Total DNA methylation in the brain in response to decitabine treatment in female rats

Original research article/Review

Balagova L.,¹✉ Buzgoova K.,^{1,2} Karailiev P.,¹ Jezova, D.¹

¹Slovak Academy of Sciences, Biomedical Research Center, Institute of Experimental Endocrinology;
²Comenius University in Bratislava, Faculty of Pharmacy, Department of Pharmacology and Toxicology, Bratislava, Slovakia

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Abstract Hypomethylating agent decitabine is being used in the treatment of certain types of leukaemia in combination with other anticancer drugs. Aberrant DNA methylation has been suggested to occur in pathological states including depression. Scarce data in male rats suggest antidepressant effects of decitabine. The main aim of our studies is to test the hypothesis that the inhibition of DNA methylation results in antidepressant effects in female rats. Before doing so, we decided to verify the effects of decitabine on DNA methylation in females. The findings demonstrate that the treatment with decitabine at the dose shown previously to inhibit DNA methylation in males, had no effect on total DNA methylation in two brain regions, namely the hippocampus and frontal cortex of female rats. In conclusion, the present study allows us to suggest that the effect of decitabine on DNA methylation in the brain is sex dependent.

Keywords 5-aza-2'-deoxycytidine – epigenetics – gender

INTRODUCTION

Decitabine is an analogue of the pyrimidine base cytosine and it has a chemotherapeutic potential given by the ability to inhibit DNA methyltransferases (Zhou et al., 2018). DNA methylation is an epigenetic mechanism catalysed by DNA methyltransferases, which occurs by covalent attachment of methyl groups to the 5-position of cytosine residues of DNA, especially at the cytosine-guanine dinucleotides. Decitabine incorporates into nascent DNA strands during DNA synthesis, while irreversibly binding DNA-methyltransferases and inactivating them (Malik and Cashen, 2014). Increased DNA methylation is generally associated with the attenuation of the extent of gene expression. Recent studies have shown that the inhibition of DNA methylation may induce antidepressant-like effects in male rats. Sales and colleagues (2011) treated healthy male rats kept under standard conditions with different doses of decitabine and they reported antidepressant effects of repeated injections at the dose of 0.4 mg/kg.

The main aim of our studies is to test the hypothesis that the inhibition of DNA methylation results in antidepressant

effects in female rats. Before doing so, we decided to verify the effects of decitabine on DNA methylation in females. We used the same dose described to be effective in male rats (Sales et al., 2011), but unlike these authors, we were working with rats exposed to low tryptophan diet known to induce depression-like behaviour.

MATERIAL AND METHODS

Female adult Sprague-Dawley rats were fed normal or low tryptophan diet for 10 days. The low tryptophan and control diets contained 0.04% and 0.2% of tryptophan, respectively (Franklin et al., 2015). Animals exposed to low tryptophan diet were treated either with decitabine (5-aza-2'-deoxycytidine) at the dose of 0.4 mg/kg or vehicle on day 8 and 9 of tryptophan depletion. Decitabine was injected intraperitoneally three times with the time interval of 5 h between the 1st and 2nd and 18 h between the 2nd and 3rd injection. On day 10 of tryptophan depletion, the animals were quickly decapitated and their organs were collected. All the procedures were

* E-mail: lucia.balagova@savba.sk

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After the isolation of DNA from the hippocampus and the frontal cortex, the DNA methylation was quantified using the DNA Methylation enzyme-linked immunosorbent assay (EIA) kit (Epigentek, USA), according to the manufacturer's instructions. The results were analysed by one-way ANOVA.

RESULTS

The treatment of female rats with decitabine had no effect on the total DNA methylation in the hippocampus. Similarly, there were no changes in DNA methylation in the frontal cortex of decitabine treated rats. The analysis by one-way ANOVA did not show significant effect of treatment either in the hippocampus ($F(2, 17) = 0.28695$, $p > 0.05$) or in the frontal cortex ($F(2, 17) = 0.14242$, $p > 0.05$). Ingestion of low tryptophan diet was not associated with the changes in total DNA methylation (Fig. 1).

DISCUSSION

The present study was designed to verify the efficacy of decitabine to inhibit DNA methylation in female rats before investigating the behavioural consequences of decreased DNA methylation. The findings demonstrate that the treatment with decitabine had no effect on total DNA methylation in two brain regions. Obviously, present experimental design is not appropriate for further investigation of the impact of reduced DNA methylation on depression-like behaviour.

Hypomethylating agent decitabine is being used in the treatment of certain types of leukaemia in different doses and in combination with other anticancer drugs (Tiong and Wei, 2019). Aberrant DNA methylation has been suggested to occur in numerous disease states, including cancer, autoimmune diseases and neurodegenerative disorders (Kader et al., 2018). Depressive disorder in humans is also associated with changes in epigenetic mechanisms including DNA methylation. In particular, increased DNA methylation levels on selected genes, such as brain-derived neurotrophic factor (BDNF) gene, have been reported in patients with depression (Chen et al., 2017).

It may be argued that no dose response relationships were evaluated in the present experiments. The reason is that this was done previously by others (Sales et al., 2011), who investigated DNA methylation and behavioural parameters in male rats treated with 6 different doses of decitabine in the range of 0.1–0.8 mg/kg. According to their results, an optimal dose was the one used in the present study, namely 0.4 mg/kg. This dose of decitabine, described to induce the inhibition of DNA methylation in males (Sales et al., 2011) was ineffective in the present study in females. Surprisingly, very little

A

DNA methylation in the hippocampus

B

DNA methylation in the frontal cortex

Figure 1. Total DNA methylation (A) in the hippocampus and (B) in the frontal cortex in female rats fed low tryptophan diet and treated with decitabine at the dose of 0.4 mg/kg or vehicle

information is available on potential sex differences in the action of decitabine or in total DNA methylation. There is a study in healthy humans showing reduced level of global methylation in women (Zhang et al., 2011). DNA methylation of an exon of the gene coding for BDNF in the prefrontal cortex was found to be higher in adult female compared to male rats maltreated during the development (Balze et al., 2013). Perinatal hypoxia-induced DNA methylation of certain genes in the hearts of adult offspring was lower in female compared to male rats. In vivo effects of decitabine treatment were not evaluated (Patterson et al., 2010).

Even though no information that allowed prediction of sex differences in the pharmacokinetics or pharmacodynamics of decitabine was available, the present study suggests that the effect of decitabine on DNA methylation in the brain is sex dependent.

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