UNIVERSITI SAINS MALAYSIA
PROJEK PENYELIDIKAN JANGKA PENDEK
LAPORAN AKHIR

KAJIAN TENTANG KEMUNGKINAN PENGARUH INHIBITOR ENZIM PENUKAR ANGIOTENSIN KE ATAS OVULASI

PENYELIDIK

DR. RITA CHATTERJEE

PENYELIDIK BERSAMA

PROF. MADYA DR. SYED MOHSIN SAHIL JAMALULLAIL
PROF. DR. AMAR CHATTERJEE

BAHAGIAN PENYELIDIKAN & PEMBANGUNAN

CANSELORI

UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) **Nama Penyelidik:** DR. RITA CHATTERJEE

Nama Penyelidik-Penyelidik Lain *(Jika berkaitan)* : 1. Prof. Madya (Dr) Syed Mohsin Sahil Jamalullail

2. Professor (Dr) Amar Chatterjee

2) **Pusat Pengajian/~~Pusat/Unit~~** : Sains Perubatan, Dept. of Pharmacology

3) **Tajuk Projek:** Kajian Tentang kemungkinan Pengaruh Inhibitor Enzim Penukar Angiotensin ke atas Ovulasi

4) (a) **Penemuan Projek/Abstrak**

*(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris** Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).*

See Appendix - 1

(b) **Senaraikan Kata Kunci yang digunakan di dalam abstrak:**

Bahasa Malaysia	Bahasa Inggeris
	Hemispaying
	Compensatory ovulation
	Compensatory ovarian hypertrophy
	Captopril (ACE-inhibitor)
	Prolactin
	Progesterone

5) **Output Dan Faedah Projek**

(a) Penerbitan *(termasuk laporan/kertas seminar)*
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

This project has fetched one research publication in Biomedical Research and a conference presentation at MSPP annual meeting held at Langkawi, 26-28 May 1995.

Reprint and abstract attached.
*appendix 2 *appendix 3

(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

The research findings contribute towards the rationality of using Captopril in the treatment of essential hypertension in women of child-bearing age, as it could block ovulation by inducing a lesion at the prolactin-progesterone system

(c) Latihan Gunatenaga Manusia

i) Pelajar Siswazah

Not applicable

ii) Pelajar Prasiswazah:

Not applicable

iii) Lain-Lain : Trained a laboratory technologist in different methods involved in this project.

6. **Peralatan Yang Telah Dibeli:**

Allotted fund has been utilised on consumable items only.

UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

5 untuk pengesahan & tandatangan Dekan

APPENDIX 1

FINAL REPORT OF THE SHORT-TERM RESEARCH PROJECT

Title: "Kajian Tentang Kemungkinan Pengaruh Inhibitor Enzim Penukar Angiotensin ke atas Ovulasi"

ACE inhibitors are often recommended as the drug of choice to ameliorate essential hypertension. Since the presence of renin-angiotemsin system in the gonadotrophs and the preovulatory follicle might have a link in ovulatory process, our study has been directed to investigate the possible impact of ACE inhibitor, if any, at the pituitary-ovarian system.

Experimental research schedule and the findings as presented in the conference and also published are as follows:

Strictly 4-day cyclic rats were subjected to surgical hemi-spaying on day 1 of the cycle, distributed into four groups and treated with either captopril (ACE inhibitor), captorpirl with prolactin, captopril with progesterone or the vehicle alone. On the following day 1 of the cycle examination of the fallopian tubes and the ovaries of the animals revealed that 70% of the captopril-treated animals failed to ovulate but maintained compensatory ovarian hypertrophy (40.9 $\pm$ 3.00 Vs 32.4 $\pm$ 2.6 mg). The remaining 30% of the same group of animals although showed the sign of ovulation and compensatory ovarian enlargement (41.8 $\pm$

2.2 Vs 32.4 ± 2.6 mg), yet the number of eggs ovulated were found to be extremely low in count (1.6 ± 0.1 Vs 12.3 ± 0.4).

Conversely, the groups of animals which had either prolactin or progesterone concurrently with captopril showed conspensatory changes in terms of ovulation and ovarian hypertrophy as documented in the vehicle-treated controls. While angiotensin helps in the synthesis and release of prolactin and the progesterone-primed preovulatory environment of the follicle is prolactin dependent, our findings of captopril-induced failure of compensatory ovulation in hemispayed rats suggest that angiotensin converting enzyme inhibitor possibly generates a lesion on the prolactin-progesterone system, an essential system to initiate ovulation.

* Presented at the 11th Scientific Meeting of MSPP held at Langkawi on May 26 - 28, 1995 (Abstract attached).

* Published in Biomed. Res. 6:129-133; 1995 (reprint attached).

Appendix 2

Biomedical Research 1995; 6(2): 129-133

Does captopril interrupt compensatory ovarian changes in hemispayed rats?

R. Chatterjee, S.S.J.Mohsin and A.Chatterjee[1,2]
Department of Pharmacology and Department of Physiology[1],School of Medical Sciences, Universiti Sains Malaysia,16150 Kubang Kerian, Kelantan, Malaysia.

Key words: Captopril, hemispaying, ovulation, ovarian hypertrophy, prolactin, progesterone, rats.

Abstract

Angiotensin 2 is shown to be linked with pituitary synthesis and release of prolactin and ovulation. Study was undertaken to investigate whether captopril, an angiotensin converting enzyme blocker, could modulate compensatory ovarian changes following hemispaying in rats.

Strictly 4-day cyclic rats were subjected to surgical hemispaying on day 1 (estrus) of the cycle, distributed into four groups and treated with captopril alone, captopril concurrently with prolactin, captopril along with progesterone and the vehicle alone. On the following day 1, 70% of the captopril-treated group failed to ovulate but maintained a comparable degree of hypertrophy of the surviving ovary. The remaining 30% animals showed compensatory ovarian changes yet the number of eggs ovulated were significantly low in count. Animals which received combined treatment of captopril and either of prolactin or progesterone presented compensatory values as observed in the vehicle - treated controls.

The results suggest that captopril possibly generates a lesion on prolactin-progesterone system which leads to anovulation in hemispayed rats.

Introduction

The existence of intrinsic renin-angiotensin system in the gonadotrophs (1,2,3) and the prolactin synthesising and releasing profile of angiotensin 2 (4,5) are well documented. Since LH receptor binding is influenced by prolactin (6,7), the preovulatory environment of prolactin within the follicle may influence the process of ovulation (8). A large number of in vitro and in vivo experimental findings now suggest that progesterone plays a key role in spontaneous ovulation (9,10,11,12).

The high concentration of angiotensin 2 in the preovulatory follicle (15,16) with its possible link in progesterone formation (17) may contribute to a role of angiotensin2 in the ovulatory process (18). However, failure of saralasin, an angiotensin 2 antagonist, to block spontaneous ovulation argued against the role

for intraovarian angiotensin 2 in ovulation (19).

Unilateral spaying triggers compensatory ovarian hypertrophy and ovulation through increased secretion of gonadotropin (20,21). Present experiments were designed to evaluate whether captopril, an angiotensin converting enzyme inhibitor, modulates ovarian changes following hemispaying.

Materials and Methods

Animals

Laboratory-bred sexually mature Wistar rats of 170 - 180 g were housed at 22°C with light on for 12 h per day. The animals had free access to food and water. Animals that had shown at least three consecutive 4-day cycles were chosen for study.

Experimental protocol

Surgical hemispaying was performed under light ether anesthesia via a flank incision between 09:00 and 11:00 h on day 1 (estrus) of the cycle. Animals were then equally distributed into four groups. Animals of group 1 received freshly prepared captopril (SQ 14,225., donated by Bristol-Myers Squibb, Pharmaceutical Res. Inst., N,J., U.S.A.) injections(sc) in saline (0.2ml) at 08:00 and 16:00 h on days 3 and 4 of the cycle. The dose was 5.0 mg/kg/day. Animals of group 2 had similar captopril schedule plus a concomitant injection(sc) of prolactin in saline (1.0 mg in 0.2 ml) at 16:00 h on day 4 (proestrus) of the cycle. Group 3 animals received captopril on days 3 and 4 and a concomitant injection(sc) of progesterone (1.0 mg in 0.2 ml) at 09:00 h on day 4 of the cycle. Animals of group 4 acted as vehicle - treated controls. On the following day 1, vaginal smears were examined to confirm vaginal cyclicity. Animals were sacrificed. At autopsy, fallopian tubes of the animals were carefully seperated from the remaining ovaries, flushed with 0.9% saline under dissecting microscope and the eggs, if any were counted after adding 0.5 ml of 1% hyaluronidase in the flushings. Ovaries were freed from their adhering tissues and weighed on a torsion balance.

Statistical analysis

All data were expressed as sample means ± SEM and analysed by Student's t test. A value of $P < 0.05$ was considered significant.

Results and Discussion

Results as presented in Table 1 show that captopril treatment on days 3 and 4 in the hemispayed cyclic rats caused anovulation in 70% of the test animals. The remaining 30% rats though ovulated yet the egg count was 1.60 ± 0.10 against the compensated control value for 12.30 ± 0.36. However, compensatory hypertrophy of the remaining ovary in the captopril - treated rats (41.80 ± 2.20 vs 43.20 ± 2.12) obviously suggests normal ovarian growth response to augmented gonadortropin stimulation. Angiotensin 2 is reported to be involved in granulosa lutein cell progesterone production (17) and also concerned with the formation and release of the pituitary prolactin (4,5), the hormone that participates in preovulatory intrafollicular rise in progesterone (22). Several lines of evidence also suggest that intrafollicular progesterone affects follicular rupture by influencing prostaglandin biochemistry (23), because a positive association between the levels of progesterone and prostaglandin $F_2\alpha$ in the ovarian tissue always exists (24). Therefore, to clarify whether captopril mediates its antiovulatory profile by direct attenuation of intraovarian progesterone formation or through attenuated synthesis and release of prolactin, the hormone that is needed for LH-receptor coupling (6,7), surgically-manipulated hemispayed rats were subjected to concomitant treatment of captopril concurrently with either prolactin or pogesterone (Table 1). Both the groups of rats, however, reacted and showed compensatory ovulation and ovarian hypertrophy of the

surviving ovary as documented in controls (Table 1). The reversal of antiovulatory profile of captopril by prolactin here does not support the concept of direct involvement of intraovarian angiotensin 2 in follicular rupture (18). Nocturnal hyperprolactinemia as evident during preovulatory phase in normal ovulatory women (25) is claimed to be essential to maintain LH-stimulated progesterone production (22). Moreover, administration of bromocriptine (that blocks prolactin secretion) to normal cyclic women results in diminished progesterone production (26,27). It is therefore possible that captopril-generated lesion at prolactin-progesterone system may be the reason for ovulation failure in hemispayed rats.

Acknowledgements

The author (RC) acknowledges the research grant provided by Universiti Sains Malaysia, Penang (322/0500/3011) that has resulted in this article. The encouraging cooperation of Dato' Professor K.J. Ratnam is highly appreciated. Technical and secretarial assistance of Siti Norliza and Memi Abdul Hamid, respectively are also appreciated.

References

1. Deshepper CF, Ganong WF. Renin and angiotensin in endocrine glands; in Martini L, Ganong WF (eds): Frontiers in Neuroendocrinology. New York, Raven Press, 1988, Vol. 10, pp 79-98.

Table 1 : Effects of captopril and captopril with prolaction or progesterone on the compensatory features of the surviving ovary in hemispayed rats®

Treatment	Wt. of operated ovary (mg)	Wt. of surviving ovary (mg)	No. of rats ovulated	No. of ova ovulated per ovary (operated side)	No. of ova ovulated per ovary (surviving side)
ULO+Vehicle (Control)	31.33±1.88	43.20± 2.12	10/10	5.33± 0.33	12.30± 0.36
ULO+ Captopril (on days 3,4)	32.41± 2.61 -	41.80 ±2.20 40.90 ±3.00	6/20 14/20	5.20± 0.46 -	1.60± 0.10* NIL*
ULO+ Captopril+ prolactin (on day 4)	30.86 ±1.77	41.17 ± 2.16	10/10	5.60± 0.40	11.25± 0.55
ULO+ Captopril+ progesterone (on day 4)	31.24± 2.16	43.20± 1.80	10/10	5.42± 0.36	11.60± 0.44

®Values are group means±SEM; *Significantly different from control means; ULO = Unilateral ovariectomy

2. Naruse K, Takii Y, Imagami T. Immunohistochemical localization of renin in luteinizing hormone-producing cells of rat pituitary. Proc Natl Acad Sci USA 1981; 78:7579-7583.

3. Steele MK, Brownfield MS, Ganong WF. Immunocytochemical localization of angiotensin immunoreactivity in gonadotrophs and lactotrophs of the rat anterior pituitary gland. Neuroendocrinology 1982; 35:155-158.

4. Canonico PL, McLeod RM. Angiotensin peptides stimulate phosphoinositide breakdown and prolactin release in anterior pituitary cell culture. Endocrinology 1986; 118:233-238.

5. Aguilera G, Hyde CL, Cat KJ. Angiotensin II receptors and prolactin release in pituitary lactotrophs. Endocrinology 1987; 111:1045-1053.

6. Morishige WK, Rothchild I. Temporal aspects of the regulation of corpus luteum function by luteinizing hormone,prolactin and placental luteotropin during the first half of pregnancy in the rat. Endocrinology 1974; 95:260-273.

7. Holt JA, Richards JS, Midgley AR Jr, Reichert LE. Effect of prolactin on LH receptor in rat luteal cells. Endocrinology 1976; 98:1005-1013.

8. Yoshimura Y, Nakamura Y, Yamada H, Ando M, Ubukata Y, Oda T, Suzuki M. Possible contribution of prolactin in the process of ovulation. Horm Res 1991; 35:22-32.

9. Mori T, Suzuki A, Nishimura T, Kambegawa A. Inhibition of ovulation in immature rats by antiprogesterone antiserum. J Endocrinol 1977; 73:185-186.

10. Synder BW, Beecham GD, Schane HP. Inhibition of ovulation in rats with epostane, an inhibitor of 3ß-hydroxy-steroid dehydrogenase (41865). Proc Soc Exp Biol Med 1984; 176:238-242.

11. Tsafriri A, Abisogun AO, Reich R. Steroids and follicular rupture at ovulation. J Steroid Biochem 1987; 27:359-363.

12. Swanson RJ, Lipner H. Mechanism of ovulation : effect of intrafollicular progesterone antiserum in rabbits. Fed Proc 1977; 36:390.

13. Lamprecht SA, Lindner HR, Strauss JF III. Induction of 20α-hydroxysteroid dehydrogenase in rat corpora lutea by pharmacological blockade of pituitary prolactin secretion. Biochim Biophys Acta 1969; 187:133-143.

14. Day SL, Kirchick HJ, Birnbaumer L. Effect of prolactin on luteal function in the cyclic rat: positive correlation between luteinizing hormone-stimulated adenyl cyclase activity and progesterone secretion; role in corpus luteum rescue of the morning surge of prolactin on day 3 of pseudopregnancy. Endocrinology 1980; 106:1265-1269.

15. Culler MD, Tarlatzis BC, Lightman A, et al. Angiotensin II - like immuno - reactivity in human ovarian follicular fluid. J Clin Endocrinol Metab 1986; 62:613-615.

16. Lightman A, Tarlatzis BC, Rzasa PJ, et al. The ovarian renin-angiotensin system: Renin-like activity and angiotensin II/III immunoreactivity in gonadotropin-stimulated and unstimulated human follicular fluid. Am J Obstet Gynecol 1987; 156: 808-816.

17. Stirling D, Magness RR, Stone R, Waltreman MR, Simpson ER. Angiotensin II inhibits luteinizing hormone-stimulated cholesterol side chain cleavage expression and stimulates basic fibroblast growth factor expression in bovine luteal cells in primary culture. J Biol Chem 1990; 265:5-8.

18. Kuo T-C, Endo K, Dharmarajan AM, Miyazaki T, Atlas SJ, Wallach EE. Direct effect of angiotensin II on in vitro perfused rabbit ovary. J Reprod Fert 1991; 82:469-474.

19. Daud AI, Bumpus FM, Husain A. Angioten-

sin II : does it have a direct obligate role in ovulation? Science 1989; 245:870-871.

20. Chatterjee A, Greenwald GS. Compensatory ovarian hypertrophy following unilateral ovariectomy of the pseudopregnant or pregnant rat. Endocrinology 1971; 88:491-496.

21. Chatterjee A. The possible mode of action of prostaglandins II. Failure of prostaglandin $F_2\alpha$ in the prevention of compensatory ovulation and ovarian hypertrophy following unilateral ovariectomy in rats. Acta Endocrinol 1972; 70:786-790.

22. McNatty KP, Sawers RS, McNeilly AS. A possible role of prolactin in the control of steroid secretion by the human Graafian follicle. Nature 1974; 250:653-655.

23. Espey LL. Simultaneous determination of ovarian prostaglandin E_2, prostaglandin $F_2\alpha$, 6-keto prostaglandin $F_1\alpha$, β-estradiol and progesterone during ovulation in the PMSG/hCG- primed immature rat. Biol Reprod, Suppl. 1986; 34: Abstr. 125.

24. Vijayakumar R, Walter WAW. Ovarian stromal and luteal tissue prostaglandins, 17β-estradiol, and progesterone in relation to the phases of the menstrual cycle in women. Am J Obstet Gynecol 1987; 156:947-951.

25. Nakamura Y, Yoshimura Y, Oda T. et al. The alteration of serum prolactin concentrations during the preovulatory period in normal subjects; in Mizuno M, Mori H, Taketani Y (eds): Role of Prolactin in Human Reproduction. Basel, Karger, 1988; pp 15-24.

26. Schulz KD, Geiger W, Pozo ED, Lose KH, Kunzig HJ, Lancranjan I. The influence of the prolactin-inhibitor bromocriptin (CB 154) on human luteal function in vivo. Arch Gynak 1976; 221:93-96.

27. Saunders DM, Hunter JC, Hasse HR, Wilson GR. Treatment of luteal phase inadequacy with bromocriptine. Obstet Gynecol 1979; 53:287-289.

Correspondence to:

Prof. Amar Chatterjee
Department of Pharmacology
School of Medical Sciences
Universit Sqains Malysia
16150 Kubang Kerian,
Kelantan, Malaysia.

DOES CAPTOPRIL INTERRUPT COMPENSATORY OVARIAN CHANGES IN HEMISPAYED RATS?

*R. Chatterjee, S.S.J. Mohsin and A. Chatterjee+
Department of Pharmacology and Department of Physiology+,
School of Medical Sciences, Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan, Malaysia.

Angiotensin 2 is shown to be linked with pituitary synthesis and release of prolactin and ovulation [1,2]. Study was undertaken to investigate whether captopril, an angiotensin converting enzyme blocker, could modulate compensatory ovarian changes following hemispaying in rats.

Strictly 4-day cyclic rats were subjected to surgical hemispaying on day 1 (estrus) of the cycle, distributed into four groups and treated with captopril alone, captopril concurrently with prolactin, captopril along with progesterone and the vehicle alone. On the following day 1, 70% of the captopril-treated group failed to ovulate but maintained a comparable degree of hypertrophy of the surviving ovary. The remaining 30% animals showed compensatory ovarian changes yet the number of eggs ovulated were significantly low in count. Animals which received combined treatment of captopril and either of parolactin or progesterone presented compensatory values as observed in the vehicle-treated controls.

The results suggest that captopril possibly generates a lesion on prolactin-progesteron system which leads to anovulation in hemispayed rats.

References:

1. Canonico & McLeod (1986). *Endocrinology*, 118:233-238.
2. Kuo et al (1991). *J. Reprod Fert*, 82:469-474.

* The author acknowledges the research grant provided by Universiti Sains Malaysia, Penang, that has resulted in this article.