Evaluation of toxic impact of the synthetic cannabinoids JWH-018 and its N-(3-hydroxypentyl) metabolite on human cell lines.

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INTRODUCTION

The emergence and abuse of synthetic cannabinoids has been rising during the last years, mostly among younger populations, as an alternative to cannabis[1]. Given their relatively recent appearance, the pharmacological and toxicological profiles of these new psychoactive substances are not well understood. Current studies suggest that they have stronger psychoactive effects compared to natural cannabinoids and their metabolites retain affinity towards CB1 receptors in the CNS[2].

In this study, the cell toxicity of JWH-018, one of the first synthetic cannabinoids appearing on the market[3], and its N-(3-hydroxypentyl) metabolite was assessed using human cell lines HEK-293T and SH-SY5Y by the MTT assay. The results were compared with those obtained with the LDH assay and the Scepter 2.0 cell counter. This device is an automated handheld device that uses the Coulter principle of impedance to detect particles or cells. This implementation of the methodology enables the accurate discrimination of cell populations according to cell size and volume[4].

RESULTS

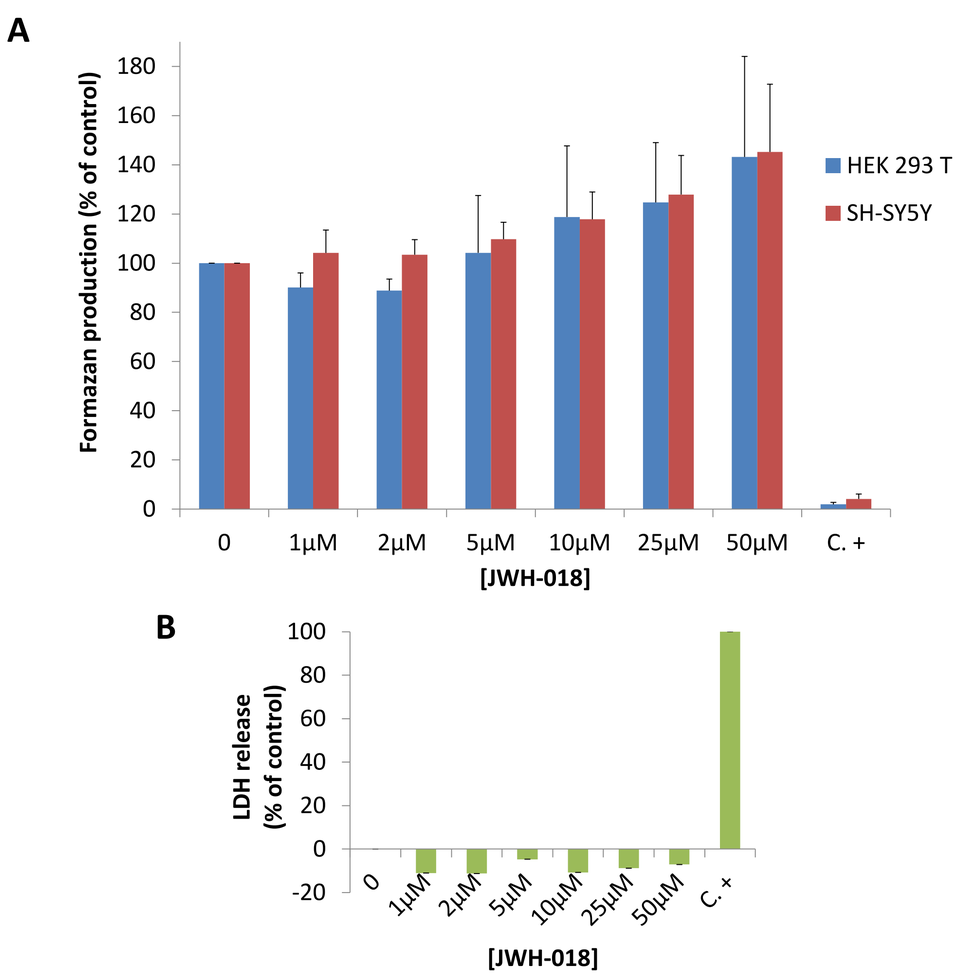

Fig. 1 - **Cytotoxic Evaluation of JWH-018 in HEK 293 T and SH-SY5Y cells.** Both sets of cells were treated with 0, 1, 2, 5, 10, 25 and 50 µM of JWH-018 for 24 h. (A) Formazan formation as determined by the MTT assay. (B) Results for the LDH assay. Data are expressed as the mean ± 1S (n = 4 or 5).

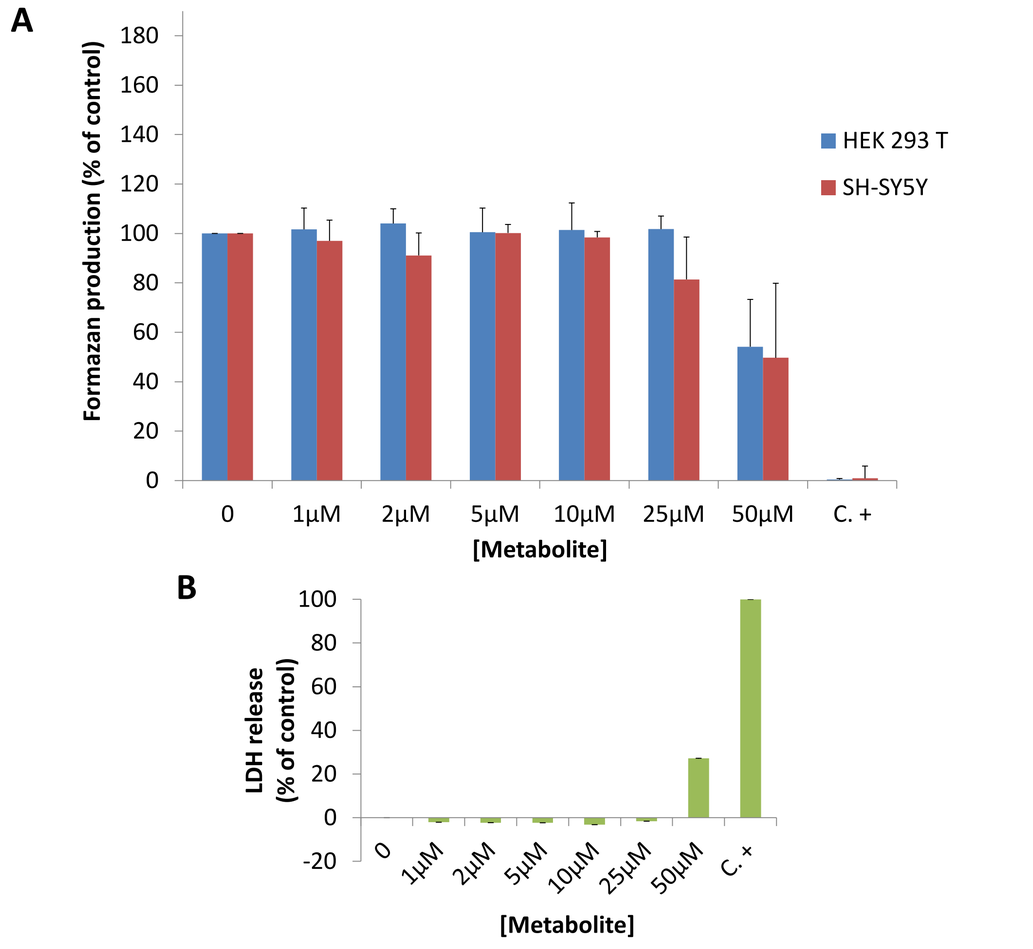

Fig. 2 - **Cytotoxic Evaluation of JWH-018 N-(3-hydroxypentyl) metabolite in HEK 293 T and SH-SY5Y cells.** Both sets of cells were treated with 0, 1, 2, 5, 10, 25 and 50 µM of JWH-018 N-(3-hydroxypentyl) metabolite for 24 h. (A) Formazan formation as determined by the MTT assay. (B) Results for the LDH assay. Data are expressed as the mean ± 1S (n = 4 or 5).

	Drug exposure concentration	
	0 µM	50 µM
	Mean cell diameter (µm)	
(A) Cells exposed to JWH-018		
	12,54 ± 0,18	12,73 ± 0,31
(B) Cells exposed to JWH-018 N-3OH-Pentyl metabolite		
	12,41 ± 0,21	10,8 ± 0,2

Fig. 3 – **Mean diameter of SH-SY5Y cells exposed to (A) JWH-018 and (B) JWH-018 N-(3-hydroxypentyl) metabolite.** The SH-SY5Y cells treated with 0 and 50 µM of each drug for 24 h were analyse by the Scepter 2.0 cell counter. The diameter measurements obtained were acceptably reproducible, displaying the coefficients of variation less than 5%.

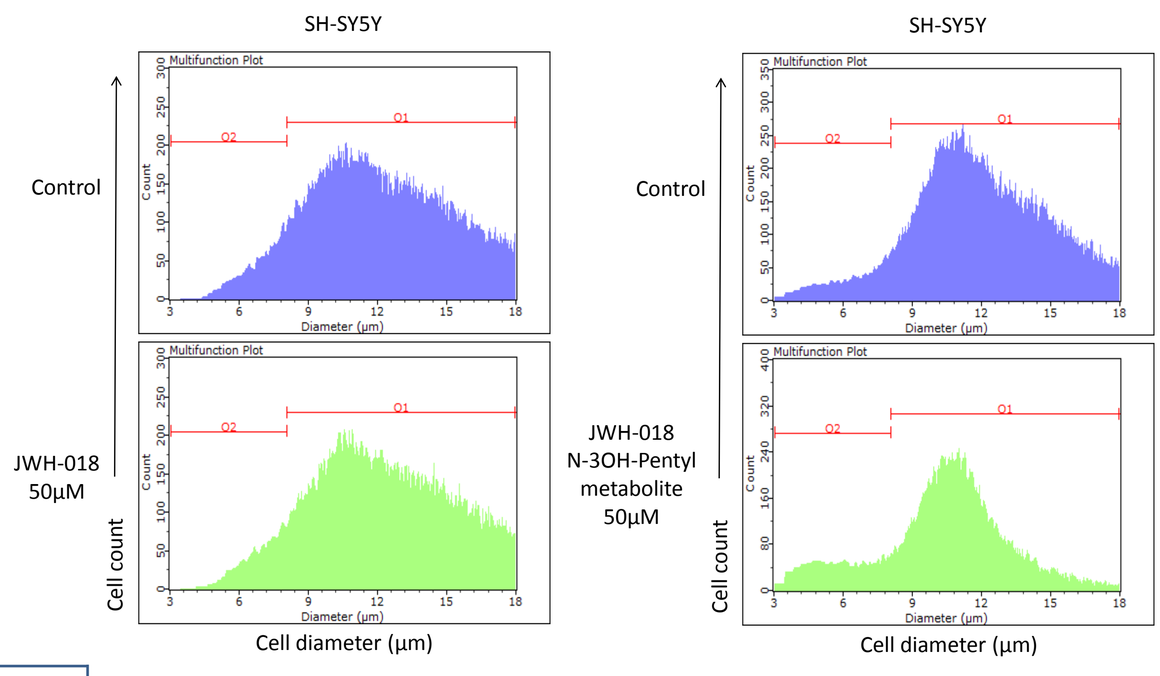

Fig. 4 – **Comparison of SH-SY5Y cell size distributions obtained with the Scepter.** Diameter distributions of SH-SY5Y cells treated with 0 and 50 µM of (A) JWH-018 and (B) JWH-018 N-(3-hydroxypentyl) metabolite for 24 h as measured by the Scepter 2.0 cell counter.

CONCLUSIONS

The JWH-018 MTT results points to no toxicity impact of this substance on human cells until a concentration of 50µM is reached. In fact, JWH-018 seems to increase cell growth up to concentrations of 50µM, the maximum concentration used in our assays. These results agree generally with literature reports. On the other hand, the JWH-018 N-(3-hydroxypentyl) metabolite shows a toxicological impact on cells above 25µM. The results of both MTT and LDH assays demonstrate the same trend. In addition, we have performed a cell volume analysis, using the Scepter 2.0 cell counter, with the aim of study JWH induced apoptosis. The preliminary results inidicate that the JWH-018 metabolite induces apoptosis while the parent JWH-018 does not.

REFERENCES

1. Vandrey, R., *et al.* (2012) *Drug and alcohol dependence* **120**, 238–41
2. Aung, M., *et al.* (2000) *Drug and alcohol dependence* **60**, 133-40
3. European Monitoring Centre for Drugs and Drug Addiction (2009) Thematic paper — Understanding the 'Spice' Phenomenon, Luxembourg: Office for Official Publications of the European Communities; doi: 10.2810/27063
4. Tahara, M. *et al.* (2013) *Biochemical and biophysical research communications* **434** 753-9