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Full-length article

Inhibitory effect of agmatine on proliferation of tumor cells by modulation of polyamine metabolism[1]

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Abstract

Aim: To assess the inhibitory effect of agmatine on tumor growth *in vivo* and tumor cell proliferation *in vitro*. **Methods:** The transplanted animal model, [^{3}H]thymidine incorporation assay, 3-[4,5-dimethythiazol-2-yl]-2,5-diphenyltetrazolium assay, and lactate dehydrogenase (LDH) release assay were performed. **Results:** Agmatine, at doses of 5–40 mg/kg, suppressed the S_{180} sarcoma tumor growth dose-dependently in mice *in vivo* and the highest inhibitory ratio reached 31.3% in Kunming mice and 50.0% in Balb/c mice, respectively. Similar results were obtained in the transplanted B_{16} melanoma tumor model. Agmatine (1–1000 μmol/L) was able to attenuate the proliferation of cultured MCF-7 human breast cancer cells in vitro in a concentration-dependent manner and the highest inhibitory ratio reached 50.3% in the [^{3}H]thymidine incorporation assay. Additionally, in the LDH release assay, spermine (20 μmol/L) and spermidine (20 μmol/L) increased the LDH release significantly, but agmatine (1–1000 μmol/L) did not, indicating that the inhibitory effect of agmatine on the proliferation of MCF was not related to cellular toxicity. In the [^{3}H]thymidine incorporation assay, putrescine (12.5–100.0 μmol/L) could reverse the inhibitory effect of agmatine on the proliferation of MCF concentration-dependently, suggesting that the inhibitory effect of agmatine on the proliferation of MCF might be associated with a decreased level of the intracellular polyamines pool. **Conclusion:** Agmatine had significant inhibitory effect on transplanted tumor growth *in vivo* and proliferation of tumor cells *in vitro*, and the mechanism might be a result of inducing decrease of intracellular polyamine contents.

Introduction

Polyamines, including putrescine, spermidine, and spermine, are required for cell proliferation and homeostasis. The intracellular pool of polyamines is precisely regulated through their biosynthesis, degradation, uptake, and excretion[1]. The disorder of intracellular polyamines plays an important role in carcinogenesis. Polyamines can promote the neoplastic transformation of normal cells, stimulate the proliferation of tumor cells, and facilitate angiogenesis in tumor tissues. Therefore, their metabolism pathway is an interesting anticancer drug target[2].

Agmatine, one of the analogs of polyamines, is the product of *L*-arginine decarboxylation and was initially believed to be present only in bacteria, plants, and invertebrates. Now it has been shown to be present in mammals[3]. The accumulated results show that agmatine has some important biological activities[4]. Among them, the inhibitory effect of agmatine on cell proliferation is of great interest.

The current results show that agmatine is able to modulate the cellular concentration of polyamines[5]. Agmatine can be hydrolyzed to putrescine and urea. Putrescine is then converted into spermidine and spermine by spermidine/spermine synthases[6]. So agmatine might have the capacity to increase the level of intracellular polyamines. In addition, agmatine has been postulated to decrease the cellular level of polyamines. There is much evidence to support this hypothesis. First, because agmatine and polyamines are

structurally analogous and derived from same precursor, *L*-arginine[7], administration of exogenous agmatine would be able to reduce the synthesis of polyamines by a back-feed way. Second, as a competitor, agmatine can retard putrescine intake by the same carrier[8]. Most importantly, besides polyamines, agmatine is the only known molecule that has the capacity to induce antizyme[9]. Antizyme is the only known endogenous protein that binds to ornithine decarboxylase, inhibiting its activity and accelerating its degradation. Indeed, when tested *in vitro*, agmatine inhibited DNA synthesis and proliferation in some cell lines[10]. Moreover, Regunathan *et al*[11] reported that agmatine inhibited proliferation of human coronary artery vascular smooth muscle cells by stimulation of imidazoline receptors. Satriano *et al*[12] claimed that agmatine dramatically decreased the ratio of DNA synthesis on mouse kidney proximal tubule cells by attenuation of the cellular polyamine level. In 2003, Gardini *et al*[13] found that agmatine inhibited the proliferation of rat hepatoma cells. These results indicate that agmatine might be an endogenous anti-proliferation factor, and whether the pharmacological effect of exogenous agmatine on cells *in vivo* is the same as *in vitro* is an interesting question.

In the present study, we investigated the inhibitory effects of agmatine on several classical tumor cells *in vivo* and *in vitro* and explored its possible mechanisms *in vitro.*

Materials and methods

Reagents and drugs Agmatine sulfate was obtained from the Beijing Institute of Pharmacology and Toxicology; cyclophosphamide was manufactured by Hengrui Pharmaceutical Co (Lianyungang, Jiangsu, China); spermine, spermidine, putrescine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and sodium dodecylsulfonate (SDS) were obtained from Sigma Chemical (St Louis, MO, USA); Roosevelt Park Memorial Institute medium (RPMI-1640) was purchased from Gibco (Carlsbad, CA, USA); and [^{3}H]thymidine was obtained from DuPont/NEN Company (Boston, MA, USA).

Animals Male Kunming, Balb/c, and C_{57} mice [20±2 g, Grade II, Certificate No SCXK (Jun) 2002-001, Experimental Animal Center of Academy of Military Medical Sciences] were used. After transplanted with tumor cells, animals were randomly distributed into different groups. The control group was administered with saline alone and the others were treated with different drugs. All drugs were dissolved in normal saline and freshly prepared on the experimental day. Both normal saline and agmatine were administered subcutaneously (sc) and cyclophosphamide was injected intraperitoneally (ip) in a volume of 10 mL/kg. All of the animals were housed and maintained in a temperature-controlled room (22 °C–24 °C) with free access to qualified food and water at all times.

Cell culture MCF-7 human breast cancer cells were maintained in RPMI-1640 medium with 10% fetal bovine serum (FBS), 100 kU/L penicillin and 100 kU/L streptomycin. Cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere and subcultured every 3 d.

Evaluation of agmatine's inhibitory effects on the growth of tumor cells *in vivo* S_{180} sarcoma and B_{16} melanoma cells were subcultured in the abdominal cavity of mice for 8 d. The resulting ascites were diluted with saline to form a suspension containing 2×10^{10} cells/L. Aliquots of cell suspensions (0.2 mL) were injected (sc) into the right armpit of the mice. From the 1st day after implantation, saline, agmatine (5–40 mg/kg, tid, sc), or cyclophosphamide (20 mg/kg, qd, ip) were administered for 10 d. At d 10, the animals were killed and the tumors were chipped from their armpits. The tumor weights (g) were measured and the mean tumor weight of every group was calculated. The anti-tumor activities of the drugs were determined by a comparison between the inhibitory ratios obtained from the treated groups and the control group. The inhibitory ratio of drugs was expressed as [(average tumor weights in saline group–average tumor weights in drug treated group)/(average tumor weights in saline group)×100].

Measurement of proliferation *in vitro* in [^{3}H]thymidine incorporation assay Proliferation of the MCF cells was assessed by [^{3}H]thymidine incorporation assay. Briefly, cells suspended in RPMI-1640 medium with 10% FBS were seeded into a 96-well cell culture plate (80 μL/well) at a density of 6000 cells/well. Then they were treated with saline (control), or different concentrations of agmatine (1, 10, 100, 200, 500, or 1000 μmol/L), respectively, at a volume of 20 μL. Drugs were added for a total period of 48 h and [^{3}H]thymidine (3.7×10^4 Bq/well) was added at 36 h of incubation. The medium was removed and the cells were washed three times with phosphate-buffered saline and then twice with ice-cold 10% trichloroacetic acid. Fixed cells were then solubilized in 0.2 mol/L NaOH (100 μL/well) and sonicated for 15 min. After mixing with scintillant liquid (1 mL) for 24 h, an aliquot (90 μL/well) was used for scintillation counting. Then the radioactivity was determined with a Multi-purpose Scintillation Counter (Columbus Instruments, Columbus, OH, USA). The mean cpm value of every group was calculated. The anti-proliferation potency of the drugs was determined by a comparison between the inhibitory ratios obtained from the

treated groups and the control. The inhibitory ratio of the drugs was expressed as [(average cpm value in control group–average cpm value in drug treated group)/(average cpm value in control group)×100%].

Measurement of proliferation *in vitro* Cell proliferation was also confirmed again by measuring with MTT assay based on the colorimetric measurement of formazan dye formed from MTT by mitochondrial dehydrogenases. Exponentially growing cells were plated at a seeding density of 7.5×10^4 cells/mL in 96-well plates (80 μL/well). Then they were treated with saline (control), or different concentrations of agmatine (100, 200, 500, or 1000 μmol/L), respectively, at a volume of 20 μL. After they were incubated with or without drugs for 44 h, 20 μL of MTT reagent (0.5 g/L) was added to each well. The plates were incubated at 37 °C for an another 4 h. At the end of the incubation, the formazan crystals formed by MTT metabolism were solubilized by the addition of 100 μL of 10% SDS to each well. After 16 h, the absorbance of the solubilized product was measured at 570 nm in a Micro-plate Reader (Molecular Devices Corporation, Sunnyvale, CA, USA). The anti-proliferation potency of the drug was determined by a comparison between the inhibitory ratios. The percentage of growth inhibition was calculated by comparison of the absorbance of the treated group versus the control [(average absorbance value in control group-average absorbance value in drug treated group)/(average absorbance value in control group)×100].

Lactate dehydrogenase release assay To assess whether the reduction of cell numbers was attributable to the cellular toxicity of polyamines or agmatine, we measured the release of lactate dehydrogenase (LDH) in cell medium after drug treatment. Cells were cultured at a seeding density of 7.5×10^4 cells/mL in 24-well plates (800 μL/well). They were treated with saline, a range of concentrations of agmatine, spermidine, or spermine, respectively, at a volume of 200 μL and incubated at 37 °C and 5% CO_2 for 48 h. Then 0.8 mL of the supernatant of each well was used for analysis by an Automatic Biochemical Analyzer (Hitachi7020, Tokyo, Japan). The release of LDH from the treated cells was compared to the control.

Statistical analysis Data were expressed as mean±SD. SAS software (SAS Inc, Raleigh, NC) was used to conduct a one-way ANOVA. $P<0.05$ was considered statistically significant.

Results

Inhibitory effects of agmatine on the growth of S_{180} sarcoma tumor cell lines in Kunming and Balb/c mice In Kunming mice transplanted with S_{180} sarcoma tumor cell lines, the S_{180} cells grew well and the average tumor weight reached 1.6 g in the normal, saline-treated group. Meanwhile, cyclophosphamide, a clinically approved anticancer agent, exhibited significant anti-tumor activity. In the cyclophosphamide-treated group (20 mg/kg, qd, ip), the average tumor weight was only 0.7 g and the inhibitory ratio was 56.3%. Agmatine also exerted a remarkable inhibitory effect on tumor growth. In the agmatine-treated groups (5–40 mg/kg, tid, sc), the tumor weights were significantly reduced (n=27, $P<0.05$) in a dose-dependent manner. The inhibitory ratio of tumor growth reached 31.3% at a dose of 40 mg/kg (Table 1).

Table 1. Anti-tumor activity of agmatine on S_{180} in Kunming mice. The S_{180} sarcoma tumor cells were inoculated (sc) into Kunming mice on d 0. The bearing mice were randomly distributed into 6 groups. Each group contained 27 mice. From d 1, they were injected with saline, cyclophosphamide, or differeent doses of agmatine, respectively, at a volume of 0.1 mL/10 g. After 10 d, tumor weight was measured. n=27. Mean±SD. $^cP<0.01$, compared with the saline-treated group. "–" represents 0.1 mL/10 g saline.

	Dose /mg·kg^{-1}	Tumor weight /g	Ratio of inhibition/%
Saline	–	1.6±0.9	
Cyclophosphamide	20	0.7±0.2^c	56.3
Agmatine	5	1.7±0.4	-6.3
	10	1.3±0.7	18.8
	20	1.1±0.6^c	31.3
	40	1.1±0.8^c	31.3

In Balb/c mice transplanted with S_{180} sarcoma tumor cell lines, we obtained similar results. The average tumor weight was 1.0 g in the normal, saline-treated group. Cyclophosphamide (20 mg/kg, qd, ip) inhibited tumor growth significantly, and the inhibitory ratio reached 50.0%. Agmatine also inhibited the growth of tumors in a dose-dependent manner (n=10, $P<0.05$). The inhibitory ratio of agmatine on tumor growth was 50.0% at a dose of 40 mg/kg (Table 2).

Inhibitory effects of agmatine on the growth of B_{16} melanoma tumor cell lines in C_{57} mice In C_{57} mice transplanted with B_{16} melanoma tumor cells, the tumor weight was 1.8 g in the normal, saline-treated group. In the cyclophosphamide-treated group (20 mg/kg, qd, ip), the tumor weight decreased to 1.0 g and the inhibitory ratio reached 44.4%. Agmatine (2.5–20.0 mg/kg, tid, sc) significantly suppressed the growth of the tumor (n=10, $P<0.05$), but the effect did not exhibit an obvious dose-dependent relationship (Table 3).

Inhibitory effect of agmatine on the proliferation of MCF cells *in vitro* In the [^{3}H]thymidine incorporation assay, the

Table 2. Anti-tumor activity of agmatine on S_{180} cell growth in Balb/c mice. In Balb/c mice transplanted with S_{180} sarcoma tumor cell lines as well, we acquired similar results. On d 0, the S_{180} sarcoma cells were inoculated (sc). The 60 bearing mice were reandomly distributed into 6 groups. Each group contained 10 mice. From d 1, they were injected with saline, cyclophosphamide, or different doses of agmatine, respectively, at a volume of 0.1 mL/10 g. After 10 d, tumor weight was measured. n=10. Mean±SD. $^bP<0.05$, $^cP<0.01$, compared with the saline-treated group. "–" represents 0.1 mL/10 g saline.

	Dose /mg·kg^{-1}	Tumor weight /g	Ratio of inhibition/%
Saline	–	1.0±0.3	
Cyclophosphamide	20	0.5±0.3^c	50.0
Agmatine	5	0.8±0.3	20.0
	10	0.6±0.2	40.0
	20	0.5±0.2^b	50.0
	40	0.5±0.3^c	50.0

Table 3. Anti-tumor activity of agmatine on B_{16} cell growth in C_{57} mice. The B_{16} melanoma cell lines were inoculated (sc) into C_{57} mice on d 0. The 60 bearing mice were randomly distributed into 6 groups. Each group contained 10 mice. From d 1, they were injected with saline, cyclophosphamide, or different doses of agmatine, respectively, at a volume of 0.1 mL/10 g. After 10 d, tumor weight was measured. n=10. Mean±SD. $^bP<0.05$, compared with the saline-treated group. "–" represents 0.1 mL/10 g saline.

	Dose /mg·kg^{-1}	Tumor weight /g	Ratio of inhibition/%
Saline	–	1.8±0.3	
Cyclophosphamide	20.0	1.0±0.4^b	44.4
Agmatine	2.5	0.9±0.5	50.0
	5.0	0.6±0.3^b	66.7
	10.0	0.9±0.6^b	50.0
	20.0	0.8±0.6^b	55.6

MCF cells grew well and the average cpm value was 3143.8 in the normal, saline-treated group after a 48-h incubation. Agmatine showed anti-proliferation activity compared with the saline-treated group in a concentration-dependent manner. The cpm value was significantly reduced (n=8, P<0.05) after pretreatment with agmatine (1–1000 μmol/L). The inhibitory ratio of cell proliferation was 50.3% at a concentration of 1000 μmol/L (Table 4).

This effect of agmatine on cellular proliferation was further proved with the MTT assay. The MCF cells grew well and the absorbance value was 0.99 in the normal, saline-treated group after a 48-h incubation. Agmatine showed anti-proliferation activity compared with the saline-treated group in a concentration-dependent manner. The absorbance value was significantly reduced (n=8, P<0.05) after the cells were administered with agmatine (100–1000 μmol/L). The inhibitory ratio of cell growth was 23.8% at a concentration of 1000 μmol/L (Table 5).

Table 4. Effect of agmatine on MCF-7 human breast cancer cells proliferation in a [^{3}H]thymidine incorporation assay. In the assay, the cells were seeded into a 96-well cell culture plate at a density of approximately 6000 cells/well. Then they were treated with saline (control), or different concentrations of agmatine (1, 10, 100, 200, 500, or 1000 μmol/L), respectively, at a volume of 20 μL. Each group contained 8 wells. Drugs were added for a total period of 48 h, and [^{3}H]thymidine (3.7×10^4 Bq/well) was added at 36 h of incubation. To evaluate the anti-proliferation effect, the cpm value was counted and the inhibitory ratio was calculated. n=8. Mean±SD. $^bP<0.05$, compared with control.

	Concentration /μmol·L^{-1}	[^{3}H]thymidine incorporation/cpm	Inhibitory ratio/%
Saline		3143.8±506.5	0
Agmatine	1	2776.8±662.3	11.7
	10	2610.0±940.3	17.0
	100	2576.3±345.3	18.1
	200	2380.0±533.8	24.3
	500	2080.0±327.0^b	33.8
	1000	1562.5±264.0^b	50.3

Table 5. Effect of agmatine on MCF-7 human breast cancer cells proliferation by MTT. The cells were seeded into a 96-well cell culture plate at a density of approximately 6000 cells/well. Then they were treated with saline (control), or different concentrations of agmatine (100, 200, 500, or 1000 μmol/L), respectively, at a volume of 20 μL. Each group contained 8 wells. Drugs were added for a total period of 48 h and MTT (2.5 g/L, 20 μL/well) was added at 44 h of incubation. To evaluate the anti-proliferation effect, the absorbance of the solubilized product was measured at 570 nm on a Micro-plate Reader (Molecular Devices Corporation, Sunnyvale, CA, USA). The percentage of inhibition was calculated by comparison of the absorbance of the treated group versus the control. n=8. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* control.

	Concentration /μmol·L^{-1}	Absorbance	Inhibitory ratio/%
Saline		1.01±0.02	
Agmatine	100	0.97±0.03^b	4.0
	200	0.91±0.03^c	9.9
	500	0.86±0.03^c	14.9
	1000	0.77±0.02^c	23.8

We then investigated the time-dependent effect of agmatine on MCF cell proliferation. MCF cells were cultured for different lengths of time in the presence of 1 mmol/L agmatine, and cell viability was evaluated by a [^{3}H]thymidine incorporation assay. Over 48 h, the inhibitory potency of agmatine strengthened gradually with prolonged time (n=8, P<0.01). The inhibitory ratio was 10%, 17%, 38%, and 62% at 12 h, 24 h, 36 h and 48 h, respectively (Figure 1).

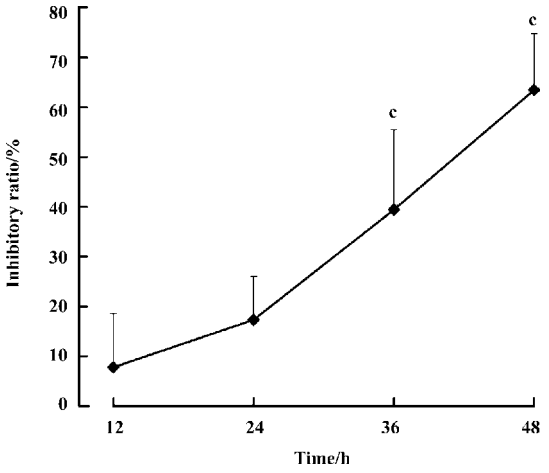

Figure 1. Time-dependent effect of agmatine on MCF-7 human breast cancer cells proliferation. MCF cells were cultured for different lengths of time in the presence of 1 mmol/L agmatine, and cell viability was evaluated by [^{3}H]thymidine incorporation assay. Over 48 h, the inhibitory potency of agmatine strengthened gradually with prolonged time. Mean±SD for 4 experiments. n=8. ^cP<0.01, compared with the saline-treated group.

Effect of agmatine on LDH release in the medium To assess whether the reduction of cell numbers was attributable to toxicity of polyamines or agmatine, we measured the release of LDH in cell medium after drug treatment. Administration on MCF cells for 48 h, spermine (20 μmol/L) or spermidine (20 μmol/L) significantly increased the activity of LDH in the medium, but agmatine (1–1000 μmol/L) did not. At a concentration of 1000 μmol/L, agmatine decreased the LDH activity significantly (n=8, P<0.05; Table 6).

Putrescine reverses agmatine's inhibitory effect on MCF cell proliferation As polyamines are essential growth factors, their dramatic intracellular decrease may be the main mechanism of the anti-proliferation action of agmatine. To check whether this is the mechanism involved, MCF cells were treated simultaneously with agmatine (1 mmol/L) and putrescine (12.5–100.0 μmol/L). Putrescine counteracted the inhibitory effect of agmatine on MCF cell proliferation in a concentration-dependent manner (n=8, P<0.05; Table 7).

Table 6. Effect of agmatine, spermidine, or spermine on lactate dehydrogenase (LDH) release in MCF-7 human breast cancer cells. To assess whether the reduction of cell numbers was attributable to toxicity of polyamines or agmatine, we measured the release of LDH in cell medium after drug treatment. The cells were plated at a seeding density of 7.5×10^4 cells/mL in 24-well plates. Then they were treated with saline (control), spermine (20 μmol/L), spermidine (20 μmol/L), or different concentrations of agmatine (1, 10, 100, or 1000 μmol/L), respectively, at a volume of 200 μL. Each group contained 8 wells. Drugs were added for a total period of 48 h and then 0.8 mL of the supernatant of each well was used for analysis by the Automatic Biochemical Analyzer (Hitachi7020, Tokyo, Japan). The percentage release of LDH from the treated cells was calculated by comparing it to the control cells. n=8. Mean±SD. ^bP<0.05, ^cP<0.01, compared with control.

	Concentration /μmol·L^{-1}	LDH release /U·L^{-1}
Saline		26.0±1.6
Agmatine	1	25.8±0.9
	10	27.0±1.2
	100	25.8±1.3
	1000	22.3±0.9^b
Spermine	20	51.0±2.2^c
Spermidine	20	28.8±0.5^c

Table 7. Reversal of agmatine's effect on MCF-7 human breast cancer cells proliferation by putrescine. The cells were seeded into a 96-well cell culture plate at a density of approximately 6000 cells/well. Then they were treated with saline (control), putrescine (100 μmol/L), agmatine (1000 μmol/L), or different concentrations of putrescine (12.5, 50.0, or 100.0 μmol/L), and agmatine (1000 μmol/L), respectively, at a volume of 20 μL. Each group contained 8 wells. Drugs were added for a total period of 48 h, and [^{3}H]thymidine (3.7×10^4 Bq/well) was added during the last 12 h of incubation. To evaluate the anti-proliferation effect, the cpm value was counted and the inhibitory ratio was calculated. n=8. Mean±SD. ^bP<0.05, ^cP<0.01, compared with control. ^eP<0.05, ^fP<0.01, compared with agmatine 1000 μmol/L. "–" represents 0.1 mL/10 g saline.

	Concentration /μmol·L^{-1}	[^{3}H]thymidine incorporation/cpm	Inhibitory ratio/%
Saline	–	2584.5±503.8	–
Putrescine	100	2960.8±1090.3	-14.6
Agmatine	1000	1224.5±241.3^c	52.6
Agmatine+putrescine	1000+12.5	1513.5±288.0^c	41.4
	1000+50	1673.3±335.8^{ce}	35.3
	1000+100	2005.0±395.5^{bf}	22.4

Discussion

The present study demonstrated the inhibitory properties of agmatine toward S_{180} sarcoma and B_{16} melanoma cells in vivo. We found that, at doses of 5–40 mg/kg, agmatine suppressed S_{180} and B_{16} cell growth in three kinds of mice *in vivo*. The highest inhibitory ratio was more than 50.0%. We applied for a Chinese patent with these results in 2002 (02125495.8). Although Gardini *et al* reported that agmatine inhibited the proliferation of rat hepatoma cells *in vitro* by modulation of polyamine metabolism in 2003[13], they did not report anything related to the inhibitory effects of agmatine on the growth of transplanted tumors *in vivo*; in addition, the paper by Gardini *et al*[13] was published much later than when we applied for the Chinese patent. It is reasonable to state, therefore, that our current results demonstrate for the first time that agmatine has an inhibitory effect on S_{180} and B_{16} cell lines *in vivo*.

Polyamines play an essential role in proliferation, differentiation, and neoplastic transformation in mammalian cells[1]. Indeed, cellular polyamine levels are higher in tumor cell lines. Conversely, the depletion of polyamines results in growth arrest of neoplastic cells *in vitro*. The polyamine-biosynthetic pathway is an inviting target for the development of agents inhibiting carcinogenesis and tumor growth. The present therapeutic agents acting on this pathway are α-difluoromethylornithine (DFMO) and polyamine analogs[2]. They influence both polyamine synthesis and degradation and are now being used in clinical trials.

Agmatine is an analog of polyamines and can modulate the cellular concentration of polyamines[14]. As an intermediate of putrescine, agmatine may be a factor for increasing the cellular concentration of polyamines. Although tumor cells and tissues have been reported to have increased polyamines levels compared with normal cells, this increase is often in the range of 2- to 3-fold. When putrescine levels are approximately 10-fold higher than those present in cancer cells, the cells undergo apoptosis[15]. It has also been reported that overloaded polyamines have toxic effects on some normal cells. The toxicity of polyamines was studied in a well-characterized neuronal system of cerebellar granule cells *in vitro*. Twenty-four-hour exposure to spermine (1–500 μmol/L) resulted in a concentration-dependent death of granule cells, with the half of lethal dose (LD_{50}) being reached at a concentration below 50 μmol/L. Putrescine was moderately toxic, with the LD_{50} at a concentration of only 500 μmol/L. The LD_{50} of spermidine was tested between concentrations of 50 and 100 μmol/L and its toxicity has been evaluated to be approximately 50% of that of spermine[16]. This was consistent with our results (data not shown). In contrast, agmatine has been postulated to decrease the cellular level of polyamines by inducing antizyme, competing with putrescine on transporter and other mechanisms.

So, in theory, agmatine might have double-edged effects on cell growth. But, to date, there has been no report that agmatine can enhance the proliferation of cells. Conversely, there is much evidence that agmatine can suppress cell proliferation, including different cells and malarial parasites[17,18]. Consistent with these studies, the present study proves agmatine has a significant inhibitory effect on cell proliferation in several classical solid tumors in a transplanted model *in vivo* and in an MCF model *in vitro*. Putrescine prevented the effect of agmatine on [^{3}H]thymidine incorporation in MCF cells. This effect of agmatine is similar to that of DFMO, which can block the synthesis of polyamines significantly. These results suggest that the effect of agmatine might be related to its influence on the synthesis of polyamine.

In addition, polyamines can interact with DNA directly[19], so, in our experiments, the decreased [^{3}H]thymidine incorporation may be caused by the inhibitory synthesis of DNA. As putrescine plays a partial role in energy supply[20], the results of the MTT assay indicate that agmatine might inhibit MCF cell proliferation by influence on energy metabolism. In addition, the effect on LDH activity in the medium showed that agmatine (1–1000 μmol/L) exhibited no cellular toxicity, whereas spermidine (20 μmol/L) and spermine (20 μmol/L) did. To sum up, these results partly demonstrate that agmatine does not increase the level of cellular polyamines and supports the conclusion that the anti-proliferation effect of agmatine is a result of polyamine limitation.

Currently, we know that agmatine's toxicity is low and its effects on tumor cells are not similar to those of classical chemotherapeutical drugs. Regarding its low toxicity, its enhancement of opioid analgesia, and its antidepressant effect[21,22], we hope that agmatine could efficiently improve life quality of cancer patients. There are still many issues to be explored, and further experiments should be carried out to confirm our results.

In conclusion, agmatine has significant inhibitory effects on transplanted tumor growth *in vivo* and proliferation of tumor cells *in vitro*. The possible mechanisms might be related to inducing decrease of intracellular polyamine contents.

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