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Short communication

Estimation of the relationship between diazepam use and risk of violent death using *post-mortem* data

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Introduction

Quantitative determination of toxic, potentially toxic drugs or toxins in different body fluids and tissues in *post-mortem* toxicology plays a major role in the interpretation process that contributes towards the determination of the cause of death (Lefrancois et al., 2021). Modern analytical techniquesapplied in forensic toxicology such as gas and liquid chromatography, mass spectrometry or infrared spectroscopy give large amount of complex and multidimensional data (Bovens et al., 2019). In order to extract relevant information, these data can be analyzed using univariate statistical tests or chemometric algorithms for multivariate data analysis. These algorithms are used for exploratory data analysis in order to identify trends/patterns in the data set or to model these trends for classification and prediction purposes (Sauzier et al., 2021). Herein, chemometric data analysis including orthogonal projections to latent structures discriminant analysis (OPLS-DA) was used to investigate the differences in a forensic data set containing concentration of diazepam and its metabolites in post-mortem blood samples from cases of natural and cases of violent death.Further, diazepam concentrationwas used to estimate the possible association between their use and the risk of violent death.

Materials and methods

Data from 293 cases autopsied at the Institute of forensic medicine, criminology and medical deontology, during the period 2019-2020 were included in the study.Based on the cause of death they were categorized in two groups, a group of natural (used as control) and a group of violent death. The latter was further dividedin group of suicides, accidents and homicides. Age and concentration of diazepam and its metabolites: nordiazepam, oxazepam and temazepam were used as variables for the chemometric data analysis.

Benzodiazepines in the *post-mortem*blood samples were extracted onBond Elut C18 solid-phase extraction cartridge. After derivatization with MTBSTFA (N-Methyl-N-tert-butyldimethylsilyltrifluoroacetamide), the GC/MS analysis was performed on Agilent GC 6890N systemusing capillary columnDB-5 MS, 30 m x 0.25 mm x 0.25 μm and helium as carrier gas.

Orthogonal projections to latent structures discriminant analysis (OPLS-DA) was applied using SIMCA 14.1 software (Umetrics, Umea, Sweden). Prior OPLS-DA, data were scaled to unit variance.

In order to study the effect of total diazepam concentration (including its metabolites) on the risk of violent death, the concentration values were categorized in four levels: 10-100 ng/mL, 100-300 ng/mL, 300-1000 ng/mL and above 1000 ng/mL. Then the relative odds

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ratio (OR) and relative risk (RR) were calculated. OR and RR greater than 1 were considered statistically significant.

Results and discussion

Two hundred and ninety three post-mortem blood samples were analyzed during the two year period: 76 natural death cases (25.94%) and 217 violent death cases (74.06%). The group of violent death included 124 accidents, 77 suicidesand only 16 homicides (excluded from further analysesdue to the low number of cases).Benzodiazepines were mostly detected in thesuicide cases (45%) while in more than 60% of natural death cases toxicological findings for benzodiazepines were negative. Diazepam was most often detected drug of the benzodiazepine group (72 cases), while bromazepam and alprazolam were detected in 7 and 4 cases, respectively.

The OPLS-DA model for comparison/classification of groups of natural death and accidents had the following cross-validation parameters: R^2X (cum) = 0.659; R^2Y (cum) = 0.512; Q^2 (cum) = 0.401 which indicates a good model. The analysis of the VIP plot of the model revealed that the mostimportant variable for class separation was age followed by presence/concentration of temazepam.

The inspection of the VIP plot of the OPLS-DA model for comparison between groups of natural death and suicides indicated that in this case the mostimportant variable for class separation wastemazepam followed by diazepam.Concentrations of temazepam in all cases were within therapeutic ranges (Schulz et al. 2012) which indicate that the presence of this metabolite can be associated with a risk of violent death. However, there was no significant association of temazepam concentration and risk of violent death.

Statistically significant RR for the association between total diazepam concentration (including metabolites) and the risk of committing suicide was found for diazepam concentration starting at 300 ng/mL. Additionally, concentration-dependent risk for committing suicide was also observed. The risk for fatal outcome in accidents was statistically significant for total diazepam concentrations > 1000 ng/mL. However, when compared with fatal accidents due to alcohol use, RR of diazepam concentrations in the range of 100-300 ng/mL is similar with RR of alcohol in concentration range0.5-0.8 g/L. According to Christophersen et al. (2020), theoretically estimated maximal therapeutic concentration for diazepam (213.6ng/mL) can be used for establishment of limit concentration in drivers. Similar value for the concentration of diazepam and its metabolites was found for the group of accidents in our study. It should be emphasized that the results obtained in this study are based on total concentration of diazepam and its metabolites. Therefore, further investigation for estimation of the limit concentration for each compound isrequired.

Conclusion

The data analyses have shown that diazepam use is often associated with violent death. The OPLS-DA models comparing natural death cases and accidentsas well asnatural death cases and suicides showed satisfactory classification performance. The VIP plot of the models revealed that age and temazepam concentration were the most important variables for class separation. The identification of temazepam as a metabolite which differs between the groups of natural death and both accidents and suicides is an important finding which can give aid in interpretation of diazepam adverse effects. The present study identified relatively strong dose dependent association between the presence of diazepam above 300 ng/mL and the risk of suicide. However,not every use of diazepam brings a risk for violent fatal outcome/death in the general population and more investigation is needed to identify the associationwith other important factors.

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