Commercial hormone replacement therapy jeopardized pro-inflammatory factors in experimental rat models

La terapia de reemplazo hormonal comercial puso en peligro los factores proinflamatorios en modelos experimentales de ratas

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Abstract

hormone replacement therapy is considered the easiest and most convenient contraceptive method. Commercially available contraceptive combination differs in their composition and concentration of combined constituents. These variations make some of these products preferred over others by consumers based on their side effects profile. The objective of the current research was to ascertain the pro-inflammatory influences of commercially available products. To do so, five groups of rats (ten rats each group) were exposed to Microgynon, Depo-Provera, Marvelon, and Yasmin compared to the control non-treated group. We measured pro-inflammatory markers including d-dimer, TNF-α (tumour necrosis factor-alpha), IL(interleukin)-6, IL(interleukin)-1B, and CRP (c-reactive protein). The results confirmed that Yasmin has induced the most deleterious effects on pro-inflammatory markers indicated by significant elevation of IL1B. In conclusion, hormone replacement therapy should be critically indicated and precaution raised inpatient with subclinical diseases, especially cardiovascular ones.

Keywords: estrogen, progesterone, contraceptive, hormone replacement, interleukin, C-reactive protein.

Resumen

la terapia de reemplazo hormonal se considera el método anticonceptivo más fácil y conveniente. La combinación anticonceptiva comercialmente disponible difiere en su composición y concentración de componentes combinados. Estas variaciones hacen que algunos de estos productos sean preferidos por los consumidores sobre otros en función de su perfil de efectos secundarios. El objetivo de la investigación actual fue determinar las influencias proinflamatorias de los productos disponibles comercialmente. Para hacerlo, cinco grupos de ratas (diez ratas cada grupo) fueron expuestos a Microgynon, Depo-Provera, Marvelon y Yasmin en comparación con el grupo de control no tratado. Medimos marcadores proinflamatorios que incluyen dímero D, TNF-α (factor de necrosis tumoral alfa), IL (interleucina)-6, IL (interleucina)-1B y PCR (proteína c reactiva). Los resultados confirmaron que Yasmin ha inducido los efectos más nocivos sobre los marcadores proinflamatorios indicados por una elevación significativa de IL1B. En conclusión, la terapia de reemplazo hormonal debe ser una indicación crítica y una precaución en pacientes con enfermedades subclínicas, especialmente cardiovasculares.

Palabras clave: estrógeno, progesterona, anticonceptivo, reemplazo hormonal, interleucina, proteína C reactiva.

Introduction

Estrogen therapy during the post-menopausal increases the "hepatic production of C-Reactive Protein (CRP)"[1], and the representation of IL-6 increases and plays an inflammatory role[2]. It has been illustrated the contrary results for Estradiol[3,4] used in postmenopause. Bestowing to an earlier conducted study progesterone increases the sequel of experimental stroke[5], however, an alternative study clarifies that progestins resolves "oxidative stress" and relegates generating of IL-6 and TNF-α[6].

In the western world, vascular diseases are a huge health care burden and a considerable increase in aging is ob-

served[7]. This risk is increased only in women alongside aging without caring about the well-known sex difference in cardiovascular diseases[8,9]. The post-menopausal decrease in ovarian hormones can also be the contributing factor, which can have impacts on many tissues alongside vasculature[7,10]. The beneficial effect of estrogen on "cerebrovascular function" has constantly been shown in observational studies and experimental animal research[10]. However, a large number of "randomized clinical trials" raised questions about the benefits of "hormone-replacement therapy (HRT)", and in fact, they detect an increase in stroke[11-13].

These diversified verdicts played a role to mention the necessity for improved comprehension of vascular actions of "ovarian hormones" alongside the understanding of "medroxyprogesterone acetate (MPA)"; a "synthetic analog of progesterone". It is usually instructed to use in a mixture with estrogen for the treatment of "perimenopausal symptoms". Having benefits, it MPA also has some back draws. According to some studies, MPA can also withstand the favorable influences of "estrogen on cardiovascular" functions and operations[14-16]. Talking about example, MPA has positive effects on estrogen biomedical metabolism, vasculature system, and advancement of atherosclerosis[14].

The pathogenesis of "cerebral ischemia"[17] is mainly caused by "cerebrovascular inflammation"[18]. The induction of promotion of inflammation mediators alongside the inclusion of inducible nitric oxide synthase and cyclooxygenase[19,20] are the lead process in "cerebrovascular inflammation". Cyclooxygenase is upregulated during cerebral ischemia which results in the generation of prostanoids, such as PGE2, and are considered to be harmful to stroke outcomes[21]. After cerebral ischemic injury[22] expression of iNOS is increased and its peak is shown in 24-48 hours. No production is thought to have either beneficial or detrimental effects following ischemia, quantities are produced and the stage of "evolution of cerebral injury"[22-24] depends on the cellular compartment.

Estrogen has constructive influences in paradigms of cardiovascular damage[10] according to experimental animal studies. While effects of progestagens "cerebrovascular inflammation" are still unknown. To answer this question, rat models were used in the in vivo progestagen treatment, which is just a reflection of known clinical blood levels[25]. During the process, the unpredicted unfavorable effects of combined HRT on stroke were seen[26], researchers assumed that progestagens, progesterone, or MPA, undo the influences of estrogen on inflammation. They also assumed the normal alteration in "endogenous estrogen" and progesterone and that caused alteration in cerebrovascular inflammation throughout the estrous cycle.

To begin, a lot of controversies exist using progesterone in systematic inflammation. To check the different effects of "estradiol and progesterone" on the "inflammatory and apoptotic responses" in rat models using commercially available estrogen-progesterone combination products, the present study was created.

Materials and methods

To carry out this research, a total of thirty healthy female "Wistar albino rats" with the age age 10-12 weeks and weight 200–260gm, were collected from the "animal house of Medical Research Institute in University of Mosul", the period of the study ranges from 1/1/2020 to 1/12/2020. Then, animals were endorsed in the "animal house of Mosul University" and were avowed under meticulous settings of temperature (24 ± 2°C), "light-dark periods" of 12 hours, and with free access to water and commercial diet[26,27,28].

Next, the "international guiding principles" for biomedical research involving animals were adopted. The animals were administered a four-week adaptation period after they were located in their new environment. The animals were divided into five groups, ten rats each. Control group, Microgynon® group, Depo-Provera group, Marvelon group, Yasmin® group. The dose administration is listed in table 1.

Table 1. Specification of used drugs.

Trade name	Composition		Manufacturer	Origin	Administration and Dosing schedules
	Progestin	Estrogen			
Microgynon pills	Levonorgestrel (0.15 mg/kg)	Ethinylestradiol (0.03 mg/kg)	Bayer	Germany	Orally for 4 days; one day break for 8 weeks
Depo-Provera injection	Medroxyprogesterone (3.5 mg/rat)		Pfizer	USA	I.M.; once weekly for 8 weeks
Marvelon pills	Desogestrel (0.15 mg/kg)	Ethinylestradiol (0.03 mg/kg)	MSD	USA	Orally for 4 days; one day break for 8 weeks
Yasmin pills	Drospirenone (0.5mg/kg)	Ethinylestradiol (0.03 mg/kg)	Bayer	Germany	Orally for 4 days; one day break for 8 weeks)
MSD= Merck and Co. Inc.					

Finally, the serum sample analysis was carried out for measured parameters using ELISA technique. The results of samples were statistically analyzed and comparison was conducted between Microgynon, Depo-Provera, Marvelon, and Yasmin group compared to the control group.

Results and discussion

Regarding CRP, all studied groups show the same level of CRP (0.16 ng/ml) which is considered a normal level.

Regarding D-dimer levels (ng/ml), non-significant differences exist between all studied groups (levels are approximate to 200ng/ml) except for the Yasmin group at 8-weeks' time point where the level show significant ($p<0.05$) elevation to reach 380.6±19 compared to control or other groups at same time points.

Regarding IL-1B levels (pg/ml), non-significant differences exist between baseline and week-4 timepoints in all studied groups (levels are approximate to 200ng/ml) except for Marvelon which showed significant differences at week-4. However, at week-8 timepoints, all studied groups show significantly higher IL-1B levels compared to baseline except for Depo-Provera which showed non-significant differences with baseline levels.

Figure 1. Hormonal upset in rats treated by commercially available contraceptive medicines; Microgenon (M), Depo-Provera (D), Marvelon (Mv), and Yasmin (Y). Data expressed as mean±SD. *$p<0.05$ Yasmin compared to baseline, #$p<0.05$ Marvelon compared to baseline, and \$$p<0.05$ Microgynon compared to baseline.

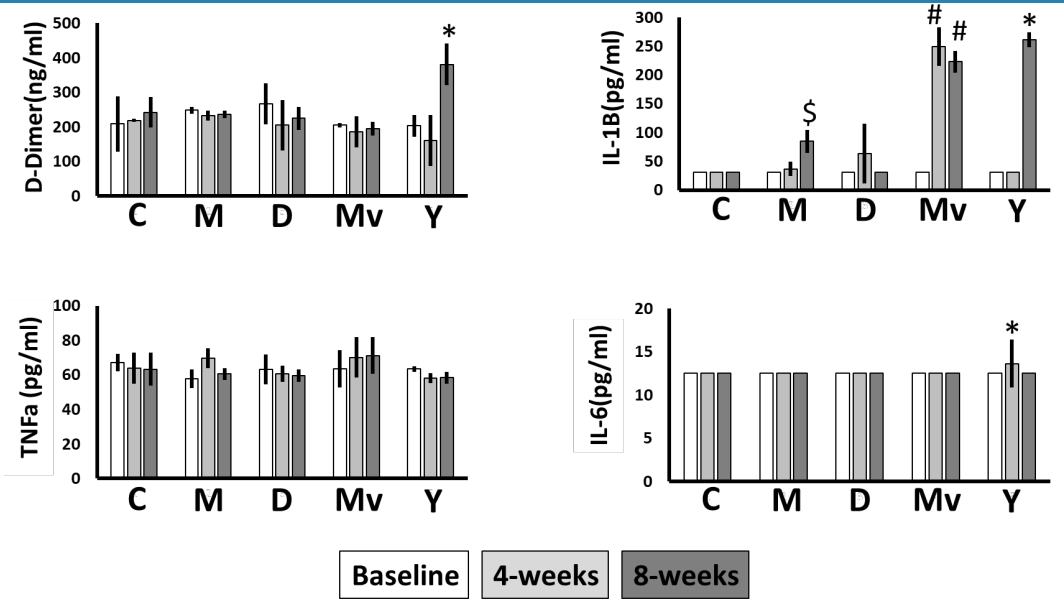
The present study confirmed that hormonal replacement therapy carries a pro-inflammatory risk. The study findings support this statement as confirmed by measured pro-inflammatory markers. However, there have been discrepancies in the outcomes when these commercial products are compared to each other. Compared to 4-weeks, the proinflammatory defect was more obvious at 8-weeks, if any. Yasmin pills have shown the most deleterious defect compared to Microgynon, Marvelon, and Depo-Provera. Yasmin induced significantly higher D-dimer and IL-6. Surprisingly, Yasmin-induced IL-6 elevation was acute since the elevation was obvious in 4-weeks, and the level reduced to baseline after 8 weeks. This effect interestingly might confirm that tolerance to Yasmin IL-6 proinflammatory effects might be produced. Yasmin induced significantly higher pro-inflammatory status, indicated by higher IL-6 at week 4 and elevated IL-1B, D-dimer at week 8. The second deleterious agent was Marvelon; which was associated

Obvious changes were seen after the treatment which continued for 4 weeks[29] and before that, two large "observational studies"[30,31] and two clinical trials [30-32] "significant elevations" in CRP in women who are taking replacement therapy (48-260% higher than in non-users). CRP hepatic formation is chiefly conducted to the promotion of inflammation cytokine Il-6[33]. Elevated levels of CRP are possibly linked with cardiovascular events in healthy subjects[34-38] and also in the subjects with established vascular disease[35,36, 39,40].

HRT was considered that increase in CRP was caused by "systematic inflammation" but it was the cause of direct hepatic passing of oral estrogen[39]. In evidential form, they showed that plasma IL-6 levels were quite similar to the estrogen users and in the non-user women who were CHD free or in the individuals whom CHD eventually developed[41-44].

Reduction of "TNF-α expression by progesterone" in a rat model of brain injury was reported by Jiang et al[45] where conflicted data with the findings of researchers were also observed. At the same time, Roof et al[46] revealed that the "membrane-stabilizing effect" can also reduce oxidative stress by progesterone. Estradiol reduces the "TNF- α expression" in female rats managed with "a combination of progesterone and estradiol"[44,47] and estradiol also protects the CNS against neurotoxic stimuli. But this finding contradicts the finding of our research, that highlighted by the anti-inflammatory role of estradiol[47].

Figure 1



Conclusion

The outcome confirmed that sex hormone medicines has jeopardized the proinflammatory marker, showing variation between the outcome when different brands were used. Yasmin has the utmost deleterious outcome compared to others while Depo-Provera was the safest product.

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