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Biological Activities of Kinetin on Animals

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Abstract: As a cytokinin growth factor kinetin (N^6-furfuryladenine) with several biological effects observed for human cells and fruit flies. It was named kinetin because of its ability to induce cell division. The mechanism of kinetin synthesis in DNA is thought to be via the production of furfural, an oxidative damage product of DNA deoxyribose and it is quenched by the adenine base converting it into N^6-furfuryladenine. Since 1994, kinetin has been thoroughly tested for its powerful antiaging effects in human skin cells and other systems. At present, kinetin is one of the most widely used components in numerous skin care cosmetics and cosmeceuticals. Kinetin which was isolated 50 years ago for the first time as a plant hormone as well as other cytokinins isopentenyladenine, zeatin and benzylaminopurine induce callus (clusters of dedifferentiated plant cells) to redifferentiate into adventitious buds. Because of some similarities in the biological phenotypes of cancer and callus cells, cytokinins and especially kinetin, affect the differentiation of human cells through a common signal transduction system. Therefore, cytokinins found their way to use in molecular medicine.

Key words: Kinetin, biological activity, antiaging, buds, plant cells

INTRODUCTION

Kinetin remains an interesting object of scientific research and a rich source of inspiration even it has been discovered 50 years. Long lasting studies have deepened the knowledge about its role and significance as well as making kinetin the best known cytokinin. Recently, discovered data showed us a great potential and broad field of kinetin activity as it plays a role in many processes, from regulation of growth and development of plants through antioxidative and antiaging properties to therapeuticutility.

Kinetin belongs to a group of plant hormones called cytokinin. These are adenine derivatives with an additional side chain at the N^6 atom. In accordance with the structure of the side chain, cytokinins are classified as isoprenoid with an isopentenyl or a hydroxyisopentenyl group or aromatic where a benzyl group occurs. Kinetin contains a furfuryl group at N^6 (Miller *et al*., 1956).

INFLUENCE OF KINETIN ON ANIMALS

Kinetin antioxidative properties: In experiments using animal cells and other organisms, it was shown that kinetin influences many processes, regulates proliferation and has antiaging and antioxidant properties. Kinetin's antioxidant and scavenger activity was confirmed *in vivo* and *in vitro*. It could act in a few different ways as a donor of hydrogen as an enzyme or as an activator of enzyme activity (Sharma *et al*., 1995; Sharmam *et al*., 1997; Rattan, 2002). Because of these properties kinetin prevents damage to DNA, proteins and other macromolecules, avoiding the accumulation of abnormal particles in organs, tissues and cells. Kinetin can act as a free radical scavenger when oxygen radicals directly abstract hydrogen from the α carbon of the amine bond of N^6-furfuryladenine (Rattan, 2002). The kinetin copper complex catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide at the reaction rate constants 2.3×10^{-7}/M/sec at pH 9.8 and 25°C. Kinetin was proved to protect DNA against the formation of 8-oxo-deoxyguanine which is the result of hydrogen peroxide generation in a Fenton reaction. Inhibition of 8oxodG formation was exhibited in a dose dependent manner with a maximum efficiency of 50% at a concentration of 100 μM (Olsen *et al*., 1999). Kinetin protects against oxidative and glycoxidative protein damage generated *in vitro* by sugars and iron/ascorbate system. Glycation is a nonenzymatic reaction of binding hexose, mostly glucose to the amine group of protein or nucleotides (Pischetsrieder *et al*., 1999). The products of these reactions are accumulated in cells during aging (Yokosuka *et al*., 2006). Inside the cell, due to a high concentration of glucose and other reactive sugars like pentoses and α-oxo-aldehydes, glycation/glycoxydation reactions progress fast (Cervantes-Laurean *et al*., 1996; Hamada *et al*., 1996). Kinetin at a concentration of 50 μM exhibits an 82% inhibition of Bovine Serum Albumin (BSA) pentosidine formation. At 200 μM, the cytokinin prevents BSA aggregation during glycation and also inhibits 59-68% Advanced Glycation End Product (AGEP) development (Verbeke *et al*., 2000).

Kinetin antiaging properties: The antiaging properties of kinetin were shown using *in vitro* cell cultures, *in vivo* on skin and fruitifies. The fruitify Zaprionus with its diet supplemented with 125-625 μM kinetin, prolonged life span due to a reduction in the age specific death rates, slowed down development and delayed maturation of insects in the larval and pupal stages. Delayed aging is reached at the cost of decreased reproductive activity and egg laying capacity. The molecular mechanism of kinetin activity is connected with an increase in catalase activity. The enzyme belongs to the oxydoreductase group, displays strong antioxidant activity and catalyzes the decomposition of hydrogen peroxide into water and oxygen. A concentration of 125 μM seems to be the most effective for antiaging and lifeprolonging effects. A higher concentration, 500 μM and above, gives toxic and lifeshortening results. The cytokinin exerts a similar effect on human cell cultures at these doses (Sharma *et al*., 1995; Sharmam *et al*., 1997). Nymphs of *Lipaphis erysimi* fed kinetin treated *Raphanus sativus* L. showed an increased activity of catalase, glutathione peroxidase and superoxide dismutase and a decrease in the activity of APTase (Rup *et al*., 2006). In *in vitro* skin cultures, mammary carcinoma and cystic disease kinetin delays the onset of several morphological and biochemical processes connected with aging. During senescence *in vitro* cell cultures became large and flattened, full of lysosomal residual bodies and oxidation modified macromolecules, debris and accumulated lipofuscin with disorganized cytoskeleton and some of them contain more than 1 nucleus. The addition of kinetin at 40-200 μM concentrations in culture media prevents fibroblasts from developing these changes. In spite of avoiding age related degeneration, kinetin can also slightly reverse the changes. Upon removal of the cytokinin, some of the aging characteristics reverse (Orr and McSwain, 1960). Some properties of kinetin were proved *in vivo* using aged skin of hairless dogs. After 50 days of daily application of solution containing kinetin a 0.01, 0.1, 1, 10 and 96.6 mM improvement in skin texture, wrinkling and pigmentation was observed. After 100 days, rejuvenation and depigmentation became more visible.

Lower concentrations of kinetin normalized hyperpigmentation and improved the aged skin structure. Throughout the treatment, no adverse effect was observed, showing that kinetin is safe for longterm therapy (Kimura and Doi, 2004).

Kinetin influence on animal cell and tissue cultures: Kinetin influences both the epidermis and the dermis in the skin in the same way. It stimulates keratinocyte proliferation and differentiation in the epidermis, increases the amount of laminin 5 at the dermalepidermal junction and influences the formation of fibrillin and elastin deposition as well as their organization perpendicularly to the dermal epidermal junction in the dermis (Vicanova *et al*., 2006). On the other hand, human keratinocyte culture exhibits significant growth inhibition in media containing 40-200 μM kinetin concentration. At the same time, it stimulates the cells to differentiate especially strongly in the presence of calcium (Berge *et al*., 2006). Kinetin retards the out growth of epithelium skin cultures at 1-0.25 mg/100 mL and increases epithelial sheet production at 0.006-0.015 mg/100 mL (Orr and McSwain, 1957). Its riboside appears to be toxic to fibroblasts, breast carcinomas and cystic disease cells at 1 mg/100 mL and results in reduced or no outgrowth in *in vitro* culture. But it is not toxic at 0.1 mg/100 mL (Orr and McSwain, 1960). Kinetin in high concentrations (100 mg/1000 mL) acts as a toxin and triggers cytoplasm vacuolization and degenerative changes in fibroblast cell cultures. At lower doses (1 and 10 mg/1000 mL) chromatin became more sensitive to acid hydrolysis which results in higher transcription activity. DNA amounts in the fibroblast nucleus increase after 24 and 72 h incubation with Kowalska (1992).

Kinetin's molecular mechanism of action in animal cells: In endothelium cells kinetin influences signaling pathways connected with the cytoskeleton. Human Dermal Microvascular Endothelial Cells (HDMEC) between the 5th and 30th passages were treated with kinetin at 50 μM. This resulted in changes in the expression of moesin, actin and rho GDP Dissociation Inhibitor (GDI) and an increased activity of rho GTPase which influences actin. Actin is a protein that forms the cytoskeleton and is connected with the rho pathway. Moesin belongs to the ERM (Ezrin/Radixin/Moesin) protein family that connects cell membrane proteins with actin underneath the membrane.

Moesin participates in signal transduction and cytoskeleton remodeling. It is modulated by phosphorylation, the phosphoinositide pathway and is controlled mutually with rho GTPase. Rho GDI expression is elevated in aged Human Umbilical Vein Endothelial Cells (HUVEC) and it suppresses rho GTPase activity. It interacts directly with ERM protein which reduces rho GDI activity, thereby activating rho GTPase (Chimini and Chavrier, 2000). Using similar pathways kinetin takes part in the regulation of cell proliferation. Cell cycle arrest in the G1 phase is connected with senescence and leads to

apoptosis. This occurs when G1specific cyclin D1 or cyclin E1, pRB, p16, p21 and p27 undergo changes. When cellular function decreases, p53 activation takes place.

This suppresses cell cycle progression, stimulates a rise of p21 and p27 and induces G1 arrest. Kinetin decreases expression of p16, p27 and p53 and increases the amount of D1 cyclin. The rho pathway as well as influencing cytoskeleton, supports cell cycle transition G1/S and thus it promotes proliferation. The rho GTPase enhances expression of p27 and thus regulates D1 cyclin. In HDMEC treated with kinetin, rho GTPase is activated, total p16, p21, p27 is reduced, the amount of cyclin D1 is enlarged and stimulation of G1/S transition is observed. Kinetin delays aging of endothelial cells and increases proliferation and metabolic capacity (Lee *et al*., 2006; Wagner *et al*., 2001). Earlier experiments showed that kinetin delayed the onset of aging of fibroblasts and helped to complete cytokinesis but it does not promote induction of the S phase. This suggests diverse activities of the cytokinin depending on the cell type (Orr and McSwain, 1960). There are also other cases where kinetin was proved to act through cGMP and Ca^{2+} connected pathways. At 50-150 μM, it inhibits platelet aggregation. It is supposed to stop Na^{+}/H^{+} exchanger activation and phospholipase C activation and at the same time prevent Phosphatidylinositol (PIP_2) metabolism and lipid signaling pathways. This results in lower intracellular alkalization and Ca^{2+} mobilization, augmented cyclic AMP synthesis and inhibition of thromboxane A2 formation which is known to be responsible for platelet aggregation. cAMP stops the Na^{+}/H^{+} exchanger and leads to reduced mobilization of intracellular Ca^{2+} and phosphorylation of P47 (Immunityrelated GTPases (IRG) family). At 70 and 150 μM kinetin decreased the amount of free radicals in collagenactivated platelet. Intra venous injection of 4-6 mg kg^{-1} of the cytokinin into mice prolonged bleeding time by approx. 1.9-2.1 fold (Hsiao *et al*., 2003; Sheu *et al*., 2003). 6Benzylaminopurine (6BAP), kinetin and zeatin induce positive inotropic ef fects in rat atria involving P2 purinoceptors, cGMP and intracellular calcium release but not using pathways connected with arginine/nitric oxide, cyclooxygenase, phospholipase C or L type calcium channels (Froldi *et al*., 1999). Kinetin as well as some other cytokinins, auxins and gibberelinins, increases rat lung, small intestine, liver and renal cortex guanylate cyclase activity 2-4 fold. The maximal stimulation of guanylate cyclase was observed at a 1 μM concentration of the plant hormones (Vesely *et al*., 1985). Cytokinins inhibits muscle creatine kinase (CKMB), activates Alanine Amino Tranferease (ALT) and Aspartate Aminotransferase (AST) and increases the level of AST, CK and LDH but they do not influence carbonic anhydrase and glucose6 phosphate dehydrogenase (Elik *et al*., 2002; Ciftci *et al*., 2003). Cytokinins are also incorporated into the rRNA, tRNA and tRNA of tobacco callus, *E. coli* and yeast cells. Specific incorporation of kinetin into *E. coli* tRNA Tyr at position 37 by the putative tRNA kinetin transglycosylase takes place. The exchange reaction occurs in the presence of protein from *E. coli*, yeast or MRC5V2 cell extracts. Likewise, enzymes of *E. coli* or MRC5V2 are able to catalyze incor poration of kinetin into yeast tRNA (Barciszewski *et al*., 1992). This shows a relationship between tRNA and enzymes in prokaryotic and eukaryotic organisms.

PERSPECTIVES

Pluripotency of cytokinins, especially in the context of recently discovered properties, makes it an attractive subject for experiments concerning a molecule with multiple roles and possible applications (Yu *et al*., 2010). The potential is largely connected to kinetin utility in cosmetics. As it is not only an antioxidant but also an antiaging compound that reduces wrinkles, regulates skin pigmentation and improves overall skin appearance, kinetin has become the object of cosmetic companies' interest (Alsokari, 2011). Numerous reports about diverse diseases that kinetin can influence has raised its possible application in medicine (Alsokari, 2011). At present kinetin could be useful in FD therapy but it can also probably exert effects on other genetic disorders (Wang *et al*., 2011). Since, cytokinins induce AML cell differentiation, they could possibly also affect other kinds of cancer cells (MaBecki *et al*., 2012).

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REFERENCES

Alsokari, S.S., 2011. Synergistic effect of kinetin and spermine on some physiological aspects of seawater stressed Vigna sinensis plants. Saudi J. Biol. Sci., 18: 37-44.

Barciszewski, J., D. Otzen, S.I.S. Rattan and B.F.C. Clark, 1992. Specific incorporation of kinetin into eukaryotic and prokaryotic transfer ribonucleic acid molecules. Biochem. Int., 28: 805-811.

Berge, U., P. Kristensen and S.I. Rattan, 2006. Kinetin-induced differentiation of normal human keratinocytes undergoing aging *in vitro*. Ann. NY Acad. Sci., 1067: 332-336.

Cervantes-Laurean, D., E.L. Jacobson and M.K. Jacobson, 1996. Glycation and glycoxidation of histones by ADP-ribose. J. Biol. Chem., 271: 10461-10469.

Chimini, G. and P. Chavrier, 2000. Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. Nat. Cell Biol., 2: E191-E196.

Ciftci, M., Y. Demir, I. Ozmen and O. Atici, 2003. *In vivo* and in vitro effects of some plant hormones on rat erythrocyte carbonic anhydrase and glucose-6-phosphate dehydrogenase activities. J. Enzyme. Inhib. Med. Chem., 18: 71-76.

Froldi, G., U. Gallo, E. Ragazzi and L. Caparrotta, 1999. 6-Benzylaminopurine: A plant derived cytokinin inducing positive inotropism by P2-purinoceptors. Planta Med., 65: 245-249.

Hamada, Y., N. Arakib, N. Koh, J. Nakamura, S. Horiuchi and N. Hotta, 1996. Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway. Biochem. Biophys. Res. Commun., 228: 539-543.

Hsiao, G., M.Y. Shen, K.H. Lin, C.Y. Chou and N.H. Tzu *et al*., 2003. Inhibitory activity of kinetin on free radical formation of activated platelets in vitro and on thrombus formation *in vivo*. Eur. J. Pharmacol., 465: 281-287.

Kimura, T. and K. Doi, 2004. Depigmentation and rejuvenation effects of kinetin on the aged skin of hairless descendants of mexican hairless dogs. Rejuvenat. Res., 7: 32-39.

Kowalska, E., 1992. Influence of kinetin (6-furfurylo-amino-purine) on human fibroblasts in the cell culture. Folia. Morphol., 51: 109-118.

Lee, J.H., K.Y. Chung, D. Bang and K.H. Lee, 2006. Searching for aging-related proteins in human dermal microvascular endothelial cells treated with anti-aging agents. Proteomics, 6: 1351-1361.

MaBecki, P.H., W. Rypniewski, M. Szymanski, J. Barciszewski and A. Meyer, 2012. Binding of the plant hormone kinetin in the active site of Mistletoe Lectin I from *Viscum album*. Biochim. Biophy. Acta. (BBA) Proteins Proteomics, 1824: 334-338.

Miller, C.O., F. Skoog, F.S. Okumura, M.H. Von Saltza and F.M. Strong, 1956. Isolation, structure and synthesis of kinetin, a substance promoting cell division. J. Am. Chem. Soc., 78: 1375-1380.

Olsen, A., G.E. Siboska, B.F.C. Clark and S.I.S. Rattan, 1999. N6-furfuryladenine, kinetin, protects against fenton reaction-mediated oxidative damage to DNA. Biochem. Biophys. Res. Commu., 265: 499-502.

Orr, M.F. and B. McSwain, 1960. The effect of kinetin, kinetin ribofuranoside and gibberellic acid upon cultures of skin and mammary carcinoma and cystic disease. Cancer Res., 20: 1362-1364.

Orr, M.F., and B. McSwain, 1957. The effects of kinetin upon epithelium and fibroblasts in tissue culture. Cancer, 10: 617-624.

Pischetsrieder, M., W. Seidel, G. Munch and R. Schinzel, 1999. N^2-(1-Carboxyethyl)deoxyguanosine, a nonenzymatic glycation adduct of DNA, induces single-strand breaks and increases mutation frequencies. Biochem. Biophys. Res. Commun., 264: 544-549.

Rattan, S.I., 2002. N^6-furfuryladenine (kinetin) as a potential anti-aging molecule. J Anti-aging. Med., 5: 113-116.

Rup, P.J., S.K. Sohal and H. Kaur, 2006. Studies on the role of six enzymes in the metabolism of kinetin in mustard aphid, *Lipaphis erysimi* (Kalt.). J. Environ. Biol., 27: 579-584.

Sharma, S.P., P. Kaur and S.I. Rattan, 1995. Plant growth hormone kinetin delays aging, prolongs the lifespan and slows down development of the fruitfly *Zaprionus paravittiger*. Biochem. Biophys. Res. Commun., 216: 1067-1071.

Sharmam, S.P., J. Kaur and S.I. Rattan, 1997. Increased longevity of kinetin-fed *Zaprionus fruitflies* is accompanied by their reduced fecundity and enhanced catalase activity. Biochem. Mol. Biol. Int., 41: 869-875.

Sheu, J.R., G. Hsiao, M.Y. Shen, C.Y. Chou, C.H. Lin, T.F. Chen and D.S. Chou, 2003. Inhibitory mechanisms of kinetin a plant growth-promoting hormone, in platelet aggregation. Platelets, 14: 189-196.

Verbeke, P., G.E. Siboska, B.F.C. Clark and S.I.S. Rattan, 2000. Kinetin inhibits protein oxidation and glycoxidation *In Vitro*. Biochem. Biophys. Res. Commun., 276: 1265-1270.

Vesely, D.L., J.L. Hudson, J.L. Jr. Pipkin, L.D. Pack and S.E. Meiners, 1985. Plant growth-promoting hormones activate mammalian guanylate cyclase activity. Endocrinology, 116: 1887-1892.

Vicanova, J., C. Bouez, S. Lacroix, L. Lindmark, O. Damour, 2006. Epidermal and dermal characteristics in skin equivalent after systemic and topical application of skin care ingredients. Ann. New York Acad. Sci., 1067: 337-342.

Wagner, M., B. Hampela, D. Bernhard, M. Hala, W. Zwerschke and P. Jansen-Durr, 2001. Replicative senescence of human endothelial cells *in vitro* involves G1 arrest, polyploidization and senescence-associated apoptosis. Exp. Gerontol., 3: 1327-1347.

Wang, L., D. Zhao, L. Di, D. Cheng, X. Zhou, X. Yang and Y. Liu, 2011. The anti-hyperplasia of mammary gland effect of Thladiantha dubia root ethanol extract in rats reduced by estrogen and progestogen. J. Ethnopharmacol., 134: 136-140.

Yokosuka, K., J.S. Park, K. Jimbo, K. Yamada and K. Sato *et al*., 2006. Advanced glycation end-products downregulating intervertebral disc cell production of proteoglycans *in vitro*. J. Neurosurg. Spine., 5: 324-329.

Yu, G.H., L.Y. Zou and J.G. Liu, 2010. Combination of acupuncture with medication for treatment of hyperplasia of mammary glands in 46 cases. J. Traditional Chin. Med., 30: 232-234.