Pharmacokinetics of Arginine and Related Amino Acids[1–3],

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ABSTRACT

Arginine (ARG) and its related amino acids (AAs) ornithine (ORN) and citrulline (CIT) find a range of applications as dietary supplements in subgroups of healthy subjects (e.g., bodybuilders) and patients with acute or chronic malnutrition. These AAs appear to be well utilized in humans with, in general, a rapid return of blood concentrations to basal values (i.e., within 5-8 h) and low absolute and relative excretion in urine (< 5% of administered dose). Based on published data for the maximum observed plasma concentrations (C_{max}) after administration of doses in the range 5 to 10 g, CIT appeared to present relatively better absorption and systemic bioavailability than ARG and ORN. The few relevant dose–ranging studies available include 1 limited to a single subject receiving 5– to 20–g doses of ornithine α–ketoglutarate and another in which 8 subjects received from 5 to 15 g of CIT. Comparison of these 2 studies further indicates that CIT has higher bioavailability than ORN. The pharmacokinetics and metabolism of these AAs are modified by the coadministration of a salt such as α–ketoglutarate that modifies AA metabolism, as has clearly been demonstrated for ornithine α–ketoglutarate. Concomitant administration of a meal leads to a 15– to 30–min delay in C_{max}. Finally, data from various pharmacokinetic studies together with basic physiology and biochemistry indicate that ARG is a net urea producer and ORN has a nitrogen–sparing effect, whereas CIT is neutral. However, most of the studies performed to date carry methodological weaknesses and are difficult to compare because of a number of confounding factors. To date, there have been no pharmacokinetic studies on the long–term administration of these AAs in healthy subjects despite the need to determine the safe upper limit of daily intake.

Arginine (ARG)[4] and the related amino acids (AAs) ornithine (ORN) and citrulline (CIT) are widely used at pharmacological dosages for various purposes in a number of situations, e.g., athletes, bodybuilders, the elderly, and immunocompromised patients (see other contributions in this supplement issue of the *Journal* for details and references). The main aim of AA supplementation in subgroups of healthy subjects is to elicit growth hormone secretion ([1](#)) or to sustain nitric oxide production ([2](#)).

Pharmacokinetic studies are useful in characterizing the behavior of the administered AA and improving our understanding of their mechanism of action in terms of the metabolites involved ([3](#)) and may be used as a tool to establish an upper limit for safe administration in humans ([4](#),[5](#)). Furthermore, when AAs are considered as drugs, which is the case in some national markets in Europe, pharmacokinetic studies are a compulsory prerequisite to any clinical trials.

This article aims to summarize the published pharmacokinetic data on ARG and related AAs and to identify influencing factors (e.g., associated salts that can influence pharmacokinetic profile, diet conditions, etc.).

Pharmacokinetic studies performed in healthy subjects using continuous enteral nutrition ([6]) have not been considered because the study conditions are not physiological. Similarly, studies performed in pathological conditions ([7]) or in experimental animal models ([8]) are not discussed.

Pharmacokinetic studies in the fasting state

Arginine. In the study by Tangphao et al. ([9]), 10 healthy volunteers (6 M, 4 F) aged 23–52 y received 10 g of L–ARG in 100 mL water. Blood samples were taken for up to 8 h in heparinized tubes. ARG administration led to a 3–fold increase in plasma ARG concentrations with large differences between subjects. The mean apparent C_{max} was ≈300 μmol/L. Interpretation of the data is complicated by the fact that subjects received a meal 2 h after the ARG load, and the article does not provide indications on the composition of the meal.

Ornithine. In a study in 6 healthy young men ([10]), ORN hydrochloride was given as a single bolus dose of 6.4 g, and 15 blood samples were collected over the following 5 h. The peak plasma ORN concentration (C_{max}) was 541 ± 85 μmol/L and occurred at 60 min (apparent T_{max}). The C_{max} for ORN was accompanied by an increase in plasma glutamate (GLU) concentration. Plasma ORN levels had not returned to basal values at the end of the study (i.e., 300 min postingestion). A limited amount of ORN was detected in urine, with the maximum at 1–2 h after ingestion (7.0 ± 1.1 μmol/h vs. 1.7 ± 0.6 μmol/h at the basal state; $P < 0.05$). Plasma urea concentrations decreased ($P < 0.01$) by ~10% after ORN ingestion.

In a different study ([11]), following an overnight fast, bodybuilders (19 to 40 y old, 9 men and 3 women) received oral doses of 40, 100, and 170 mg/kg L–ORN hydrochloride in a random fashion with a 1–wk washout between doses. Blood samples were drawn at baseline and at 45 and 90 min postadministration. Because only 2 postadministration samples were collected, it is not meaningful to report the apparent T_{max}. The reported C_{max} was 605 μmol/L after 170 mg/kg (which is equivalent to 133 mg of ornithine/kg, or 11.4 g for a mean male body weight of 82.5 kg; the subjects showed a range of body weights from 61.7 to 107.6 kg). Four of the men had an abnormally high body mass index, which probably reflects a high fat–free mass because the study population were bodybuilders. However, there was no attempt to analyze body composition.

Citrulline. CIT is a nonprotein AA that is present in substantial amounts in watermelon (*Citrullis vulgaris*), with a mean content of 2.1 mg/g fresh weight, ranging from 0.5 to 3.6 mg/g according to variety ([12]). A pharmacokinetic study ([13]) was performed in 6 healthy adults receiving 3.3 kg wet weight of the red fruit of a ripe watermelon; the study is limited by uncertainty as to the actual amount of CIT ingested, but the mean CIT intake was probably ~7 g. Plasma AAs were measured at 0, 1, and 2 h postadministration. The apparent C_{max} for CIT was 593 μmol/L (range 386–1069 μmol/L); plasma ARG increased from 65 μmol/L at baseline to 199 μmol/L (128–251 μmol/L) at 2 h postadministration, whereas glutamine and other AAs remained unchanged. Urinary excretion of CIT over the 4 h following the test was 165 μmol/mmol creatinine.

Moinard et al. ([14](#)) studied 8 young male healthy adults (age: 27.6 ± 1.5 y; BMI = 22.3 ± 0.5 kg/m²) who received 2, 5, 10, or 15 g CIT in random order on separate occasions, with a 15-d washout between doses. Blood was drawn 10 times over an 8-h period for plasma AA measurement. The 24-h urine samples were collected before and after CIT administration. Only the plasma levels of CIT, ARG, and ORN were affected by CIT administration. The T_{max} of CIT was 0.72 ± 0.08 h, and the C_{max} was 2756 ± 70 μmol/L following the 10-g administration. Urinary excretion over the 8 h following administration was minimal (i.e., <5% of ingested dose), even at the highest CIT dose level.

Influence of the associated salt on pharmacokinetics

Ornithine α-ketoglutarate. In the study described above ([10](#)), subjects also received 10 g of ornithine α-ketoglutarate (OKG) corresponding to 6.4 g of ORN. Compared with ORN hydrochloride, OKG administration led to a slight increase in apparent T_{max} (i.e., 75 min) and a nonsignificant decrease in C_{max} (494 ± 91 μmol/L). Compared with ORN, OKG administration led to lower GLU production but higher plasma concentrations of proline (PRO), ARG, and CIT. Furthermore, at 60 min after the OKG load but not after the ORN load, there were significant linear correlations between plasma levels of ORN and the concentrations of ARG, PRO, and GLU. Thus, ORN and OKG interact so that OKG generates a different metabolic profile than ORN alone. The mechanism underlying this action is described elsewhere ([3,15,16](#)).

Arginine α-ketoglutarate. Ten healthy trained adult men (30-50 y) fasted for 8 h before receiving 4 g of arginine α-ketoglutarate (AKG). Blood samples were taken for 8 h following AKG ingestion. After ingestion, apparent ARG T_{max} was 1 h, and the incremental increase in ARG was modest, rising from ~75 μmol/L to ~140 μmol/L ([17](#)). It is not clear whether the low plasma ARG increase observed in this study compared with others was the result of the low ARG dose or because ARG was combined with AKG or because the subjects followed physical training (which may modify intermediary metabolism). Also, the subjects were given orange juice during the trial, and this may be an additional confounding factor.

Influence of feeding on pharmacokinetics

Influence of a meal. In a study ([18](#)), 10 healthy young adults (5M/5F; mean age 27 y) received 10 g of OKG immediately after a standardized breakfast (125-mL cup of tea, 5 g of proteins, 1 g of lipids, and 19.3 g of carbohydrate). The control group (including 6 of the 10 subjects given the OKG load) received only water after the test meal. Venous blood was sampled for up to 420 min after the OKG dose. The apparent T_{max} was 90 min (mean of $n = 10$ subjects), indicating that absorption of a meal delayed the T_{max} by ~15 min. Plasma ORN returned to basal values after 5 h. Urinary excretion of ORN increased during the 24 h following OKG administration compared with the previous 24 h (240 ± 83 μmol/24 h vs. 76 ± 24 μmol/24 h, respectively, $P < 0.001$). In addition, OKG administration induced an increase in GLU + GLN and PRO and a decrease in plasma branched-chain AAs (BCAA) and aromatic AAs. Finally, insulin secretion in this study was fairly high ([18](#)) compared with that in studies performed in fasted subjects receiving the same OKG load ([10](#)). Consequently, the subjects suffered hypoglycemia (<4 mmol/l) at 1 h postload. In addition, 1 subject received on different occasions (with 15-d washout periods) 0, 5, 10, 15, and 20 g of OKG, and the data showed that there was a nonlinear relation between dose and C_{max}.

Influence of glucose intake. In a study on the effect of simultaneous energy administration on OKG metabolism ([19](#)), 4 healthy young volunteers who were given 3 test loads: 10 g OKG, 75 g glucose, and a combination of both. In the presence of glucose, the apparent T_{max} of ORN was increased to 120 min, again underlining the role of gastric emptying in ornithine pharmacokinetics. The C_{max} was ~700 μmol/L after OKG load and 400 μmol/L after OKG plus glucose (the difference was not statistically significant because of the large coefficients of variation). As expected from previous studies ([10](#),[18](#)), administration of OKG alone led to an increase in plasma GLU, PRO, and ARG, but this effect was attenuated or even abolished by glucose. Both OKG and glucose resulted in decreased plasma BCAA levels, and the effect was additive when OKG and glucose were given together. This additive effect could reflect different targets for OKG and glucose: cellular uptake, transamination rate, and protein synthesis (an insulin-mediated effect).

Comparative bioavailability of arginine, ornithine, and citrulline

Ideally, bioavailability should be determined from the areas under the plasma concentration-time curves (AUC) after both oral and intravenous dosage. Unfortunately, most of the studies published did not report the AUC. In the absence of AUC data, C_{max} values can be compared that indicate the extent of absorption, but it should be borne in mind that this is an approximate measure, with a number of confounding factors, not only those identified above, but also differences in methods of dosage and preanalytical treatment of samples, which may affect the results ([5](#),[20](#)).

However, although approximate, the C_{max} comparison is interesting: single oral doses of ~10 g resulted in C_{max} values of ~300 μmol/L for ARG, 600 μmol/L for ORN, and 2800 μmol/L for CIT. These results reflect the physiological metabolism of these AAs in the splanchnic systems because ARG is highly metabolized in both the intestine and the liver ([21](#)), as is ORN, albeit to a lesser extent ([22](#)), whereas CIT is not metabolized in the gut and is not taken up by the liver ([23](#)).

In addition, it is important to consider the ability of these different AAs to generate metabolites of interest. ARG controls NO synthesis ([24](#)), ORN (as OKG salt) is a powerful generator of GLN, polyamines, and PRO ([15](#)), whereas CIT regulates the de novo synthesis of ARG and therefore controls ureagenesis and NO production ([23](#)).

Another perspective to take into account is the behavior of these AAs with regard to urea production. Arginine is a net urea producer (ARG → ORN + urea), whereas ORN has a net nitrogen-sparing effect (ORN + $2NH_4^+$ → ARG). Exogenous CIT is almost urea neutral because it is neither taken up nor released by the liver.

Effects of long-term administration on pharmacokinetics

OKG has been administered to elderly subjects for 1–3 mo without any metabolic side effects ([25](#)). However, no assessments of long-term administration (i.e., over 1 y and more) or pharmacokinetic studies have been performed.

Interestingly, Tangphao et al. ([26](#)) undertook repeated pharmacokinetic studies (every 4 wk) over a 12-wk course of oral administration 3 times per day of 5 or 7 g ARG. Despite the fact that this study was performed in hypercholesterolemic subjects, its relevance to the topic addressed (i.e., definition

of safe upper limits of intake) is sufficiently important to be reported here. There was no modification of the postdose AUC of ARG over time, despite the fact that the predose plasma ARG concentrations increased significantly with time. This apparent discrepancy could result from the fact that predose concentrations reflected the 3 loads of the previous day, whereas the pharmacokinetic study was performed after a single 5- to 7-g load. The fact that subjects were hypercholesterolemic is a confounding factor. Cholesterol interferes with caveolin–nitric oxide synthase binding by maintaining nitric oxide synthase in a nonfunctional form ([27]). However, nitric oxide synthesis accounts for only 5% of ARG disposal, and therefore, it is unlikely that accumulation of ARG in plasma over the course of the treatment in hypercholesterolemic subjects results from modifications in the conversion of ARG to nitric oxide. However, because of their regulatory properties ([3],[23],[24]), it is likely that long-term administration of these AAs modulates gene expression, leading to activation of catabolic processes: in rabbits, chronic administration of ARG has been shown to decrease plasma ARG ([24]). Thus, pharmacokinetic changes can reflect decreased bioavailability or increased drug clearance with time ([24]). Therefore, it is essential that long-term studies be conducted.

In conclusion, pharmacokinetic studies have been performed for ARG, ORN, and CIT. Data on C_{max} following single oral doses indicate that the bioavailability is CIT > ORN > ARG. However, only 1 study ([14]) fulfilled the criteria for modern pharmacokinetic analysis, and there are no valid data from dose-ranging studies.

Data on AUC variations with dosage and duration of treatment, time to return to basal concentrations, and level of urinary output can be useful endpoints in helping to define the upper limit of safe intake. Such endpoints would become even more relevant if considered in the context of long-term administration (i.e., >1 y), but unfortunately, no adequate long-term studies have yet been performed on ARG, ORN, or CIT.

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FOOTNOTES

[4] Abbreviations used: AA, amino acid; AKG, arginine α–ketoglutarate; ARG, arginine; AUC, area under the plasma concentration–time curve; CIT, citrulline; GLU, glutamate; ORN, ornithine; OKG, ornithine α–ketoglutarate; PRO, proline. ⬆

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