The synthetic cannabinoid XLR-11 and the impaired control of mitochondrial function by the endocannabinoid system as its underlying mechanism of nephrotoxicity

Synthetic cannabinoids (SCs) comprise a diverse group of new psychoactive substances (NPS) designed to activate at least one of the main cannabinoid receptors (i.e. CB1R, CB2R). Variations of SC have rapidly surfaced over the past few years, being marketed and used for recreational purposes. In fact, according to the latest European Monitoring Centre for Drug and Drug Addiction's Drug Report, 179 new SCs have been detected since 2008, currently accounting for around 45 % of all NPS seizures in Europe.

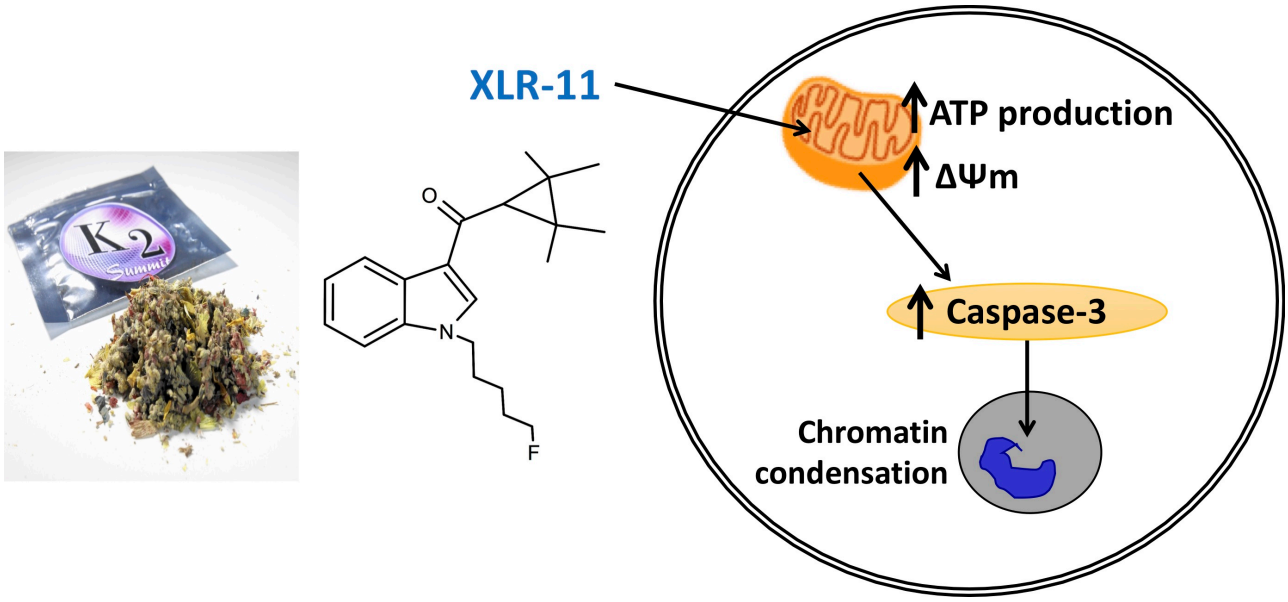

Fig. 1.

SCs are structurally similar to tetrahydrocannabinol (THC, the main psychoactive molecule of cannabis), but their higher affinity towards cannabinoid receptors results in stronger psychoactive effects, as well as aggravated adverse consequences (e.g. anxiety, hallucinations, seizures, hypertension, tachycardia). Most important, several intoxications and deaths have been reported following SC use, turning its widespread recreational use into a major public health concern. Specifically, a direct link has been established between SC use and the onset of acute kidney injury (AKI), in particular for XLR-11, an SC commonly detected in the toxicological analysis of patients with SC-associated AKI. However, the pathophysiology of AKI among SC users remains unknown.

XLR-11 is usually found in herbal smoking blends and, although it has already been banned in a few countries worldwide, it still escapes legal control under the United Nations Drug Control Conventions.

This work represents the first *in vitro* assessment of SC nephrotoxicity, as a lead approach to identify their cellular targets during such process. Results clearly supported that XLR-11, at biologically relevant concentrations (i.e. in the nanomolar range), targets the mitochondrial function in a cell model of human proximal tubule (HK-2) cells. We specifically evidenced that:

> XLR-11 induced a temporary hyperpolarization of mitochondrial membrane and increased ATP production, which was found to be occur with Bax translocation from cytosol into mitochondria. These phenomena further triggered energy-dependent apoptotic cell death pathways, as indicated by increased caspase-3 activity and chromatin condensation;
> These processes were shown to depend on the activation of cannabinoid receptors (CBRs). These results were evidenced by using specific antagonists of CBRs, as well as by exposing HEK293T cells, which do not express CBRs, to XLR-11. ATP production proved to be an exception, as it followed a CBR-independent pathway;
> Use of specific inhibitors of endocannabinoids biosynthesis further evidenced the involvement of the endocannabinoid system in the regulation of these processes' homeostasis. Interestingly, binding of XLR-11 to CBRs compromised that regulation.

Overall, our findings showed that XLR-11-induced *in vitro* nephrotoxicity was primarily driven by deregulation of mitochondrial function, with subsequent triggering of apoptotic cell death pathways. We further identified the involvement of the endocannabinoid system in the regulation of this process, as our data evidenced that XLR-11 binding to CBRs impaired endocannabinoid-mediated regulation of mitochondrial function homeostasis. Nevertheless, the exact mechanisms involved require further clarification.

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Publication

The synthetic cannabinoid XLR-11 induces in vitro nephrotoxicity by impairment of endocannabinoid-mediated regulation of mitochondrial function homeostasis and triggering of apoptosis.
Silva JP, Carmo H, Carvalho F
Toxicol Lett. 2018 May 1