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Review

Role of arginine and its methylated derivatives in cancer biology and treatment

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Abstract

Both L-arginine supplementation and deprivation influence cell proliferation. The effect of high doses on tumours is determined by the optical configuration: L-arginine is stimulatory, D-arginine inhibitory. Arginine-rich hexapeptides inhibited tumour growth. Deprivation of L-arginine from cell cultures enhanced apoptosis. The pro-apoptotic action of NO synthase inhibitors, like NG-monomethyl-L-arginine, is manifested through inhibition of the arginase pathway. NG-hydroxymethyl-L-arginines caused apoptosis in cell cultures and inhibited the growth of various transplantable mouse tumours. These diverse biological activities become manifest through formaldehyde (HCHO) because guanidine group of L-arginine in free and bound form can react rapidly with endogenous HCHO, forming NG-hydroxymethylated derivatives. L-arginine is a HCHO capturer, carrier and donor molecule in biological systems. The role of formaldehyde generated during metabolism of NG-methylated and hydroxymethylated arginines in cell proliferation and death can be shown. The supposedly anti-apoptotic homozygous Arg 72-p53 genotype may increase susceptibility of some cancers. The diverse biological effects of L-arginine and its methylated derivatives call for further careful studies on their possible application in chemoprevention and cancer therapy.

Introduction

L-arginine (Arg), an essential amino acid, is required for normal growth of microbes, plants and animals. Deprivation of this amino acid from the culture medium or other sources of nutrition causes serious disturbances in cellular and organ function leading to total destruction. On the other hand, excessive doses of Arg also influence cell function, including cell death and cell proliferation. Substantial information has been obtained in the past decades on the role of Arg in tumour growth and in tumour therapy.

Effect of Arg deprivation and supplementation on tumour cell proliferation

Arg, an essential amino acid, is required to maintain normal metabolism and proliferation of cells in culture [1]. Attempts to influence tumour cell proliferation by changes in amino acid balance were based on such observations. The role of the enzyme arginase, which decreases the amount of Arg, was thoroughly investigated in this respect and also used in the therapy of human tumours [2]. According to Umeda *et al.*[3], the proliferation of both HeLa cells in vitro and rat Novikoff hepatoma in vivo

could be decreased by arginase, causing relative Arg deficiency. Otsuka [4] has shown that an enzyme, very similar to arginase inhibits DNA synthesis in normal rat liver. The proliferation promoting activity of L-Arg is also underscored by the fact, that Arg is converted by arginase to L-ornithine, which is the precursor of various polyamines essential for cell proliferation [5]. Tanaka *et al.*[6] have demonstrated the death of 3T3 cells after Arg deprivation. Wheatley *et al.*[7–10] analysed the effect of deprivation of eleven essential amino acids on several tumour cell lines and found that apoptotic-like cell death occurs as a consequence of this manipulation. The cell lines died considerably more quickly during Arg deprivation than in the absence of any other essential amino acids. Moreover, when co-cultures of normal and tumour cells were deprived of Arg the normal cells survived and the tumour cells died. According to these observations, Arg deprivation causes selective death of cultured malignant cells. Lamb and Wheatley [11] have also shown, that Arg deprivation most probably impairs the control of DNA synthesis at the G_1 checkpoint, which normally inhibits its initiation of DNA synthesis under unfavourable conditions.

Arg imbalance was also produced by excess of Arg supplementation in the diet. Brittenden *et al.*[13] Suggested a possible therapeutic effect of Arg-rich diet in malignant disease, in combination with anti-cancer chemotherapy. Ogilvie *et al.*[14] found that excess Arg combined with doxorubicin chemotherapy extended disease-free interval and survival time of dogs with lymphoma. According to the studies of Hester and Fee [15] on squamous cell carcinoma in the CH3/KM mouse the mechanism of action of high amounts of Arg may be the stimulation of host immune surveillance. However, Robinson *et al.*[16] found that Morris hepatoma-bearing rats fed with Arg-rich diet did not show any alteration in tumour growth or cytokine production. The role of Arg in carcinogenesis has been challenged by the experiments of Weinberger *et al.*[17] who found that high doses of Arg glutamate decreased the carcinogenic activity of various acetamine-derivatives in rats.

Interesting data were reported on Arg-induced apoptosis of pancreatic acinar cells both in vitro and in vivo [18] providing a model of acute pancreatitis. The possible therapeutic use of Arg against pancreatic acinic cell carcinoma has not been examined yet.

Arg-rich hexapeptides were identified from peptide libraries that inhibit the interaction of vascular endothelial growth factor to its receptor. These hexapeptides inhibit the proliferation of human umbilical vein endothelial cells and also block the angiogenesis induced by vascular endothelial growth factor in vivo, in the chick chorioallantoic membrane and in the rabbit cornea. One of the hexapeptides blocks the growth and formation of metastases of HM7 human colon carcinoma cells in nude mice [19]. These results may serve as leads for development of anticancer drugs.

Arg imbalance was established in our early in vivo experiments [20]. High doses of L-Arg, D-Arg and DL-Arg (400–500–1000 mg/kg body weight intraperitoneally or orally) were administered to Wistar rats bearing subcutaneous Yoshida's sarcoma or to Swiss mice bearing subcutaneous Ehrlich carcinoma for 9–15 days (table 1). D-Arg inhibited the growth of Yoshida's sarcoma significantly (50%, $p < 0.05$), when applied in a daily dose of 500 mg/kg, orally. Intraperitoneal administration of the same dose to Ehrlich carcinoma bearing mice resulted in a 20%, statistically not significant, inhibition.

L-Arg, however, enhanced the growth of Yoshida's sarcoma, when given intraperitoneally or orally in a dose of 400 mg/kg. The same tendency, namely significant (40%) enhancement was seen after intraperitoneal treatment (400 mg/kg) of Ehrlich carcinoma bearing mice.

Table 1: Effect of D-Arg, L-Arg and DL-Arg on the growth of transplantable animal tumours

Treatment	Tumour	*Number of animals*		Daily dose (mg/kg)	Duration of treatment (days)	Inhibition %	*Remarks*
		Treated	Control				
D-Arg[1]	Yoshida	6	6	500 p.o.	12	50	$p < 0.05$
D-Arg [1]	Ehrlich s.c.	10	10	500 i.p.	10	20	$p < 0.1$
L-Arg[3]	Yoshida	10	10	400 i.p.	10	-40	Enhancement $p < 0.05$
L-Arg[3]	Yoshida	10	10	400 p.o.	9	-30	Enhancement $p < 0.1$
L-Arg[2]	Ehrlich s.c.	10	10	500 i.p.	12	-40	Enhancement $p < 0.05$
DL-Arg[3]	Ehrlich s.c.	10	10	500 i.p.	12	-	-

[1]Serva (USA), [2]Ajinomoto (Japan), [3]Reanal (Hungary) Animals were sacrificed 24 hours after the last treatment and tumour weight was measured. Inhibition or enhancement is given as per cent of the control tumours. Abbreviations:i. p. – intraperitoneal; p. o. – peroral; s. c. – subcutaneous; Arg – arginine

Intraperitoneal application of 500 mg/kg DL-Arg to mice, inoculated with Ehrlich carcinoma had neither inhibitory nor enhancing effect on tumour growth. Various pathways of metabolism of this amino acid may explain the mode of action of Arg imbalance. Among these methylation and hydroxymethylation appear to be of special importance.

Effect of methylated, hydroxy and hydroxymethylated Arg on cell death and proliferation

Arg is a highly reactive compound, both as a free amino acid and as a constituent of a protein. The structure of proteins can be altered by specific enzymatic modification of the side chains. One of these protein-modifying reactions is methylation, resulting in the addition of methyl groups to the guanidine residues of Arg. Methylated Arg also occur in free form, possibly resulting from enzymatic hydrolysis of methylated proteins in vivo (fig. 1).

Mono-di- and trimethyl Arg, hydroxymethyl Arg, N-omega-hydroxy L-Arg, N-nitro-L-Arg methyl ester, Nitro-Arg were studied regarding the possible effect on cell death and cell proliferation of these compounds.

Tyihák *et al.*[21] demonstrated that monomethyl Arg and dimethyl-Arg inhibit significantly the growth of tobacco tissue cultures in concentrations of 10–100 ppm in agar nutrient medium, NG-methylated Arg added to agar-medium also significantly inhibited the growth of the roots of lettuce seedlings. Further investigations along this line carried out by Szende *et al.*[22] have shown that NG-hydroxymethyl Arg inhibited dose-dependently and significantly the proliferation of HT-29 human colon carcinoma cells, P-388 mouse lymphoma cells and PC-3 human prostate carcinoma as well as K-562 human erythroleukaemia cells in culture. The cells of the treated cultures showed morphological signs of apoptosis in a high percentage. In our in vivo experiments [20] hydroxymethylated Arg was administered to Swiss mice inoculated with Ehrlich ascites tumour. The daily dose of NG-hydroxymethyl Arg was 400 mg/kg intraperitoneally, based on previous acute toxicity studies. After 10 days of NG-hydroxymethylated Arg treatment complete inhibition of the growth of Ehrlich ascites tumour was observed.

C57B1 mice, inoculated with Lewis lung tumour intramuscularly and made tumour free by amputation of the tumorous leg 10 days after tumour transplantation, were treated for seven days with 400 mg/kg NG-hydroxymethyl Arg, daily, intraperitoneally. The treatment started 24 hours after amputation. The animals were sacrificed on the 8^{th} day after starting the treatment. Lung metastasis number and volume were determined. The average metastasis number in the treated animals was 27, in the controls 54. The average volume of the lung nodules was 34 mm^3 in the treated and 50 in the control mice.

The anti-proliferative and apoptosis-inducing effect of Arg-derivatives was confirmed by the studies of Singh *et al.*[5] who found that N-omega-hydroxy-L-Arg inhibited the proliferation of the high-arginase-expressing MDH-MB-468 cells and induced apoptosis after 48 hours. It has also been shown by Washo-Stultz *et al.*[23] that N-nitro-Arg methyl ester sensitised cells to apoptosis induced by sodium deoxycholate.

L-Arg, nitro-Arg and methyl-Arg have been found to induce increase in cytosolic Ca concentration in cultured NIT-1 cells [24], leading to depolarisation of the plasma membrane potential, a phenomenon common during the process of apoptosis.

NG-methyl-L-Arg [25] N-nitro-L-Arg methyl ester [23], N-hydroxy-L-Arg [5] and L-NG-methyl-Arg [26] all proved to be inhibitors of nitric oxide synthase. Nitric oxide synthase converts L-Arg to produce NO, which "Janus-faced" compound certainly may influence both cell proliferation and cell death.

Role of formaldehyde in the mechanism of action of methylated arginines

Another possible mode of action of methylated and hydroxymethylated Arg can be deduced from the fact that these molecules are formaldehyde generators. It has been demonstrated by Hullán *et al.*[27] that NG-hydroxymethyl Arg as a biomolecule is one of the compounds that are responsible for the endogenous formaldehyde level. The guanidine group of L-Arg can bind one, two or three molecules of CH_2O and in the reaction mono-, di- and trihydroxymethylated Arg derivatives are formed. This process is catalysed by the enzyme transmethylase [28]. The hydroxymethylated derivatives of Arg are relatively stable compounds. Arg is suitable to carry the endogenous CH_2O in form of hydroxymethyl group in biological systems. The hydroxymethyl groups are attached to the guanidine group by reversible bindings [29]. Although little is known about the demethylation of NG-methylated Arg, NG-hydroxymethyl-L-Arg generates a direct CH_2O-yielding activity, which may be responsible for its apoptotic effect [22,30]. It has also been shown in our recent experiments [31] that the administration of analytically pure formaldehyde to cell cultures causes dose dependently apoptosis (1–10 μg/ml) or stimulation of DNA synthesis and cell proliferation (0.1–0.01 μg/ml). The calculated quantity of formaldehyde released by demethylation processes from hydroxymethyl Arg is in the above mentioned range and the formaldehyde-mediated biological action of this compound has to be taken into consideration.

Lysine-arginine antagonism

The lysine-arginine antagonism widely shown in nature is also represented in apoptosis resistant cell lines that contain A-to G alteration is the death domain, encoding L-arginine instead of L-lysine in codon 441 [32].

Arg 72-p53 genotype and cancer

Over-representation of the homozygous Arg 72-p53 genotype in cervical carcinoma patients has been reported [33]. However, Tachezy *et al.*[34] did not find increased risk for human papilloma virus-associated cervical tumour development associated with Arg 72-p53.

Conclusions

Although data in the literature are pointing to the anti proliferative effect of both Arg depletion and supplementation, Arg proved to be essential for tumour cell growth. This observation raises the question, whether decreasing the concentration of Arg in nutrients and consequently in blood serum or the administration of D-arginine may lead to retardation of tumour growth in humans, too. Arg, because of its strong basic guanidine group, plays an important role in molecular interactions in biological systems, such as interaction between Arg and formaldehyde, both of which are normal components of cells and biological fluids. As a result, hydroxymethyl derivatives of Arg are formed. These compounds may be the source of formaldehyde generation. Arg may be considered a formaldehyde capturer, carrier and generator molecule. These functions may also play role in the biological activity of Arg and its methylated and hydroxymethylated derivatives. An interesting therapeutic possibility worthy of further investigation may be the administration of methylated and hydroxymethylated Arg in order to induce tumour cell death or to prevent tumour cell proliferation.

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