAIDs and especially the immunosupressants display a vast array of important side effects, which could significantly influence one's quality of life or even could precipitate blood loss, infection or death.

On this basis, overtime, there has arisen a need for alternative therapies to help alleviate and treat the painful symptoms of arthritis and possibly slow down disease progression. Recently the use of nutraceuticals as over the

counter therapeutic options for arthritis sufferers has met unprecedented interest. The current scientific knowledge has proven that despite of the fact that the use of such remedies is based on an empirical approach, a lot of them are frequently referred to as chondroprotectors and are truly endowed by a significant therapeutic potential. Some nutraceuticals are worth mentioning due to the accumulated sound scientific evidence as possible alternative treatments for arthritis. Such substances are cod-liver oil and polyunsaturated fatty acids (PUFAs), glucosamine, chondroitin, Green tea extracts, Asian folk remedies and more recently, creatine / Kre-Alkalyn® etc.

Research conducted by All American Pharmaceutical & Natural Foods Corporation (AAP) and Medical Universities it has contracted, have investigated a number of these key natural nutrients to quantify their effectiveness when exposed to cartilage and muscle cells forming the joints. Additional research focused on ways in which to ensure maximum bio-effectiveness from each source. Methods of extraction and the conditioning of each of the nutrients were utilized and compared, using patented and patent pending technologies that AAP has developed over the last 12 years.

On the basis of the well-described protective effects of creatine and PUFA on cartilage, All American Pharmaceutical has developed a new patents pending formula to combine their therapeutic potential in a single product. In order to assess its efficacy in a controlled scientific experiment, human chondrocytes (joint tissue cells) were used as an in vitro model of joint injury and chondroprotection. Fluoroquinolone antibiotics 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 buffered creatine formulations were tested in a comparative fashion using ciprofloxacin-induced cytotoxicity in SW1353 human chondrocytes as an in vitro model of joint-injury.

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/mol), 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. 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/mol 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 Kre-Celazine® ameliorated the cytotoxicity of the antibiotic as evident from the data summarized in table 7.1. and figure 7.1. The chondroprotective effects were more pronounced at the higher creatine level (1 mmol/L).

The combined treatment of SW1353 cells with ciprofloxacin and the buffered creatine was associated with far more pronounced protection of the chondrocytes. In all treatment groups the combination of ciprofloxacin+buffered creatine was associated with significantly higher cell viability as compared to the effects of the drug alone. 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.

Under identical conditions the chondroprotecive effect of polyunsaturated fatty acids was also assessed. Thereby the co-administration of ciprofloxacin and 5 µg/mL untreated or treated fats ameliorated the cytotoxicity of the antibiotic as evident from the data summarized in table 1. and figure 1. The chondroprotective effect was significantly more pronounced when TF were co-administered. The combined treatment of SW1353 cells with ciprofloxacin and 10 µg/mL of both TF and UF was associated with far more pronounced protection of the chondrocytes. In all treatment groups however, the combination of ciprofloxacin+TF was invariably superior in its cytoprotective effects as compared to the untreated conventional formulation. The results presented unambiguously indicate that the described TF formulation is characterized with superior chondroprotective activity under the chosen experimental conditions.

The main conclusions that could be drawn from this study are as follows:

Both creatine and PUFA's proved to afford a significant chondroprotective effect in an in vitro model, in corroboration of the compelling evidence in scientific literature for their beneficial role in arthritis.

The products processed using the patented and patents pending technological approach of All American Pharmaceutical display an obvious advantage over the non-conditioned nutrients as far as chondroprotective effects are concerned.

These findings unambiguously indicate that combining the prominent effects of these conditioned PUFAs and pH correct creatine in a single formula is an attractive option for nutraceutical supplementation of individuals suffering from symptoms

resembling arthritis. Both natural ingredients act on cartilage in different ways and thanks to the, so called, synergistic effect each ingredient contributes to an increased combined efficacy vs. the non-combined products.

Appendix 7. Cytoprotective effects of creatine (conventional or buffered) in an *in vitro* model of ciprofloxacin induced-joint toxicity. Experimental data.

Table 7.1. 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).

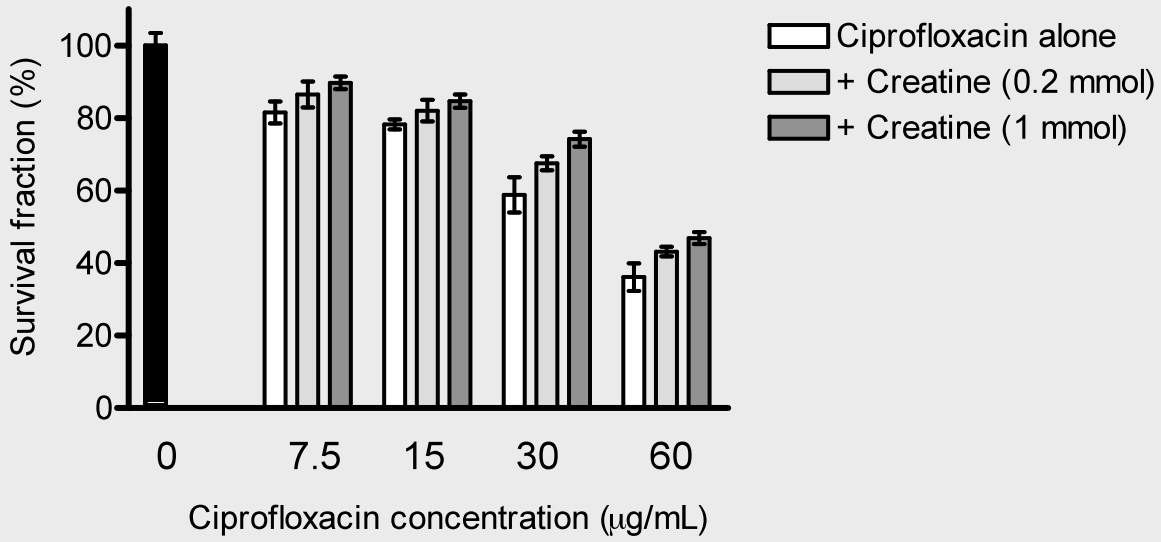


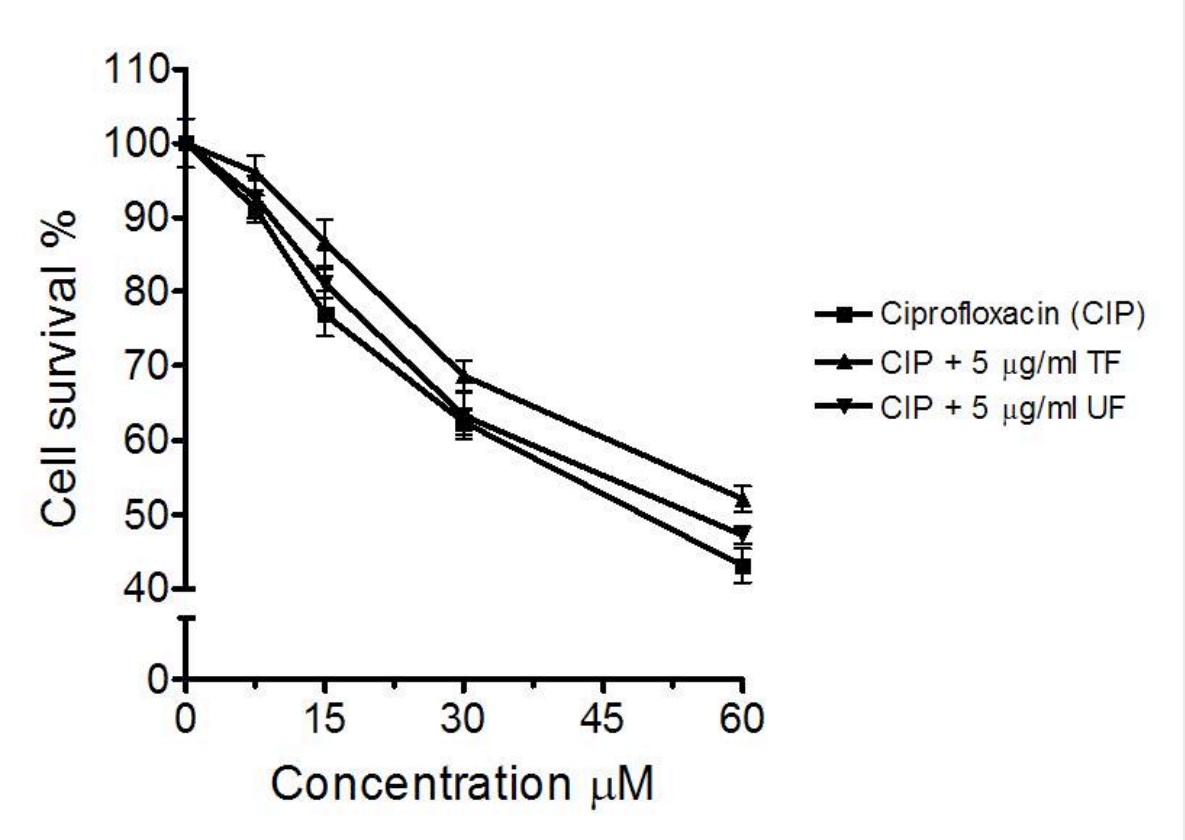
Fig. 7.1. Cytoprotective effects of the conventional creatine formulation against ciprofloxacin-induced cytotoxicity in SW1353 chondrocytes, as assessed by the MTT-dye reduction assay after 72 h incubation. Each column represents the arithmetic mean ± sd (n=6).

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

Table 1.

Concentration (μg/ml)	Cell viability (% of untreated control)					
	Ciprofloxacin (alone)		Ciprofloxacin + 5 μg/ml TF		Ciprofloxacin + 5 μg/ml UF	
	mean	sd	mean	sd	mean	sd
0.0	100.0	3.3	100.0	3.3	100.0	3.3
7.5	91.2	1.8	96.0*	2.3	92.7	2.9
15.0	77.0	3.0	86.6*#	3.1	81.1	1.9
30.0	62.4	1.7	68.7*#	2.0	63.3*	3.1
60.0	43.1	2.3	52.1*#	1.7	47.2*	1.1

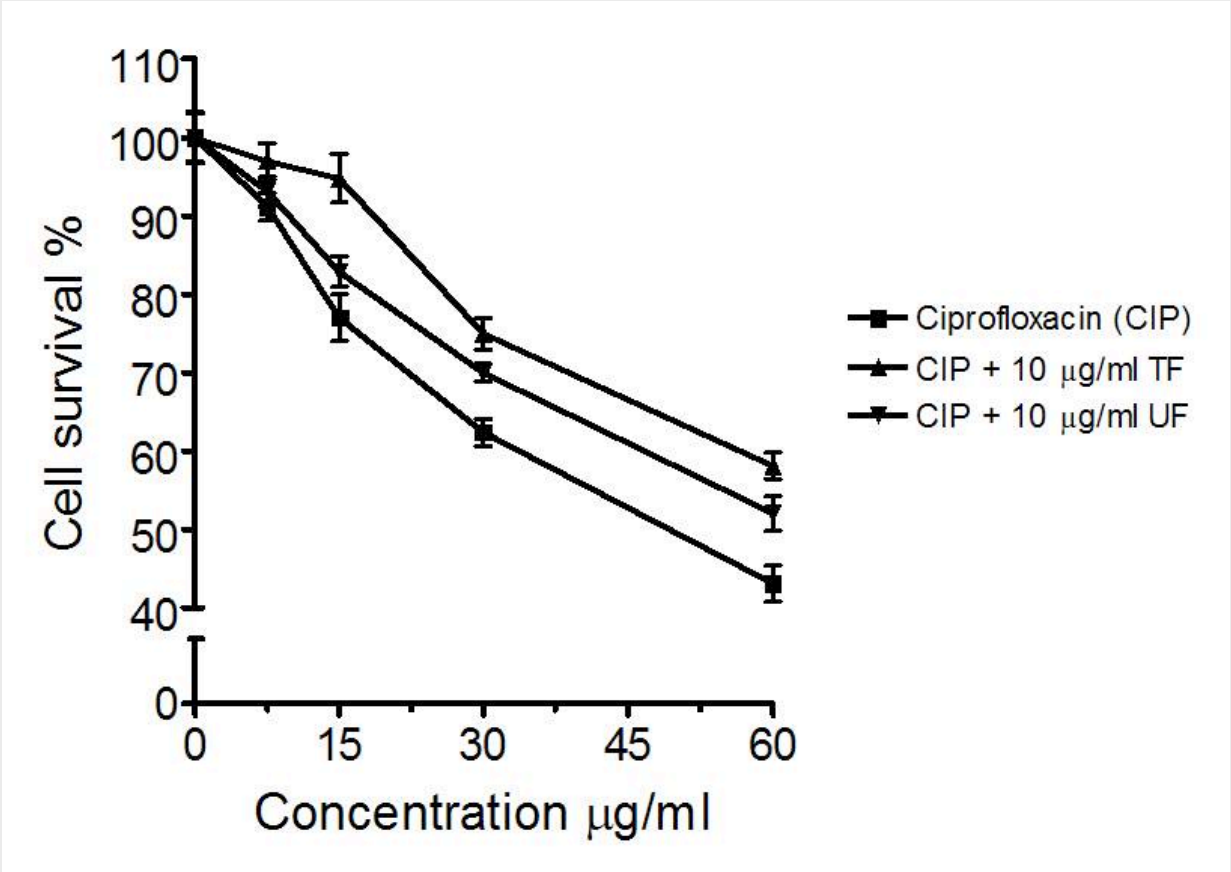
* significantly different vs ciprofloxacin alone ($p<0.05$, students t-test); #significantly different vs. ciprofloxacin + UF ($p<0.05$, students t-test);



Cytoprotective effects of TF vs UF (5 µg/ml) against the chondrotoxic effects of ciprofloxacin in HTB-94 chondrocytes (MTT test).

Concentration (µg/ml)	Cell viability (% of untreated control)					
	Ciprofloxacin (alone)		Ciprofloxacin + 10 µg/ml TF		Ciprofloxacin + µg/ml UF	
	mean	sd	mean	sd	mean	sd
0.0	100.0	3.3	100.0	3.3	100.0	3.3
7.5	91.2	1.8	97.0*#	2.3	93.1	1.9
15.0	77.0	3.0	94.8*#	3.1	82.9	2.0
30.0	62.4	1.7	75.0*#	2.0	70.0*	1.1
60.0	43.1	2.3	58.1*#	1.7	52.0*	2.2

* significantly different vs ciprofloxacin alone ($p<0.05$, students t-test); #significantly different vs. ciprofloxacin + UF ($p<0.05$, students t-test);



Cytoprotective effects of TF vs UF (10 µg/ml) against the chondrotoxic effects of ciprofloxacin in HTB-94 chondrocytes (MTT test).

Table 7.2. 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 buff. creatine	94.1	1.3	1.15
+ 1 mmol/L buff. creatine	95.2	2.4	1.17
Ciprofloxacin 15 μg/ml	78.2*	1.4	-
+ 0.2 mmol/L buff. creatine	89.1*#	4.3	1.14
+ 1 mmol/L buff. creatine	93.9*#	3.2	1.20
Ciprofloxacin 30 μg/ml	58.8*	4.8	-
+ 0.2 mmol/L buff. creatine	72.5*#	1.1	1.23
+ 1 mmol/L buff. creatine	78.3*#	3.7	1.33
Ciprofloxacin 60 μg/ml	36.1*	3.8	-
+ 0.2 mmol/L buff. creatine	50.8*#	5.7	1.41
+ 1 mmol/L buff. 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).

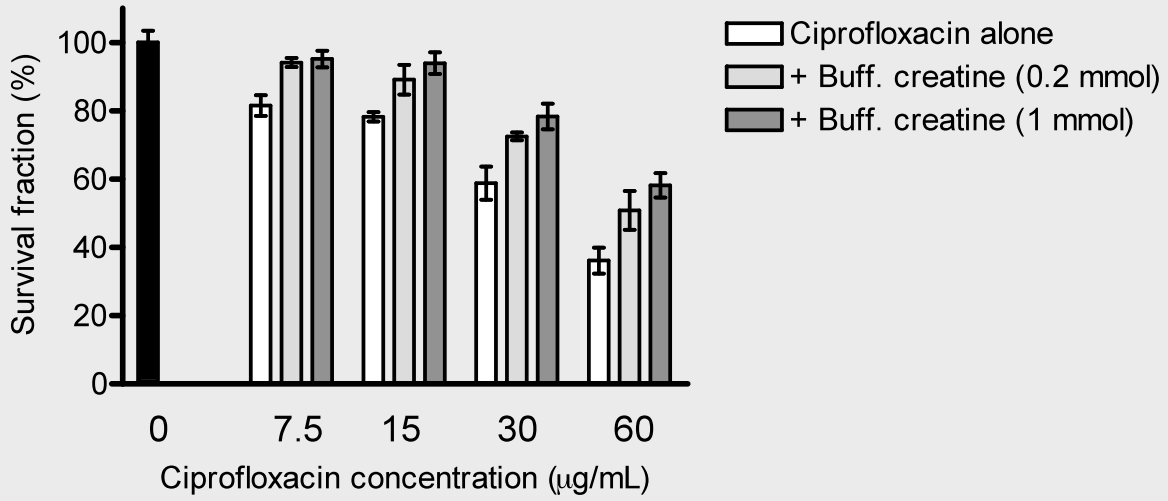


Fig. 7.2. Cytoprotective effects of the buffered creatine formulation against ciprofloxacin-induced cytotoxicity in SW1353 chondrocytes, as assessed by the MTT-dye reduction assay after 72 h incubation. Each column represents the arithmetic mean ± sd (n=6).