Induced Dominant Lethals in Female Mice: Effects of Triaziquone and Phenylbutazone

Although the induction of dominant lethals in female mice may be just as important in testing the mutagenicity of certain types of chemicals as the more usual assay in males, females are considered less suitable[1]. This is mainly due to the fact that a variety of non-genetic factors are capable of interfering with the ovum and ovulation, with fertilization and cleavage and with implantation. On the other hand, oocytes entering metaphase I and progressing to metaphase II are particularly susceptible to the induction of dominant lethals. Since, in contrast to the spermatocyte, the female gamete at this stage is not subject to selection before fertilization, it may be especially well suited for the detection of primary mutagenic events[1]. It has repeatedly been demonstrated that chemically induced dominant lethals in females are in fact of a genetic nature, i.e. due to chromosomal aberration[2–5].

Triaziquone was shown by RÖHRBORN and BERRANG[6] to induce dominant lethals by interfering either with the dictyate stage of oocytes in young female C3H mice or with the oogonia of embryos in utero. Further experiments suggested that the pre-ovulatory and ovulatory stages, i.e. oocytes entering metaphase I and metaphase II, were particularly sensitive. In order to define the stages that were affected by the treatment[7], ovulation was 'synchronized' by treating the females with gonadotrophin[3]. Since this procedure seemed to us to have a number of disadvantages in routine testing, we attempted to time ovulation by careful observation of the oestrus cycle only.

In the experiments described, the effect of triaziquone treatment was compared with that of phenylbutazone, a drug known to cause neither dominant lethals nor cytogenetic changes in male germ cells of the mouse[8,9].

Materials and methods. Groups of 9-week-old female mice of the CFLP strain (Laboratory Animals, Carworth Europe, Huntingdon, England) were each injected i.p. with 0.25 mg/kg of triaziquone (2,3,5-triethyleneiminobenzoquinone-1,4) in 1 ml of saline, either at pro-oestrus (Experiment 1) or at late pro-oestrus/oestrus (Experiment 2). Within 45 min of the administration of the drug, 3 of the treated females were mated with one untreated male of the same age and breed. 15.5 h later, the females were examined for the presence of a vaginal plug; only those that had copulated were retained in the experiments. For comparison, groups of NMRI-mice obtained from our own breeding unit were also treated in experiment 2.

In Experiment 3, untreated CFLP males and females were mated, again in a ratio of 3:1, and the females in

Table I. Experiment 1: Dominant lethal effect of 0.25 mg/kg triaziquone administered to female CFLP-mice in pro-oestrus

		Controls	Triaziquone
No. of fertilized ♀♀		24	31
Corpora lutea	total	258	268
	$\bar{X}$	10.75	8.65[a]
	s (±)	2.95	3.37
Implantations	total	260	296
	$\bar{X}$	10.83	9.55
	s (±)	2.88	2.90
Live implants	total	242	203
	$\bar{X}$	10.08	6.55[a]
	s (±)	3.33	3.62
Dead implants	total	18	93
	%	6.9	31.4[b]

[a] Student's t-test, $p \leqq 0{,}01$ [b] χ^2-test. $p \leqq 0{,}01$.

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[3] G. RÖHRBORN, in *Chemical Mutagenesis in Mammals and Man* (Eds. F. VOGEL and G. RÖHRBORN; Springer-Verlag, Berlin 1970) p. 294.
[4] G. RÖHRBORN, O. KÜHN, I. HANSMANN and K. THON, Humangenetik *11*, 316 (1971).
[5] G. RÖHRBORN and I. HANSMANN, Humangenetik *13*, 184 (1971).
[6] G. RÖHRBORN and H. BERRANG, Mutation Res. *4*, 231 (1967).
[7] W. KUHLMANN, in *Chemical Mutagenesis in Mammals and Man* (Eds. F. VOGEL and G. RÖHRBORN; Springer-Verlag Berlin 1970), p. 180.
[8] L. MACHEMER and R. HESS, Experientia *27*, 1050 (1971).
[9] R. RATHENBERG and D. MÜLLER, Agents Actions *2*, 180 (1972).

Table II. Experiment 2: Dominant lethal effect of 0.25 mg/kg triaziquone administered to CFLP and NMRI female mice at leta pro-oestrus and oestrus

		CFLP mice		NMRI mice	
		Controls	Triaziquone	Controls	Triaziquone
No. of fertilized	♀♀	37	39	37	33
Corpora lutea	total	515	512	456	387
	$\bar{X}$	13.92	13.13	12.32	11.73
	s (±)	2.36	1.96	1.53	2.59
Implantations	total	462	435	403	346
	$\bar{X}$	12.49	11.15	10.89	10.48
	s (±)	2.88	2.31	2.47	2.05
Live implants	total	430	331	367	257
	$\bar{X}$	11.62	8.49[a]	9.92	7.79[a]
	s (±)	3.07	2.40	2.94	2.55
Dead implants	total	32	104	36	89
	%	6.9	23.9[b]	7.9	23.0[b]

[a] Student's t-test, $p = \leqq 0.01$. [b] χ^2-test, $p \leqq 0{,}01$.

Table III. Experiment 3: Effects of 0.01 and 0.25 mg/kg triaziquone administered to female CFLP mice on the first day post coitum

		Controls	Triaziquone 0.01 mg/kg	Controls	Triaziquone 0.25 mg/kg
No. of fertilized	♀♀	39	36	37	3[b]
Corpora lutea	total	494	435	463	7
	$\bar{X}$	12.67	12.08	12.51	2.33[a]
	s (±)	2.98	2.78	3.00	7.00
Implantations	total	488	407	453	15
	$\bar{X}$	12.51	11.31	12.24	5.00[a]
	s (±)	3.04	3.10	2.84	6.93
Live implants	total	452	338	402	1
	$\bar{X}$	11.59	9.39[a]	10.86	0.33[a]
	s (±)	3.14	3.35	3.41	0.58
Dead implants	total	36	69	51	14
	%	7.4	17.0[b]	11.2	93.3[b]

[a] Student's t-test, $p \leqq 0{,}01$. [b] χ^2-test, $p \leqq 0{,}01$.

which a vaginal plug was found, were injected with triaziquone 0.01 or 0.25 mg/kg i.p. 15.5 h later, i.e. on the first day post-coitum.

Experiment 4 was conducted on CFLP mice at oestrus in the same way as Experiment 2, except that the animals were treated with phenylbutazone (Butazolidin®). The drug was given by the oral route in a dose of 400 mg/kg in 10 ml/kg of 2% carboxymethyl-cellulose. This dose corresponds to about $^1/_3$ of the oral LD_{50} in this species.

Throughout the experiments, the animals were kept under the usual 12 h darkness-light conditions at a temperature of 22 ± 1 °C. 14 days after the detection of the vaginal plug, the pregnant animals were sacrificed and the corpora lutea and living and dead implants counted. Deciduomata as well as dead embryos were classed as dead implants.

The reproduction parameters were analysed statistically. The statistical significance of the differences in the numbers of corpora lutea, total implants and live implants was evaluated by the t-test and that of the conception rate and the number of dead implants by the χ^2-test. P-values of $\leqq 0.01$ were considered as significant.

Results. When given at pro-oestrus, triaziquone had no influence on the conception rate, although the number of corpora lutea was significantly decreased. The implantation rate did not change, but significantly fewer live implants were found and there was a corresponding increase in the proportion of dead implants (Experiment 1, Table I). Treatment of the females in late pro-oestrus (Experiment 2) affected neither the number of corpora lutea nor the overall implantation rate, but there was a significant shift in the ratio of dead and live implants. The result obtained in the two different strains of mice were nearly identical (Table II).

When 0.25 mg/kg of triaziquone was given on the first day post-coitum, there was a marked decrease in the conception rate, in the number of corpora lutea and in both the overall implantation rate and the number of living implants. The proportion of dead implants amounted to 93%. By contrast, a dose of 0.01 mg/kg did not affect conception, nor was there any change in the implantation rate. The number of living embryos, however, was significantly decreased and the number of dead implants correspondingly greater (Experiment 3, Table III).

Treatment with phenylbutazone had no effect on any of the parameters examined (Experiment 4, Table IV).

Table IV. Experiment 4: Effect of 400 mg/kg phenylbutazone administered orally to female CFLP mice at oestrus

		Controls	Phenylbutazone
No. of fertilized ♀♀		13	14
Corpora lutea	total	129	156
	$\bar{X}$	9.92	11.14
	s (±)	5.16	2.54
Implantations	total	143	151
	$\bar{X}$	11.00	10.79
	s (±)	3.74	3.09
Live implants	total	116	138
	$\bar{X}$	8.92	9.86
	s (±)	5.47	2.85
Dead implants	total	27	13
	%	18.9	8.6

Discussion. A significant reduction in the number of live embryos and a corresponding increase in the number of dead implants in relation to the total implantation rate may be taken as evidence of a dominant lethal effect[1,10]. Post-implantation loss is held to be a more relevant criterion of mutagenicity than pre-implantation loss of zygotes. The latter, estimated from the difference between the numbers of corpora lutea and total implants, is easily modified by non-genetic (i.e. hormonal and cytotoxic) factors.

Alkylating agents, and even more so gonadotrophin treatment, are known to give rise to superovulation, which in itself is likely to increase pre-implantation loss[3]. Since only fertile matings were considered, the difficulty of deciding whether sterility is attributable to the death of the ovum after fertilization or to inhibition of ovulation and fertilization was circumvented[2].

The susceptibility of oocytes changes rapidly during the pre- and postovulatory stages of meiosis and, at least in experiments with X-rays, it was regarded as essential that the treatment should be exactly timed[11,12]. In

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studies with chemical agents, it seems sufficient to consider the sensitive stages broadly, since any effect occurring at the time will depend on the local availability of reactive compound. Pro-oestrus is the earliest stage at which mating can take place, and ovulation begins about 8 h thereafter. During this time, oocytes enter metaphase I and metaphase II; 26 h after early mating the first zygotes in mitosis I are found[7].

The experiments with triaziquone demonstrated that treatment at late pro-oestrus and oestrus (Experiment 2) was precisely enough timed to induce a high number of dominant lethals without affecting conception, ovulation and the implantation rate. Practically identical results were obtained in two different strains of mice. By and large, our results are comparable with those obtained by RÖHRBORN[3] with triaziquone after hormonal pretreatment.

In Experiment 3, in which treatment is assumed to have been administered between ovulation and mitosis I, cytotoxic effects interfered markedly with fertility, particularly at the higher dose of triaziquone (0.25 mg/kg).

In contrast to the alkylating agent, phenylbutazone had no effect when given at the sensitive stage of oocyte development; in this respect the results of the present experiment are in keeping with the negative results obtained in other types of mutagenicity study on somatic or gonadal cells[8,9,13].

Zusammenfassung. Bei weiblichen Mäusen induzierte Triaziquon unter konventionellen Laboratoriumsbedingungen dominante Letalfaktoren, wenn die Substanz im Pro-Oestrus oder im Oestrus verabreicht wurde. Weniger gut geeignet erwies sich diese Applikation wenige Stunden nach Kopulation, da embryotoxische Wirkungen auftraten. Im Gegensatz zu Triaziquon führte Phenylbutazon – weiblichen Mäusen im Oestrus verabreicht – nicht zu dominanten Letaleffekten.

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Le Lumomagnéson: marqueur fluorescent de l'os

«ANTOINE MIZAUD, médecin de Paris, paraît avoir remarqué le premier vers le milieu du seizième siècle, l'action de la garance sur les os. Mais il faut avouer que l'observation de MIZAUD était entièrement oubliée, lorsque plus d'un siècle et demi après lui, BELCHIER et DUHAMEL appelèrent, sur le fait important dont il s'agit, l'attention des anatomistes». C'est en ces termes que FLOURENS[1] rappelle la première observation de marquage in vivo du squelette et, en même temps, évoque l'intérêt de cette technique.

Mais c'est durant les vingt dernières années, que de grands progrès se sont produits dans la mise au point de divers marqueurs fluorescents de l'ostéogénèse. Outre l'alizarine extraite de la garance, on a vu successivement les tétracyclines[2], la quercétine[3], les porphyrines[4,5], la calcéine verte[6], les différents Procions[7,8], la calcéine bleue[9] et enfin le xylenolorange[10].

Cette grande variété et la gamme de couleurs disponibles réduisent sans doute la valeur d'une recherche de nouveaux marqueurs du calcium en voie de dépôt dans le tissu osseux. Cependant, le squelette comporte d'autres sels minéraux dont le rôle éventuel est trop souvent imprécis et dont le mode d'incorporation est mal connu. Parmi ceux-ci, nous savons que le magnésium est, quantitativement, le quatrième cation de l'os. La moitié du magnésium total du corps humain se trouve dans le squelette. La mise en évidence de ce minéral au moment de sa pénétration dans l'os pourrait donc présenter un certain intérêt.

Certains auteurs[11,12] considèrent le Lumomagnéson[13] comme le produit le mieux adapté à la détection du magnésium en fluorométrie de solutions aqueuses. Nous avons pensé utiliser cette propriété en administrant le Lumomagnéson à l'animal vivant avec l'espoir d'y marquer en fluorescence le magnésium au moment de son dépôt dans le tissu osseux. Toutefois, avant d'entreprendre l'expérimentation in vivo, il était nécessaire de déterminer avec certitude si le Lumomagnéson était réellement sélectif dans la mise en évidence, in vitro, du magnésium, par fluorométrie. Les essais ont démontré qu'en fait ce produit détecte parfaitement le magnésium mais qu'il décèle également les traces de calcium quoiqu'en produisant une fluorescence moins intense que pour le magnésium. Des mesures quantitatives précises n'ont pas été effectuées, le seul fait qualitatif de la non sélectivité rigoureuse étant suffisant pour ne pas considérer le Lumomagnéson comme détecteur exclusif du magnésium, mais bien, à la fois, du magnésium et du calcium.

Technique. Le Lumomagnéson est un sel sodique du 2-hydroxy-3-sulfo-5-chlorophénylazo-1-acid barbiturique, trés soluble dans l'eau. La solution à 50 mg par ml fut utilisée. Dix jeunes chiens (âgés de 3 à 6 mois) ont reçu des injections par diverses voies. Les quantités suivantes de Lumomagnéson ont été injectées: par vois i. p., de 50 à 300 mg/kg d'animal; par voie i. v. de 10 à 20 mg/kg; par voie i. m., de 50 à 80 mg/kg. Les injections n'ont provoqué aucune réaction chez les animaux d'expérience. Le produit a été parfaitement toléré. Les chiens ont été sacrifiés à des intervalles variant de 2 h. à 15 jours après l'injection. Les pièces osseuses ont subi les manipulations de routine pour l'enrobage au méthécrylate de méthyle (passages successifs au méthanol, chloroforme et toluol).

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