RESEARCH PROJECT

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Title: Cytotoxic and genotoxic potential of Nitazeni and related metabolites

Introduction

The production and use of new psychoactive substances (NPS) is continuously increasing worldwide and in Europe it has become a primary concern of governance and citizens. This is not surprising, given the fact that psychoactive substances cause acute and chronic diseases, with a significant cost for society and are also responsible for a high number of deaths.

In particular, in recent years the number of molecules belonging to the class of new synthetic opioids (NSO) in the illicit drug market has increased. These compounds, initially studied as analgesic drugs which were then abandoned in most cases and are now mostly synthesized in clandestine laboratories and sold as alternatives to heroin. Opioid abuse continues growth. In 2019, in the USA, the number of opioid drug abuse reached 9.3 million cases. Furthermore, the recent trend of illicit use and abuse of NSO, produced in bulk and sold at low prices, has contributed to the current epidemic of opioid overdose deaths in many countries.

Among NSOs, the group of 2-benzylbenzimidazoles, called "Nitazenes", has been linked to many cases of intoxication and mortality in Europe, Canada and the United States. They are analogues of benzimidazole derivatives, invented in 1950 in the laboratories of the Swiss pharmaceutical giant CIBA, in an attempt to synthesize safer analgesics. While several benzimidazole derivatives were later patented, the 2-benzilbenzimidazole analgesics (isotonitazene; etonitazene; metonitazene, clonitazene etc.) have never been clinically approved.

The recent appearance in Europe of isotonitazene in the NSO market and, subsequently, of other structurally related 2-benzilbenzimidazoles supports the need to provide new research tools and implement toxicological data to understand the risks related to the diffusion and use of these powerful analgesics on a national and international scale. Furthermore, their involvement in serious adverse events may go undetected because these substances are not routinely screened in European toxicology laboratories. Among other things, the detection of Nitazeni in biological samples is difficult and their legal status is often an unclear gray area, which represents a real threat to public health.

Alongside the significant emerging problem, represented by the adverse effects and the appearance of absolutely atypical and difficult to manage acute toxicity pictures, an aspect of great importance toxicological, but also social, is represented by the possible genotoxic, mutagenic and carcinogenic

effect. Of great importance is the impact that mutations can have on human health. In fact, the key role that mutational events play in the development of many pathological processes, such as hereditary anomalies and chronic- and neuro-degenerative pathologies, such as cancer and Alzheimer's, has been recognized.

In recent years we have tested numerous molecules belonging to different classes of NPS and many of these have proven to be genotoxic, which supports the importance of evaluating and investigating this aspect also for nitazenes.

The **objective** of this project will therefore be to evaluate the cytotoxic and genotoxic potential in vitro, using an innovative flow cytometry protocol, validated in our laboratory and published, of some molecules belonging to the 2-benzilbenzimidazole class (histonitazene, etonitazene, metonitazene and clonitazene), recently identified and selected by the Department Italian for Anti-Drug Policies (DPA) and by other European National Alert Centers.

The study will also include the evaluation of the genotoxicity of the metabolites of the molecules under study, produced in vitro via enzymatic systems.

Finally, the research will focus on identifying the possible mechanism underlying the observed effects, possibly demonstrated by analyzing the potential involvement of stress oxidative and inflammation.

References

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TRAINING PROGRAM

The project aims to enable the researcher to acquire toxicological skills specifications, to be acquired at the laboratory of the Tutor.

In detail, the training plan is aimed at:

1. Training regarding knowledge of the issues relating to NPS and NSO and of the importance of the toxicological evaluation of a xenobiotic, in particular in terms of genotoxicity.

2. Acquisition of the skills necessary for maintaining cells in suspension and their main applications in in vitro toxicological assays, with particular attention to the TK6 cells.

3. Training of the use of the Guava easyCyte 5HT flow cytometer equipped of a blue laser operating at 488 nM (present in the proposing tutor's laboratory) and competent in this regard to flow cytometric methods of analysis of the main cellular end-points, such as viability, cell proliferation and apoptosis.

4. Acquisition of the skills necessary for the evaluation of these parameters also using spectrophotometric techniques and optical microscopy.

5. Training of using advanced methodologies and instruments to evaluate the genotoxicity of a xenobiotic and its metabolites, by flow cytometry, as an innovative alternative to optical microscopy.

6. The fellow will also acquire the skills necessary for the evaluation of oxidative stress and of the main biomarkers of inflammation, through the use of different methodological approaches and instrumental such as flow cytometry, immunofluorescence microscopy and spectrophotometry.