Letter to the Editor

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Expression levels of antioxidant genes in human SH-SY5Y cells long term exposed to methadone

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Dear Editor

Introduction

Methadone maintenance treatment (MMT) has been found to be an effective harm-reduction approach to treating drug abusers. It has been reported that methadone induces NADH/NADPH-mediated reactive oxygen species (ROS) synthesis [1]. A few studies reported that cancer risk increases in patients on MMT [2, 3]. The human neuroblastoma SH-SY5Y cells endogenously expressing the PDYN and OPRM1 genes [3], and also SH-SY5Y cells expressed the CYP1A, CYP2D6 and CYP3A enzymes which are necessary for methadone metabolism [4]. Therefore, this cell line was chosen by researchers as a model cell types for experiments investigation the effects of methadone and morphine.

Very recently we reported that the mRNA levels of several antioxidant genes significantly down-regulated in the human neuroblastoma SH-SY5Y cells exposed to different concentrations of methadone [5]. The expression levels of seven genes (GSTP1, CAT, SOD1, SOD2, NQO1, NQO2, and GSTM2) decreased at 72 h. We have shown that the number of down-regulated genes increased as a function of exposure time [5]. In previous report the SH-SY5Y cells were exposed to methadone for 1–72 h. There is no data on the effect of long term exposure of methadone on the mRNA levels of antioxidant genes. Therefore, the present study was carried out.

Materials and methods

Details in experimental design were reported in our previous report [5]. Human SH-SY5Y cell line was obtained from National Cell Bank of Iran (the Pasteur Institute of Iran). The cells were exposed to methadone at final concentrations 2.5 and 10 μM of methadone. The conditions of cell culture were the same as reported in our previous report [5]. Treated cells were incubated at 37°C for 18 days. It should be noted that cells were passage after every 3 days and methadone was immediately added. The control cells were maintained in methadone-free medium. The control cells were maintained in methadone-free medium. The SH-SY5Y cells also were treated with co-treatment of 10 µM methadone and 1 mM N-acetyl-cysteine (NAC; C₉H₁₇-NO₃S) in the media for 18 days. This study was approved by the Shiraz University Ethics Committee.

Total RNA was extracted by RNX-Plus kit (CinnaGene, Iran) following the manufacturer’s instructions. All samples had high quality of RNA (OD₂₆₀/₂₈₀ = 1.8–2.1). RNA samples were used for cDNA synthesis using the PrimeScript RT regent Kit (Takara, Japan). Quantitative real-time PCR conditions and specific primers were described previously [5]. PCR reactions were done in 20 μL final volume containing 25 ng cDNA. The PCR set at 95°C for 30 s, followed by 40 cycles of 95°C for 10 s, and 60°C for 45 s. Relative differences in gene expression between groups were expressed using cycle time (Ct) values. These Ct values were first normalized with that of the “TATA box-binding protein” (TBP) in the same sample and then expressed as fold changes with control (set to 1.0). Obtained results of gene expression were analyzed using 2−ΔΔct method.
Data were presented as mean (SE) of three independent experiments. The differences of the mRNA levels between the study groups were investigated using Mann Whitney-U test.

Results

Table 1 showed the mRNA alterations in cells after exposure to methadone. The mRNA levels of \( \text{CAT} \), \( \text{SOD1} \), \( \text{GSTO1} \), and \( \text{GSTP1} \) were significantly decreased in both 2.5 and 10 μM methadone treated cells compared with untreated control cells. The mRNA levels of \( \text{SOD2} \), \( \text{NQO1} \), \( \text{NQO2} \), and \( \text{GSTM2} \) showed no alteration at 2.5 μM while they were decreased at 10 μM of methadone. Only the level of \( \text{GSTM3} \) significantly increased in treated cells. Therefore the cells exposed for 18 days with methadone, showed decreased in 8 mRNA (out of 9).

The mRNA levels of the investigated genes in cells exposed to NAC were not significantly different compared to the mRNA levels expressed by the same genes in control cells. The mRNA levels revealed differences between cells exposed to methadone and cells co-treatment with methadone plus N-acetyl-cysteine. In cases that methadone, increased the level of mRNAs, methadone plus N-acetyl-cysteine, result in decreased mRNA levels and vice versa (data not shown).

Discussion

Very recently it has been reported that after 72 h treatment with methadone results seven down-regulated genes (out of nine examined genes) [5]. The present finding confirms our suggestion. The present data suggested that methadone can inhibits the expression of a majority of antioxidant genes in long term exposure. Previous studies indicating that methadone increases the production of ROS [1] and the present study confirms that methadone via production of ROS regulate the antioxidant mRNA levels. It is speculated that among patients on MMT the incidence of oxidative stress related diseases may be higher than general population. Epidemiologic studies investigating the incidence of oxidative stress related disease in MMT patients seems to be useful. On the other hand, other molecular studies should be done in order to show the molecular bases for the above mentioned down-regulation of the antioxidant genes, after the SH-SH5Y cells treated with methadone.

Conclusion

This finding suggested that methadone may act through inducing ROS production and in long-term exposure to methadone is associated with down-regulation of eight genes (out of nine genes).

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Conflict of interest statement: No competing interests are declared by any of the authors.

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