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Examination of the effect of melatonin use before hysterosalpingography on ovarian follicle reserve in rats

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Summary

Objective: To examine the effects of lipiodol, melatonin, and radiation used during hysterosalpingography (HSG) on rat ovarian follicle reserve. *Materials and Methods:* A total of 50 Wistar rats with regular estrous cycles were randomly divided into five groups. Group 1 was the control group. The other groups, X-ray was applied (G2), 0.1 ml lipiodol was applied to each uterine horn (G3), 20 mg/kg intraperitoneal melatonin application was followed by 0.1 ml lipiodol administration to each uterine horn after 15 minutes (G4), 20 mg/kg melatonin was administered to ligamentum suspensorium ovarii, followed by 0.1 ml lipiodol application to each uterine horn after 15 minutes (G5), respectively. The rats in G2, G3, G4, and G5 were exposed three times to whole body radiation. Then, all rats were re-opened and left oophorectomy was performed. Left ovarian samples were fixed in formaldehyde. Primordial, primary, secondary, and tertiary follicles were counted in the preparations, and all were added to calculate the ovarian follicle reserve. *Results:* Primordial, primary, and ovarian follicle reserves were found significantly lower in G2, in comparison to other groups. Atretic follicle values were significantly higher in G2 compared to G3, and in G3 compared to the other groups. Regression of angiogenesis within corpus luteum was found significantly lower in G2, compared to G3, and in G3 compared to other groups. All values in G1, G4, and G5 were similar. *Conclusion:* Use of lipiodol and melatonin during HSG procedure prevents the negative effects of radiation on ovarian follicle reserve.

Key words: HSG; Lipiodol; Melatonin; Ovarian follicle reserve; Rat.

Introduction

Infertility is a serious problem for both of women and men. Three examinations have been deemed essential in the evaluation of infertile couples. These include 1) proving the presence of ovulation, 2) semen analysis, and 3) examination of the condition of fallopian tubes [1, 2]. Hysterosalpingography (HSG), magnetic resonance imaging (MRI), hysterosalpingo-contrast-sonography using echovist (HyCoSy), and laparoscopy can be used in the fallopian tube evaluation procedure [3-6]. The grounds on which the researchers explain why they use methods other than HSG are HSG procedure causes pain and the reproductive organs are exposed to high levels of ionized radiation [7-9]. However, HSG procedure has such advantages as being a simple, inexpensive, and simple to use technique in a polyclinic setting. Use of a fat-soluble solution-like lipiodol has a positive effect on pregnancy [10-12]. Other techniques have not yet been proven to have a pregnancy increasing effect as lipiodol. Consequently, it may be acknowledged that HSG is presently an indispensable technique in infertility patients. During HSG, the patient is exposed to ionized radiation. Radiation increases oxygen radicals (ROS) in the cell. ROS (superoxide, hydroxyl, peroxyl, alkoxyl, and singlet oxygen radicals) exercise a carcinogen effect by damaging the cell DNA [13]. They destroy the cell membrane, lisosome membrane, and membranes of such cell organelles as endoplasmic reticulum, break down cells, and lead to necrosis [14, 15]. This event is called lipid peroxidation. Lipid peroxidation stimulates collagen production in cell cultures. Fibrosis develops in the tissues [16, 17]. Melatonin, produced by the pineal gland, is a hormone that has a strong antioxidant effect, and that modulates the ovarian function in humans [18]. Melatonin is effective on hydroxyl radical, singlet oxygen, peroxyl radical, and superoxide anion, from the oxygen radicals. It protects the nucleus DNA, membrane lipids, and cytosolic proteins against oxidative stress [19]. Additionally, it supports SOD, GSH-Px, glutathione reductase, and glycose-6-phosphate dehydrogenase of the antioxidant system [20]. It has an inhibitor effect on nitric oxide synthetase [21]. Furthermore, melatonin is absorbed easily and rapidly passing through the morphophysiological barriers (blood-brain barrier, placenta, etc.) irrespective of the route by which it is administered. It protects the cells of the organ it penetrates into against oxidative stress. It also has a protective effect on mitochondria, which is a cell organelle [22].

There is no study on whether ovarian follicle reserve changes in rats due to X-ray exposure during HSG that will decrease or not with the use of lipiodol and melatonin given

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both intraperitoneally and into suspensorium ovarii. Therefore, the aim of the current study was to investigate the protective effects of melatonin against X-ray on ovarian follicle reserve in rats during HSG.

Materials and Methods

Following the approval the Ethics Committee of Firat University, all surgical procedures and caring protocols used were planned and conducted in accordance with "Guide for the Care and Use of Laboratory Animals" in this study. Experiments were performed in a total of 50 albino Wistar female rats at the animal laboratory of Firat University. Rats were five months old, weighed from 180-200 grams, and had regular cycles. They were kept at a temperature from 21-23°C, with relative humidity (55-60%), under controlled photo-periods (12:12 hours light:dark), were fed with a standard rodent bait, and drank tap water. Feeding of the rats was suspended 18 hours prior to the experiment, only water was allowed. The rats that were detected to be in the estrus phase via vaginal cytology and were anesthetized with chloral hydrate (400 mg/kg, IP). Each rat was placed on the operating table in a supine position and abdomen was opened through a midline incision. Rats were randomized into five groups of ten. G1: the group in which the abdomen was opened and closed without any X-ray irradiation, G2: the group in which the abdomen was opened and closed, and X-ray was applied, G3: the group in which the abdomen was opened and 0,1 ml. Lipiodol amp. was applied to each uterine horn, G4: the group in which the abdomen was opened and 20 mg/kg Melatonin was administered intraperitoneally, followed by 0,1 ml lipiodol application to each uterine horn after 15 minutes, and G5: the group in which the abdomen was opened and 20 mg/kg Melatonin was administered to ligamentum suspensorium ovarii, followed by 0,1 ml lipiodol application to each uterine horn after 15 minutes.

After their abdomens were closed, the rats in G2, G3, G4, and G5 were exposed to whole body irradiation three times leaving two-minute intervals in between (total dose = 15-20 miliRad, double table, and double tube). During the experiment, blood pressure, heart rate, and body temperature of the rats were monitored. After three hours, the abdomens of all rats were re-opened and remaining ovaries were removed. Ovarian tissue was fixed in 10% formaldehyde for histological examination, and buried into paraffin blocks, from which 4-µm cross-sections were prepared. The cross-sections were then stained with hematoxylin eosin. Primordial, primary, secondary, and tertiary follicles were counted in the preparations examined under light microscopy, as suggested by Souza *et al.* [23]. Total follicle reserve was calculated by the sum of all. Atretic follicle count was made. Corpus luteum and corpus albicans were counted and the total number of corpuses was calculated. Regression of angiogenesis in corpus luteum was examined. Presence of fibrosis on the ovarian stroma was examined. An ordinal scale was formed for regression of angiogenesis in corpus luteum and presence of fibrosis (non e= 0p, present = 1p, and markedly present = 2p). Cystic follicles in the ovary were counted. SPSS 9.0 software was used in the statistical analyses. One Way Anova was employed in the statistical analyses of continuous and ordinal data. Post Hoc Tukey HSD was used, and $p < 0.05$ was considered significant.

Results

Primordial follicles, primary follicles and ovarian reserve in G2 were found significantly lower than those in the other groups ($p < 0.05$, Tukey HSD test) (Figure 1).

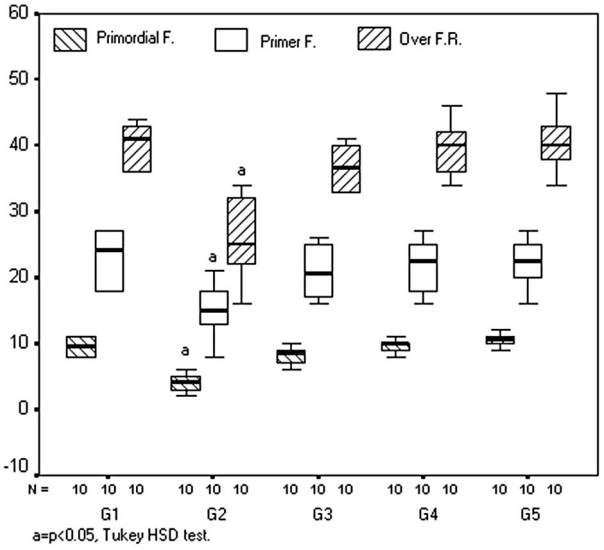

Figure 1. — Stacked box graph pertaining to the ovarian follicle reserve and its elements.

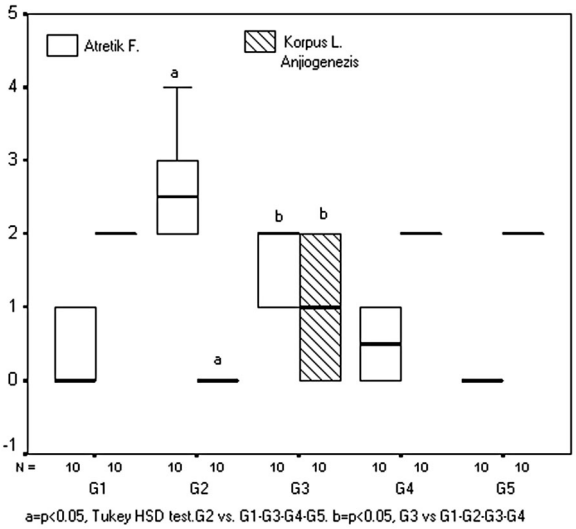

Figure 2. — Atretic follicle values and changes in angiogenesis in corpus luteum.

Atretic follicle values were found significantly higher in G2, relative to G3, and in G3, relative to other groups ($p < 0.05$, Tukey HSD test). Regression of angiogenesis in corpus luteum was lower in G2, compared to G3 and in G3, compared to other groups ($p < 0.05$, Tukey HSD test). Addition of melatonin to lipiodol reduced atretic follicle count, and had a positive effect on regression of angiogenesis in corpus luteum (Figure 2).

Corpus luteum, corpus albicans, and total number of cor-

Table 1. — *Parameters that were found similar in all groups. Values are expressed as mean ± SD. NS = p > 0.05, One Way Anova.*

Parameter	G1	G2	G3	G4	G5	*p*
Secondary follicle (number)	3.2±0.9	2.8±0.8	3.2±0.9	3.4±0.8	3.3±0.8	Ns
Tertiary follicle (number)	4.5±0.5	4±0.6	4.5±0.5	4.5±0.7	4.4±0.7	NS
Corpus luteum (number)	4.7±0.5	3.8±2.2	4.3±0.6	4.7±0.8	5±0.8	NS
Corpus albicans (number)	0.2±0.4	0.4±0.5	0.01±0.1	0.3±0.5	0.4±0.5	NS
Total	4.9±0.7	4.2±1.8	4.4±0.8	5±1	5.3±0.9	NS
Stromal fibrosis (point)	0±0	0±0	0±0	0±0	0±0	NS
Cystic follicle (microscopic)	0±0	0±0	0±0	0±0	0±0	NS

puses, as well as fibrosis and cystic follicle development were found similar in all groups ($p > 0.05$, Tukey HSD test). Parameters investigated and found similar in groups are shown in Table 1.

Discussion

Radiation that emerges during HSG process reduces primordial follicles, primary follicles, ovarian follicle reserve, and regression of angiogenesis in corpus luteum, while increasing atretic follicle count. Use of lipiodol exclusively or together with melatonin prevents the harmful effects of radiation. In the present study, primordial follicles, primary follicles, and ovarian follicle reserve significantly decreased, and atretic follicle values significantly increased in rats whose HSGs were taken. Sugino [24] noted that the oxygen radicals and the antioxidant system in the ovary had a part in many events of reproductive physiology (follicle development, oocyte maturation, ovulation, corpus luteum function, and follicular atresia development). Oxygen radicals in the ovary are normally produced by neutrophils and macrophages, and reside in corpus luteum and follicles. Oxygen radicals (ROS) inhibit oocyte development, and increase degenerated oocyte count, as well as apoptosis [24]. In case of an ovulation event in the ovary, follicles grow in size, and go through several steps (primordial, primary, secondary, and tertiary follicle). In this process, the follicle that is in advanced stages becomes more resistant to oxygen radicals, as Cu-Zn SOD and Mn-SOD are generated in cumulus oophorus and theca cells [25, 26]. Furthermore, the amount of melatonin, a strong antioxidant, in the tertiary follicle rises three-fold of its amount in the serum. Therefore, the present authors found that numbers of primordial and primary follicles, the most susceptible follicles to ROS, were significantly lower.

Poppy seed oil in lipiodol (ethiodol=ethiodized poppy seed oil) is composed of various oil molecules. It contains a high rate of linoleic acid ($C_{18}H_{32}O_2$) and omega series of the polysaturated fatty acids [27]. Linoleic acid is a strong antioxidant, taking part in many positive events in the body. It is anti-carcinogenic. It modulates immune functions, and prevents atherosclerosis, diabetes, and obesity [28]. The reason why the present authors observed less ovarian follicle damage in the groups, they used lipiodol which may include the useful effects of linoleic acid. Melatonin is a very strong antioxidant. The fact that there was less ovarian follicle damage in the melatonin groups may be attributed to the antioxidant effect of melatonin, as it detoxifies ROS that arise during radiation [29]. Koc *et al.* [30] found melatonin effective in preventing liver damage in rats to whom they administered 5 and 10 mg/kg/ip melatonin before exposure to total body radiation. The authors found that the damage in ovarian follicles was significantly lower in the melatonin group. Sener *et al.* [31] exposed 32 male Spraque Dawley species rats to total body radiation, and found that tissue malondialdehyde (MDA) level and myeloperoxidase (MPO) activity decreased, while glutathione (GSH) level increased in many tissues (liver, lung, colon, and small intestines) in cases to whom melatonin (10 mg or 20 mg/kg/ip) was administered.

Atretic follicle values in G1, G4, and G5 were similar, while those in G3 were higher. That is, addition of melatonin to lipiodol brings about a significant decline in atretic follicle count. Oxygen radicals are produced by neutrophils and macrophages in the ovary. As melatonin also inhibits neutrophil activity, this may explain why atretic follicle count in the melatonin group was less than that in the lipiodol group [31]. Lipiodol is used in chemoembolization cases, as well [32-35]. Lipiodol caught by liver cells are retained there for a long time. It has an anti-proliferative and cytotoxic effect on human hepatoma cells. Its cytotoxic effect is more marked on macrophages and cancer cells, but is less so in normal human liver cells [32, 33]. The lipiodol used during HSG in this study may be being retained by the ovarian cells. This may be the reason why the ovarian reserve in G3, G4, and G5 was found better than that in G2.

Lipiodol increases the intra-cellular amounts of anti-tumoral agents like Cisplatin, used in chemoembolization cases, in liver cells, and the success rate of chemoembolization increases [34, 35]. Lipiodol used in this study increased the intra-ovarian amount of melatonin, which in turn reduced the damage in the ovaries. Melatonin was applied to Lig. suspensorium ovarii in G5. Although the values in G4 and G5 were statistically similar, mean values in

G5 were higher. This may be attributed to the higher amount of intra-ovarian melatonin in G5.

Regression of angiogenesis in corpus luteum was significantly lower in the group whose HSG was taken, in comparison to other groups. In normal rat ovary, capillaries that emerge in C. luteum regress. Vascular endothelial growth factor (VEGF) has the major role in the emergence of these structures in the corpus luteum. One of the main stimulants of VEGF is hypoxia [36]. Radiation causes radiation arteritis (ulcerated plaques in the artery) in major arteries (coronary, pulmonary, thoracic aorta, brachial, renal, and ilio-femoral), and hypoxia and ischemia in the organ fed by the concerned artery [37-39]. Rats in G2 may have developed severe radiation arteritis in the ilio-femoral or uterine arteries, relative to G3, G4, and G5. Even if radiation arteritis with the same severity had developed in G2, hypoxic effect was more marked there, as it was deprived of the useful effects of lipiodol and melatonin. The present results are consistent. Hypoxia-induced factor-1 (HIF-1) is activated in both the ovary and other organs in case of acute or chronic hypoxia [40]. HIF-1 alpha and hypoxic environment bring about regression and apoptosis in follicles, and result in an increase in atretic follicles and a decrease in follicular reserve [40]. The increase in atretic follicles and fibrosis, observed in G2, may be associated with the apoptotic effect of chronic hypoxia [40]. Melatonin has a positive effect on microvascular perfusion, as it supports the endothelium [41]. Restoration of microvascular perfusion will lessen the effect of hypoxia (HIF-1 alpha, VEGF). This may be one of the reasons why there was less damage in the melatonin group. Sato *et al.* [42] applied PGE1 infusion from the hepatic artery (HA) or superior mesenteric artery (SMA) to cases who had hepatic resection, and found that the application lasted two days less by HA route. Application of antioxidants closer to the target organ increases their effects. In the present study the authors administered melatonin both by intraperitoneal route (G4) and to Lig. suspensorium ovarii (G5). Values in G5 were found better than those in G4, although the difference was not significant. Lipiodol antioxidant is a drug that exercises a cytotoxic effect on the macrophage. Melatonin antioxidant has a microvascular-regulating, neutrophil migration-reducing and mitochondria-protecting effect. Lipiodol used in HSG procedure reduces the damage in the ovary through its antioxidant and anti-macrophage effect, while melatonin, whose intra-ovarian concentration is increased by lipiodol, further enhances this positive effect through its features listed above. Lipiodol and melatonin have a synergistic effect.

Conclusion

Use of lipiodol and melatonin during HSG procedure prevents the negative effects of radiation on ovarian follicle reserve.

References

[1] Crosignani P., Rubin B.: "Guidelines to the prevalence, diagnosis and management of infertility". *Hum. Reprod.*, 1996, 1775.

[2] Ayida G., Chamberlain P., Barlow D., Koninckx P., Golding S., Kennedy S.: "Is routine diagnostic laparoscopy for infertility still justified? A pilot study assessing the use of hysterosalpingo-contrast sonography and magnetic resonance imaging". *Hum. Reprod.*, 1997, *12*, 1436.

[3] Krysiewicz S.: "Infertility in women: diagnostic evaluation with hysterosalpingography and other imaging techniques." *A.J.R. Am. J. Roentgenol.*, 1992, *159*, 253.

[4] Woodward P.J., Wagner B.J., Farley T.E.: "MR imaging in the evaluation of female infertility". *Radio Graphics*, 1993, *13*, 293.

[5] Campbell S., Bourne T.H., Tan S.L., Collins W.P.: "Hysterosalpingo contrast sonography (HyCoSy) and its future role within the investigation of infertility in Europe". *Ultrasound Obstet. Gynecol.*, 1994, *4*, 245.

[6] Swolin K., Rosencrantz M.: "Laparoscopy vs. hysterosalpingography in sterility investigations. A comparative study". *Fertil. Steril.*, 1972, *23*, 270.

[7] Unterweger M., De Geyter C., Frohlich J.M., Bongartz G., Wiesner W.: "Three-dimensional dynamic MR-hysterosalpingography; a new, low invasive, radiation-free and less painful radiological approach to female infertility". *Hum. Reprod.*, 2002, *17*, 3138.

[8] Van der Weiden R.M., van Zijl J.: "Radiation exposure of the ovaries during hysterosalpingography. Is radionuclide hysterosalpingography justified?" *Br. J. Obstet. Gynaecol.*, 1989, *96*, 471.

[9] Yang K.T., Chiang J.H., Chen B.S., Liang C.H., Lee S.F., Liao S.C.: "Radionuclide hysterosalpingography with technetium-99m-pertechnetate: application and radiation dose to the ovaries" *J. Nucl. Med.*, 1992, *33*, 282.

[10] Schwabe M.G., Shapiro S.S., Haning R.V. Jr.: "Hysterosalpingography with oil contrast medium enhances fertility in patients with infertility of unknown etiology". *Fertil. Steril.*, 1983, *40*, 604.

[11] Yun A.J., Lee P.Y.: "Enhanced fertility after diagnostic hysterosalpingography using oil-based contrast agents may be attributable to immunomodulation." *A.J.R. Am. J. Roentgenol.*, 2004, *183*, 1725.

[12] Watson A., Vandekerckhove P., Lilford R., Vail A., Brosens I., Hughes E.: "A meta-analysis of the therapeutic role of oil soluble contrast media at hysterosalpingography: a surprising result?" *Fertil. Steril.*, 1994, *61*, 470.

[13] Richard M.J., Arnaud J., Jurkovitz C., Hachache T., Meftahi H., Laporte F., *et al.*: "Trace elements and lipid peroxidation abnormalities in patients with chronic renal failure. " *Nephron.*, 1991, *57*, 10.

[14] Welbourn C.R., Goldman G., Paterson I.S., Valeri C.R., Shepro D., Hechtman H.B.: "Pathophysiology of ischaemia reperfusion injury: central role of the neutrophil". *Br. J. Surg.*, 1991, *78*, 651.

[15] Grace P.A.: "Ischaemia-reperfusion injury". *Br. J. Surg.*, 1994, *81*, 637.

[16] Bedossa P., Houglum K., Trautwein C., Holstege A., Chojkier M.: "Stimulation of collagen alpha 1(I) gene expression is associated with lipid peroxidation in hepatocellular injury: a link to tissue fibrosis?" *Hepatology*, 1994, *19*, 1262.

[17] Lee K.S., Buck M., Houglum K., Chojkier M.: "Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by oxidative stress through c-myb expression" *J. Clin. Invest.*, 1995, *96*, 2461.

[18] Wurtman R.J., Axelrod J., Chu E.W.: "Melatonin, a pineal substance: effect on the rat ovary". *Science*, 1963, *141*, 277.

[19] Reiter R., Tang L., Garcia J.J., Munoz-Hoyos A.: "Pharmacological actions of melatonin in oxygen radical pathophysiology." *Life Sci.*, 1997, *60*, 2255.

[20] Pablos M.I., Reiter R.J., Ortiz G.G., Guerrero J.M., Agapito M.T., Chuang J.I., Sewerynek E.: "Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light". *Neurochem. Int.*, 1998, *32*, 69.

[21] Bettahi I., Pozo D., Osuna C., Reiter R.J., Acuna-Castroviejo D., Guerrero J.M.: "Melatonin reduces nitric oxide synthase activity in

rat hypothalamus." *J. Pineal Res.*, 1996, *20*, 205.
[22] Reiter R.J., Tan D.X., Qi W., Manchester L.C., Karbownik M., Calvo J.R.: "Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo". *Biol. Signals Recept.*, 2000, *9*, 160.
[23] Souza A.Z., Fonseca A.M., Izzo V.M., Clauzet R.M., Salvatore C.A.: "Ovarian histology and function after total abdominal hysterectomy." *Obstet. Gynecol.*, 1986, *68*, 847.
[24] Sugino N.: "Reactive oxygen species in ovarian physiology". *Reprod. Med. Biol.*, 2005, *4*, 31.
[25] Tamate K., Ishikawa M., Sengoku K., Abe M., Nakata T., Shimizu T., Taniguchi N.: "Role of oxygen radical and superoxide dismutase in ovulation in the rat". *Nippon Sanka Fujinka Gakkai Zasshi*, 1992, *44*, 1.
[26] Suzuki T., Sugino N., Fukaya T., Sugiyama S., Uda T., Takaya R., *et al.*: "Superoxide dismutase in normal cycling human ovaries: immunohistochemical localization and characterization". *Fertil. Steril.*, 1999, 72, 720.
[27] Abe S., Otsuki M.: "Styrene maleic acid neocarzinostatin treatment for hepatocellular carcinoma". *Curr. Med. Chem. Anti-Canc. Agents*, 2002, *2*, 715.
[28] Brown J.M., McIntosh M.K.: "Conjugated linoleic acid in humans: regulation of adiposity and insulin sensitivity". *J. Nutr.*, 2003, *133*, 3041.
[29] Vijayalaxmi, Reiter R.J., Tan D.X., Herman T.S., Thomas C.R. Jr.,: "Melatonin as a radioprotective agent: a review". *Int. J. Radiat. Oncol. Biol. Phys.*, 2004, *59*, 639.
[30] Koc M., Taysi S., Buyukokuroglu M.E., Bakan N.: "Melatonin protects rat liver against irradiation-induced oxidative injury". *J. Radiat. Res.*, 2003, *44*, 211.
[31] Sener G., Jahovic N., Tosun O., Atasoy B.M., Yegen B.C.: "Melatonin ameliorates ionizing radiation-induced oxidative organ damage in rats". *Life Sci.*, 2003, *74*, 563.
[32] Durand-Fontanier S., Simon A., Duroux J.L., Descottes B., Delage C.: "Lipiodol ultra-fluid: an antitumor agent-in vitro study". *Anticancer Res.*, 1999, *19*, 4357.
[33] Chou F.I., Lui W.Y., Chi C.W., Chan W.K.: "I-131-lipiodol cytotoxicity in hepatoma cells". *Proc. Natl. Sci. Counc. Repub. China. B.*, 1994, *18*, 154.
[34] Kishimoto S., Miyazawa K., Fukushima S., Takeuchi Y.: "In vitro antitumor activity, intracellular accumulation, and DNA adduct formation of cis-[((1R,2R)-1,2-cyclohexanediamine-N,N') bis (myristato)] platinum (II) suspended in lipiodol" *Jpn. J. Cancer Res.*, 2000, *91*, 99.
[35] Ikeda M., Maeda S., Shibata J., Muta R., Ashihara H., Tanaka M., *et al.*: "Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma" *Oncology*, 2004, *66*, 24.
[36] Geva E., Jaffe R.B.: "Role of vascular endothelial growth factor in ovarian physiology and pathology". *Fertil. Steril.*, 2000, *74*, 429.
[37] Rubin D.I., Schomberg P.J., Shepherd R.F., Panneton J.M.: "Arteritis and brachial plexus neuropathy as delayed complications of radiation therapy" *Mayo Clin. Proc.*, 2001, *76*, 849.
[38] Kieran S.M., Cahill R.A., Sheehan S., Barry M.C.: "Radiation-induced femoral arteritis". *Ir. Med. J.*, 2004, *97*, 179.
[39] Bigot J.M., Mathieu D., Reizine D.: "Radiation arteriopathies". *Ann. Med. Interne*., 1983, *134*, 411.
[40] Ferrara N., Frantz G., LeCouter J., Dillard-Telm L., Pham T., Draksharapu A., *et al.*: "Differential expression of the angiogenic factor genes vascular endothelial growth factor (VEGF) and endocrine gland-derived VEGF in normal and polycystic human ovaries" *Am. J. Pathol.*, 2003, *162*, 1881.
[41] Bertuglia S., Marchiafava P.L., Colantuoni A.: "Melatonin prevents ischemia reperfusion injury in hamster cheek pouch microcirculation". *Cardiovasc. Res.*, 1996, *31*, 947.
[42] Sato T., Yasui O., Kurokawa T., Asanuma Y., Koyama K.: "Appraisal of intra-arterial infusion of prostaglandin E1 in patients undergoing major hepatic resection report of four cases". *Tohoku J. Exp. Med.*, 2001, *195*, 125.

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