Guanidino compounds after creatine supplementation in renal failure patients and their relation to inflammatory status

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Abstract

Background. Specific guanidino compounds have been described as uremic toxins and their concentrations are increased in renal failure due to diminished glomerular filtration, whereas the guanidino compound creatine is used as a performance-enhancing substance in athletes. The present study investigates the effects of creatine supplementation on plasma guanidino compounds in a chronic haemodialysis population.

Methods. Twenty male haemodialysis patients were included in a placebo-controlled cross-over trial. Patients were treated with creatine (2 g/day) or placebo during two treatment periods of 4 weeks, separated by a washout of 4 weeks. Plasma guanidino compounds and routine biochemical parameters were determined, as well as the prognostic inflammatory and nutritional index (PINI).

Results. Upon creatine supplementation, guanidinoacetate concentrations decreased by 15%, due to inhibition of creatine synthesis. Concentrations of α-keto-δ-guanidinovaleric acid increased three-fold and argininosuccinate doubled. Guanidinosuccinate concentrations did not change, but correlated inversely with CRP ($r = -0.736; P = 0.002$) and correlated positively with plasma urea concentration ($r = 0.54; P = 0.02$).

Conclusions. Creatine supplementation in haemodialysis patients significantly altered the concentration of specific guanidino compounds. Guanidinosuccinate correlated positively with plasma urea and negatively with inflammation markers.

Keywords: creatine; guanidino compounds; neurotoxins; urea cycle

Introduction

Guanidino compounds are generated \textit{in vivo} as a result of protein and amino acid metabolism. In general the guanidino compounds acquire the guanidino group from arginine, through two metabolic pathways [1–5]. The major metabolic route (Figure 1) consists of transamidination of arginine to glycine, yielding guanidinoacetate, with subsequent methylation to creatine and further metabolism to creatinine. The transamidination of glycine to guanidinoacetate is mediated through the enzyme L-arginine-glycine transamidase (AGAT; EC 2.1.4.1), while other substrates ($\beta$-alanine, $\gamma$-aminobutyric acid and $\delta$-aminovaleric acid) can also be transamidated through this enzyme yielding $\beta$-guanidinopropionic acid ($\beta$-GPA), $\gamma$-guanidinobutyric acid ($\gamma$-GABA) and $\delta$-guanidinovaleric acid. Furthermore, the $\alpha$-amino group of arginine can be transaminated and acetylated, yielding $\alpha$-keto-$\delta$-guanidinovaleric acid (α-K-$\delta$-GVA) and $\alpha$-$N$-acetylgarginine (α-NAA). Argininc acid (ArgA) can be formed through hydrogenation of α-K-$\delta$-GVA [3,4]. The biosynthesis of guanidinosuccinate (GSA) and methylguanidine (MG) has been proposed to be a consequence of the action of oxygen radical species on arginosuccinic and creatinine [6].

The plasma concentrations of several guanidino compounds are altered in patients with renal failure, mainly as a consequence of reduced excretion and to a minor extent due to increased synthesis. In particular major increases in creatinine, guanidine, guanidinosuccinate and methylguanidine concentrations have been reported. Several studies have suggested their contribution to the uremic syndrome [7–10]. The four major uremic guanidino compounds mentioned above, blocked $\gamma$-aminobutyric acid (GABA)- and glycine-evoked depolarization in mouse spinal cord neurons and were found to be convulsants in experimental conditions [7–10]. More recently, several guanidino compounds were shown to interfere with leukocyte activity and homocysteine protein binding, possibly contributing to cardiovascular disease, which is one of the...
Guanidino compounds after creatine supplementation in renal failure patients

Fig. 1. Summary of the major pathways of the guanidino compound metabolism, relevant to uraemia. The guanidino compounds acquire the guanidino group from arginine, either through transamidation or through the urea cycle (see the introduction for more detailed description of the individual pathways). 1. Transamidation. The synthesis of guanidinoacetate is catalyzed by L-arginine-glycine transamidase (AGAT). 2. Guanidinoacetate methyltransferase (GAMT). 3. Acetylation. 4. Arginase. 5. Ornithine transcarbamoylase. 6. Argininosuccinate synthase. 7. Argininosuccinase. 8. Putative pathways leading to the synthesis of guanidinosuccinate and methylguanidine. These compounds can be synthesized by the action of oxygen radical species (ROS) on creatinine and argininosuccinate. There exists a tight metabolic coupling between guanidinosuccinate and urea. 9. Non-enzymatic degradation. 10. Hydrogenation. 11. Transamination.

major causes of morbidity and mortality in patients with chronic renal disease [11,12].

Creatine has become one of the most popular performance-enhancing dietary supplements in professional and amateur athletic populations [13–16]. Safety concerns of high-dose creatine ingestion have been raised, based on case reports. More specifically no data exist on the effects of creatine supplementation on the guanidino compound metabolism in patients with renal failure [17,18], although creatine administration might be considered in view of the presence of muscle wasting [19] and the disturbed guanidino compound metabolism in these patients.

The purpose of this study was to investigate the safety aspects of dietary creatine supplementation in haemodialysis patients with special interest in the effects of creatine on plasma guanidino compound concentrations. We hypothesized that the guanidino compound concentrations could change due to the arginine-sparing effect of creatine, and we related these concentrations to markers of inflammation, as inflammation can profoundly disturb the amino acid metabolism. We did not aim to investigate the ergogenic effects of creatine in this study population.

Materials and methods

Study design and population

Twenty male maintenance haemodialysis patients, who had been dialyzed for at least 3 months were recruited. Dialysis was performed with a low-flux cellulose triacetate dialyzer (Sureflux-L; Nipro, Osaka, Japan) for 4 to 5 h three times weekly. Exclusion criteria were acute illness, life expectancy <3 months, low compliance due to cognitive, social, or psychiatric problems and inability to provide informed consent. All patients were treated with folic acid, pyridoxine and vitamin B12 orally three times a week. No vitamin C supplements were used in the study protocol. The study was approved by the local ethical committee and written informed consent was obtained from all participants.

The study followed a double-blind, placebo-controlled, cross-over design. Patients received 2 g creatine or placebo daily in the evening during two treatment periods of 4 weeks, in random order, and separated by a washout period of 4 weeks. The creatine dosage (2 g) was selected on the basis of pilot experiments and is used in healthy athletes as a maintenance dose, after initial creatine loading.

Creatine monohydrate (CreaPure®) was obtained from Degussa Bioactives (Freising, Germany). Placebo tablets contained Fast Flo lactose (Foremost Farms, Baraboo, WI, USA). Treatment duration, blood flow, dialysate flow and membrane surface area were determined by the attending nephrologists, but no changes were made during the study period. Single-pool Kt/Vurea was calculated at the start and end of the study, where $K = \text{dialyzer urea clearance}$, $T = \text{duration of dialysis}$ and $V = \text{urea distribution volume at the end of dialysis}$. 
Biochemical determinations

Plasma samples were deproteinized by adding an equal volume of a trichloroacetate solution (200 g/l). Two hundred microlitres of supernatant were analysed by a Biotronic LC 5001 (Biotronik, Maintal, Germany) amino acid analyser adapted for guanidino compound determination. The guanidino compounds were separated over a cation-exchange column using sodium citrate buffers, and detected fluorimetrically after ninhydrin derivatization as previously reported in detail [20,21]. Detected compounds include creatine, creatinine, α-keto-δ-guanidinovaleric acid, guanidinoacetate, guanidinosuccinate, arginine,arginic acid, homoarginine, guanidine, methylguanidine and γ-guanidinobutyric acid.

Plasma urea concentrations and aspartate-aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, creatine kinase and glutamyl transferase (GGT) activities were determined on a Modular P analyser (Roche Diagnostics). Plasma samples were deproteinized by adding an equal volume of a trichloroacetate solution (200 g/l). Two hundred microlitres of supernatant were analysed by a Biotronic LC 5001 (Biotronik, Maintal, Germany) amino acid analyzer adapted for guanidino compound determination.

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GGT activities were determined on a Modular P analyser (Roche Diagnostics, Mannheim, Germany). α1-acid glycoprotein, albumin and prealbumin were determined nephelometrically using commercial reagents (Dade Behring, Marburg, Germany) on a BN II nephelometer. CRP was determined turbidimetrically on a Modular P analyser (Roche Diagnostics).

The prognostic inflammatory and nutritional index (PINI) was calculated as \([\alpha1\text{-acid glycoprotein (mg/l)} \times \text{CRP (mg/l)})/[(\text{albumin (g/l)}) \times \text{prealbumin (mg/l)})].\]

Statistics

Data are expressed as median (interquartile range). Variables were log-transformed and standardized prior to further analysis to improve normality in parametric analyses. In order to estimate the effect of creatine treatment on the concentrations of the different guanidino compounds in this cross-over study, a mixed effect model was fitted treating patient and treatment period as random effects to account for the repeated measurements. Age, body mass index (BMI) and nutritional status (PINI score) were included in the mixed models as covariates, but were found to be non-significant and removed from further analyses. The associations between guanidino compounds and markers of inflammation were investigated using Spearman correlations with only baseline concentrations prior to treatment being analysed to avoid influence of creatine treatment. The analyses were performed using S-plus (S-plus 7.0, Insightful, Seattle, WA). Differences were considered significant at \(P < 0.05\).

Results

Baseline patient characteristics

Table 1 summarizes the baseline patient characteristics. None of the patients suffered from an acute illness throughout the study. Baseline guanidino compound concentrations were comparable to those previously described in dialysis patients, using the same analytical methodology [22]. Routine biochemistry was within the expected range for dialysis patients throughout the study, excluding intercurrent infection, liver or other diseases. No changes in Kt/V were observed during the study period.

Effect of creatine supplementation on guanidino compounds

Plasma creatine concentrations increased 13-fold in response to creatine supplementation, indicative of good patient compliance and intestinal absorption.

Figure 2 illustrates the estimates of the mixed effect linear model examining the effects of creatine supplementation on plasma guanidino compounds.

Significant negative standardized estimates were observed for guanidinoacetate and positive estimates were found for α-keto-δ-guanidinovaleric acid, argininc acid and methylguanidine. GAA was found to decrease significantly by 13% after creatine supplementation (Figure 3) with an inverse linear relationship between changes in plasma creatine and plasma GAA (\(\Delta\text{creatinine}/\Delta\text{GAA}; r = -0.55; P = 0.02\)). Guanidinosuccinate, arginine, homarginine and guanidine concentrations were found to be unchanged by creatine supplementation.

Low-grade inflammation and guanidino compounds in uraemia

Baseline guanidinosuccinate concentrations were inversely correlated with markers of inflammation. A negative correlation was found between guanidinosuccinate and CRP (\(r = -0.74; P = 0.001\)), α1-acid glycoprotein (\(r = -0.62; P = 0.007\)) or PINI score (\(r = -0.72; P = 0.002\)), though a positive correlation was observed between guanidinosuccinate and prealbumin concentrations (\(r = 0.45; P = 0.05\)) or urea concentrations (\(r = 0.54; P = 0.02\)). No correlation with arginine or albumin concentrations (\(r = 0.27; P = \text{NS}\)) was found.

The other guanidino compounds did not correlate with inflammation or nutritional status (data not shown).

Discussion

The present study is the first one to examine the effects of exogenous creatine supplementation on guanidino
compound metabolism in haemodialysis patients. Significant changes in the concentrations of specific plasma guanidino compounds were observed when oral creatine supplements were given in this placebo-controlled cross-over trial.

In our patients with renal impairment, several-fold increases in specific guanidino compounds were observed due to the impaired renal function and altered metabolism, whereas some of these guanidino compounds can be related to uraemic toxicity [22].

In line with animal studies and studies in healthy volunteers [23–25], we were able to demonstrate a reduction in plasma guanidinoacetate by creatine supplementation in haemodialysis patients. Whereas baseline guanidinoacetate concentration is slightly increased in haemodialysis patients [26], our data suggest that creatine supplementation can reduce guanidinoacetate concentrations by repression of the AGAT enzyme, the first and committed step during creatine synthesis in vivo, even under conditions where the guanidino metabolism and one-carbon metabolism are disturbed. In this regard, creatine should be considered as an important regulator of creatine synthesis and by extension of other guanidino compounds under conditions with normal and reduced renal function. Differences in response to creatine treatment were observed between patients, where the decrease in guanidinoacetate was related to the increase in creatine concentrations.

In our haemodialysis patients, creatine supplementation was found to increase α-keto-δ-guanidinovaleric acid and argininic acid concentrations. It has been suggested that α-keto-δ-guanidinovaleric acid could be synthesized in vivo by transamination from arginine and could be further metabolized to argininc acid [4]. In hyperargininemic patients, decreased guanidinosuccinate and increased α-keto-δ-guanidinovaleric and argininic acid concentrations (♦ indicates mean estimate, whereas bars represent the 95% confidence intervals).
concentrations have been described [27,28], which further supports the idea that the altered guanidino compound pattern is secondary to an increased arginine availability after creatine supplementation [25], but are unlikely to generate harmful side effects at the usual dosages (5–20 g) of creatine; the attained concentrations in our haemodialysis patients were much higher. Therefore, safety aspects of creatine should not be generalized under conditions with reduced renal function.

Concentrations of guanidinosuccinate are very low in healthy volunteers, but increase up to 500-fold in patients with renal failure. The metabolic pathways of guanidinosuccinate remain incompletely understood [6,29–31]. The relation between guanidino compound metabolism and inflammation has not been investigated thoroughly. A close metabolic coupling between guanidinosuccinate and urea has been suggested whereas the relationship between arginine and guanidinosuccinate remains unclear [31]. In our study population, we did not demonstrate significant changes in guanidinosuccinate concentrations after creatine supplementation (Figure 2), and no relation between arginine and guanidinosuccinate concentrations was observed. A positive relation between guanidinosuccinate and urea concentrations was found, supporting the guanidino cycle hypothesis [31].

We did observe, however, a strong correlation of baseline inflammatory and nutritional markers with guanidinosuccinate concentrations. After creatine supplementation, this relationship remained significant. As creatine supplementation influences the guanidino compound concentrations, only baseline data were used in further analyses.

Low-grade inflammation in uremia could influence the metabolism of the different guanidino compounds or amino acids. As inflammation in uremia is associated with malnutrition, the negative association of the inflammatory markers and the positive association of the nutritional markers with guanidinosuccinate concentrations could be explained by the actual nutritional status, food intake and resulting amino acid metabolism. The synthesis of guanidinosuccinate is proportionally linked to the rise in the serum urea level in patients with renal failure. Urea has been described as a specific inhibitor of argininosuccinate, the fourth urea cycle enzyme (Figure 1, enzyme 7) that leads to an increased concentration of argininosuccinate [6]. Further metabolization of argininosuccinate to guanidinosuccinate is mediated through the action of oxygen radicals. Inflammation could influence guanidinosuccinate concentrations through a disturbed amino acid metabolism with elevated urea concentrations and increased argininosuccinate availability.

The observed relationship between guanidinosuccinate concentrations and inflammation could, however, also be explained through inhibition of the immune response. Guanidinosuccinate and guanidinopropionate can inhibit neutrophil superoxide production [30,32] and a mixture of guanidino compounds was found to suppress the natural killer cell response to interleukin-2 [33].

In a recent study, Glorieux et al. [34] investigated the relationship between specific guanidino compounds and stimulated leucocytes. Methylguanidine and guanidine stimulated the proliferation of undifferentiated HL-60 cells, whereas the antiproliferative effect of calcitriol was neutralized in the presence of methylguanidine and guanidinosuccinic acid. Methylguanidine and guanidinoacetic acid enhanced the lipopolysaccharide-stimulated intracellular production of tumour necrosis factor-α by normal human monocytes. Though several hypotheses can be formulated, further research is necessary as the regulation of these pathways at a molecular level remains unknown.

Although these in vivo data cannot fully establish a causal relationship between guanidino compounds and inflammation, our data in haemodialysis patients suggest a significant relationship in vivo, possibly contributing to the cardiovascular morbidity and mortality.

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Conflict of Interest Statement. None declared.

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