Cancer Chemother Pharmacol (1985) 14: 74–78

Cancer Chemotherapy and Pharmacology

Contragestational profile of the tumor-inhibiting agent, L-alanosine, in the rat and the hamster

G. Galliani*, A. Assandri, D. Barone, M. Grandi, G. Mistrello, and G. C. Lancini

Lepetit Research Laboratories, Via Durando 38, I-20158 Milano, Italy

Summary. *L-Alanosine [L-2-amino-3(N-hydroxy-N-nitrosamino)propionic acid], a tumor-inhibiting agent, induces pregnancy arrest after single or multiple SC or PO administration to rats and hamsters. Its contragestational effects are dose- and route-dependent, with no important differences in species-sensitivity or administration schedules. L-Alanosine is maximally effective shortly (3–4 days) after implantation. Both placenta and fetus appear to be target tissues. Consistent with previous in vitro findings, adenine but not aspartic acid counteracts the contragestational action of L-alanosine. The 'contragestational test', i.e., the effect on conceptus growth, appears to be an interesting approach for learning more about the antiproliferative activity of an antineoplastic agent.*

Introduction

Antimetabolites and other cytotoxic agents interfering with proliferating cell systems can have both antitumor and contragestational activities [8, 15, 16, 18, 22]. Study of their effects on conceptus growth is an interesting tool for learning more about their action on replication processes, which may be applicable to their effects on 'abnormal' cell replication [1].

L-Alanosine [L-2-amino-3-(*N*-hydroxy, *N*-nitrosamino) propionic acid], an antimetabolite produced by *Streptomyces alanosinicus* [2, 12], has been shown to have marked inhibiting activity against some experimental tumors [13, 14, 17]. No studies aimed specifically at its potential contragestational activity in mammals have been reported [11, 19].

The in vitro mechanism of action of alanosine has been extensively investigated. It has been reported that in Novikoff rat hepatoma cells the compound arrests cell division by interfering with the biosynthesis of adenine nucleotides [7, 20], whereas in *Candida albicans* it acts as an antagonist of L-aspartic acid, inhibiting the synthesis of pyrimidine nucleotides [3, 4].

The present paper deals with studies conducted to assess L-alanosine's contragestational activity in hamsters and rats.

The effects of adenine and aspartic acid on the contragestational action of L-alanosine were also investigated.

Offprint requests to: G. Galliani

* *Present adress:* Roussel Maestretti, Pharmacology Dept., Viale G. Sasso 18, I-20131 Milan (Italy)

Materials and methods

Female Syrian golden hamsters (120–160 g) bred in our facilities and Sprague-Dawley rats (220–280 g) purchased from Charles River Italy were used.

The animals were kept in separate air-conditioned rooms at 60% relative humidity with a 14-h light:10-h darkness photoperiod. They had free access to standard chow and tap water. The females were mated and the morning that sperm was found in the vaginal smear was designated day 1 of pregnancy. In each case, L-alanosine (Na salt) was given dissolved in saline in volumes of vehicle of 2 ml/kg/day (SC) or 3–5 ml/kg/day (PO). Control animals were given the vehicle only.

Antifertility profile. The contragestational profile of L-alanosine was assessed by an experimental design previously described [5]. Briefly, hamsters and rats were given single SC injections during the first 11–13 days of pregnancy or single or multiple (5 days) SC and PO administrations during the most effective time of gestation. The effects on pregnancy were ascertained on day 14 in hamsters and on day 16 in rats. At autopsy the uterine horns were examined and the number of implantation sites and live fetuses were counted. Since L-alanosine was shown to have no preimplantation activity (see Table 1), only those animals with implantation sites were included in the data. Results were expressed as percent of pregnancies terminated and percent of live fetuses. The ED_{50} values reported are the doses (mg/kg/day) necessary to terminate pregnancy in 50% of the animals.

Effect of adenine and aspartic acid. Pregnant rats and hamsters were given single effective doses of L-alanosine during the most effective time of gestation. At the same time as L-alanosine administration, the animals received various doses of adenine (Sigma Chem. Co., St. Louis, Mo) or aspartic acid (Merck Darmstadt, Federal Republic Germany) IP, suspended in saline containing 2% Tween 80. These two latter drugs were also given at various intervals before and after L-alanosine treatment.

The effects on pregnancy were determined as described above.

Results

The contragestational effects of 10–25 mg/kg of L-alanosine in single SC doses during the first 11–13 days of gestation in rats and hamsters are shown in Table 1 and Fig. 1.

Table 1. Lack of preimplantation activity of L-alanosine in hamsters and rats

Species	Treatment on day (of pregnancy)	Dose (mg/kg) SC	No. of treated animals	No. of animals with implants	No. of implantation sites (mean ± SE)
Hamster	1	25	8	7	13.7 ± 0.5
	1	10	5	4	13.3 ± 1.8
	1	(Vehicle)	5	4	11.8 ± 1.2
	3	25	10	9	13.8 ± 0.6
	3	10	5	5	12.0 ± 0.7
	3	(Vehicle)	10	9	12.7 ± 1.0
Rat	1	10	8	6	9.8 ± 2.1
	1	(Vehicle)	8	7	10.4 ± 1.8
	3	10	6	6	10.6 ± 1.3
	3	(Vehicle)	6	6	10.8 ± 1.9
	5	10	6	5	11.8 ± 0.7
	5	(Vehicle)	6	5	10.3 ± 2.0

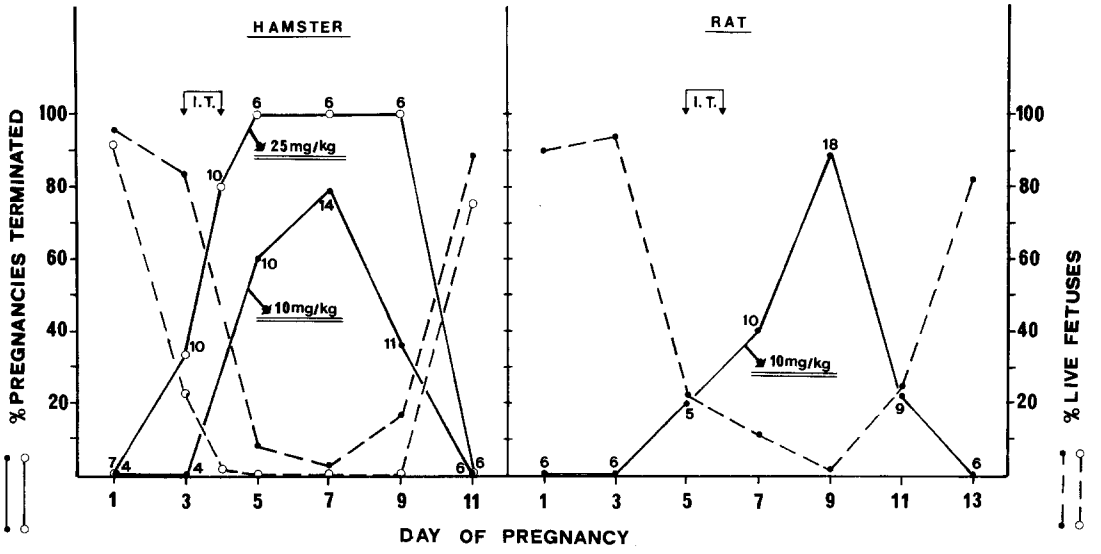

Fig. 1. Antifertility-inducing activity of a single injection of L-alanosine to hamsters and rats. On the *abscissa*, the day when the single injection was given. The number of animals is shown next to each value. I.T. = implantation time

When administered during the preimplantation period L-alanosine did not affect tubal transport of fertilized ova or the implantation of blastocysts. In fact, there were no significant differences in either the number of animals with implants or in the number of implantation sites between the treated and control animals (Table 1).

The data depicted in Fig. 1 refer to the postimplantation effect of L-alanosine. In both species, the maximal contragestational effect occurs 3–4 days after implantation, i.e., on day 7 in the hamster and on day 9 in the rat. The effectiveness of L-alanosine (10 mg/kg) decreases gradually when given before or after the day of maximal conceptus vulnerability. The results obtained in hamsters after two different doses (10 and 25 mg/kg) clearly indicate that the effects of L-alanosine on pregnancy are both related to time of gestation and dose-dependent.

Table 2 shows the dose-response effects for L-alanosine after single and multiple SC injection during the most effective period of gestation to hamsters and rats. A good dose-response relationship was obtained in both species, both as percentage of pregnancies terminated and percentage of live fetuses. The contragestational activity was practically the same for rats and hamsters, with ED_{50} values of about 7–7.5 mg/kg after a single SC injection and of 1.5–2 mg/kg/day after multiple SC administration (Table 3).

Given orally, L-alanosine was one-third to one-fifth as effective as when given parenterally (Table 3).

The results that show the influence of adenine on L-alanosine's antifertility effetcs are summarized in Table 4. As can be seen, adenine counteracts the embryocidal effect of L-alanosine in a dose- and time-dependent manner. The optimum time for the administration of the purine appears to be between the 2nd and the 8th h after L-alanosine, whereas later administrations (24–48 h) were completely ineffective. Similar results were obtained in the hamster.

Aspartic acid given in different doses, at different times, and by different routes of administration did not affect the

Table 2. Effects of L-alanosine on pregnancy after single and multiple injection during the most effective time of gestation

Species	Treatment on day(s) (of pregnancy)	Dose (mg/kg/day) SC	No. of animals	Pregnancies terminated (%)	Live fetuses (%)	ED_{50} (mg/kg/day)
Hamster	7	20	6	100	0	
	7	10	14	78.6	2.2	
	7	5	8	12.5	35.1	~ 7.5
	7	2.5	9	0	73,1	
	7	(Vehicle)	9	0	95.1	
	4– 8	5	6	100	0	
	4– 8	2	6	50	19.3	~ 2
	4– 8	1	5	0	66.7	
	4– 8	(Vehicle)	5	0	90.5	
Rat	9	20	8	100	0	
	9	10	16	87.5	3.1	
	9	5	6	33.3	32.0	~ 7
	9	2.5	4	0	62.7	
	9	(Vehicle)	7	0	91.1	
	6–10	5	5	100	0	
	6–10	2	16	80	1.6	
	6–10	1	6	16.7	32.3	~ 1.5
	6–10	0.5	4	0	78.8	
	6–10	(Vehicle)	8	0	93.1	

Table 3. Influence of species, route, and schedule of treatment on contragestational effects of L-alanosine

Species	Treatment on day(s) (of pregnancy)	Route	~ ED_{50}	
			mg/kg/day	Total dose (mg/kg)
Hamster	7	SC	7.5	7.5
	7	PO	30	30
	4– 8	SC	2	10
	4– 8	PO	10	50
Rat	9	SC	7	7
	6–10	SC	1.5	7.5
	6–10	PO	4	20

antifertility action of L-alanosine in either rats or hamsters (Table 5).

Discussion

L-Alanosine given by single or multiple SC, or PO administrations induces pregnancy arrest in the both rats and hamsters.

Consistent with what has been reported for other antimetabolites [8], L-alanosine has no preimplantation activity and exerts its maximum effectiveness shortly after implantation. In fact, in the rat its activity peaks on day 9, that is, 3–4 days after blastocyst nidation. The same antifertility activity was observed in the hamster. In this species, in which implantation occurs on days 3–4, the day of maximum conceptus vulnerability was the 7th day of gestation. At the doses given (25 and/or 10 mg/kg), the drug was effective when administered up to the 9th day of pregnancy in hamster and the 11th day in rats. Later administrations had little or no effect.

The data obtained with L-alanosine given by single- or multiple-dose regimens demonstrate that its contragestational effect is dose- and route-dependent, with no important differences in species-sensitivity or administration schedules. In fact, the doses required to induce pregnancy arrest in 50% of the animals (ED_{50}) were 7 mg/kg following a single SC injection for the rat and 7.5 mg/kg for the hamster. After multiple administrations they were 1.5 mg/kg day (7.5 mg/kg, SC, total) for the rat and 2 mg/kg/day (total 10 mg/kg) for the hamster. Administered orally, L-alanosine was one-third to one-fifth as effective as when administered parenterally, which is consistent with its low oral availability due to an important metabolic first pass that yields mostly inactive products [9, 10].

This antifertility spectrum of activity suggests the following.

First, L-alanosine is contragestational through a specific effect on conceptus development. The antifertility doses are far enough from the lethal ones [14, 21] to exclude any possible toxic effect on the mother, and the compound induces pregnancy arrest only when given within the period of gestation in which the proliferative activity of the cells of the conceptus is particularly intense. In this context, it has already been reported for the hamster that the processes of cellular proliferation in the placenta are particularly active during the early postimplantation days and are essentially complete by the 8th day, while the most intense phase of embryonal development occurs between the 7th and 10th days of pregnancy [6]. Thus, the marked antifertility effect of L-alanosine when given up to day 9 suggests that both the placenta and the fetus are target tissues for its contragestational action. The same is presumably true for the rat, in view of the similar times of pregnancy at which contragestational effects are observed in the two species.

Second, we demonstrated that the total dose required to arrest pregnancy with single or multiple administration (once a

Table 4. Effectiveness of adenine in maintenance of pregnancy in animals treated with L-alanosine during the most effective contragestational time of gestation

Species	L-Alanosine[a] (mg/kg) SC	Adenine		No. of animals	Pregnancies terminated (%)	Live fetuses (%)
		mg/kg IP	Time before or after L-alanosine administration (h)			
Rat	10	(Vehicle)	–	23	91.3	2.1
	10	50	0	5	80.0	5.0
	10	100	0	6	16.7	47.2
	10	200	0	10	0	74.1
	10	200	− 2	12	50	17.5
	10	200	0	15	0	71.9
	10	200	+ 2	5	0	83.8
	10	50	+ 2	6	50	22.3
	10	200	+ 8	15	0	90.7
	10	50	+ 8	6	0	63.6
	10	200	+24	10	100	0
	10	200	+48	6	83.3	3.1
	(Vehicle)	200	–	12	0	89.8
	(Vehicle)	(Vehicle)	–	12	0	91.5
Hamster	10	(Vehicle)	–	10	70	5.3
	10	100	0	6	16.7	19.3
	10	100	+ 2	7	0	65.5
	10	100	+ 8	6	16.7	20.4
	10	100	+24	5	60	4.7

[a] Given on day 9 (rats) or day 7 (hamsters)

Table 5. Failure of aspartic acid to maintain pregnancy in animals treated with L-alanosine during the most effective contragestational time of gestation

Species	L-alanosine[a] (mg/kg) SC	Aspartic acid		No. of rats	Pregnancies terminated (%)	Live fetuses (%)
		mg/kg IP	Time before or after L-alanosine treatment (h)			
Rat	10	(Vehicle)	–	23	91.3	2.1
	10	50	0	5	80	1.7
	10	100	0	6	83.3	1.3
	10	200	0	5	100	0
	10	500	0	6[c]	100	0
	10	200[b]	0	6	83.3	7.8
	10	500[b]	0	6	83.3	1.4
	10	200	− 2	12	91.7	3.5
	10	200	0	5	100	0
	10	200	+ 2	6	100	0
	10	200	+ 8	5	80	2.0
	(Vehicle)	200	–	5	0	90.4
	(Vehicle)	500	–	4[d]	0	92.3
	(Vehicle)	(Vehicle)	–	12	0	91.5
Hamster	10	(Vehicle)	–	5	80	3.1
	10	50	0	4	75	2.1
	10	100	0	4	100	0
	10	200	0	10	100	0

[a] Given on day 9 (rat) or day 7 (hamster)
[b] Given SC
[c] Two of six animals died
[d] One of four animals died

day for 5 consecutive days) during the most effective period of gestation is pratically the same. This finding, plus the short plasma half-life of L-alanosine [10] and the evidence that its action is fully expressed within 24 h (see below) indicate that drug concentration and time of exposure contribute equally to the biological response. Similar relationships between schedule of treatment and activity have been shown in leukemia-bearing mice [14; and unpublished data from this laboratory].

Third, the doses of L-alanosine that affect conceptus development during the period of maximum effectiveness are much lower than those necessary to inhibit the growth of experimental tumors in rodents. For example, doses at least 10 times higher than 50% of the contragestational doses are required to achieve a significant increase in the survival time of L 1210 and P 388 leukemia-bearing mice [14; and our unpublished data]. Studies on other known antineoplastic agents are in progress to assess the 'sensitivity' of conception products to their antiproliferative action.

In vitro studies have shown that in mammalian cells L-alanosine acts by inhibiting the conversion of IMP to AMP and its effect can be reversed by adenine, through the pathway that utilizes adenine phosphoribosyl transferase to synthesize AMP [7, 20]. Moreover, the antagonism of the inhibition of cell growth caused by alanosine in yeast but not in eukaryotic cells by aspartic acid has also been demonstrated [3, 7]. Our in vivo data confirm that adenine is able to counteract the contragestational action of L-alanosine but not aspartic acid. We found that the relieving effect is optimum when the purine is given 2–8 h after L-alanosine and that conceptus development is irreversibly compromised within 24 h after antimetabolite administration.

In view of this overall experimental evidence obtained with L-alanosine, we conclude that the contragestational test might be a useful tool in the search for and development of antineoplastic agents.

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Received April 24, 1984/Accepted August 17, 1984