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THE BIOLOGICAL ROLE OF α-KETOGLUTARIC ACID IN PHYSIOLOGICAL PROCESSES AND ITS THERAPEUTIC POTENTIAL

BIOLOGICZNA ROLA KWASU α-KETOGLUTAROWEGO W PROCESACH FIZJOLOGICZNYCH I JEGO POTENCJAŁ TERAPEUTYCZNY

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Abstract

In this article we present the results of recent studies on the mechanism of action and biological role of α-ketoglutaric acid (AKG) in animals including developmental period of life. AKG is an intermediate in the Krebs cycle, which generates energy for life processes. Administration of AKG has been shown to be beneficial for proper development and function of the skeletal system during growth of young organisms, as well as in adulthood. In the form of a dietary supplement it also contributes to inhibition of osteoporosis in women. Moreover, it promotes the growth of muscle mass and accelerates wound healing. AKG has a significant impact on the morphology of the gastrointestinal tract in healthy animals and animals with damaged gastrointestinal tract mucosa. It is also a promising substance for the treatment of patients with short bowel syndrome, as it stimulates beneficial changes in intestinal morphology. Recent research has also revealed that AKG has neuroprotective effects.

Key words: α-ketoglutaric acid, glutamine, dietary supplements, intestinal absorption

Streszczenie

W niniejszym artykule zostały przedstawione wyniki badań z ostatnich lat ukazujące mechanizm działania oraz biologiczną rolę kwasu α-ketoglutarowego (AKG) z uwzględnieniem zwierząt w okresie rozwojowym. AKG jest produktem pośrednim w cyklu Krebsa. Wykazano, że podawanie AKG ma dobroczynny wpływ na rozwój układu kostnego u młodych organizmów oraz jego funkcjonowanie w dorosłym życiu. W formie dodatku żywieniowego przyczynia się także do zahamowania procesu osteoporozy u kobiet. Dodatkowo wpływa na wzrost masy mięśniowej oraz przyspiesza proces gojenia się ran. AKG wykazuje również istotny wpływ na morfologię przewodu pokarmowego co czyni z tej substancji obiecującą formę terapii pacjentów z zespołem krótkiego jelita. Ostatnie badania wykazały również neuroprotekcyjne działanie AKG.

Słowa kluczowe: alfaketoglutaran, glutamina, suplement diety, wchłanianie jelitowe

BIOSYNTHESIS OF AKG

α-Ketoglutaric acid is an organic chemical compound containing both a carboxyl and a ketone group. For this reason, it is referred to as a dicarboxylic acid (derivative of glutaric acid). The carboxyl group of AKG has acidic properties, while the ketone group influences the reactivity of the compound. Other names for this molecule are also used in the literature, e.g., 2-ketoglutaric acid and 2-oxoglutaric acid. AKG is characterized by good solubility in water and is relatively stable in aqueous solutions; it is also non-toxic [1]. AKG is one of the compounds participating in the Krebs cycle (Fig. 1) in the course of which it undergoes oxidative decarboxylation catalyzed by the α-ketoglutarate dehydrogenase complex.

AKG is a key molecule in the metabolism of proteins [2] (Fig. 2). It functions as the main acceptor of amino groups in the transamination of amino acids. The products of these transformations are glutamate and ketoacid. Glutamate then undergoes oxidative deamination, which leads to the complete removal from the body of the amino group in the form of ammonia. The hydrocarbon acceptor in this reaction is NAD^+ or $NADP^+$. A result of this transformation is alpha-ketoglutarate, and NH^{4+}. When the level of glutamate is low, the last reaction is reversed, leading to a decrease in the level of α-ketoglutarate and, consequently, to impaired energy metabolism of the brain. Ketoacid, the second product of deamination, can be used for energy generation or be used as a substrate for the synthesis of fatty acids or glucose.

From among the many well-known biochemical pathways leading to the synthesis of AKG [3, 4], noteworthy are fermentation and enzymatic transformation by microorganisms (e.g., *Arthrobacter paraffineus, Bacillus megaterium, Candida lipolytica, Pseudomonas fluorescens, Serratia marcescens, Pichia inositovora and Yarrowia lipopytica*). For example, a natural strain of bacteria *Y. lipopytica* cultured on glycerol is able to produce 0.35 $g\ L^{-1}\ h^{-1}$ AKG [5].

Chemical compounds that are derivatives of AKG, such as ornithine alpha-ketoglutarate (OKG) and arginine alpha-ketoglutarate (AAKG), also deserve mention. These compounds have an AKG salt in their structure. OKG is composed of one molecule of α-ketoglutarate and two molecules of ornithine. This formula provides the stability of salt and a pH close to the physiological range. Ornithine is necessary for the proper course of

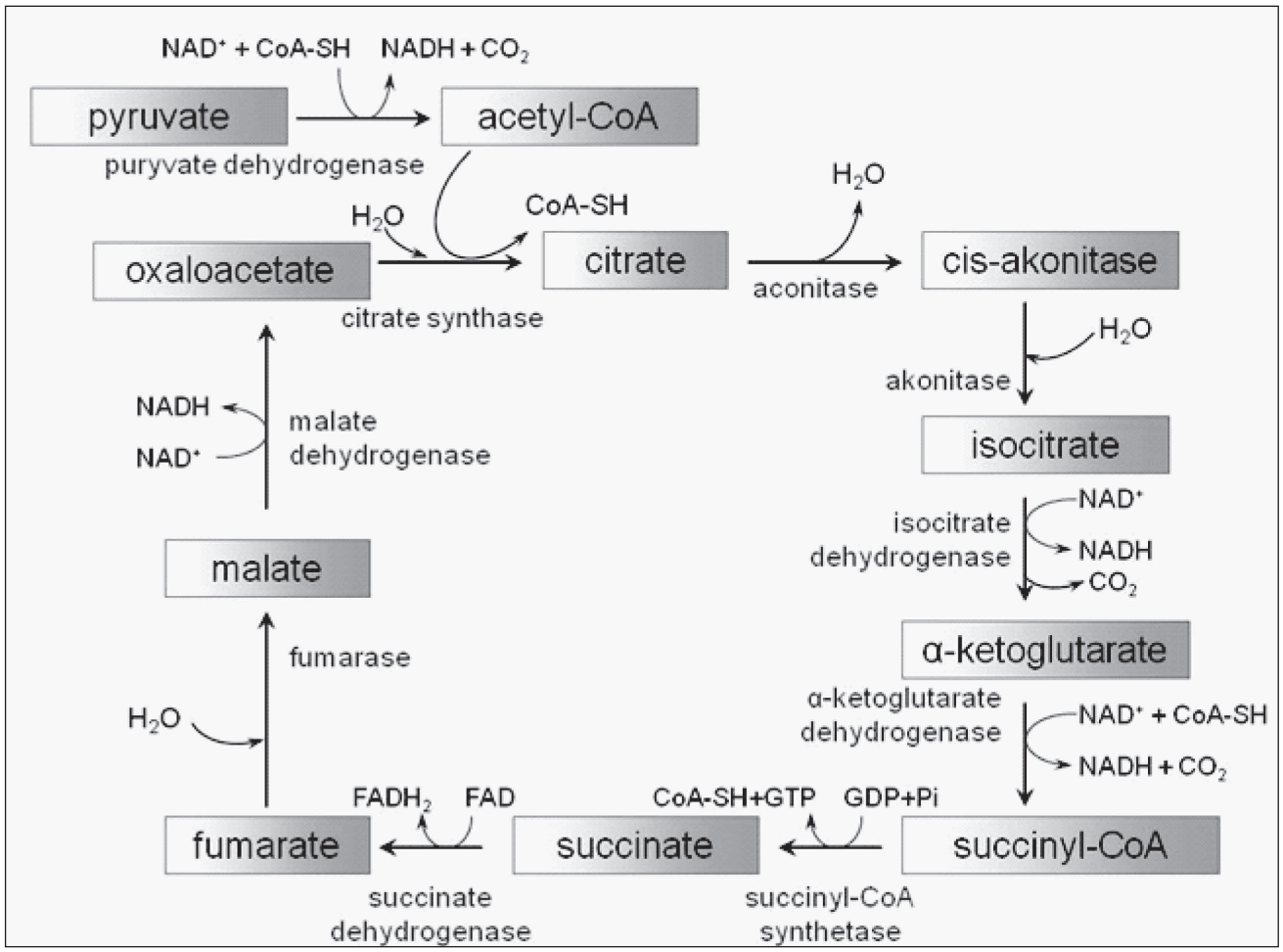

Fig. 1. Diagram of the Krebs cycle.

Ryc. 1. Schemat cyklu Krebsa.

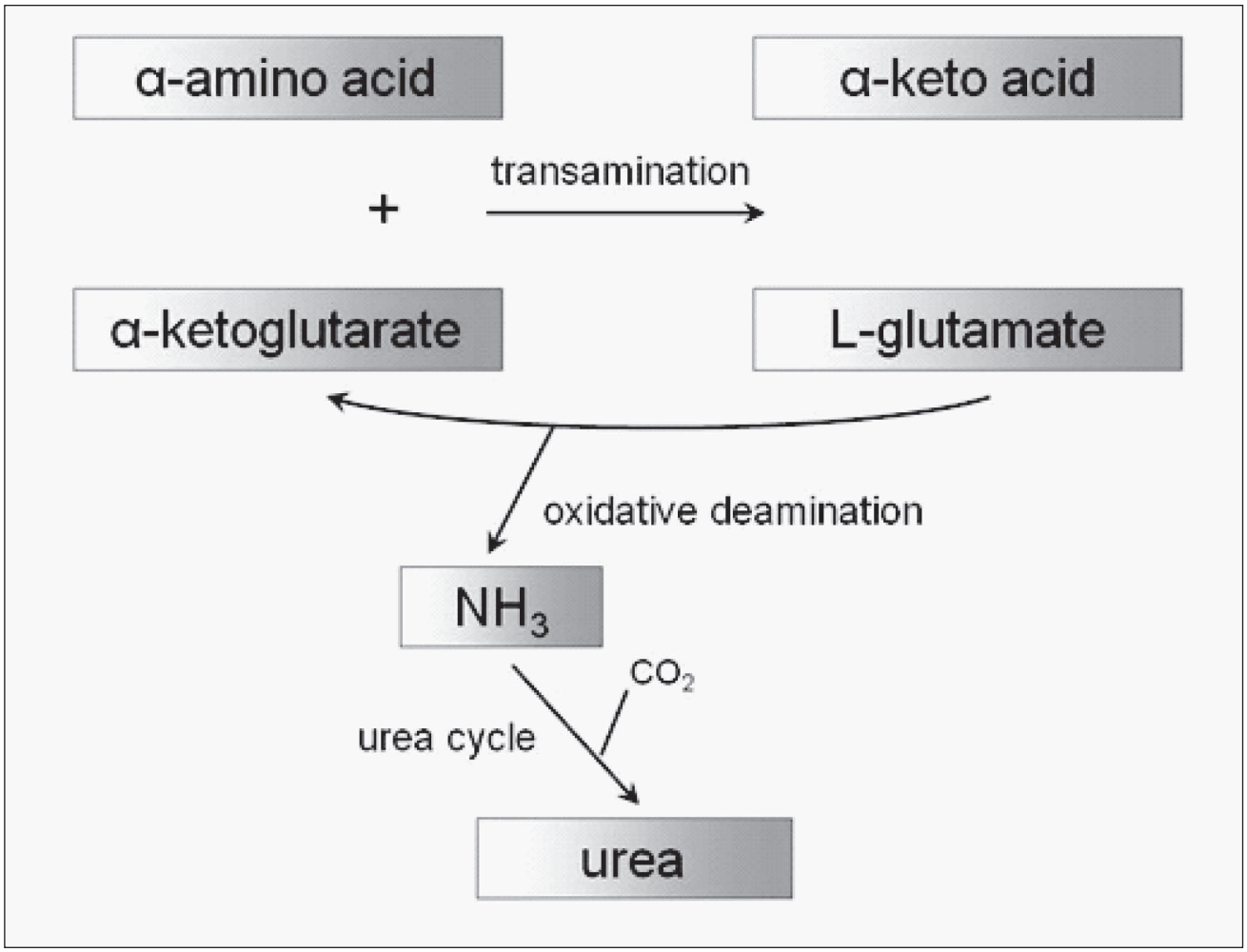

Fig. 2. Diagram of protein metabolism.

Ryc. 2. Schemat metabolizmu białek.

the urea cycle. The combination of α-ketoglutarate and ornithine determines the direction of changes leading to the synthesis of glutamine, proline, arginine and polyamides. As a result of combining AKG and arginine, secretion of nitric oxide in the body increases.

MECHANISM OF ACTION AND THE AKG ABSORPTION

The vast majority of AKG in the human body is metabolized in the enterocytes of the intestinal mucosa [6, 7]. The most intensive absorption of AKG takes place in the small intestine, the slowest occurs in the colon [7]. Sodium-dicarboxylate cotransporter (NADC-1) is responsible for transport of AKG in the body.

AKG utilization in the body depends to a large extent on the route of administration. Studies in growing pigs have shown that AKG administered intraduodenally (i.d.) is significantly less absorbed than administered intravenously (i.v.) and intragastrically (i.g.) [8]. These results also indicate that the intraduodenal route of administration increases the utilization of nutrients in the intestine, despite the low AKG utilization after intraduodenal infusion. Intraduodenal administration of AKG with standard complete nutrient solution affects the whole body energy expenditure and the distribution of nutrients in oxidation processes. A considerable percentage of infused AKG is retained in the body irrespective of the route of administration. Only 10% of the AKG administered enterally enters the circulation [9, 10]. Eighty percent of the AKG is metabolized by enterocytes into CO_2 during the first pass by the intestinal epithelium. Filip et al. [11] have demonstrated that the AKG that escaped the first-pass metabolism in the intestine converts to glutamine and proline, and then is deposited or incorporated in other tissues and organs, such as the liver, brain, muscle and bones. Dąbek et al. [12] showed that when AKG was administered to pigs via the portal vein its concentration increased within 5 minutes and then it was rapidly eliminated from the blood (half-time less than 5 min). Better absorption of AKG in the initial segment of the small intestine was also demonstrated. Interestingly, when different doses of AKG were administered, identical elimination curves of this metabolite from the peripheral and visceral circulation [12] were obtained.

It is noteworthy that recent studies [13] indicate the participation of AKG in inhibiting the activity of ATP

synthase, which is complex V of the mitochondrial electron transport chain. The influence of AKG on the activity of this enzyme was studied in adult *Caenorhabditis elegans*, and significant extension of its lifespan was demondtrated. α-KG inhibits ATP synthase and, similarly as ATP synthase knockdown, inhibition by α-KG leads to reduced ATP content, decreased oxygen consumption, and increased autophagy in both *C. elegans* and mammalian cells.

THE ROLE OF AKG IN BONE FORMATION

Studies in recent years have shown a significant role of AKG in bone development in young organisms. In experiments carried out on lambs, Tartar et al. [14] have shown the influence of long-term oral administration of AKG with the addition of NaOH on the development of bone and its functions. The geometrical and mechanical properties of bone were compared and evaluated. The study showed no effect of AKG on body weight or the growth rate of lambs, but a significant positive impact of AKG on the length and weight of the ribs was found. In lambs receiving AKG orally for the first 14 days of life, the length and weight of the ribs increased significantly, 3.2% and 8.2%. Additionally, AKG was shown to increase the mineral density of the cortical bone (7.1%) and to improve the mechanical endurance of the ribs in terms of the moments of maximum elastic strength and ultimate strength (by 10% and 8%). These results indicate that AKG has a long-term, significant impact on skeletal development and helps to improve chest mechanics and functioning in young animals. On the other hand, Harisson et al. [15] showed significantly higher dencities of trabecular, and cortical bone and better mechanical properties of the femur in lambs fed AKG (1 to 14 days old) compared with control animals. Although previous studies have shown that enteral administration of AKG increases the level of plasma IGF-1 (insulin growth factor 1), which is one of the main factors responsible for regulating the function of bone cells [16], the study of Harisson et al. [15] showed that the influence of AKG on the investigated bone is independent of the plasma IGF-1 concentration. It was also shown that the administration of AKG in the early postnatal period increased the length of the sixth rib, and its final strength, maximum elastic strength, and Young's modulus compared with controls [17]. In addition, Andersen et al. [17] researching the long-term (169 days) effect of AKG on bone in pigs, showed a sex-specific effect of AKG on the growth of piglets. In female piglets AKG stimulated growth, while in the case of male piglets, the opposite effect was observed. The results of these studies clearly show that the administration of AKG in the early postnatal period is beneficial to bone development during growth and its correct functioning later in life.

AKG is a precursor in the synthesis of glutamate, which, in turn, is transformed into glutamine. Glutamate and glutamine are used as an oxidative fuel in the placenta during pregnancy [18]. Tartar et al. [19] investigated whether the administration to pregnant sows feed with AKG and β-hydroxy-β-methylbutyrate (HMB) for the last two weeks of pregnancy affects long-term bone growth in the offspring. The results showed increased birth weight and final weight (90 days) of piglets from mothers fed a diet with AKG or a diet combining AKG and HMB, as well as increased daily weight gain compared with controls. AKG administered to pregnant sows increased the plasma levels of aspartate, serine, glutamine, proline, glycine, alanine, valine, ornithine, lysine. and arginine in newborns. In contrast, the combination diet, AKG and HMB, induced increases in the concentration of amino acids such as aspartate, threonine, serine, glutamine, proline, glycine, citrulline, valine, methionine, cystine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, lysine, histidine, and arginine above the control values. These studies have also shown that AKG may affect serum GH and IGF-1 levels. It has been shown to increase the GH level in newborns, as well as in 90-day-old pigs in the group born by sows receiving combined treatment with AKG and HMB. Higher levels of IGF-1 were observed in the serum of neonates of mothers fed AKG, as well as AKG + HMB, and in 90-day-old piglets IGF-1 levels were higher in all groups. Furthermore, both these metabolites exerted, individually or in combination, effects on bone tissue metabolism, which were expressed as increased values of the bone formation marker BAP (bone alkaline phosphatase) in newborns and 90-day-old piglets. The addition of AKG to the prenatal diet influenced the weight of the femur and, in the case of the combination of AKG and HMB, it lengthened the femur of newborns by nearly 2% compared with the control group. The cross-sectional area increased in both experimental groups and the highest second moment of inertia was reached in pigs fed the combined diet. Maximum elastic and ultimate strength was reached in all of the groups. There was also a significant effect of AKG and HMB on body weight. The mechanism through which additives influence body weight and daily weight gains may be via enhanced by amino acid metabolism. The results of these studies also demonstrated a significant increase in the glutamine concentration in the plasma of newborns in all experimental groups. Oral administration of OKG increases the trabecular and cortical bone mineral density of the tibia in turkeys [20]. AKG as at source of glutamate and glutamine is a precursor of proline, a necessary amino acid in the formation of collagen fibers. Supplementation with the sodium salt of AKG significantly increases collagen synthesis in young growing piglets, both before and after weaning [21]. The impact of the calcium salt of AKG on the C-terminal cross-linked telopeptide of type I collagen (CTX) was also demonstrated in postmenopausal women with osteopenia [22]. AKG-Ca administration caused a significant increase in serum CTX levels and preserved the bone mass in the lumbar spine. The influence of AKG on osteopenia has also been studied on animal models gastrectomides rats [23]. It was shown that the administration of AKG in drinking water for 8 weeks prevents loss of calvarial, trabecular and cortical bone in animals subjected to gastrectomy. In contrast, there was no effect of AKG on mineral content and bone density. However, the important role of α-ketoglutarate sodium salt on the mineralization of the tibia of female rats during the development of osteopenia and when this condition was established was demonstrated [24]. In the first case (during development of the disease), AKG not only stoped

the degradation of bone tissue, but also stimulated tissue mineralization. In the second case (in an advanced stage of the disease), AKG significantly reduced the intensity of the disease. The above research results demonstrate the effectiveness of AKG in the prevention and treatment of osteoporosis and osteopenia.

THE BIOLOGICAL ROLE OF OKG

It has been shown that a diet supplemented with OKG is of significant importance in the treatment of sarcopenia [25]. Research has shown that the combination of diet, OKG, hormone therapy, and physical activity is able to inhibit disease and reduce muscle atrophy. Vaubourdolle et al. [26] showed that the enteral administration of OKG in rats inhibits muscle weight loss by decreasing muscle protein hypercatabolism. In addition, OKG had a significant impact on the muscle glutamine pool. Other experiments on young Wistar rats confirmed the effectiveness of OKG [27]. That study was aimed at determining the effect of OKG, AKG, and ornithine on restoring glutamine pools. After fasting for 24 hours, the animals, which had burned 20% of the body surface area, it administered enterally diet supplemented with OKG or alone ornithine, or only AKG. It was investigated the amino acid concentration in the plasma, liver and intestinal mucosa. OKG proved to be most effective, demonstrating the metabolic interactions between the components of this molecule. The activity of OKG is due to a specific interaction between α-ketoglutarate and ornithine. OKG has also found use in the treatment of catabolic states (generated as a result of burns to the body) and in malnourished elderly patients. Studies indicate that OKG is conducive to wound healing in burned patients with pressure ulcers and in the course of treatment following reconstructive surgery. OKG at 10 g administered enterally twice a day for 24 days significantly affected the level of ornithine, proline and phenylalanine in the blood plasma of patients following trauma [28].

It is also worth mentioning that OKG is used in sports supplementation in pre-training preparations. The precursor of ornithine in the body is arginine, which is involved in the degradation of amino acids, as well as in the removal of ammonia from the body. Preparations containing OKG are supposed to save stocks of arginine and improve the synthesis of nitrogen oxide. Supplementation with amino acids can increase training tolerance. The effect of diets with the addition of keto acids was studied in healthy young male adults [29]. The men received a mixture of Na-AKG and Ca-AKG in drinking water. The study group demonstrated significantly less general stress and emotional exhaustion compared with the control group. Subjects in the treatment group were able to tolerate higher training volumes and reach higher power output and peak muscle torque. The mixture of keto acids favoranly influenced training and improved tolerance to exercise.

ROLE OF AKG IN THE DEVELOPMENT OF THE GASTROINTESTINAL TRACT

In *in vitro* studies conducted on intestinal porcine epithelial cells (IPEC-1) it was shown that AKG activates mTOR signaling, increasing protein synthesis in the cells [30]. This can spare glutamine, which is one of the main sources of energy for intestinal epithelial cells [31]. AKG given in the diet significantly inhibits the adverse effects induced by lipopolysaccharide (LPS) in the gastrointestinal tract of piglets, among others, decreases in the protein levels and ratio of phosphorylated mTOR to total mTOR in duodenal, jejunal and ileal mucosa, as well as the adverse effects on intestinal morphometry, i.e., AKG reduces the ratio of villus height to crypt depth [32]. Supplementation of 1% AKG to the basal diet of young pigs (24 days old) also reduced liver damage that was induced by administration of LPS [33]. AKG resulted in a decrease plasma glutamate concentration concomitantly with a reduction in hepatic concentrations of glutamate, glutamine, leucine, asparagine, lysine, alanine, serine, threonine, valine, and phenylalanine. Improvement in liver morphology and its anti-oxidative capacity were also observed. An effect of AKG on the morphology of the gastrointestinal tract was also shown in adult rats [34]. AKG supplementation resulted in a significant increase in duodenal crypt depth [34]. AKG also caused a significant linear, dose-dependent increase in enterocyte length (diets supplemented with 0, 3, or 6 g/kg feed of AKG for 14 consecutive days) [35]. These studies indicate that the effects of AKG on the structure of the intestine were dose-dependent: AKG at a dose of 3 g/kg resulted in a reduction in the height of villi and microvilli, while at 6 g/kg, these changes were statistically insignificant. Long-term research by Pierzynowski et al. has demonstrated the ability to improve the structure of the gastrointestinal tract by dietary supplementation with AKG. This molecule appears to be effective in the development of the GI system.

The Dumas research team [36] studied the effects OKG on intestinal adaptation after massive (80%) small bowel resection in rats. Enteric supplementation of OKG contributed to an increase in the villus height-to-crypt depth ratio. Moreover, thanks to OKG glutamine pools and protein content in muscles were restored. OKG has a stimulating effect on the growth of children receiving long-term total parenteral nutrition [37]. OKG was added to the parenteral solution during the first 5 months, and during the following 5 months, control patients received a solution without the additive. In the first period there was significant acceleration of growth and concentrations of IGF-1, glutamine and glutamate in the blood plasma of children. These observations, as mentioned before indicate a significant relationship between supplementation with AKG and IGF-1 level. Studies have confirmed the beneficial effects of AKG on the growth of the intestine after resection and may be important for the development of new therapies for the treatment of short bowel syndrome.

Studies by our team have shown the impact of AKG on the exorcine pancreatic function of weaned piglets [38]. AKG was administered to animals in feed. Significant effects of AKG were observed at doses of 100 and 250 mmol/kg b. wt. An insignificant and short-lived effect of AKG stimulating the secretion of pancreatic enzymes was shown. It was observed that AKG is inhibited by the so-called gastric phase

and intestinal exocrine secretion and has no impact on the so-called cephalic secretion phase. It was further found that the effect of AKG may be of two types, the molecule can inhibit the secretion of juice, and, as a source of hydrogen ions (after buffering), it can stimulate the secretion of pancreatic juice. The results of these and the above experiments have led our team to plan the continuation of research with the use of AKG in newborn animals. Young organisms need to rapidly develop the gastrointestinal tract, which is the gateway for the nutrients necessary for the development of the entire organism.

THE NEUROPROTECTIVE EFFECT OF AKG

The neuroprotective effect of AKG is the result of its antioxidant properties and participation in the tricarboxylic acid cycle. Moreover, AKG is responsible for reducing the concentration of free ammonia in the body, thus reducing the risk of cerebral ischemia. Cerebral ischemia causes significant changes in the hippocampus. Hippocampal neurons are very sensitive to reduced oxygen levels. Kovalenko et al. [39] demonstrated the significant effect of AKG on the neuron in the CA1 area of the hippocampus in a model of cerebral ischemia induced by occlusion of carotid artery in the Mongolian gerbil. Animals were receiving AKG in drinking water for 7 to 21 days after surgical occlusion. Hypoxia led to the death of 90% of the neuronal cells and to major damage to the remaining pool of neurons. The death of neurons in the CA1 was associated with the development reactive astrogliosis and a significant decrease in the level of neuronal cell adhesion. Treatment with AKG of sick animals caused an increase in neuronal survival by 20%-50%, as well as a decrease in astrogliosis. These results suggest that the acid AKG prevents damage to nerve cells, may also be effective in inhibiting angiogenesis. Bruegge et al. [40] have shown that AKG analogues can be used to stimulate erythropoiesis and angiogenesis; these analogues are called 'HIF-stabilizers'.

SUMMARY

The results of previous studies show a beneficial effect of AKG on bone formation and the function of the skeletal and muscular system. There is evidence that OKG reduces protein catabolism in skeletal muscle and increases protein synthesis in the liver and intestine. In addition it increases the concentration of proteins that are markers of nutritional status. It seems to be an effective activator for wound healing and tissue regeneration. Also of interest are results regarding the influence of AKG on the development of the gastrointestinal tract and its neuroprotective effect. There are also studies showing a role of AKG in the treatment of some pulmonary disorders, as well as in the treatment of some types of cancer. The results presented in this paper show that AKG is a very interesting a compound with possible applications in many areas, including a large potential in medicine of developmental period.

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