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Impact of imatinib administration on mouse ovarian follicle counts and intra-ovarian protein levels related to follicular quality

이마티닙 투여가 생쥐 난포의 양 및 난포의 질과 관련된 난소 내 단백질 수준에 미치는 영향에 대한 연구

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Abstract

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Following the development of cancer management, cancer survivors have increased, and their concerns for future fertility have grown. Compared to the harmful effects of conventional chemotherapeutics on reproduction, new chemotherapeutics show inconclusive evidence about its deleterious effects on reproduction. Women treated with cyclophosphamide (Cp) are at a high risk of premature ovarian

failure. One of the mechanisms of Cp-induced ovarian damage is the apoptotic pathway in ovarian follicles. However, few studies have been reported the exact effect of imatinib, a tyrosine kinase inhibitor, on ovarian function. Thus, this study aimed to investigate the impact of imatinib on the quantity and quality of mice ovarian follicles.

We injected saline, cyclophosphamide (Cp, 50 or 75 mg/kg), or imatinib (7.5 or 15 mg/kg) into female B6D2F1 mice (eighteen mice for each group). One week later, ovaries were collected. Nine mice in each group were used for follicle count. In multiple ovarian sections stained by Mayer's hematoxylin-eosin solution, we counted the number of various types of follicles and the proportion of good quality follicles, which was defined as intact spherical shape of follicles with oocytes. The other nine mice in each group were used for the western blot analysis. Levels of six proteins related to follicular function, apoptosis, and vascularization (AMH, BCL-xL, BAX, A-SMase, caspase-3, and α-SMA) were quantified within whole ovaries. We compared the ovarian follicle counts and expression of proteins among the groups.

A significant decrease in primordial follicle count was observed in the group administered imatinib 7.5 mg/kg ($p = 0.001$) and 15 mg/kg ($p = 0.010$), as well as in the group administered Cp 75 mg/kg ($p = 0.002$), compared to the saline control group. In contrast, no differences in primary and antral follicle counts were observed between the group treated with imatinib 7.5 mg/kg or 15 mg/kg and the saline control group. Although administration of Cp 50 mg/kg or 75 mg/kg significantly decreased the proportion of good quality primordial follicles, administration of imatinib 7.5 mg/kg or 15 mg/kg did not reduce the proportion of good quality primordial follicles. AMH level was similar in the groups treated with imatinib 15 mg/kg and Cp 50 or 75 mg/kg, compared to the saline control group. Anti-apoptotic BCLX-L and pro-apoptotic BAX and A-SMase levels were also similar in the five groups. However, Caspase 3 and α-SMA levels were significantly higher in the imatinib 7.5 or 15 mg/kg and Cp 50 or 75 mg/kg groups than those in the saline control group.

In conclusion, the administration of imatinib to mice

significantly reduced primordial follicle count and increased protein levels of Caspase 3 and α-SMA. Our findings suggest that imatinib has potential ovarian toxicity via apoptotic process, as like in Cp.

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Keywords: imatinib; cyclophosphamide; tyrosine kinase inhibitor; primordial follicle; caspase; fertility.

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Chapter 1: Introduction

Imatinib, a tyrosine kinase inhibitor, is widely used in patients with chronic myeloid leukemia (CML) or gastrointestinal stromal tumors (1,2). Although the detrimental effect of chemotherapeutics on future fertility is a major concern for female cancer survivors, it is largely unknown whether imatinib causes ovarian damage (3).

The main target of imatinib is an oncogenic protein breakpoint cluster region-Abelson murine leukemia (BCR-ABL). Imatinib also inhibits other tyrosine kinases such as ABL, KIT, and platelet-derived growth factor receptor (PDGFR) (4-6). Within the ovary, KIT ligand and PDGF have been shown to independently promote primordial follicle activation, transition of primordial to primary follicle, oocyte growth, granulosa cell proliferation, and follicle survival (7-9). Because imatinib inhibits the c-kit pathway, which is essential for ovarian follicle development, it may have a negative impact on ovarian follicle survival (10).

To date, studies on ovotoxiciy of imatinib in human are scarce. In a case series research, amenorrhea is usually not induced in

women taking imatinib orally, and successful conception commonly occurs (11). However, a few case reports suggested that imatinib may affect the loss of the ovarian reserve. One case report showed that long-term administration of imatinib (for 2 years) might lead to primary ovarian insufficiency (12). In another case report, imatinib user showed a severely impaired ovarian response to exogenous gonadotropin stimulation, but showed a normal ovarian response after stopping imatinib (13).

Ovotoxic effect of imatinib in several animal experiments are still inconclusive. In zebrafish model, imatinib feeding once, twice, or three times per day caused irreversible suppression of ovarian folliculogenesis with frequency dependence (14).

In mice model, long-term injection of imatinib (for 4-6 weeks) induced diminished ovarian reserve (15). In that report, mice treated with imatinib could yield in-vivo fertilized zygotes through ovarian stimulation, but development of zygotes in-vitro and implantation of subsequent blastocysts were severely hampered (15).

Intraperitoneal injection of imatinib to the human ovary-xenografted mice increased follicular atresia and induced bizarre-shaped follicles without oocyte (16). Meanwhile, in

leukemic mice model, administration of imatinib orally for two months did not affect the numbers of primordial, primary, and secondary follicles (17).

Up to our knowledge, the effect of imatinib on ovarian function is still controversial. Thus, we aimed to investigate the effect of imatinib injection once to mice on the quantity and quality of ovarian follicles, and levels of six proteins; anti-müllerian hormone (AMH), BCL-xL, BAX, A-SMase, caspase-3, and and α-SMA.

Cyclophosphamide (Cp), an alkylating drug used in cancer treatment protocols, is a well-known ovotoxic agent by inducing follicle loss. We also tested the deleterious effect of Cp injection on ovarian follicles and compared the results to those by imatinib injection.

Chapter 2: Materials and Methods

1. Mice

Six- to seven-week-old B6D2F1 female mice (Orient Bio, Seongnam, Korea) were used. They were raised in controlled sterile conditions at 22°C with a 12-h light/dark cycle, and had free access to autoclaved pellet diet and water. The experimental protocols and animal handling procedures were ethically performed with the approval of the Institutional Animal Care and Use Committee (IACUC) of the Seoul National University Bundang Hospital approved this study (IACUC No. 53-2019-014).

2. Experimental design

After one week of adaptation, 90 mice were divided into five groups, and each agent was injected intraperitoneally once to the mice: 0.1 mL of normal saline (control group), Cp (Cp monohydrate; Sigma Aldrich, St Louis, MO, USA) 50 mg/kg, Cp 75 mg/kg, imatinib (Enzo Life Sciences, Farmingale, NY, USA) 7.5 mg/kg, or imatinib 15 mg/kg. Cp was dissolved in phosphate buffered saline (PBS) and prepared at different

concentrations. The dose of imatinib was based on equality of the average human oral dose of 400-800 mg/day to treat CML (assumed as 8-16 mg/kg in a human of 50 kg) (14,18). Imatinib powder was dissolved in PBS to obtain an imatinib stock solution. The final volume of intraperitoneal injection was all set at 0.1 mL. One week later, we sacrificed mice by cervical dislocation and collected bilateral ovaries.

3. Histological examination and follicle counts

Nine mice in each group were used, thus eighteen ovaries in each group were obtained. All ovaries were fixed in 4% buffered paraformaldehyde and embedded in a paraffin block, and then cut into 4 μm sections serially, resulting in at least five sections per ovary. The ovarian sections were stained by Mayer's hematoxylin-eosin solution (Merck-Serono, Darmstadt, Germany) for histologic examination. Ovarian follicles were evaluated by two senior experts. Under a light microscope (Nikon, Tokyo, Japan) at x400 magnification, ovarian follicles were classified into four types as defined in a previous study (19):

1. Primordial follicle: single layer of flattened pre-

granulosa cells

2. Primary follicle: single layer granulosa cells including cuboidal forms

3. Secondary follicle: at least two layers of cuboidal granulosa cells

4. Antral follicle: multiple layers of cuboidal granulosa cells with antrum

Each follicle was evaluated for the integrity according to the following criteria as mentioned in a previous study (20):

1. G1 (good quality) follicle: intact spherical follicle and oocyte

2. G2 (fair quality) follicle: granulosa cells pulled away from the edge of follicles, but with intact oocyte

3. G3 (poor quality) follicle: disruption and/or loss of granulosa-theca cells, with pyknotic nuclei and/or missing oocyte.

Representative histological images of ovarian follicles are shown in Figure1.

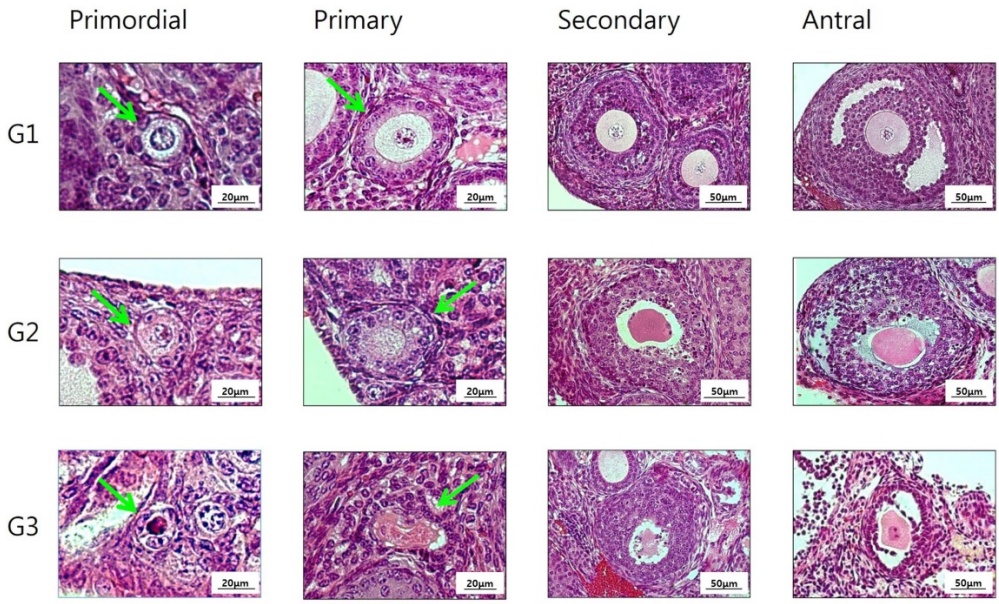

Figure 1. Representative histologic images of mouse ovarian follicles. Stained by Mayer's hematoxylin-eosin. G1: good quality, G2: fair quality, and G3: poor quality.

4. Western blotting

Another nine mice in each group were used for the western blot analysis. Ovaries were suspended in a lysis buffer (20 mM Tris-HCl pH 8.0, 137 mM NaCl, 1% Nonidet P-40 and 10% glycerol) supplemented with protease inhibitors (0.5 mM PMSF, 0.025 mM N-CBZ-L-phenylalanine chloromethyl ketone, 0.025 mM N-p-tosyl-lysine chloromethyl ketone and 0.025 mM L-1-tosylamide-2-phenyl-ethylchloromethyl ketone). After centrifugation at 4°C for 10 min at 10,000 x g, the pellets were discarded and the supernatant was obtained.

After boiling for 5 min, we loaded 50 μg of protein onto a 12% SDS-polyacrylamide gel and performed electrophoresis at 120 V for 1.5 h. The resolved proteins were transferred onto nitrocellulose membranes at 100 V for 2 h. After incubation in a blocking buffer (5% non-fat milk, 0.05% tween-20 in 20 mM TBS pH 8.0) for 1 h at room temperature, the blots were incubated overnight at 4°C with appropriate primary antibodies: AMH (1:400, sc-166752, Santa Cruz Biotechnology, CA, USA), BCL-xL (1:100, sc-271121, Santa Cruz Biotechnology), BAX (1:300, sc-7480, Santa Cruz Biotechnology), A-SMase (1:200, ab83354, Abcam, Cambridge,

UK), cleaved caspase-3 (1:500, 5a1e, Cell Signaling Technology, Danvers, MA, USA), and α-SMA (1:200, sc-53142, Santa Cruz Biotechnology).

In Figure 2, representative bands from the western blot analysis are presented. Specifically, the bands for BCL-xL appear to be double, and this phenomenon is common for the phosphorylated BCL-xL (21).

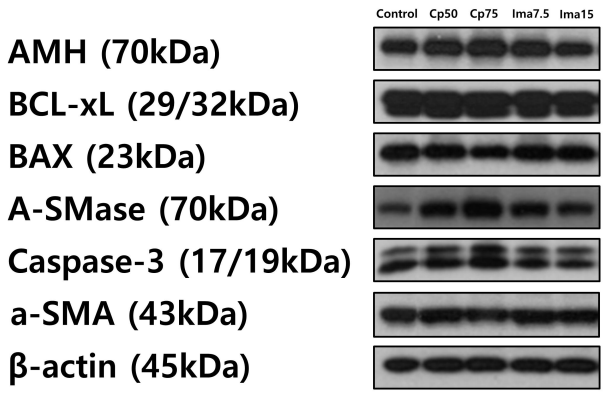

Figure 2. The expressions of six proteins within whole ovaries by western blot.

BCL-xL is an anti-apoptotic marker and BAX is a pro-apoptotic marker. A-SMase is an enzyme known to increase the levels of the pro-apoptotic sphingolipid ceramide; thus, it acts as a pro-apoptotic marker. Caspase-3 is a well-known marker for late type apoptosis. α-SMA, detected in tissues with disrupted blood vessels, is a marker for vessel damage.

Next, the blot was incubated with anti-rabbit secondary antibodies conjugated with horseradish peroxidase (1:1000, catalog # A4914, Sigma-Aldrich). Scion Image for Windows (Scion Corporation, Worman's Mill, CT, USA) was used for analyzing the chemiluminescence signal. For each protein, eight or nine replicates were used. In the experimental groups, each protein level was expressed as relative to the protein level of saline control group. Therefore, the protein level of the saline control group was always 1.0.

5. Statistical analysis

We used IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 5.0 (GraphPad software, CA, USA). Ovarian follicle counts were compared by using one-way ANOVA followed by Tukey's multiple-comparison test. All samples were tested for normality before ANOVA. A p-value <0.05 was considered statistically significant.

The protein levels were compared by using the Kruskal-Wallis test followed by the Mann-Whitney U test. A p-value <0.01 was considered statistically significant.

Chapter 3: Results

The detailed ovarian follicle counts including the proportion of G1 follicle in the five groups are presented in Table 1.

Notably, the number of primordial follicles was significantly lower in the group treated with imatinib 7.5 mg/kg (13.9 ± 7.0 vs. 23.3 ± 8.0; p=0.001), as well as in the group treated with imatinib 15 mg/kg (15.5 ± 7.9 vs. 23.3 ± 8.0; p=0.010) than the control group. The Cp 75 mg/kg treated group also showed decreased number of primordial follicles than the control group (13.8 ± 10.7 vs. 23.3 ± 8.0; p=0.002), but Cp 50 mg/kg treated group did not.

Table 1. Ovarian subtype follicle counts and the proportion of good quality (G1) follicle in mice treated with cyclophosphamide (Cp) or imatinib.

	Control	Cp 50 mg/kg	Cp 75 mg/kg	Imatinib 7.5 mg/kg	Imatinib 15 mg/kg	p-value†
No. primordial	23.3±8.0	19.6±8.1	13.8±10.7**	13.9±7.0***	15.5±7.9**	0.001
G1 primordial (%)	64.5±11.9	36.0±14.5***	40.0±22.1***	51.7±23.2	58.7±12.9	<0.001
No. primary	23.6±7.9	22.7±7.6	24.6±11.7	23.6±6.6	21.7±7.2	0.506
G1 primary (%)	67.0±12.5	55.0±21.4	53.2±18.8*	60.5±15.5	59.1±11.6	0.174
No. secondary	49.1±10.4	49.2±12.1	45.8±24.6	50.8±9.8	36.1±10.2***	0.004
G1 secondary (%)	51.7±12.8	38.2±11.2**	37.6±11.8**	46.7±9.2	38.4±12.8**	0.002
No. antral	14.1±6.1	16.0±8.9	20.0±10.3	18.1±4.2	18.6±6.4	0.107
G1 antral (%)	86.6±12.0	75.0±14.8*	73.2±14.5**	79.6±13.1	86.4±6.9	0.015

Mean±SE. †; by one-way ANOVA. *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$ when compared with control group (by Tukey's multiple-comparison).

For better understanding, ovarian follicle counts in the five groups are depicted in Figure 3. Primary and antral follicle counts were all similar amongst five groups (Figure 3-B, 3-D). Secondary follicle count was significantly lower in the imatinib 15 mg/kg treated group than the other four groups (p=0.001 for each) (Figure 3-C).

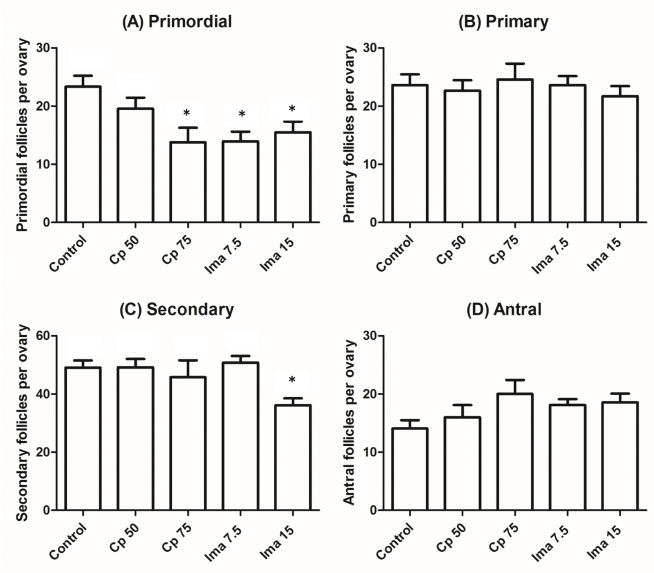

Figure 3. Ovarian subtype follicle counts in the five groups. Data are expressed as the mean±SE. Control: 0.1-mL of normal saline. Cp 50: cyclophosphamide 50 mg/kg. Cp 75: cyclophosphamide 75 mg/kg. Ima 7.5: imatinib 7.5 mg/kg. Ima 15: imatinib 15 mg/kg. *; $p<0.05$ by one-way ANOVA followed by Tukey's multiple-comparison test.

The proportion of G1 follicles in the five groups are depicted in Figure 4. Although administration of imatinib 7.5 or 15 mg/kg significantly decreased the number of primordial follicles, the proportion of G1 primordial follicle was well preserved, as shown in Figure 4-A. Similarly, the proportion of G1 primary and antral follicles was also preserved in two imatinib treated groups, but the proportion of G1 secondary follicles was not (Figure 4-C).

Interestingly, administration of Cp 50 or 75 mg/kg significantly decreased the proportion of G1 follicles in almost all types, compared to the control group (Figure 4).

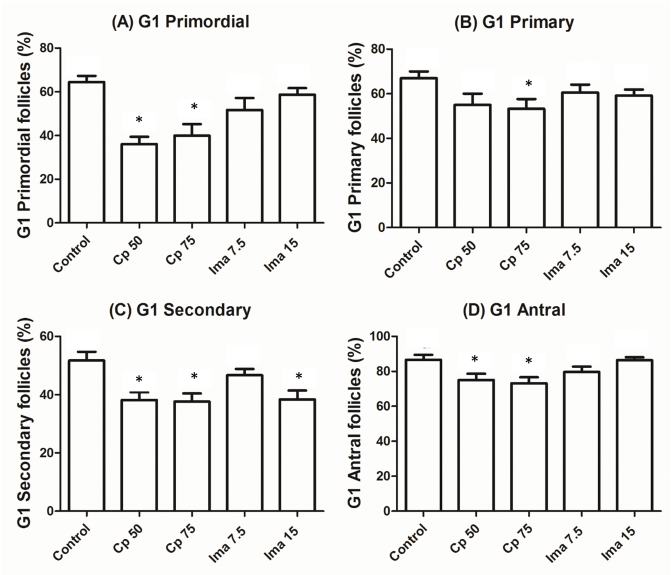

Figure 4. The proportion of good quality (G1) subtype follicle in the five groups. Data are expressed as the mean±SE. Control: 0.1-mL of normal saline. Cp 50: cyclophosphamide 50 mg/kg. Cp 75: cyclophosphamide 75 mg/kg. Ima 7.5: imatinib 7.5 mg/kg. Ima 15: imatinib 15 mg/kg. *; $p<0.05$ by one-way ANOVA followed by Tukey's multiple-comparison test.

As summarized regarding primordial follicles, administration of imatinib 7.5 or 15 mg/kg significantly reduced the number of primordial follicles but preserved well the proportion of G1 primordial follicles. The administration of Cp 50 mg/kg did not reduce the number of primordial follicles but reduced the proportion of G1 primordial follicles. The administration of Cp 75 mg/kg significantly reduced both the number of primordial follicles and the proportion of G1 primordial follicles.

Levels of six ovarian proteins in the five groups are depicted in Figure 5. The ovarian AMH levels in Cp 50 mg/kg, Cp 75 mg/kg, and imatinib 15 mg/kg treated groups were similar to control group, but its level was rather high in imatinib 7.5 mg/kg treated group ($p < 0.001$). The ovarian levels of anti-apoptotic BCL-xL, and pro-apoptotic BAX and A-SMase were all similar in the five groups. However, ovarian caspase-3 protein levels were significantly higher in Cp 50 mg/kg, Cp 75 mg/kg, imatinib 7.5 mg/kg, and imatinib 15 mg/kg treated groups, compared to the control group. Also, α-SMA were significantly higher in the group treated with imatinib or Cp than those in the saline control group.

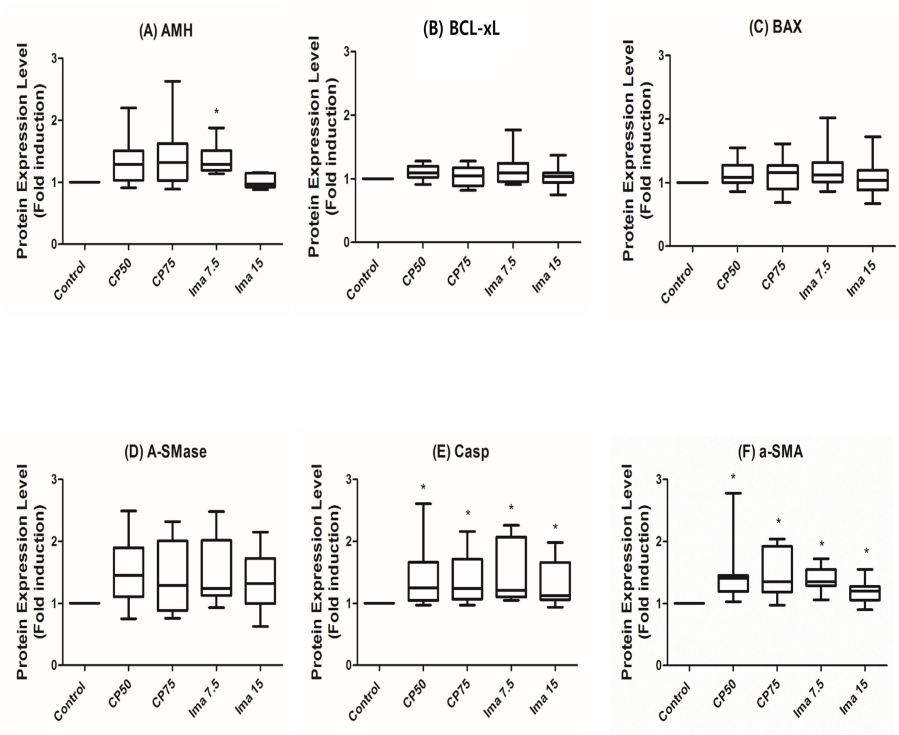

Figure 5. Box-Whisker bars to demonstrate relative protein levels within whole ovaries. Control: 0.1-mL of normal saline. Cp 50: cyclophosphamide 50 mg/kg. Cp 75: cyclophosphamide 75 mg/kg. Ima 7.5: imatinib 7.5 mg/kg. Ima 15: imatinib 15 mg/kg. *; $p < 0.01$ when compared to the saline control group by the Mann-Whitney U test.

Chapter 4: Discussion

In the present study, we examined ovotoxicity of imatinib as well as Cp in mice model. Administration of imatinib 7.5 and 15 mg/kg significantly reduced the number of primordial follicles, a representative of ovarian reserve. Increased ovarian caspase-3 and α-SMA levels were observed in imatinib administrated mice. These findings suggest that imatinib induces primordial follicle loss, possibly via apoptotic mechanism operated with caspase-3 and vascular damage.

Decreased number of primordial follicle was also observed in the Cp 75 mg/kg treated group, while increased ovarian caspase-3 levels were observed both in Cp 50 and 75 mg/kg treated groups. Caspase-3 is major downstream effector enzyme in late type apoptosis process, and increased caspase-3 induced by Cp has also been reported (22).

It has been reported that imatinib induces loss of primordial follicles in animal models (14-16), which is consistent with our findings. Furthermore, we here found, for the first time, imatinib exerts an ovotoxic effect through apoptotic mechanism operated with caspase-3, as like in Cp. Given that there was no

difference in ovarian expression of BCL-xL, BAX, and A-SMase among the imatinib or Cp treated groups, and control, BCL-xL, BAX, and A-SMase do not seem to work on apoptotic mechanism that cause primordial follicle reduction.

Imatinib has been proposed as an agent to prevent primordial follicle loss caused by cisplatin (23). The ovoprotective effect was presumed because imatinib could inhibit the c-Abl-TAp63 pathway, which is one of the main mechanisms of cisplatin-induced follicular apoptosis. However, subsequent studies have challenged these results and there is controversy about the ovoprotective effect of imatinib. Therefore, given that our study demonstrates imatinib's ovotoxicity, use of imatinib to protect ovarian damage induced by anticancer drugs should be careful.

So-called 'burnout' hypothesis has been suggested as a mechanism for ovarian damage by Cp (24,25). In 'burnout' hypothesis, Cp inhibits the dormancy of primordial follicle pool by destructing growing follicles. This induces premature activation of primordial follicles, thereby reducing primordial follicles. We here found that Cp reduced the primordial follicle count without affecting primary, secondary and antral follicle count. Therefore, 'burnout' phenomenon appear not to occur by

Cp (at least by the dose we studied). Luan et al. also demonstrated that Cp specifically depletes primordial follicles by direct induction of apoptotic cell death, rather than depleting primordial follicle pool through activation or destroying growing follicles (22).

We here also found that imatinib reduced the primordial follicle count, but not affected other growing follicle counts. In same context, 'burnout' phenomenon appear not to occur by imatinib (at least by the dose we studied).

We here found that imatinib reduces the primordial follicle count, as like in Cp. However, the extent of primordial follicle damage by imatinib might be modest, as compared with Cp, because the proportion of G1 follicles was maintained in the imatinib treated groups.

This could be clinically important, because the ovarian reserve may be recovered after discontinuation of imatinib, if G1 follicles are maintained during imatinib administration.

Further studies are needed to determine how long does the decrement of primordial follicles last after imatinib administration and when does decrement of primordial follicles recover after imatinib discontinuation.

In the present work, ovarian AMH levels were well preserved after one-week exposure of imatinib or Cp. This could be explained by well-preserved primary and secondary follicles after imatinib or Cp administration, which is main sources of AMH production. Meanwhile, it has been reported that serum AMH levels drop within 3 days after Cp administration, and then rebound to levels equivalent to 7 days after Cp treatment (22). They found that healthy granulosa cells in follicles replace damaged granulosa cells and secrete AMH. A further research is needed on how ovarian AMH level changes over time after imabinib administration.

Our study has several limitations. Although we demonstrated the ovotoxicity of imatinib, underlying mechanism of ovotoxicity induced by imatinib should be further elucidated. We here exposed mice for one week by a single dose of imatinib. Further studies are needed to evaluate the effects of various exposure durations or doses of imatinib.

Chapter 5: Conclusion

In conclusion, imatinib administration to mice negatively effects on primordial follicle counts. This detrimental effect might be induced via caspase-3-dependent apoptosis and vascular damage, as like in Cp. Potential ovotoxicity induced by imatinib treatment should be further studied both biologically and clinically.

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국문 초록

이마티닙 투여가 생쥐 난포의 양 및 난포의 질과 관련된 난소 내 단백질 수준에 미치는 영향에 대한 연구

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암 치료의 발전으로 인해 암 생존자가 증가하고 있고, 그들의 생식 능력 보존에 대한 갈망 또한 증가하고 있다. 기존의 고식적인 항암제가 생식 능력에 유해한 영향을 주는 것으로 잘 알려져 있는 반면에, 새로운 항암제들의 생식 능력에 대한 영향은 연구가 부족한 실정이다. 항암제 사이클로포스파미드 (cyclophosphamide) 를 투여한 여성의 경우 조기 난소 기능 부전의 위험도가 크게 증가한다. 그 기전의 하나로 사이클로포스파미드로 인한 난포의 세포자멸사 기전이 (apoptotic pathway) 많이 연구되어 왔다. 하지만, 티로신 키나제 억제제 (tyrosine kinase inhibitor) 계열 항암제인 이마티닙 (imatinib) 의 난소 기능에 대한 연구는 현재까지 매우 부족하

다. 따라서, 본 연구는 이마티닙 투여가 생쥐 난포의 양과 질에 미치는 영향을 탐색하고자 하였다.

암컷 B6D2F1 생쥐에 식염수, 사이클로포스파미드 (Cp, 50 or 75 mg/kg), 이마티닙 (7.5 or 15 mg/kg)을 각각 주입하였다. 각 그룹에 대해 18마리의 생쥐가 배정되었고 주입 후 1주일 뒤에 난소들이 채취되었다. 각 그룹의 9마리 생쥐에서 얻는 난소를 여러 층으로 자르고 헤마톡실린과 에오신 염색 (hematoxylin-eosin solution) 후, 다양한 종류의 난포 수와 양질의 난포 비율을 확인하였다. 양질의 난포는 온전한 구형으로 난자를 갖고 있는 난포로 정의되었다. 각 그룹의 다른 9마리 생쥐에서 얻은 난소에서 난포의 기능, 세포자멸사, 혈관 기전과 관련있는 6 종류의 단백질 (AMH, BCL-xL, BAX, A-SMase, caspase-3, and α-SMA)이 웨스턴 블랏 (western blot) 분석을 통해 정량화 되었다. 그룹들 사이에서 난포의 수와 발현되는 단백질이 비교되었다.

대조군과 비교시 이마티닙 그룹 (7.5 mg/kg , 15 mg/kg)에서 원시난포의 수가 유의하게 적었고 사이클로포스파미드 75 mg/kg 그룹에서도 유의하게 원시 난포의 수가 적었다. 다른 종류의 난포를 살펴보면, 이마티닙 그룹과 대조군간 일차난포 (primary follicle) 와 동난포 (antral follicle)의 수는 차이가 없었다. 대조군과 비교했을

때 사이클로포스파미드 그룹에서 양질의 원시난포 비율이 유의하게 감소하였는데, 이마티닙 그룹에서는 양질의 원시난포 비율이 감소하지 않았다. 실험군을 대조군과 비교시 AMH, 항세포자멸사 (anti-apoptotic) BCLX-L, 향세포자멸사 (pro-apoptotic) BAX와 A-SMase 단백질 양은 비슷했다. 그러나, 이마티닙 그룹과 사이클로포스파미드 그룹에서 카스파제 3와 α-SMA 단백질은 대조군과 비교시 유의하게 증가하였다.

결론적으로, 생쥐에게 이마티닙을 투약하면 원시난포의 수가 유의하게 줄고 난소에서 카스파제 3와 α-SMA 단백질이 유의하게 늘었다. 이마티닙은 사이클로포스파미드처럼 세포자멸사 기전과 혈관 손상을 통한 잠재적인 난소 독성을 갖는 것으로 보인다.

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주요어 : 이마티닙, 사이클로포스파미드, 티로신키나제억제제,
원시난포, 카스파제, 생식능력.
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