Alpha-Ketoglutarate

Subjects: Allergy

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The generation of peroxynitrite (ONOO−) is associated with several diseases, including atherosclerosis, hypertension, neurodegeneration, cancer, inflammation, and sepsis. Alpha-ketoglutarate (αKG) is a known potential highly antioxidative agent for radical oxidative species such as peroxides.

Keywords: alpha-ketoglutarate (αKG) ; peroxynitrite (ONOO−) ; reactive oxygen and nitrogen species (RONS)

1. Introduction

αKG is widely known as an intermediate of the Krebs cycle and the natural ubiquitous collector of amino groups in body tissues. It has a potent ‘sparing’ effect on endogenous glutamine pools and a synergistic effect on the synthesis of glutamine. We have seen that αKG dramatically increases the synthesis of arginine, proline, and polyamines and reduces oxidative stress, which also play a key role in metabolic adaptation before and after surgery [1]. αKG is involved as a co-substrate in 2-oxo-glutarate-dependent dioxygenase and hypoxia inducing factor (HIF-1) and as a substrate of the Jumonji C domain-containing lysine demethylases (KDM2-7). Besides these functions, αKG is involved in the energy-generating process, wherein αKG is led by the formation of NAD^+ over $NADH^+$ to form carbon dioxide and succinyl-CoA or by an overflow of $NADH^+$ to generate glutamate by up-regulating the glutamate dehydrogenase pathway. αKG can be formed enzymatically by the oxidative decarboxylation of isocitrate (isocitrate dehydrogenase), by the glutamate–pyruvate pathway (glutamate–pyruvate transaminase), and by the reversible transfer of amino groups from glutamate to oxalate by glutamate–oxaloacetate transaminase in either the inner-mitochondrial-membrane or cytoplasmic form.

αKG is known to react with H_2O_2 non-enzymically to form succinate and carbon dioxide in several biological systems, including cell cultures [2] in vitro and in vivo [3] and even in cell culture media alone [4]. We recently reported that αKG reduced oxidatively modified proteins in the presence of cigarette smoke radicals, estimated from the content of carbonyl proteins [5]. Additionally, our group reported that oral supplementation with αKG effectively increased the energy level and reduced the oxidative stress situation during surgery, as measured by the content of oxidatively modified proteins, compared to the placebo group, which resulted in a better recovery and lower hospitalisation time [1].

Peroxynitrite is a biological oxidising and nitrating agent generated physiologically by the superoxide anion radical ($O_2^{\bullet-}$) and $NO^{\bullet}$ [6]. The nitration of tyrosine residues on proteins seems to take place in the normal ageing process [7], but also in the progress of a variety of diseases such as atherosclerosis, hypertension, neurodegeneration, inflammation, cancer, and sepsis [8][9]. For example, protein tyrosine nitration was shown to take place in the apoptosis of cultured motoneurons [10] and in an amyloid lateral sclerosis animal model during its progression [11].

2. Discussion

Recent years have witnessed an avalanche of new knowledge implicating free radicals in virtually every aspect of biology and medicine. It is now axiomatic that the regulated accumulation of RONS contributes to organismal health and well-being. RONS serve as signalling molecules involved in cell growth, differentiation, gene regulation, replicative senescence, and apoptosis [12].

Peroxynitrite is a strong oxidant that can be formed in vivo by the reaction of $O_2^{\bullet-}$ and $NO^{\bullet}$. The discovery of peroxynitrite as a biological oxidant was seeded by combined data from the physiological and chemical literature [13]. Peroxynitrite is able to mediate oxidation and/or nitration in aqueous phases but also in hydrophobic milieux after free diffusion through membranes [9] to initiate lipid peroxidation and nitration, protein tyrosine nitration, and DNA modifications.

Antioxidant enzymes, such as superoxide dismutase (SOD), control the steady-state levels of peroxynitrite by reducing any overproduced $O_2^{\bullet-}$, e.g., from macrophages, to H_2O_2. Uric acid, either endo- or exogenous, is a known antioxidative substance that scavenges peroxynitrite [14]. It is known that αKG is a potent antioxidative acting substance that reduces

H_2O_2 to water and succinate. This compound is predominately formed in the inner mitochondria but is also formed quantitatively in cytoplasm. We showed for the first time that αKG is able to directly reduce peroxynitrite to succinate at physiological pH, as revealed by NMR. It appears that the affinity to reduce $ONOO^-$ is very high because of the exponential decrease in the chemiluminescent signal. Using an enzymatic reaction in the quantification of αKG, $ONOO^-$ was also able to eliminate αKG and not interfere with the enzymatic enzymes.

The nitration of tyrosine usually generates an additional negative charge and adds a relatively bulky substituent to the protein, which may affect the local charge distribution and/or conformation [9]. In vivo nitration of proteins in the presence of peroxynitrite is predominately estimated using the tyrosine residues of proteins such as bovine serum albumin (BSA), haem proteins, SOD, cytochrome c, or fibrinogen kinase glutathione S-transferase [15].

We used BSA as the example protein for the nitration of tyrosine residues because of its high level of tyrosine residues per protein. The nitration of tyrosine residues on BSA with $ONOO^-$ was reduced by αKG: between 0 and 24 mM αKG showed a negative linear function in preventing the nitration of tyrosine residues. Combining these results with the αKG enzymatic reaction mix in the spectrophotometric assay, we speculate that the enzymatic active centre might be protected from $ONOO^-$ by its substrate αKG. We suggest that this also takes place in other enzymes in which αKG is a co-factor in the inner mitochondrial membrane, as well as in the cytosol.

αKG is the obligate co-substrate of Fe(II)/2-oxoglutarate-dependent dioxygenases (OGDD), a superfamily of enzymes that catalyse the oxidative decarboxylation of αKG, producing succinate and CO_2 from O_2 [16]. Prolyl hydroxylation of hypoxia-inducible factor (HIF)-α, as catalysed by the Fe(II)/2-oxoglutarate (AKG)-dependent prolyl hydroxylase domain (PHD) enzymes, has a hypoxia-sensing role in animals [17].

Furthermore, the binding of prolyl-hydroxylated HIF-α to PHD2 is ~50-fold hindered by prior αKG binding; thus, when αKG is limiting, HIF-α degradation might be inhibited by PHD binding [17]. Given that αKG is a limiting co-substrate for PHD activity during normoxia and that 2-oxoglutarate (αKG) levels depend on amino acid availability, it is possible that PHD activity depends not only on oxygen but also on amino acid availability. This suggests a global metabolic sensor function for PHDs, which could be signalling not only to HIF-α but also to mTOR [18]. We demonstrated first that αKG substitution has clear anticancer activity in vivo [19][20]. αKG was also able to reduce tumour growth and intra-tumoral perfusion [21][22]. Those findings were verified by another research group [23]. Furthermore, nitro-tyrosine residue levels on rat myocytes pretreated with an antioxidative solution containing αKG were significantly lower than those for control rats without pre-treatment [2].

αKG is a molecule involved in multiple metabolic and cellular pathways. Any loss of αKG, e.g., by peroxynitrite or hydrogen peroxide, might result in multiple dysfunctions due to αKG's several functions as an energy donor, a precursor in amino acid biosynthesis, a signalling molecule, and a regulator of epigenetic processes and cellular signalling via protein binding. In vitro and in vivo antioxidative activities, protection against oxidative stress, and increased energy levels in αKG-supplemented humans were obtained in multiple studies [1][2][19].

αKG demonstrates a high potential to reduce peroxynitrite to body-suitable products such as succinate and nitrite, and it may protect against the nitration of mitochondrial and cytosolic proteins at neutral pH in cells and in blood. Nevertheless, further investigations are needed.

References

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