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Original Article

Impact of Oral Contraceptive on Induction of Ischemic Stroke in Female Rats

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ABSTRACT

Background and Aim: There are evidences for role of oral contraceptives on the induction of ischemic stroke in clinical practice, but pathophysiological mechanism underlying these close relationships remains unknown. Furthermore, the impact of oral contraceptive on induction of ischemic stroke in female rats has not been previously studied. This study investigated the induction of ischemic stroke by dose-dependent oral contraceptive in rats. **Methods:** Wistar 30 rats were randomly chosen and divided into three groups: High dose group-1 received daily oral contraceptive (OC), ethinylestradiol (20 mg/kg orally for 16 days) and low dose group-2 (5 mg/kg orally for 16 days) while control group received daily oral saline. The number of neurons per slice in the CA1 hippocampal region and the changes of neurological score as behavioral testing were measured in each group. **Results:** We demonstrated the number of neurons per slice in the CA1 hippocampal region in both high and low dose of oral contraceptive decreased in female rats, in particular, in high group was significantly decreased compared with control group. In addition, a neurological deficit was evident in dose-dependent of oral contraceptive. Further studies are needed to confirm the pathological mechanisms by oral contraceptives but our data provide that oral contraceptive, ethinylestradiol may induce ischemic stroke in dose-dependent.

Key words: Female rats, Ischemic stroke, Oral contraceptive.

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INTRODUCTION

Stroke constitutes a serious socioeconomic and healthcare problem in women because it is the principal cause of incapacity and the first cause of death in this section of the population in several countries. The incidence of cerebral infarction and myocardial infarction is lower in females than in males, but only up to menopause, when, especially after > 65 years of age, the differences disappear. This is attributable to the protective role of the natural estrogens during the fertile part of the woman's life. The estrogens have anti-atherogenic and neuroprotective effects and, before menopause, account for a risk profile that is less atherogenic. The risk factors that have higher importance in young women are migraine and oral contraceptive use as well as etiologies specifically associated with pregnancy, birth and puerperium, or even diseases that present more commonly in women (systemic lupus erythematosus, fibromuscular dysplasia).[1] Oral contraceptive use has been associated with a small but significant increase in ischemic stroke risk in many,[2-4] but not all, studies. This was a particular concern with early OC preparations that contained high doses of estrogen.[5] Newer OC preparations containing less than 50 µg of ethinyl estradiol are associated with a lower risk of stroke than high-dose preparations. A meta-analysis of 16 epidemiologic studies found that a non-smoking, normotensive woman's annual stroke risk would be expected to increase from 4.4 to 8.5 per 100,000 with use of low estrogen oral contraceptives. Thus, treatment of 24,000 women would lead to one additional ischemic stroke each year.[6,7] However, it is unknown whether experimentally oral contraceptive can induce ischemic stroke. We investigated the impact of oral contraceptive on the induction of ischemic stroke in female rats.

MATERIALS AND METHODS

Animals

The nulliparous Wistar female rats (8 weeks old; 200-250g) were provided by Laboratory Animal Centre of the Pyongyang University of Medical Sciences and adapted in a lab environment before experiments for a week. During the experiment, feed and water were available to rats at any time. The temperature was maintained at 20±2°C and the humidity was 55%. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Basic Medicine, Pyongyang University of Medical Sciences.

Monitor Stages of the Estrous Cycle

The vaginal smears from rats daily between 9:00 and 10:00 AM were collected and cell types were

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microscopically identified. Only rats showing at least three consecutive normal (4 day) estrous cycles were used for experiments.

Administration of Oral Contraceptive

30 rats were randomly chosen and divided into three groups: High dose group-1 received daily oral contraceptive (OC), ethinylestradiol (20 mg/kg orally for 16 days) and Low dose group-2 (5 mg/kg orally for 16 days) while Control group received daily oral saline. The administration of OC and saline was begun in proestrus (higher levels of circulating ovarian hormones).

Histopathology

Rats were anesthetized with isoflurane and perfused with formalin: acetic acid and methanol (FAM) as described. The head was removed and immersed in FAM at 4°C for 1 day. The brains were then removed from the skull, and coronal brain blocks were embedded in paraffin; coronal sections were stained with hematoxylin and eosin. Hematoxylin and eosin stained sections were visualized at 40X magnification under a Nikon microscope equipped with a Sony CCD camera coupled to an MCID image analyzer. For each animal, normal neurons were counted in the CA1 region of each hippocampus by an investigator blinded to the experimental conditions. Coronal brain sections were made at the level of 3.8 mm from bregma. For each section, 18 fields per sections were obtained, and three slides per rat were counted. The data are presented as the mean count from three slides.

Behavioral Testing

Each animal underwent neurological testing using a 33-point score on 2 occasions before experiment to ascertain a "normal" score. This 33-point neurological test was developed from a series of tests described by Hunter and colleagues [8] and consisted of 11 separate tests which assess limb function and mobility. On the 16th day of experiment, animals were tested to monitor any motor impairment as a result of the procedure.

Statistical Analysis of Data

Results were expressed as the mean and SEM. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 16.0 and the differences between the means assessed using Dunnet's multiple range test. P value of <0.05 was taken as the level of statistical significance.

RESULTS

Table 1 showed that the number of neurons per slice in the CA1 hippocampal region in each group. As shown in Table 1, the number of neurons per slice in High group was significantly decreased compared with control group (P<0.01).

A neurological deficit was evident in the both high and low group on the 16th day of experiment but the deficits were not significantly different between High and Low group (Figure 1).

A significant decrease of neurological score was seen in both high and low group compared to control group (respectively P<0.01 and P<0.05).

Table 1: The number of neurons per slice in the CA1 hippocampal region.

	The number of neurons per slice
Control	1268±31
High	785.1 ± 39**
Low	1034±56△

Each value represents the mean ± SEM of 10 rats per group. **P<0.01 as compared with control group. △P<0.01 as compared with high group

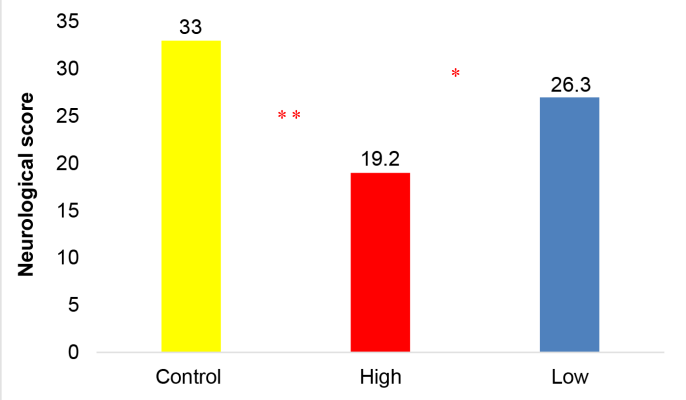

Figure 1: The changes of neurological score.
Each value represents the mean ± SEM of 10 rats per group. **P<0.01 and *P<0.05 as compared with control group.

DISCUSSION

Drugs can sometimes cause ischemic or hemorrhagic stroke. Some drugs, e.g. the oral contraceptives, can increase the tendency to form clots; others, such as cocaine, may cause a cerebral vasculitis. However, oral contraceptives are widely used, but there are limited data on the cerebrovascular risks associated with these medications.

Since 1962, many studies have been devoted to the relationship between oral contraceptives and stroke. They are all case-control or cohort epidemiological studies and thus contain the difficulties and biases that are inherent in these types of studies. Until now there is no evidence to induce ischemic stroke by oral contraceptive use experimentally. Thus, we sought to determine whether use of high and low-dose oral contraceptive, ethinylestradiol influences the risk for stroke. In this study, high dose group-1 received daily oral contraceptive (OC), ethinylestradiol (20 mg/kg orally for 16 days) and low dose group-2 (5 mg/kg orally for 16 days) while control group received daily oral saline. The administration of OC and saline was begun in proestrus (higher levels of circulating ovarian hormones). As a result of our study, the number of neurons per slice in the CA1 hippocampal region in High group was significantly decreased compared with control group (P<0.01). On the order hand, the number of neurons in Low group was significantly increased compared with high group (P<0.05). A neurological deficit was evident in both high and low group on the 16th day of experiment but the deficits were not significantly different between high and low group. Oral contraceptive use may be associated with an increase in the risk of ischemic stroke.

CONCLUSION

Our data are consistent with a substantial increase in the risk of ischemic stroke associated with oral contraceptive use.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

OC: Oral Contraceptive.

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