

TOXICOLOGY OF LAPACHOL IN RATS: EMBRYOLETHALITY

GUERRA, M. de O., MAZONI, A. S. B., BRANDÃO, M. A. F. and PETERS, V. M.

Centro de Biologia da Reprodução, Universidade Federal de Juiz de Fora, C.P. 328, CEP 36001-970, Juiz de Fora, Minas Gerais, Brazil

Correspondence to: Martha de Oliveira Guerra, R. São Mateus, 711, Ap. 103 C, CEP 36025-001, Juiz de Fora, MG, Brazil, e-mail: moguerra@cbr.ufjf.br

Received July 7, 1999 – Accepted March 9, 2000 – Distributed February 28, 2001

ABSTRACT

Lapachol is a naphthoquinone with therapeutic potential against enterovirus, Chagas disease and is also used as an antimalarial and antiinflammatory agent. In order to study teratogenic potential of Lapachol, pregnant Wistar rats were treated with 0.5 ml of distilled water (control group); 0.5 ml of hydroalcoholic solution (vehicle group) and 10 mg of Lapachol in 0.5 ml of hydroalcoholic solution (treated group) by oral gavage from the 8th to the 12th day of pregnancy. The following variables were observed: maternal body weight on days 1, 6, 15 and 21 and food intake on days 2, 6, 15 and 21 of pregnancy. The number of live and dead fetuses and the sites of resorptions were counted. The ovaries were weighed and the *corpora lutea* were counted. Data were analyzed by ANOVA-one way, Dunnett test and the chi square test. Significance level test $\alpha = 0.05$. Results have shown that mothers were unaffected but there were a 99.2% of fetus mortality, indicative of a strong abortifacient effect of Lapachol in rats.

Key words: Lapachol, embryotoxicity, rat.

RESUMO

Toxicologia do Lapachol em ratas: embrioletalidade

O Lapachol é uma naftoquinona com potencial terapêutico contra enterovírus, doença de Chagas e como antimalárico. Para estudar o potencial embriotóxico do Lapachol, ratas originalmente Wistar, grávidas, foram tratadas com 0,5 ml de água destilada (grupo controle); 0,5 ml de solução hidroalcoólica (grupo veículo) e 10 mg de Lapachol em 0,5 ml de solução hidroalcoólica (grupo tratado) por gavagem, do 8^o ao 12^o dia de gestação. As seguintes variáveis foram observadas: peso corporal materno nos dias 1, 6, 15 e 21, consumo de ração nos dias 2, 6, 15 e 21 de gestação. O número de fetos vivos e mortos e os locais de reabsorção foram contados. Os ovários foram pesados e os corpos lúteos, contados. Os dados foram analisados por ANOVA “one way”, teste de Dunnett e Qui-quadrado. O nível de significância dos testes foi $\alpha = 0,05$. Os resultados mostram que as mães não foram afetadas, mas ocorreram 99,2% de mortes fetais, o que indica um potente efeito embriotóxico do Lapachol para o rato.

Palavras-chave: Lapachol, embriotoxicidade, rato.

INTRODUCTION

Lapachol is a naphthoquinone (2-hydroxy-3-(3-methyl-2 butenyl)-1,4 naphthoquinone), extracted from Pau d'arco (Family Bignoneaceae) (Santana *et al.*, 1968).

A great spectra of therapeutic activities have been attributed to Lapachol or its derivatives such as prevention of cercarial skin penetration of *Schistosoma mansoni* (Pinto *et al.*, 1977); trypanosomicidal (Austin, 1974; Lopes *et al.*, 1978), antiinflammatory (Almeida, 1990) and antineo-

plastic agents (Santana *et al.*, 1968; Rao, 1974; Linardi *et al.*, 1975); antimalarial against erythrocytic stages of *Plasmodium falciparum* (Carvalho *et al.*, 1988) and use against enteroviruses (Pinto *et al.*, 1987).

In spite of these therapeutic possibilities, only a few papers have been published about the toxicology of Lapachol (Santana *et al.*, 1968; Morrison *et al.*, 1970; Austin, 1974). So it seems important to screening the toxic effect on embryo after administration of Lapachol to rats as a part of the studies on the toxicology of this phytoterapic.

MATERIAL AND METHODS

Three-month old female Wistar rats, weighing 175 ± 10 g, obtained from the Centro de Biologia da Reprodução vivarium were maintained in temperature-controlled quarters with a light – dark period of 12:12 hs. Food and water were available *ad libitum*.

Female rats were caged with fertile male. The following day was designated day 1 of pregnancy if spermatozoa or a seminal plug was present in the vagina.

Inseminated females were randomly assigned to one of the following groups: control, vehicle or treated.

Animals of control group were treated with 0.5 ml of distilled water; vehicle group was treated with 0.5 ml of hydroalcoholic solution and the treated one received 10 mg of 0.5 ml of Lapachol hydroalcoholic solution. All treatments were done by oral gavage from the 8th to the 12th day of pregnancy – period of greater sensibility to teratogens in rats (Manson & Kang, 1994).

The dose of Lapachol was calculated on the basis of the therapeutic doses given to humans (25 mg/kg), and the DL-50 which was 1.600 mg/kg of rat body weight (Santana *et al.*, 1968).

In order to observe maternal toxic effect, clinical criteria were adopted (Manson & Kang, 1994) and the following variables were collected: maternal body weight on days 1, 6, 15 and 21 of pregnancy; food intake on days 2, 6, 15 and 21 of pregnancy. Locomotor activities, piloerection, vaginal bleeding and any other unusual behavior were also observed.

Rats were killed on day 21 of pregnancy by an overdose of ether inhalation. The uterus of each

animal was exposed and the number of live and dead fetuses were counted.

The sites of resorption were counted and recorded. Live and dead fetuses were examined to detect external malformations.

The ovaries were weighed and the *corpora lutea* were counted.

Statistical analysis were performed using ANOVA-one way Dunnett test and the chi square test to evaluate continuous and discontinuous data, respectively. Significance level test was $\alpha = 0.05$.

The project was approved by CBR ethical committee.

RESULTS

Rats of all experimental groups had no alteration of locomotor activities and did not present piloerection. There were no maternal deaths and the food intake was similar among the groups (Table 1).

No maternal body weight loss was observed during the gestation period (Table 2).

Mean of implants and corpora lutea were similar among the experimental groups (Table 3).

The implantation index (total implantation/total corpora lutea \times 100) was 89.8%, 91.8% and 95.4%, in the control, vehicle and Lapachol treated groups, respectively. There was no significant difference among the groups.

There were 2%; 0.99% and 99.2% of resorptions and a mean of 10 ± 2 (14); 11 ± 2 (9) and 1.0 live fetus in the control, vehicle and lapachol treated groups, respectively. There was a high significant difference among treated, control and vehicle groups ($p < 0.01$).

The live fetus had half the weigh of control and vehicle fetuses.

DISCUSSION AND CONCLUSIONS

Since no clinical signs of maternal toxicity were observed, except for the food intake reduction in the Lapachol treated rats, it is possible to assume that Lapachol was not toxic to the mothers considering the dose used (Manson & Kang, 1994).

The reproductive performance of mothers can be considered normal since the implantation index, the mean of implants and corpora lutea were similar in all experimental groups.

TABLE 1
Food intake in control, vehicle and Lapachol (10 mg/rat) treated pregnant rats.

Groups	Food intake (g)/days of pregnancy			
	2	6	15	21
Control	18.77 ± 5.37 (14)	17.05 ± 2.57 (14)	20.46 ± 3.59 (11)	21.37 ± 3.06 (14)
Vehicle	15.78 ± 1.03 (9)	17.78 ± 1.82 (9)	19.77 ± 3.24 (9)	22.36 ± 2.10 (9)
Lapachol	14.50 ± 3.32 (11)	15.06 ± 1.42 (11)	16.47 ± 4.16 (11)*	21.95 ± 2.09 (11)

Results are expressed in Mean ± SD (n). * p = 0.020.

TABLE 2
Body weight (g) in control, vehicle and Lapachol (10 mg/rat) treated pregnant rats.

Groups	Maternal weight (g)/days of pregnancy			
	1	6	15	21 *
Control	171.54 ± 12.19 (14)	184.45 ± 13.86 (14)	215.86 ± 29.12 (14)	194.69 ± 13.43 (14)
Vehicle	182.83 ± 11.66 (9)	199.48 ± 11.13 (9)	206.01 ± 13.68 (9)	211.10 ± 15.49 (9)
Lapachol	177.17 ± 14.16 (11)	192.92 ± 12.25 (11)	198.76 ± 13.06 (11)	205.44 ± 14.86 (11)

Results are expressed in Mean ± SD (n). * The weight was obtained after uterus, ovaries and conceptuses were removed.

TABLE 3
Mean of corpora lutea and implants in control, vehicle and Lapachol (10 mg/rat) treated pregnant rats.

Groups	Corpora lutea	Implants
Control	12 ± 1 (14)	11 ± 2 (14)
Vehicle	12 ± 1 (9)	11 ± 2 (9)
Lapachol	12 ± 1 (11)	11 ± 1 (11)

Results are expressed in Mean ± SD (n).

The conception index correlates the implantation site and the number of corpora lutea (Ford, 1982) – the higher the index the greater the number of ovulation that results in oocytes fertilization and in blastocysts implantation. When the difference between corpora lutea and implantation sites is greater than 10% there is a possibility of reproductive failure (Wilson, 1980).

The development from implanted blastocyst ongoing is coordinated by a sum of events depending mostly on the progesterone secreted by corpora lutea.

The blood concentration level of progesterone was correlated to the number and volume of corpora lutea (Kato *et al.*, 1979), so it is reasonable

to assume that progesterone level can be normal since the mean number of corpora lutea was similar among experimental groups.

Taken together, the data above mentioned suggest that the Lapachol treatment did not cause general or reproductive maternal toxicity.

Except for one mother Lapachol treated that had one live fetus, all the others presented 99% of resorbed fetus. Since resorption is an *in situ* autolyse of embryos or fetuses (Kalter, 1980) it is possible to assume that Lapachol is very toxic to the embryos during the early organogenic period. As mothers were unaffected, there was a high incidence of fetal mortality and the single live fetus was 50% lighter than the control ones, it is possible

to assume that the phytoterapic is embryocid and it is necessary to further evaluate the possibility of lower doses be teratogenic.

In conclusion, in spite of the great therapeutic potentiality of Lapachol it is necessary to evaluate its teratogenic potential and other toxic effects carefully before the clinical use in humans because it is a strong abortifacient in rats and the possibility of it having the same effect in human cannot be discarded.

Acknowledgments — Thanks are due to Prof. Rita de Cassia da Silveira e Sá for translating the manuscript, to CAPES for the financial support and to Prof. Alaide Braga de Oliveira (Faculdade de Farmácia, Universidade Federal de Minas Gerais) who kindly supplied the Lapachol.

REFERENCES

- ALMEIDA, E. R., 1990, Antiinflammatory action of lapachol. *J. Ethnopharmacol.*, 29: 239-241.
- AUSTIN, F. G., 1974, *Schistosoma mansoni* chemoprophylaxis with dietary lapachol. *Am. J. Trop. Med. Hyg.*, 23 (3): 412-419.
- CARVALHO, L. H., ROCHA, E. M. M., RASLAN, D. S., OLIVEIRA, A. B. & KRETTLY, A. U., 1988, *In vitro* activity of natural and synthetic naphthoquinones against erythrocytic stages of *Plasmodium falciparum*. *Brazilian J. Med. Bio. Res.*, 21: 485-487.
- FORD, W. C. L., 1982, The effect of 6- deoxy-6 fluoroglucose on the fertility of male rat and mice. *Contraception*, 25(5): 534-543.
- KALTER, H., 1980, The relationship between congenital malformations and prenatal mortality in experimental animals, pp. 29-44. *In*: I. Potter & E. B. Hook (ed.), *Human embryonic and fetal death*. Academic Press, New York.
- KATO, H., MORISCHIGE, W. K. & ROTCHILD, I., 1979, A quantitative relationship between the experimentally determined number of conceptuses and corpus luteum activity in pregnant rat. *Endocrinology*, 105: 846-850.
- LINARDI, M. C. F., De OLIVEIRA, M. M. & SAMPAIO, R. P., 1975, Lapachol derivative active against mouse lymphocytic leukemia, p. 388. *J. Med. Chem.*, 18(11): 1159-1161.
- LOPES, J. N., CRUZ, F. S., DOCAMPO, R. *et al.*, 1978, *In vitro* and *in vivo* evaluation of the toxicity of 1,4-naphthoquinone and 1,2-naphthoquinone derivatives against *Trypanosoma cruzi*. *Ann. Trop. Med. Parasitol.*, 76(2): 523-531.
- MANSON, J. M. & KANG, Y. J., 1994, Test methods for assessing female reproductive and developmental toxicology, pp. 989-1034. *In*: A. W. Hayes (ed.), *Principles and methods of toxicology*. 3.ed., Raven Press, New York.
- MORRISON, R. K., BROWN, D. E., OLESON, J. J. & COONEY, D. A., 1970, Oral toxicology studies with lapachol. *Toxicol. Appl. Pharmacol.*, 17: 1-11.
- PINTO, A. V., PINTO, M. C. R., GILBERT, B. *et al.*, 1977, *Schistosoma mansoni*: blockage of cercarial skin penetration by chemical agents: I naphthoquinones and derivatives. *Trans. R. Soc. Trop. Med. Hyg.*, 71(2): 133-135.
- PINTO, A. V., PINTO, M. C. F. R., LAGROTA, M. H. C. *et al.*, 1987, Antiviral activity of naphthoquinones. I. Lapachol derivatives against enteroviruses. *Rev. Lat-amer. Microbiol.*, 29: 15-20.
- RAO, K. V., 1974, Quinone natural products: Streptonigrin (NSC-45383) and Lapachol (NSC-11905) structure-activity relationships. *Cancer Chemother Rep.* 2, 4(4): 11-17.
- SANTANA, C. F., de LIMA, O. G., D'ALBUQUERQUE, I. L. *et al.*, 1968, Observações sobre as propriedades antitumorais e toxicológicas do extrato do liber e de alguns componentes do cerne do pau-d'arco (*Tabebuia avellanedae*). *Rev. Inst. Antibiot.* (Recife), 8(1/2): 89-94.
- WILSON, J. G., 1980, Environmental effects on intrauterine death in animals, pp.12-24. *In*: I. Potter & E. B. Hook (ed.), *Human embryonic and fetal death*. Academic Press, New York.