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Heroin and tapentadol promote accelerated senescence of SH-SY5Y human neuroblastoma cells at subtoxic concentrations

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Abstract

Background: Opioids are widely used in clinical practice due to their analgesic efficacy. However, their potential for abuse and deleterious effects represents a major global health concern [1]. Importantly, there is growing evidence suggesting that long-term opioid use may promote inflammatory responses and oxidative stress, altering molecular pathways involved in cell senescence, a feature often associated with accelerated ageing and cellular functional decline [2,3]. **Objective:** This work aimed to elucidate the impact of both recreationally and therapeutically used opioids (i.e., heroin and tapentadol, respectively) on neuronal cell ageing. **Methods:** SH-SY5Y human neuroblastoma cells were exposed to 1 nM and 1 µM of either heroin or tapentadol for 72h, and cell senescence was evaluated by measuring β-galactosidase activity (a widely used marker of senescent cells) using a commercially available kit (Abcam, USA). At the same time point, reactive oxygen species (ROS) levels were assessed using dichlorofluorescein (DCFH-DA). Notably, we have previously shown these opioids' concentrations to be below toxicity thresholds. 25 nM doxorubicin was used as a positive control. In parallel, SH-SY5Y cells were chronically exposed, every 2-3 days, for a total of 30 days (between passages 21 and 25), to the same opioid concentrations. Genomic DNA was collected every other passage, and relative telomere length was measured through quantitative real-time PCR. **Results:** We observed increased β-galactosidase activity in opioid-exposed cells compared with untreated controls, in a concentration-dependent manner. Specifically, heroin increased this enzyme's activity by 1.25- and 1.33-fold (for 1 nM and 1 µM, respectively), while tapentadol increased it by 1.19- and 1.29-fold at 1 nM and 1 µM, respectively. None of the opioids, at the tested concentrations, altered ROS levels after 72h exposure. From passages 21 to 25, telomere length decreased by 6% in control cells. However, opioid treatment exacerbated progressive telomere shortening, with reductions of 15.0 and 28.5% for tapentadol, and 10.0 and 27.1% for heroin at 1 nM and 1 µM, respectively. **Conclusions:** Overall, our preliminary data indicate that opioid use promotes early signs of cellular senescence and accelerated ageing (i.e., telomere shortening) in SH-SY5Y human neuroblastoma cells. Nonetheless, additional assays are ongoing to further characterize the effects of these opioids' chronic exposure on neuronal cells.

Keywords: opioids; cell senescence; telomere shortening

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References

1. Vearrier, D. et al. Clinical Pharmacology, Toxicity, and Abuse Potential of Opioids. *J Clin Pharmacol* **2021**, *61(2)*, S70-S88. https://doi.org/10.1002/jcph.1923
2. Henriques, A.R.T. et al. The impact of opioids on the hallmarks of ageing. *Mech Ageing Dev* **2024**, *222*, 111994. https://doi.org/10.1016/j.mad.2024.111994
3. Zhu, X. et al. Inflammation, epigenetics, and metabolism converge to cell senescence and ageing: the regulation and intervention. *Signal Transduct Target Ther* **2021**, *6(1)*, 245. https://doi.org/10.1038/s41392-021-00646-9