A NEW CLASS OF NON-HORMONAL CONTRAGESTATIONAL AGENTS: PHARMACODYNAMIC-PHARMACOKINETIC RELATIONSHIPS.

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CONTENTS

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1. INTRODUCTION

As a part of a research program designed to find new potential antifertility agents, new non-hormonal, non-prostaglandin-like compounds belonging to the class of 2-pheynyl-triazole [5,1-a] isoindoles Ia and the corresponding dehydro-isoquinolines Ib were identified in our laboratories.

These new structures were shown to be effective at non toxic doses in several animal species as post-implantation, early pregnancy terminating agents (1-7). Starting from these leader compounds, studies designed to clarify simultaneously both their spectrum of activity and the structure-activity relationships were undertaken.

Following this initial explorative phase, keeping in mind that an antifertility agent must be highly effective over a period of time sufficently long to block a dynamic process such as pregnancy, selected compounds were studied in depth in order to find out the relationships between their bioavailability and their effectiveness.

This manuscript reviews the multi-disciplinary research which led to the selection of the first generation compounds that have not only the very high potencies but also the diverse kinetic characteristics to make them suitable for potential use in animals and in humans.

2. STRUCTURE-ACTIVITY RELATIONSHIPS

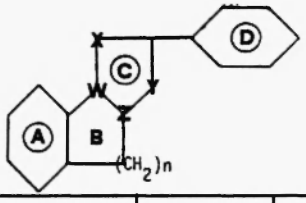
The discovery that tricyclic triazole derivatives Ia and Ib are active as post-coital, post-implantation pregnancy terminating agents and the promising results obtained with selected compounds of these classes in various animal species including monkeys (1-7) prompted us to undertake a broad chemical investigation aimed at optimizing the biological activity (8-11).

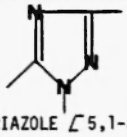
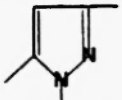
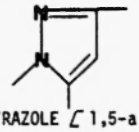
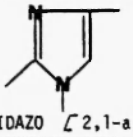
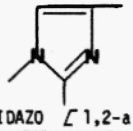
The primary screening tests were run in both pregnant rats and hamsters, with the compounds given subcutaneously dissolved in an oily vehicle for five consecutive days during the most effective time of gestation, which is days 4-8 for the hamster and 6-10 for the rat.

Our initial efforts were directed at the synthesis of several classes of fused pentaatomic heterocyclic derivatives, some of which with the triazole moiety replaced by pyrazole or imidazole fused differently with ring B are shown in table 1.

The data indicates that in all classes studied there is progressive enhancement of activity going from isoindoles to dihydro-isoquinolines to isoquinolines, the only two exceptions to this general rule being compounds of subclasses Ia (rat) and IIIa (hamster). Triazole [5,1-a]

TABLE 1: Structure-activity relationships of 2-pheynyl triazole, pyrazole, imidazole isoindoles and omologues.



Subclass		n	ED_{50} (mg/kg/d, s.c.)[a]	
			RAT	HAMSTER
I a	 TRIAZOLE [5,1-a]	1	1.8	3.5
I b		2	16	1
I c		2,Δ-5,6	2.5	0.25
II a	 PYRAZOLE [5,1-a]	1	~ 75	10
II b		2	20	3.5
II c		2,Δ-5,6	5	1
III a	 PYRAZOLE [1,5-a]	1	20	5.5
III b		2	5	5.5
III c		2,Δ-4,5	1.8	2.2
IV a	 IMIDAZO [2,1-a]	1	~ 75	~ 30
IV b		2	12.5	2.7
IV c		2,Δ-5,6	3	0.25
V b	 IMIDAZO [1,2-a]	2	>20	16
V c		2,Δ-4,5	12	4

[a] dose required for interrupting pregnancy in 50% of animals

isoindole Ia, and the corresponding dihydro-isoquinoline derivatives Ib, tend to show the highest contragestational activity in both animal species. Isoindoles Ia were found to be more active in rats, whereas the dihydro-isoquinolines Ib, were more effective in hamsters.

This species specificity was reduced and the potency increased when ring B was aromatized (n=2,Δ-5,6) to give isoquinoline derivatives of subclass Ic.

In the following stage of this project the effects of several substituents (R) in the phenyl ring D were systematically investigated. Some of these derivatives are shown in table 2. In both species the greatest increase in activity for isoindole and dihydro-isoquinoline derivatives was achieved with *meta*-alkoxy substitutions (compounds 2 and 6), while para-chloro and *para*-pheny substituents led to very potent compounds (12 and 13) in the isoquinoline class (12, 13).

Parallel kinetic investigations of 12 and 13 showed that both derivatives have an excessively long bioavailability, which together with their poor oil solubility is unfavourable for possible use in humans. These same characteristics however were thought useful for veterinary application, so that compound 12 (DL 717) was developed as the first non-hormonal pregnancy terminating agent for the bitch (13).

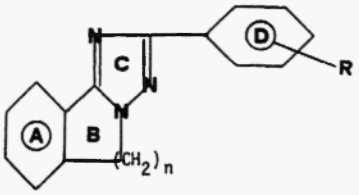
Since it was apparent (table 1) that the bridge linking the benzo ring A to the triazole moiety strongly affects the activity of these structures, the corresponding open chain triazoles, in which an *ortho*-alkyl substituent replaces the methylene chain, were synthesized (table 3).

The main advantage of this structural modification, together with the maintenance of a very high activity, was the marked increase in oil solubility and its kinetic profile shorter than that of the triazole [5,1-a] isoquinoline derivatives Ic.

Based on the contragestational activity in the hamster of quite a large number of substituted 3,5-diphenyl-1H-1,2,4-triazoles, VI, some of which are listed in table 3, the following qualitative structure-activity relationships can be deduced:

1) The presence of an alkyl group in the *ortho* position (R) of the phenyl ring A is essential for the activity.
   A maximum was observed with the ethyl derivative, whereas branching (R=CH $(CH_3)_2$) and elongating (R=n-C_4H_9) the alkyl chain led to less potent compounds. A second substituent generally has a negative influence, except when R_2 is Cl or CH_3.
2) In a series in which ring A contains an *ortho* (R) methyl or ethyl substituent and the structure of ring D is modified, an enhancement of activity was observed with the *meta*-alkoxy and 3,4 methylendioxy derivatives, as compared to the parent compounds 14 and 17.

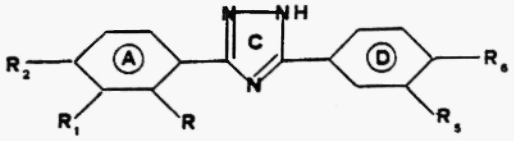
TABLE 2: Structure-activity relationships of substituted triazole [5,1-a] isoindoles and isoquinolines.



Code number	n	R	ED_{50} (mg/kg/d)	
			RAT	HAMSTER
1	1	H	1.8	3.5
2	1	m-OC_2H_5	1.1	0.38
3	1	p-Cl	7.5	1
4	1	p-C_6H_5	>5	0.7
5	2	H	16	1
6	2	m-OC_2H_5	5.5	0.09
7	2	p-Cl	20	0.065
8	2	p-C_6H_5	20	0.05
9	2,Δ-5,6	H	2.5	0.25
10	2,Δ-5,6	m-OCH_3	3.0	0.10
11	2,Δ-5,6	m-OC_2H_5	n.d.	0.125
12	2,Δ-5,6	p-Cl	1.0	0.025
13	2,Δ-5,6	p-C_6H_5	0.5	0.016

n.d. not determined.

TABLE 3: Structure-activity relationships of substituted 3.5-diphyenyl 1H-1,2,4-triazoles [VI].



Code number	R	R_1	R_2	R_5	R_6	ED_{50} (mg/kg/d) Hamster
14	CH_3	H	H	H	H	0.3
15	H	CH_3	H	H	H	>20
16	H	H	CH_3	H	H	>20
17	C_2H_5	H	H	H	H	0.15
18	$CH(CH_3)_2$	H	H	H	H	10
19	n-C_4H_9	H	H	H	H	17
20	CH_3	H	H	OCH_3	H	0.08
21	C_2H_5	H	H	OCH_3	H	0.04
22	C_2H_5	H	H	OC_2H_5	H	0.04
23	CH_3	H	Cl	OCH_3	H	0.04
24	CH_3	H	CH_3	OCH_3	H	0.04
25	C_2H_5	H	H	H	OCH_3	0.15
26	C_2H_5	H	H	OCH_3	OCH_3	0.05
27	C_2H_5	H	H	O-CH_2-O		0.03

Other substitutions such as Cl, CH_3, CF_3, $N(CH_3)_2$, resulted in significant loss of activity.

3) Methyl substitution at the nitrogen in position 2 of the triazole, caused a complete disappearance of the activity.
4) The effects of substituents at the two phenyl rings are additive. This last additive property permitted us to obtain several compounds retaining a very high pregnancy terminating activity,up to 8 times greater than that of the parent compound, 14. This high potency was confirmed in the rat for a number of selected compounds, among which 3-(2-ethylphenyl)- 5-(3-methoxyphenyl)-lH-1,2,4 triazole, 21, referred to as DL 111, was chosen for deeper evaluation as a potential candidate for human application (14-16).

3. CONTRAGESTATIONAL PROFILE.

Extensive studies of selected compounds in various species allowed us to build up a general picture of the spectrum of activity and the mechanism of action of these new classes of non-hormonal contragestational agents (1-7, 12-18).

According to the subject of this mini-review, the main experimental evidence is considered only briefly here and exemplified by the two compounds selected as candidates for the human (DL 111) and veterinary (DL 717) fields. The physico-chemical, kinetic and metabolic factors influencing the biological spectrum of activity are discussed in sections 4 and 5.

This class of compounds induces pregnancy arrest at non-toxic doses in all the species studied, including the mouse, hamster, rat, rabbit, dog, rhesus monkey and baboon.

Table 4 shows the data obtained after single and/or multiple subcutaneous (s.c.), intramuscular (i.m.), intravaginal (vag) and oral (p.o.) administrations of DL 111 and DL 717.

The spectrum of activity of DL 111 is characterized by very high effectiveness after multiple parenteral treatment, with good intravaginal and relatively low oral activity. The influence of the vehicle (oily or aqueous) and formulation (solution or suspension) in the vaginal or oral administration appears to be negligible. It is noteworthy that an interesting degree of activity could be achieved with suitable (foaming) vaginal suppositories (dog). The contragestational profile of DL 717 is, instead, characterized by high activity after a single parenteral injection and poor intravaginal and oral effectiveness. Actually when administered orally as aqueous suspensions the activity of DL 717 is pratically suppressed (hamster).

TABLE 4

Pregnancy terminating activity (ED_{50}) after single (mg/kg) and multiple (mg/kg/day) subcutaneous (s.c.), intramuscular (i.m.), intravaginal (vag.) and oral (p.o.) administration of DL 111 and Dl 717 to various species.
Compounds were given dissolved in oily vehicles or as indicated in the footnotes.

SPECIES	TREATMENT DAY (S) OF GESTATION	ROUTE	ED_{50} DL 111	DL 717
Hamster	4	s.c.	0.4	0.07
	4-8	s.c.	0.04	0.025
	4-6	vag.	1.5 or 0.7[a]	5[a]
	4-8	p.o.	4 or ∿10[a]	8 or >100[a]
Rat	7	s.c.	30	4.5
	6-10	s.c.	0.6	1
	6-10	vag.*	8	> 15[a]
	6-10	p.o.	25 or ∿ 40[a]	>250[a]
Mouse	6	s.c.	12	3
	4-8	s.c.	0.3	0.5
	4-8	p.o.	12.5	>200[c]
Dog	20	i.m.	10	∿0.5
	19--23	i.m.	0.15	-----
	18--24	vag.*	2.5 or ∿0.6[b]	-----
	18--24	p.o.	10	-----
Rabbit	7	s.c.	15	-----
	6-10	s.c.	1.5	-----
Baboon	35 or 36	i.m.	10-25**	-----
	34--38	i.m.	2-5***	-----

a) suspended in methocel;
b) foaming suppositories;
*) compounds given twice a day;
**) pregnancy termination obtained in 6 of 9 animals;
***) pregnancy termination obtained in 5 of 5 animals.

The compounds are devoid of pre-implantation activity i.e., they did not affect the implantation of blastocysts. The optimal time for treating pregnant animals with them is during the early stage of embryonic development. The period of efficacy can be lengthened either by increasing the dosage during the post-implantation period or by giving compounds able to guarantee effective levels of active principle until the period of maximal effectiveness. These findings are clearly illustrated in relation to the dose and time of pregnancy by the effects obtained in hamsters with DL 111 and DL 717 given s.c. in single doses during the

first seven days of gestation (see Fig. 1). The comparison between different species indicated that a slow rate of embryonic growth corresponds with a longer post-implantation period during which the compounds can exert their contragestational effect, i.e., a few days in hamsters, and rats, several days in dogs, and some weeks in the baboon. The compounds display their antifertility effect through a slow and progressive action leading to the degeneration and subsequent resorption or expulsion of the products of conception. The patterns of pregnancy arrest in hamsters and rats treated with a single dose of DL 111 during the most effective time are shown in figure 2. It can be seen that a significatively lower weight of the conceptuses is apparent by 24 h (hamster) to 48 h (rat) after treatment, but .complete arrest of pregnancy occurred only after 2 days in the hamster and after 5 days in the rat. Similar time-courses of action were also observed after multiple doses (Fig. 2). After pregnancy arrest, the animals promptly return to normal reproductive behavior and function, and subsequent pregnancies and progeny are normal; no abnormalities are observed in offspring of mothers given sub-effective doses. The compounds exert their contragestational effect by a direct action on the conceptuses. The biochemical mechanisms priming the cellular events that lead to pregnancy arrest are under evaluation. (14,17,18)

4. PHARMACOKINETICS-ACTIVITY RELATIONSHIPS

In accord with i) the peculiar spectrum of activity confined to the early post-implantation phase ii) the pattern of pregnancy arrest,which implies a sustained time-course of action (see section 3), and iii) the 100% efficacy needed in the pharmacological response,the degree of activity of these new contragestational agents becomes strictly dependent upon their ability to reach the site of action, achieve effective levels and remain at these levels for a sufficiently long time to produce their biological effect.

In this context, the study of the pharmacokinetics of DL 111 in the rat and the hamster (20, 21) shows how, for this class of compounds, the optimization of the biological response depends to different extents on the two factors, time and concentration, that describe bioavailability. When given intravenously, DL 111 behaves like a very short-lasting molecule, rapidly cleared from the body after it has been metabolized. In both animal species, unchanged DL 111 has a half-life of about 15 minutes, so that within 2 hours over 90% of total plasma levels is accounted for as metabolites (fig. 3). After subcutaneous or intramuscular treatment, depending on the type vehicle, i.e. oily or aqueous, the

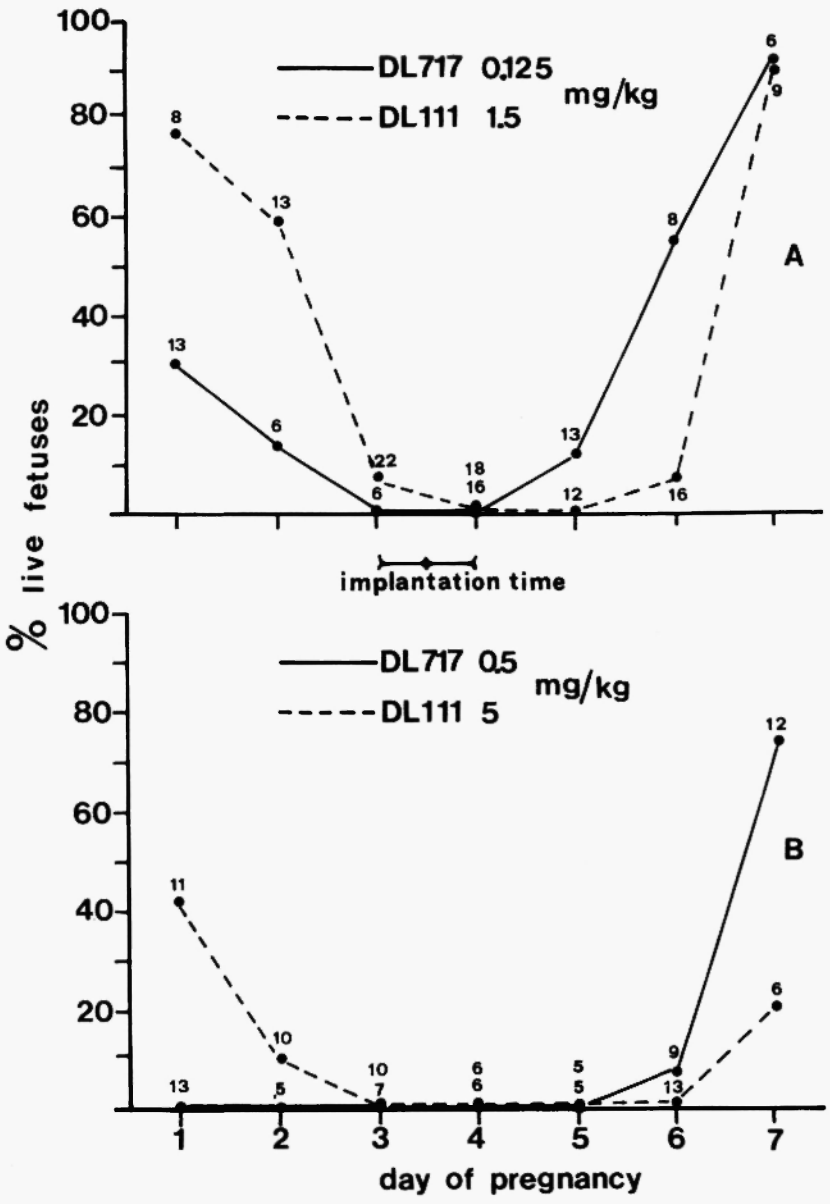


Fig. 1: – Antifertility effect of single s.c. injection of two dose-levels of DL 111 and DL 717 in the hamster. The abscissa represents the day when the single injection was given. The number of animals is shown next to each value.

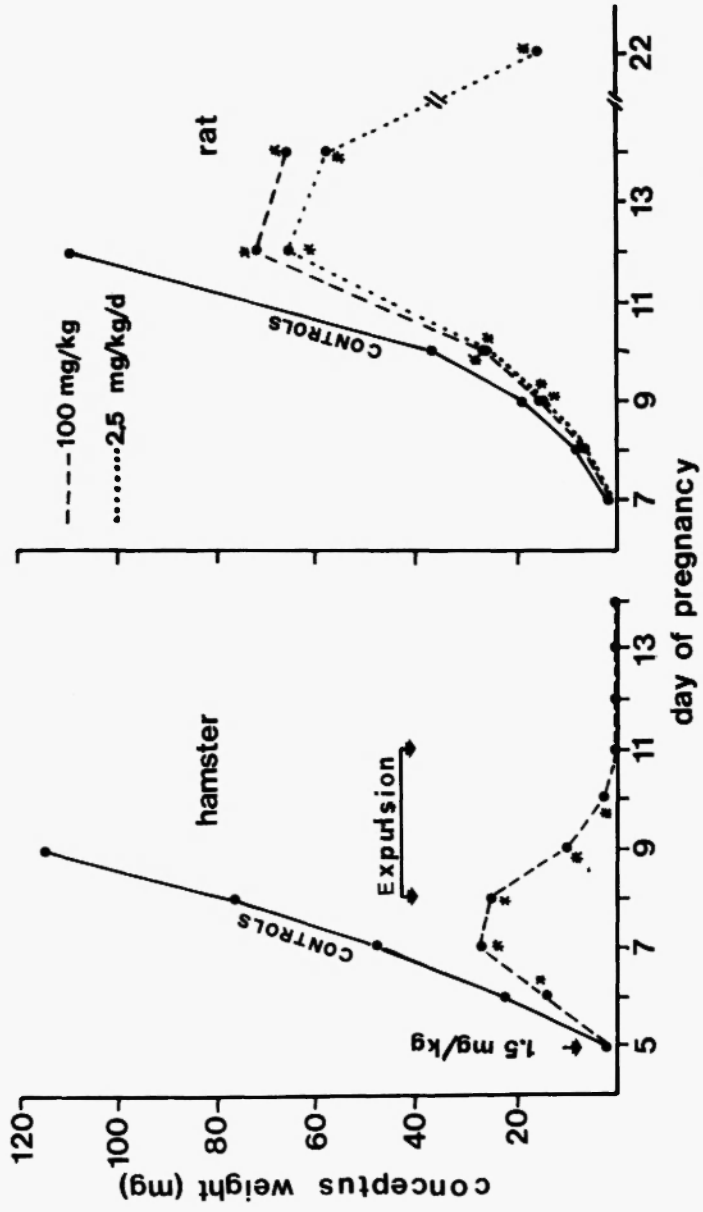


Fig. 2. — Single conceptus weights (mean values of 5–18 animals) in hamsters and rats treated s.c. with single or multiple 100% effective doses during the period of maximal effectiveness. Significantly different from corresponding controls at *p 0.01 (Student "t" test).
Significantly different from corresponding controls at *p 0.01 (Student "t" test).

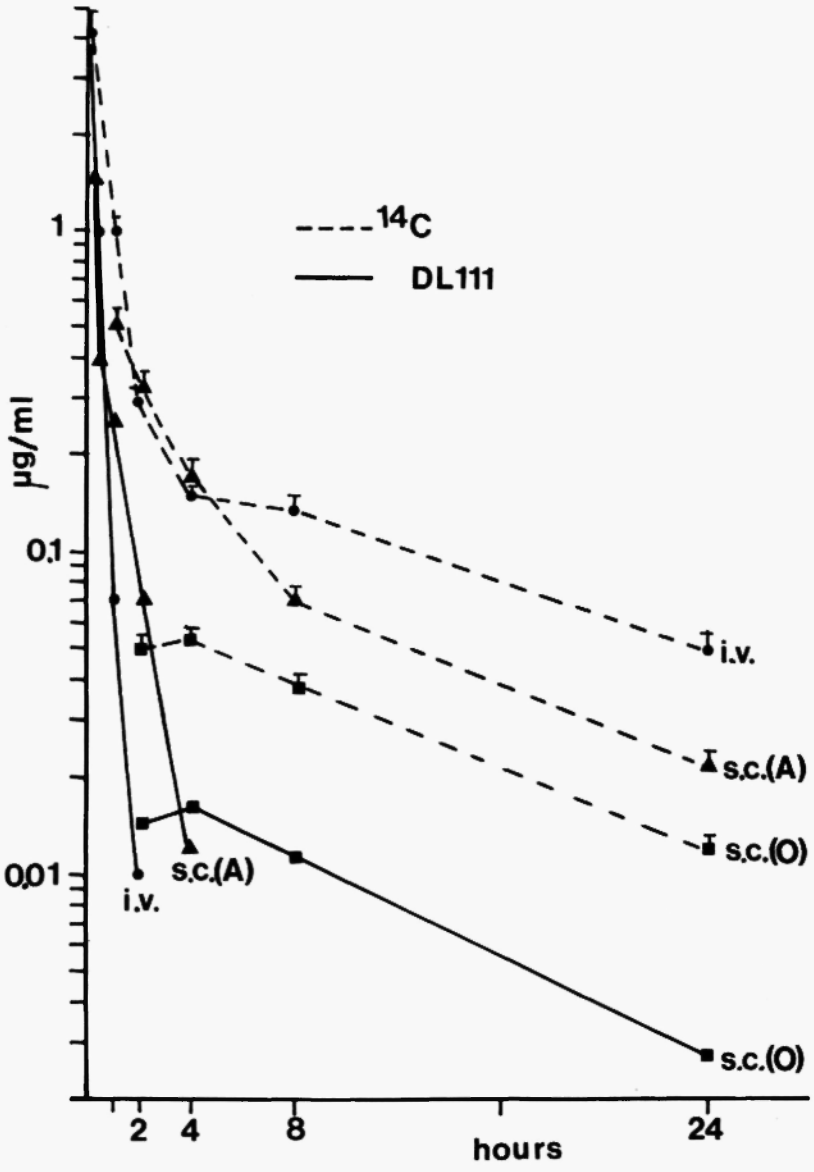


Fig. 3: – Plasma levels of total radioactivity (dashed line) and of unchanged DL 111 in hamsters given [5-^{14}C]-DL 111 i.v. (2.5 mg/kg) and s.c. dissolved in oily (1 mg/kg) or aqueous (2.5 mg/kg) vehicles.

rate of absorption from the site of injection determines the bioavailability rate,i.e. more or less sustained kinetic profiles and more or less high peak plasma levels (fig. 3).

Attempts to evaluate the kinetic parameters from the available plasma concentration data (table 5) show that for the i.m. treatments either the elimination rate constant values or those of the areas subtended to the plasma curves (AUC) are underestimated because of the delay in absorption (Table 5). As foreseen, when kinetic and activity data are compared the close correlation existing between duration of availability and efficacy becomes apparent. The single subcutaneous or intramuscular treatment in a slow release formulation (oily) is the most effective, whereas other routes (i.v.) or vehicles (aqueous) that are characterized by fast absorption have markedly less pregnancy terminating activity (table 5).

A further improvement in effectiveness is obtained when DL 111 is administered subcutaneously in oily vehicles in multiple dose regimens. For example, in the hamster the maximum activity is achieved with two doses of 0.05 mg/kg/d given on day 4 and 5 of gestation, while in the rat the doses required for the optimal response are five, given daily (0.5 mg/kg/d) from day 6 of gestation (table 6). Since the kinetic profiles of the unchanged compound (active principle) in the two species are almost identical, in the rat, the greater gain in activity observed after multiple treatment appears to be related to the longer time-course of action required for pregnancy arrest. This overall evidence leads to the conclusion that, the ideal kinetic profile would guarantee effective levels of active principle at the site of action for the length of time needed to interrupt gestation, i.e. 2 days in hamsters, 5 days in rats.

As for the optimal dose regimen, in the case of DL 111, the need of daily intervals of administration, is consistent with the data of distribution to the site of action (21). In fact, probably because of its high lipophilicity, liposolubility and protein binding, the levels of DL 111 were found to be higher and to last longer in the utero-embryo-placental complex of rats than in plasma, thus ensuring through the concentration plateau a constant exposure over 16 hours following a single subcutaneous injection (table 7).

In a sub-human species, the baboon, where the plasma kinetics of DL 111 after intramuscular administration is somewhat more prolonged than in the rat, the optimal schedule of treatment, ensuring both the highest degree of efficacy and a more uniform pattern of pregnancy arrest, was a multiple daily dose (16).

Since it is apparent that with a longer presence of active principle lower doses are far more active than much higher ones for a short period of time, it can be deduced that single doses of highly lipophilic com-

TABLE 5: Pharmacokinetics and activity data for the hamster after i.v. and s.c. administration of DL 111 dissolved in aqueous or oily vehicles.

Route and (vehicle)	dose (mg/kg)	peak-time [a] (h)	peak-level [a] (µg/ml)	half-life (h)	dose/AUC ($L \cdot h^{-1}$)	ED_{50} [b] (mg/kg)
i.v. (aqueous)	2.5	0.05	3.95	0.22	261	>10 [c]
s.c. (aqueous)	2.5	0.5	0.36	0.75	410	15.5
s.c. (oily)	1	4	0.017	7.4	682	0.4

[a] observed, [b] dose required for pregnancy arrest in 50% of animals after a single injection at the most effective time of pregnancy (day 4); [c] inactive at the dose tested.

TABLE 6: Pregnancy terminating activity (ED_{50}) after single and multiple subcutaneous administration of DL 111 dissolved in an oily vehicle.

SPECIES	Days required for pregnancy arrest	Treatment Day of gestation	ED_{50} [a] (mg/kg/day)	ED_{50} [a] total dose (mg/kg)
Hamster	2	4	0.40	0.40
		4 - 5	0.05	0.10
		4 - 6	0.04	0.12
		4 - 8	0.04	0.20
Rat	5	7	35	35
		6 - 7	15	30
		8 - 9	12	24
		6 - 8	2	6
		8 -10	1.8	5.4
		6 -10	0.5	2.5

[a] dose required for interrupting 50% of pregnancy.

TABLE 7: Concentration of DL 111X and total ^{14}C in plasma and the utero-embryo-placental complex (UEPC) of pregnant rats (day 8 of gestation) given s.c. 5 mg/kg of [5-^{14}C]-DL 111-IT dissolved in oily vehicle.

HOURS AFTER ADMINISTRATION	DOSE ABSORBED (%)	PLASMA LEVELS of DL 111 and [^{14}C] (ng/mL)	UEPC - levels of DL 111 and [^{14}C] (ng/g)	ED_{50} (mg/kg/day)	
				single dose	multiple dose
				day 7	days 6-10
4	29.6	80 [260]	210 [340]	35	0.50
8	46.1	100 [380]	260 [510]		
16	----	60 [360]	230 [480]		
24	83.9	15 [320]	130 [420]		

pounds, since they are released very slowly from the i.m. oily depot, would provide the sustained effective levels over the period required to induce pregnancy arrest. This assumption was verified for DL 717, the compound selected for terminating pregnancy in the bitch. The pharmacokinetic profile (total ^{14}C) of DL 717 (fig. 4) is characterized, like that of DL 111, by rapidly decreasing plasma levels after intravenous administration (aqueous vehicle), (see fig. 3) but, as expected, by very prolonged steady-state plasma levels (about 4 days) after intramuscular injection (oily vehicle). It should be noted that, proportionally to dose, levels of total ^{14}C after both i.v. and p.o. administration are, within 48 h, higher than those measured after s.c. injection, which disagrees with the very low oral activity of DL 717, suggesting, the existence of one important metabolic first-pass as there is for analogues (see section 5).

The kinetics of DL 717 and DL 111 after i.m. administration are consistent with the time-related effects observed in hamsters after single doses given at different stages of gestation (section 3, fig. 1). In fact, in comparison with DL 111, DL 717, being released more slowly from the site of injection, is much more effective when given during the pre-implantation period (days 1-3), when the possibility to display contragestational activity depends upon the ability to maintain effective levels until the phase of blastocyst nidation.On the contrary, in the post-implantation period (days 5-7) when the conceptus sensitivity is decreasing, the activity of DL 717 falls off more rapidly than that of DL 111.

5. INFLUENCE OF FIRST PASS METABOLISM

In all the chemical classes developed, most of the evaluated pregnancy terminating compounds have low or very low oral activity, even when administered in multiple dose regimens: the decreased activity as compared with subcutaneously administered compound differed up to from 2 to 3 orders of magnitude (12).

Data in the hamster for a series of triazole isoindoles and isoquinolines (12) excluded that lack of absorption from the gastrointestinal tract might be the cause of their very low oral activity. This is at least true when the compounds can be administered in solution, whereas suspensions of poorly liposoluble molecules, i.e. DL 717, are very poorly bioavailable, leading to a further reduction of their oral contragestational activity (see table 4). The experimental evidence obtained in metabolism studies suggests that the marked variations in activity after oral administration are mainly related to minor or major metabolic first-passes (19).

Compound 6, DL 204, was investigated in depth in this respect. The metabolic pathways for DL 204 (19), as reported in table 8 , consist of

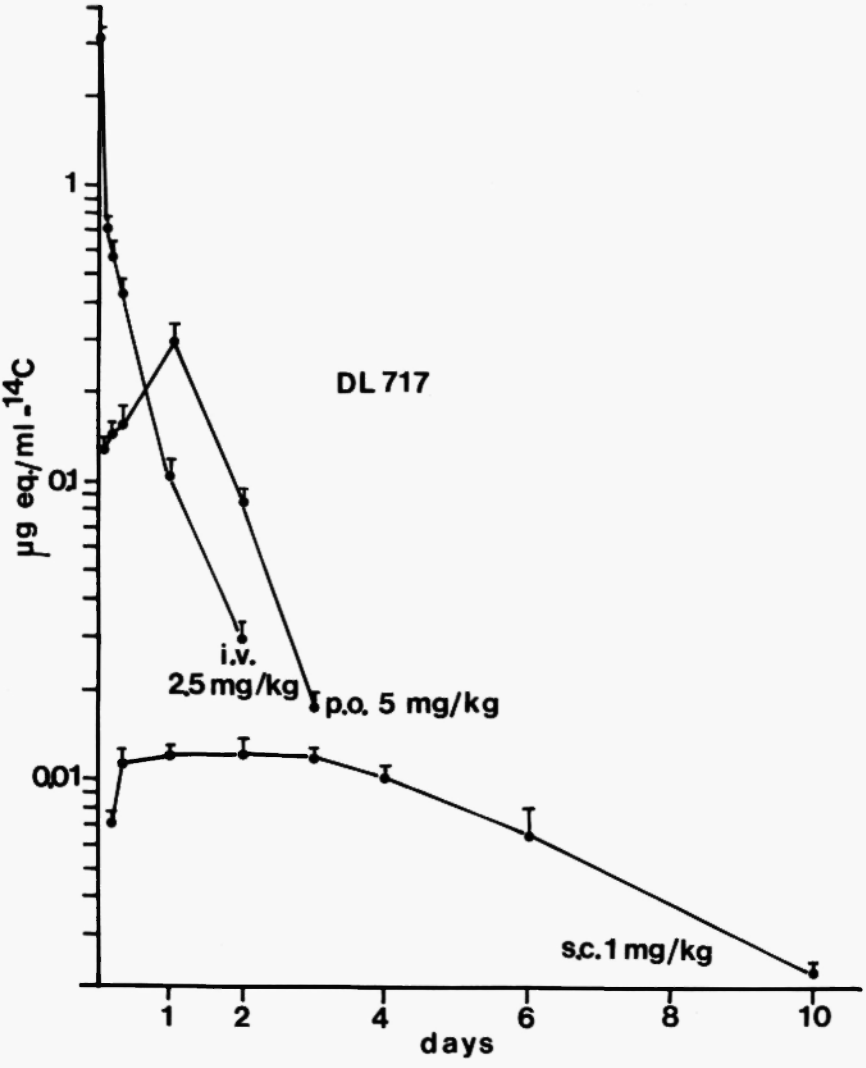
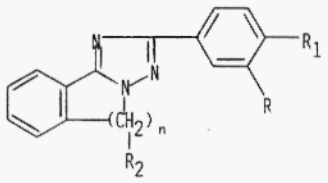


Fig. 4: – Plasma levels of total radioactivity in hamsters given i.v. (2.5 mg/kg) p.o. (5 mg/kg) and s.c. 1 mg/kg, [2-^{14}C]-DL 717. The compound was given dissolved in an aqueous vehicle (i.v.), and in an oily one (p.o. and s.c.).

TABLE 8: Pregnancy terminating activity (ED_{50}) in hamsters after multiple (4th-8th day) s.c. or p.o. administration.



COMPOUND	N	R	R_1	R_2-(C_6)	ED_{50} (MG/KG/D) S.C.	P.O.	RATIO P.O./S.C. (RT)
6.(DL 204)	2	OC_2H_5	H	H	0.09	65	722
MET. 1	2	OH	H	H	2.5	--	--
MET. 2	2	OH	OH	H	>2.5*	--	--
MET. 3	2	OH	H	OH	>2.5*	--	--
5	2	H	H	H	1	100	100
7	2	H	Cl	H	0.065	2.5	38
8	2	H	Ø	H	0.05	0.5	10
2	1	OC_2H_5	H	H	0.38	30	79
1	1	H	H	H	3.5	10	2.9
3	1	H	Cl	H	1	20	20
4	1	H	Ø	H	0.7	5	7
11	2.Δ5,6	OC_2H_5	H	H	0.125	20	160
9	2.Δ5,6	H	H	H	0.25	40	160
12.(DL 717)	2.Δ5,6	H	Cl	H	0.025	8	320
13.(L14105)	2.Δ5,6	H	Ø	H	0.016	0.2	12

* COMPLETELY INACTIVE AT THE DOSE SNOWN.

three main phase I reactions: (a) 0-dealkylation; (b) hydroxylation at C-6 of ring B and (c) hydroxylation at C-4 of the aromatic ring D. The pregnancy terminating activity of the various metabolites of DL 204 as tested in rats and hamsters, was much lower or even absent, leading to the conclusion that the parent compound must be regarded as the active principle.

The key role of the metabolic first-pass in the oral effectiveness of a series of these contragestational-agents was also apparent in a study designed for developing orally highly active compounds less subject to metabolism (12). Several available analogues of DL 204, isoindoles, dehydroisoquinolines and isoquinolines, properly modified at the three sites of metabolism were selected and tested orally in the hamster.

The results showed that: (a) for all the test-compounds the loss of activity attributable to the oral route was less than for DL 204; (b) the isoindole class was the least affected by metabolic deactivation; (c) in both the isoindole and dihydroisoquinoline classes a lower ratio of oral to subcutaneous activity was obtained by removing the ethoxyl group and by inserting a chlorine in the para position, but the best improvement in activity was observed with the two p-phenyl derivatives; (d) all isoquinolines, including DL 717, had unfavorable ED_{50} p.o./ED_{50} s.c. ratios, the only exception being the highly active p-phenyl derivative, L 14105. With regard to 3,5-diaryl-s-triazoles, as reported for the rat and the hamster, none, including DL 111, has interesting oral activity, which is on average from 1/25 to 1/100 that obtained by subcutaneous administration (11).

An alternative route of treatment, practically unaffected by the hepatic metabolic first-pass (22) and particularly suitable for treatment of women, is the intravaginal one. Comparative activity data obtained with DL 111 and DL 717 given by this route of administration indicate, however, that the degree of vaginal absorption is strongly affected by the physico-chemical properties of the products.

The intravaginal activity of DL 111, a compound with a high liposolubility (50 mg/ml in sesame oil) and moderate water-solubility (10 μg/ml), independent of the vehicle (oily or aqueous) or formulation (solution or suspension) was already good, and with the use of suitable foaming suppositories could be increased to values comparable to those measured after s.c. administration. In contrast, since DL 717, is practically insoluble in water and poorly liposoluble (0.5 mg/ml in sesame oil), it had to be given intravaginally as a suspension, and as a result was not very effective (table 4).

6. DISCUSSION

Discovery and development of a new pharmacological lead proceed through a series of steps from chemical synthesis to biological evaluation to kinetic and metabolic analysis, so that in most instances the relationships between chemical structure, biological properties and bioavailability are interpreted retrospectively.

In the development of our new contragestational agents, it was appar-

ent early that the achievement of the biological effect would be strictly dependent upon prolonged availability. Thus, in the primary screening, the period of treatment in pregnancy (most effective time), the route (subcutaneous) and the schedule of treatment (multiple daily doses) chosen, were those least affected by kinetic and metabolic factors.

Structure-activity relationships studies in two species with marked differences in sensitivity (rat, hamster) permitted us to ascertain the key portions of the molecules and the types of substituents that could either improve the activity or reduce the species specificity. This initial phase of research led to the selection of several compounds with high pregnancy terminating activity after multiple parenteral administration.

Concurrently, the contragestational activity of some of the most interesting products, possessing different physico-chemical properties, was measured at various stages of gestation, when given in different schedules, by different routes and in different vehicles.

The main evidence that emerged, when related to the pharmacokinetic profiles and physico-chemical properties of each molecule, indicated the following kinetic-activity relationships:

a) The effectiveness of the compounds was shown to be dependent on time of gestation, route, vehicle and schedule of administration.
b) The ideal time-course of the compounds at the site of action requires sustained kinetics, while short-exposures even to high concentration are not very effective in interrupting the embryonic development.
c) The maximal effectiveness can be obtained when the exposure of the products of conception to the drug action lasts for the whole length of time needed to arrest pregnancy.
d) The period of efficacy during pregnancy can be lengthened by administering long-lasting compounds before the period of maximal effectiveness.
e) The difference in potency between species appears to be due to differences in species sensitivity rather than to diverse kinetics.
f) The loss of contragestational activity observed when the compounds are given orally is mainly due to a metabolic first-pass; chemical modifications at the site of metabolism in a molecule succeed in improving the oral activity.

Based on their high potency, low acute toxicity and peculiar pharmacokinetic profiles, DL 111 and DL 717 have been selected, the first as a potential contragestational agent for women, the second for the control of the dog population.

The choice of DL 111, a highly liposoluble and relatively short-lasting molecule (i.m. in oily bases) was justified by its high parenteral and intravaginal activities, which, even though they required multiple ad-

ministrations, have the advantage of versatility of use and faster clearance from the body after abortion has occurred.

The peculiar characteristic of DL 717, besides its high lipophilicity, that determines its prolonged availability after single intramuscular doses, is its poor liposolubility. This latter property actually meets the practical needs for application to the bitch, since it makes it possible to prepare formulations (oily suspensions) able to guarantee proper levels of active principle for a prolonged period of time, extending the effectiveness up to the first days of gestation (23).

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