Creatine kinase and blood pressure: Clinical and therapeutic implications

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Acute effect of beta-guanidinopropionic acid and creatine: study protocol

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ABSTRACT

Background Beta-guanidinopropionic acid (βGPA), a creatine analogue, can be obtained without prescription on the internet and used by sportsmen to increase muscle mass and endurance capacity. However, to our knowledge, there are no published data on the effects and side effects of this substance. In contrast, creatine is used to improve short-duration/high-intensity exercise. Although many studies assessed the effect of creatine on muscle performance, none of those studies reported the effect on hemodynamic parameters.

Objective In this study, we will assess 1) the tolerability of acute oral administration of βGPA 100 mg versus placebo in healthy male volunteers and 2) if tolerated, βGPA 100 mg during 1 week. In phase 2 we will compare βGPA with placebo and creatine 5 g.

Methods Study design: Randomized, double-blind, placebo-controlled, study.
Study population: Healthy male volunteers, 18-50 years old. 1) 16 subjects; 2) 24 subjects.
Intervention: 1) Oral administration of βGPA 100 mg or placebo. 2) One week of daily oral administration of βGPA 100 mg, creatine 5 g, or placebo.
Main study parameters/endpoints Tolerability for βGPA assessed with a questionnaire; hemodynamic parameters, including blood pressure, heart rate, cardiac output, and total peripheral resistance; biochemical parameters in serum, including creatine kinase, βGPA, creatine, glucose, insulin, sodium, potassium, creatinine, and urine, including βGPA, creatine, creatinine, urea, sodium, potassium, after acute oral administration of βGPA and one week of intervention with βGPA or creatine.
Background

Beta-guanidinopropionic acid (βGPA or N-(aminoiminomethyl)-beta-alanine; C₄ H₉ N₃ O₂) can be obtained without prescription on the internet for human use. This amino acid is a naturally occurring creatine analogue, a structural isomer to creatine (C₄ H₉ N₃ O₂), which has an identical molecular formula (Figure 1).² It is is used by sportsmen to induce greater endurance capacity and promote weight loss.

The amino acid βGPA can be generated in vivo via transamidation of β-alanine.²³ The physiological concentration (without external supplementation) in human plasma is reported to range from 0.02 to 1.40 µmol/liter.⁴⁻⁶ Clearance is probably renal, as the structural isomer creatine is cleared renally as well and increasing plasma concentrations were reported in chronic renal failure, ranging from 0.18 to 180 µmol/liter.⁴,⁷,⁸

βGPA acts as a competitive inhibitor of cellular creatine uptake, attenuating the flux through the creatine kinase (CK) reaction.¹⁻⁹ CK catalyzes the rapid and reversible transfer of a phosphate group from creatine phosphate to ADP, thereby forming creatine and ATP.¹⁰ The flux through the CK reaction is linearly related to the concentration of creatine.¹¹ βGPA is also phosphorylated by CK in cytoplasm, but both βGPA and phosphorylated βGPA are “inefficient substrates” for the CK reaction: in vitro Vmax values are <1% of the Vmax values of creatine and phosphocreatine.¹²,¹³ Therefore, βGPA may modulate the energy status of tissues, from fast activity bursts to slow endurance performance (Table 1).
Table 1. Characteristics of skeletal muscle fiber types.

<table>
<thead>
<tr>
<th>Type II</th>
<th>Type I</th>
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<tbody>
<tr>
<td>High CK</td>
<td>Low CK</td>
</tr>
<tr>
<td>Predominantly glycolytic</td>
<td>Predominantly oxidative</td>
</tr>
<tr>
<td>Mitochondria poor</td>
<td>Mitochondria rich</td>
</tr>
<tr>
<td>Capillary rarefaction</td>
<td>High density of capillaries</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Burst exercise</td>
<td>Endurance capacity</td>
</tr>
<tr>
<td>Low GLUT-4 expression</td>
<td>Higher GLUT-4 expression</td>
</tr>
<tr>
<td>Insulin Resistant</td>
<td>Insulin sensitive</td>
</tr>
<tr>
<td>Less glucose uptake</td>
<td>High glucose uptake</td>
</tr>
<tr>
<td>Glucose and fatty acid stored as lipid</td>
<td>Glucose and fatty acid utilisation</td>
</tr>
<tr>
<td>Obesity prone</td>
<td>Lean</td>
</tr>
<tr>
<td>Hypertension prone</td>
<td>Normotension</td>
</tr>
</tbody>
</table>

CK, creatine kinase; GLUT-4, insulin-dependent glucose transporter protein 4. BGPA has been shown to induce a shift from type II to type I fiber predominance, comparable with the effects of endurance exercise.

Animal studies, in which rats or mice received βGPA (1 to 2%) in the diet, showed skeletal muscle fiber type and metabolic enzyme transitions qualitatively similar to the adaptations of endurance training. These alterations included a transition from type II to type I muscle fiber type predominance, with a concomittant increase in markers of oxidative metabolism, improved glucose tolerance, increased plasma membrane fatty acid transporter expression, and a commensurate decrease in glycolytic potential. Furthermore, it was anecdotally reported in animal studies that chronic intervention with βGPA (1%) in the diet resulted in hemodynamic alterations comparable with the effect of endurance training. Importantly, in these rodent studies, the animals appeared healthy after administration of very high doses of βGPA of more then 1% daily in the diet for more than 8 weeks (i.e. 200 milligrams βGPA in 20 grams food daily to an animal weighting 200 to 300 grams, or 600 to 1000 mg/kg animal weight), and the effect was reversible within 1 month after withdrawal. However, despite its human use, there is a lack of scientific data on the effects of βGPA on hemodynamic and biochemical parameters in humans. To our knowledge there are no US FDA reports on any side effect (www.fda.gov).
Creatine, the structural isomer of βGPA, is one of the most popular dietary supplements in the world. In contrast to βGPA, it is used to improve sport performance during short-duration/intensity exercise. Numerous studies have assessed the effect of creatine on muscle performance in healthy athletes and on health improvement a variety of clinical conditions. However, there is lack of data on the effect of creatine on hemodynamic parameters. Therefore, we will assess the effects of βGPA and creatine on biochemical and hemodynamic parameters.

**OBJECTIVES**

**Phase 1: βGPA in healthy male volunteers versus placebo**
The primary objective will be to assess the tolerability of oral administration of a single dose of βGPA. Other objectives include the assessment of the acute effect of oral administration of a single dose of βGPA on hemodynamic parameters, including blood pressure, heart rate, cardiac output, and total peripheral resistance, and to assess the acute effect of βGPA on biochemical parameters.

**Phase 2: βGPA in healthy male volunteers (if Phase 1 is uneventful) versus placebo and creatine**
The primary objective will be the assessment of tolerability of one week of intervention with βGPA. Other objectives will be to assess the effect of one week of oral administration of βGPA and creatine on hemodynamic parameters, including blood pressure, heart rate, cardiac output, and total peripheral resistance, and biochemical parameters.

**METHODS**

We will include 40 male volunteers (16 in phase 1, 24 in phase 20). Exclusion criteria include glucose, lipid spectrum, thyroid, kidney, or liver abnormalities, history of cardiovascular disease including TIA and stroke; CK-increasing drugs including statins; neuromuscular or endocrine disorders; vasculitis; HIV infection; infectious hepatitis; and bleeding disorders.

In phase 1 the volunteers will be randomly assigned by a computer-generated random number system to receive a single dose of βGPA (100 mg) or an identical looking placebo. The participants and the investigator will be blinded. Treatment allocation will be coordinated by an independent investigator. We will proceed to phase
2 if 100 mg is confirmed to be the NOAEL DOSE (no more adverse effects than placebo; safety measures we took to calculate this dose included calculation of HED using a close allometric relationship, and application of the tenfold safety factor). If 100 mg is not NOAEL we will assess, depending on the adverse effects observed (no overt or surrogate toxicity present), a single dose of 50, 10, or 1 mg/day, in that order in Phase 1. If 1 mg is not NOAEL, we will terminate the study for further ex vivo tests. If the NOAEL dose is established in phase 1, we proceed to phase 2. In phase 2 the volunteers will be randomly assigned by a computer-generated random number system to receive βGPA in the NOAEL DOSE, creatine 5 gram, or placebo during 1 week. As the capsules of βGPA and creatine can not be made identical, we will use the double-dummy design. All subjects take two sets of treatment: βGPA 100 mg or the equivalent placebo and creatine 5 g or the equivalent placebo. The participants and the investigator will be blinded.

Test products: βGPA

In accord with the definition for food supplements in the legislation of the European Union, we consider βGPA as well as creatine food supplements, as both substances are naturally occurring amino acids with a physiological effect (please see background). BGPA is a white, crystalline tasteless powder, soluble in water. BGPA powder is ordered at SeqChem (Sequoia Research Products). There are no reports or bans on this product or the company to our knowledge, presented on the FDA website using the FDA search engine, or online with search engine Google, as for February the 20th 2012. BGPA, creatine, and identical placebo capsules will be manufactured by the Pharmacy & Pharmacology department of the Slotervaart Hospital, Amsterdam. This department is GMP certificated (ISO 9001:2001). BGPA is sold in the U.S. and not in European countries. According to the legal guidelines of the European Union, criteria of international organs, generally accepted criteria, or national criteria are approved when a supplement is not listed in the legislation of the European Union. According to de U.S. FDA guidelines: We first qualified the supplier by establishing the reliability of the supplier, with the methods as mentioned above. Next, the substance was tested for purity, and for cyanide compounds. Cyanide was not expected to be present. However, the cyano-group in cyanamide, one of the compounds used in the formation of βGPA, provides a possible source of cyanide. We established in our tests in Amsterdam a purity of more than 99% (detection limit) and a cyanide level lower than 1 p.p.m (detection limit) (Supplement 1 and 2). Cyanide occurs in many food items, with high concentrations in cassava roots, almonds and apricot kernels (“marsepein”)
containing up to 1000-3000 mg/kg (1 part per thousand) (www.vwa.nl). The maximum allowed level of cyanide in food items is found in Annex II of Guideline 88/388/EEC (http://ec.europa.eu/food/fs/sfp/addit_flavor/flav09_en.pdf) and is 1 mg/kg in food or drinks, with the exception of the Dutch treats “noga” and “marsepein” or similar products, where 50 mg/kg is allowed (EEC, 1988; www.vwa.nl and http://ec.europa.eu/food/fs/sfp/addit_flavor/flav09_en.pdf). Because of concerns with these high levels, the Dutch Food Consumer Product Safety Authority has thereafter established a maximum daily cyanide intake of 0.05 mg/kg/day (www.vwa.nl; http://www.vwa.nl/actueel/nieuws/nieuwsbericht/10782), this is a total intake of 3.75 mg/day in a 75 kg man. With 100 mg GPA with <1 p.p.m. cyanide, the contribution to the daily intake in a 75 kg man will be <0.0001 mg/day or <0.00001 mg/kg/day.

Dose calculation
We used the FDA guidance on Estimating the Maximum Safe Starting Dose in Initial Clinical Trials in Adult Healthy Volunteers. This guidance outlines a process for deriving the maximum recommended starting dose (MRSD) for first-in-human clinical trials in adult healthy volunteers, and recommends a standardized process by which the MRSD can be selected. The purpose of this process is to ensure the safety of the human volunteers.28,32

NOAEL determination
In animal studies βGPA was administered through the diet in concentrations of 1% or more during 8 weeks without apparent adverse effects.13-16 In animals weighting 200 grams, eating estimated 20 grams per day, we calculated a “no observed adverse effect level” of 1000 mg/kg/day. Furthermore, in a patent application, Meglasson et al. recommended a human dose of 1 to 500 mg/kg/day based on his research in mice and rhesus monkeys.33 In this paper rhesus monkeys weighting 9 kg were treated with oral βGPA 48 mg/kg/day (432 mg per monkey per day) during 2 weeks without apparent adverse events.

Conversion of the NOAEL to HED
We converted the oral NOAELs in rats and monkeys (resp. 1000 mg/kg/day and 48 mg/kg/day) to human equivalent oral doses (HED) based on an algorithm proposed by the FDA based on body surface area.32 This algorithm proposes a conversion factor from rat to human of 0.16 times the rat dose; and of monkey to men of 0.32 the monkey
dose (in mg/kg/day; for a man of 60 kg) resulting in HEDs of resp. 160 mg/kg/day and 15 mg/kg/day for a man of 60 kg.

**Safety factor**

A safety factor should be applied to the HED to increase assurance that the first dose in humans will not cause adverse effects. The use of the safety factor is based on the possibility that humans may be more sensitive to the toxic effects of a substance than predicted by the animal models, that bioavailability may vary across species, and that the models tested do not evaluate all possible human toxicities, or cannot be expressed by animals or easily measured, such as headache or nausea. We conservatively chose 15 mg/kg/day oral dose for our final calculations of the human dose, because this is the lowest dose, and because of the closer allometric relationship between monkey and man. FDA advises a safety factor of at least 10. Based on an average weight of a male volunteer of 75 kg, we calculated a starting oral dose for the phase 1 study of 75*1.5 mg/day=112.5 mg/day. We will start with 100 mg/day.

**Creatine**

For creatine, we will use an oral dose 5 g per day. For healthy omnivoric males the average daily rate of creatine synthesis is estimated to be 1.3 g. Most studies on creatine supplementation, in order to increase sport performance, use a loading dose of 20 gram creatine during 5 days to increase muscle creatine content by 20%. Thereafter, a maintenance dose of 3-5 grams is used. With this dose no side effects are reported. Thus, we will use a dose of 5 gram, as it would be a sufficient dose to increase the daily rate of creatine synthesis, and this dose is frequently used by sportsmen.35,36,37

**STUDY MEASURES**

**Phase 1**

The participants will be asked to come to the hospital for visit 2 after an overnight fast. After baseline measurements they will receive 1 capsule of βGPA 100 mg, or placebo. Furthermore, the participants will receive a questionnaire to assess health status at 1h, 2h, 6h, 12h, 24h and after 1 week after the ingestion of βGPA. We have no data on the speed of the resorption of βGPA (C4 H9 N3 O2). However, the acute effects of the ingestion of the structural isomer creatine (C4 H9 N3 O2) on plasma creatine concentration have been reported.38 We consider the creatine data useful for our estimation. In the report
by Schedel et al, it was shown that the peak plasma level after the oral ingestion of 20 g creatine occurred after 2.5 hours. Six hours after the ingestion of 20 grams of creatine, plasma concentration was 50% of the maximum concentration. Thus, we will assess hemodynamic and blood and urine laboratory parameters during the first 8 hours after ingestion of βGPA, after 12, and after 24 hours. A timeline is shown in Figure 2.

**Systemic cardiovascular hemodynamics and laboratory studies**

(At baseline and at t=30, t=60, t=90, t=120, t=180, t=240, t=360, t=480 minutes, t=12h, t = 24h). Supine and sitting resting blood pressure; heart rate, cardiac output and total peripheral resistance will be measured, using a Bmeye Nexfin blood-pressure monitor for continuous non-invasive finger arterial blood pressure measurement, with an adjusted cuff size on the non-dominant arm, at heart level.

Laboratory studies will include serum βGPA, resting serum CK (after 3 days without heavy exercise), glucose, insulin, lipid profile, creatine, creatinine, liver enzymes (ASAT, ALAT, gamma GT), TSH (subclinical hypothyroidism as a cause of high CK), sodium, potassium, timed urine collection (βGPA, creatine, creatinine, urea, sodium, potassium).

**Figure 2. Time line during 24 hours after the ingestion of a single dose βGPA or placebo.**

Study time line in phase 1. After the baseline measurements the participant is asked to ingest a capsule with βGPA 100 mg or placebo. Hemodynamic and biochemical parameters are recorded during 24 hours after the ingestion.
Phase 2

The participant will come to the hospital for assessment of medical history, physical examination including weight and height, blood pressure, an electrocardiogram, and parameters of systemic cardiovascular hemodynamics, and fasting laboratory blood and urine studies. The participants will be asked to come to the hospital for visit 2 after an overnight fast. After baseline measurements they will receive the first capsule with βGPA 100 mg, creatine 5 g, or placebo in the hospital. They will receive capsules for 2 days and they will be instructed to consume the capsule on each day within 5 minutes before breakfast on a fixed time. On day 4 and day 7 the participant will be asked to come to the hospital after an overnight fast. At those visits the participants will ingest the capsules in the hospital. The participants will receive a questionnaire to assess tolerability at home during the week of intervention and 2 weeks after the intervention. At the hospital visits on days 4 and 7 of the intervention period the investigator will assess tolerability with a questionnaire. On days 4 and 7 of the intervention period, we will assess hemodynamic and laboratory parameters. A timeline is shown in Figure 3.

**Figure 3. Time line during phase 2.**
Study time line for 1 participant. After the baseline measurements the participants will be randomized between βGPA, placebo, creatine 5 g during one week with a follow-up period of two weeks.
**Systemic cardiovascular hemodynamics and laboratory studies**

At baseline and at day 4 and 7 of the intervention supine and sitting resting blood pressure; heart rate, cardiac output and total peripheral resistance will be measured, using a Bmeye Nexfin blood-pressure monitor for continuous non-invasive finger arterial blood pressure measurement, with an adjusted cuff size on the non-dominant arm, at heart level, ambulatory 24-hour blood pressure monitoring. Labatory studies will include serum βGPA, resting serum CK (after 3 days without heavy exercise), glucose, insulin, lipid profile, creatine, creatinine, liver enzymes (ASAT, ALAT, gamma GT), TSH (subclinical hypothyroidism as a cause of high CK), sodium, potassium, urine collection (creatine, creatinine, urea, sodium, potassium) at baseline; and serum βGPA, serum CK, glucose, insulin, creatine, creatinine, liver enzymes (ASAT, ALAT, gamma GT), sodium, potassium, timed urine collection (βGPA, creatine, creatinine, urea, sodium, potassium) at day 4 and 7 of the intervention.

**Data analysis and statistics**

The main study parameter is the tolerability of βGPA after oral administration in healthy male volunteers versus placebo. Other parameters are hemodynamic parameters (Resting blood pressure; heart rate, cardiac output and total peripheral resistance, measured non-invasively) after oral administration of βGPA in healthy male volunteers versus placebo and creatine. Biochemical parameters, including resting serum CK, βGPA, creatine, creatinine, glucose, insulin, liver enzymes (ASAT, ALAT, gamma GT), sodium, potassium, timed urine collection during 24 hours (standard urine assessment, sodium, potassium, βGPA, creatine, creatinine, urea).

This is a first-in-man study with βGPA, with allometric data available from other species. According to the EMEA guidelines we will include 8 subjects in each arm to assess tolerability of a single dose of βGPA versus placebo and we will include 8 subjects to assess tolerability of βGPA versus placebo during one week.
REFERENCES


