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Clozapine cases with fatal, toxic or therapeutic concentrations

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Summary. The neuroleptic drug clozapine is used in the treatment of schizophrenia and is characterized by not having the extrapyrimidal side-effects usually shown by neuroleptics. Unfortunately clozapine has other side-effects, which limit its use. This study presents methods for the analysis of clozapine and desmethylclozapine in whole blood and tissue. Case histories and pathology findings are described for 3 autopsy cases with fatal concentrations of clozapine, 5 with toxic concentrations and 2 with therapeutic concentrations together with the concentrations found in a living person.

Key words: Clozapine – Desmethylclozapine – Whole blood – Liver – Concentrations

Zusammenfassung. Das Neuroleptikum Clozapin, welches für die Behandlung der Schizophrenie benutzt wird, ist dadurch charakterisiert, daß es nicht die extrapyramidal-motorischen Nebenwirkungen hat, welche Neuroleptika gewöhnlich aufweisen. Die Anwendung von Clozapin ist hingegen durch andere Nebenwirkungen stark begrenzt. Die vorliegende Untersuchung stellt Methoden zur Analyse von Clozapin und seinem Metaboliten Desmethylclozapin in Vollblut und Gewebe vor. Vorgeschichte und pathologische Befunde bei 3 Autopsiefällen mit tödlichen Konzentrationen von Clozapin sowie Ergebnisse von 5 Fällen mit toxischen Konzentrationen und 2 Fällen mit therapeutischen Konzentrationen werden zusammen mit den Medikamentspiegeln bei einer lebenden Person vorgestellt.

Schlüsselwörter: Clozapin – Desmethylclozapin – Vollblut – Leber – Toxische Konzentration

Introduction

The neuroleptic drug clozapine has had a special history in Denmark. It was first marketed in 1974, but was withdrawn the year after due to cases of agranulocytosis described in Finland [1]. However, it very soon became obvious, that a great number of patients suffering from schizophrenia, who had previously been treated with clozapine could not be treated by traditional neuroleptics [2]. The drug was therefore remarketed in 1983, but only for use in psychiatric institutions. Finally in 1987 psychiatric specialists outside the psychiatric wards were allowed to prescribe the drug. Since 1983 the use of clozapine in Denmark has increased between 15 and 25% per year [2]. This has resulted in cases of poisoning by clozapine and as only few such cases have been reported, tissue concentrations, case histories and pathology findings are presented and compared to cases with toxic and therapeutic concentrations of clozapine.

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Materials and methods

This study includes 10 autopsy cases and 1 case from a living person. The autopsies were performed at the Institute of Forensic Pathology and all samples were analysed at the Institute of Forensic Chemistry in Copenhagen.

In the autopsy cases liver and whole blood were analysed but if blood was not available muscle was analysed. It is our experience that whole blood and muscle concentrations are of the same order [3]. Liver samples were screened for basic compounds by capillary gas chromatography (GC) with a nitrogen-phosphorous detector and with retention index calculation and library search. Positive findings were quantitated by GC with alternative confirmation by thin layer chromatography (TLC). Whole blood samples were analysed by high performance liquid chromatography (HPLC) with a UV-detector and with alternative confirmation by GC.

In the case of the living person, urine was analysed as described above for liver.

Quantitative determination by HPLC. Whole blood (2 g) was extracted with 35 ml diethylether after addition of 2 ml water and 2 ml 1 *M* carbonate buffer, pH 9.8. After 15 mins in an icebath, the ether phase was removed and washed with 1 ml 0.1 *M* carbonate buffer, pH 9.8. After discarding the carbonate phase, the ether phase was shaken with 5.0 ml 0.05 *M* sulfuric acid. After the addition of 1.0 ml carbonate buffer (pH 9.8) the acid fraction was extracted with 2.4 ml chloroform and a 2.0 ml aliquot was evaporated to dryness at 40°C under nitrogen. The residue was dissolved in 200 µl solvent for HPLC.

Table 1. Fatal poisonings by clozapine

Case no.	Sex	Age	Daily dose of clozapine	Whole blood (mg/kg)		Liver (mg/kg)		BAC[a] mg/g
				Clozapine	Desmethyl clozapine	Clozapine	Desmethyl clozapine	
1	M	42	1000 mg	3.6	n.a.	28	n.a.	0
2	M	42	?	2.6[b]	1.1[b]	19	18	0.21
3	F	27	?	13	3.1	85	50	0

[a] Blood alcohol concentration
[b] Muscle
n.a. = Not analysed

HPLC conditions. Column: RP-18 endcapped 5 µm, Merck cat. 50995. Solvent: Acetonitrile/methanol/10 m*M* phosphate buffer pH 7.0 (400/300/150), Column flow: 0.5 ml/min. Oven temp. 50°C, UV detector 215 nm.

The recovery rate was determined using 10 whole bloods spiked with 5 µmol/kg or 0.5 µmol/kg. Recoveries of 98% ± 5.5% and 94% ± 3.1%, respectively for clozapine and 88% ± 5.6% and 68% ± 5.0%, respectively for desmethylclozapine were obtained. (5 µmol clozapine ~ 1.63 mg and 5 µmol desmethylclozapine ~ 1.56 mg).

Gas chromatography

A mixture of 2 g whole blood, 100 µl 2N NaOH and 500 µl butylacetate with lecithine (0.1 mg/ml) was agitated for 30 sec. After centrifugation for 10 mins, 100 µl of the butylacetate phase was transferred to a microvial for gas chromatography.

GC conditions. NPD 325°C, Split injection 275°C, Column: HP5, 25 m × 0.32 mm × 0.52 µm. Flow 2 ml/min., Temp.: 230° for 10 min., 5°/min. to 265°, 265° for 8 min., 5°/min. up to 310°, 310° for 6 min.

The recovery rate was determined using 10 whole bloods spiked with 5 µmol/kg or 0.5 µmol/kg. Recoveries of 92% ± 2% and 82% ± 2.1%, respectively for clozapine and 80% ± 3.6% and 37% ± 1.5%, respectively for desmethylclozapine were obtained.

Screening on liver. The extraction from liver tissue for the GC screening was carried out as described above for the whole blood HPLC determination with the following modifications: 100 µl 1 m*M* lidocaine (internal standard) was added to 4 g homogenized liver tissue with water (1:1), and 1 ml water plus 0.5 g solid $NaHCO_3$/Na_2CO_3 was added instead of the buffer solution. The final residue was dissolved in 200 µl methanol for GC screening with automatic retention index calculation and library search on a Hewlett Packard GC 5890 with a GC-Chemstation, and a HP Ultra 2 column (25 m × 0.32 mm × 0.52 µm) with temperatures of 160° for 1 min, 10°/min to 320° and 320° for 8 min, and a column flow of 2 ml/min. A split-injection of 300°C was used and a NP Detector at 325°C. Positive findings were quantified by GC with the relevant reference standards. Confirmation was performed on a TLC plate (Whatmann 4806-711), sprayed with 0.1 *M* NaOH in methanol and dried. Samples were eluted with methanol and visualized with Mandelin's Reagent.

Results

The cases consisted of 8 men and 3 women aged 21–47 years old, with a median age of 41 years.

No alcohol was detected in 8 of the autopsy cases and in the other 2 cases only low concentrations of blood alcohol were found. (BAC 0.21 mg/g in case 2 and 0.36 mg/kg in case 4).

The fatal cases are shown in Table 1.

In case no. 1 (male, 42 years old) the patient was prescribed 1000 mg clozapine daily, which is a higher dose than the recommended daily dose in Denmark (maximum 600 mg). The deceased had been manic depressive and was in a mental institution. He was found breathing heavily but was dead on arrival at hospital. At the autopsy a moderately enlarged heart was found. Microscopy of the heart showed no pathological changes. The cause of death was evaluated to be acute heart failure, possibly provoked by the high concentration of clozapine.

Case no. 2 (male, 42 years old) was atypical compared to the other clozapine cases. There was no history of mental disease or clozapine medication. The person was known to be an alcohol and medicine abuser. Due to chronic pains from an oesophagus ulcer he was under methadone treatment. He was found dead in the muddy banks of a stream. The cause of death was not determined with certainty by the autopsy, but death by drowning could be a possibility with clozapine as a contributing factor. Microscopical investigation of the heart was not performed.

Case no. 3 (female, 27 years old) the patient had been suffering from schizophrenia and was hospitalized. She was found unconscious in her bed with a rapid pulse and tendencies to cramps. She had last been seen alive 1 1/2 h earlier. She was given a gastric lavage but died 10 h later. The autopsy revealed nothing of importance and clozapine was evaluated to be the cause of death. The concentrations found were so high, that it was considered to be suicide. Microscopical investigation of the heart was not performed.

In the next five cases we found toxic concentrations of clozapine (Table 2) and 4 of the patients were known to be schizophrenic (cases no. 4, 5, 7 and 8)

Case no. 4 (male, age 44 years) was a day-patient at a psychiatric institution. He had an attack of asthma and was dead on arrival at the hospital. At the autopsy and by microscopy of the lungs and heart, changes compatible with asthma bronchiale were found in the lungs, but the myocardium showed no pathological changes. The cause of death was evaluated to be an asthmatic attack in combination with a relatively large intake of clozapine.

Case no. 5 (female, 41 years old) is the only other case besides case no 1, where information was available

Table 2. Cases with toxic concentrations of clozapine

Case no.	Sex	Age	Daily dose of clozapine	Whole blood (mg/kg)		Liver (mg/kg)		BAC[a] mg/g
				Clozapine	Desmethyl clozapine	Clozapine	Desmethyl clozapine	
4	M	44	?	0.9	n.a.	6.9	n.a.	0.36
5	F	41	350 mg	0.8	0.1	16	5.0	0
6	M	27	?	0.6	0.1	17	4.7	0
7	F	47	?	0.5	n.a.	5.9	n.a.	0
8	M	28	?	0.4	n.a.	15	n.a.	0

[a] Blood alcohol concentration
n.a. = Not analysed

Table 3. Cases with therapeutic concentrations of clozapine

Case no.	Sex	Age	Daily dose of clozapine	Whole blood (mg/kg)		Liver (mg/kg)		BAC[a] mg/g
				Clozapine	Desmethyl clozapine	Clozapine	Desmethyl clozapine	
9	M	42	?	0.1	n.a.	1.5	n.a.	0
10	M	21	?	0.2	0.1	0.8	0.5	0
11	M	30	1 × 2 size unknown	0.2	0.1	n.a.	n.a.	0

[a] Blood alcohol concentration
n.a. = Not analysed

Table 4. Findings by other authors

Case no.	Accident/ Suicide	Sex	Age	Amount clozapine taken	Blood (mg/kg)		Liver (mg/kg)		Author
					Clozapine	Desmethyl clozapine	Clozapine	Desmethyl clozapine	
1	Accident	M	40	2 × 300 mg	1.4	1.6	6.5	4.4	Sticht et al. [4]
2	Suicide	M	29	?	9.5	0.5	33	1.9	
3	Accident	M	22	3 × 100 mg	4.5				Vesterby et al. [5]
4	Suicide	M	21	2000 mg	5.8		43		Meeker et al. [6]

about the daily intake of clozapine, namely 350 mg. She was last seen alive a few hours before she was found dead, and the cause of death was not found at the autopsy. Microscopical investigation of the heart was not performed.

Case no 6 (male, 27 years old) was a psychotic alcohol and drug addict, and the cause of death in this case was methadone poisoning. Microscopical investigation of the heart was not performed.

Cases no 7 and 8 were both patients who were strapped in bed. In case no. 7 (female, 47 years old) the patient was suffering from pneumonia. The pulse stopped suddenly and artificial respiration plus heart massage was given but in vain. At the autopsy slight bronchopneumonia was found. Microscopy of the heart showed no pathological changes. The cause of death was not established. Acute heart failure due to clozapine might be of importance.

In case no 8 (male, 28 years old) there had been 2 earlier suicide attempts. The patient was found dead with his head in a pillow. At the autopsy severe congestion of the lungs was found. Microscopical investigation of the heart was not performed. The cause of death was not established, and as in case number 7 it might be acute heart failure due to clozapine.

The last 3 cases were considered to have therapeutic concentrations of clozapine (Table 3)

In case no. 9 (male, 42 years old) the patient had been in a mental hospital several times, and 4 hours before being found dead he had telephoned, to say that he felt sick and had cramps. However, only low concentrations of clozapine were found and by the autopsy severe coronary changes were found to be the cause of death. Microscopical investigation was not performed.

Case no. 10 (male, 21 years old) suffered from schizophrenia and epilepsy. He was found unconscious in a train and in possession of a piece of cannabis and a bottle of methadone. The cause of death was found to be methadone poisoning. Microscopy of the heart showed no pathological changes.

The results in case no 11 are from a living person (male, 30 years old). Unfortunately we do not know how much clozapine had been prescribed.

Discussion

Findings by other authors are presented in Table 4. Sticht and co-workers [4] found a blood concentration of 1.4 mg/kg of clozapine in a case considered to be an accident and 9.5 mg/kg in a suicide case, which is consistent with our findings. Vesterby and co-workers [5] found 4.5 mg/kg in a case, which they considered to be an accident, and finally Meeker and co-workers [6] described a suicide case, where 2000 mg clozapine was taken, and here a clozapine blood concentration of 5.8 mg/kg was found, which is in agreement with our findings.

Gossweiler [7] made a survey of the side-effects of clozapine related to intake and found that in cases (n = 16) where less than 600 mg had been taken, slight side-effects were seen in 81% of the cases and severe side-effects in 19%. In 22 cases, where the dose had been between 600 and 1500 mg he found 41% with slight side-effects and 59% with severe side-effects, and finally in 28 cases, where the dose taken had been 1500–5000 mg the corresponding side effects were found in 33% and 67%, respectively. Even in cases where only a small overdose had been taken, severe symptoms such as drop in blood pressure, coma and depression of breathing had been seen.

According to Sticht and co-workers [4] cases of clozapine poisoning are clinically characterized by cardiac arryhthmia, depression of breathing, unconsciousness and cramps. This is confirmed by our findings including heavy breathing (case no. 1), unconsciousness (case no. 3), sudden lack of pulse (case no. 7) and cramp (cases no. 3 and 9). Lapierre and co-workers [8] have described hyperthermia as a frequently occurring side-effect.

In the present series no symptoms or objective findings compatible with agranulocytosis were found.

Histological changes compatible with myocarditis were found in 3 cases (cases 4, 5, 6), in 2 of these (cases 4, 5) there was infiltration of histocytes and lymphocytes in the interstitium and in case 6 eosinophils predominated. Changes compatible with myocarditis were not seen in any of the 4 cases in the present study, where microscopy was performed.

In 3 cases with a toxic clozapine concentration (cases 5, 7 and 8) the cause of death was not established. The mechanism of death in these cases might – by exclusion – be cardiac arrythmia possibly due to a toxic/allergic effect of clozapine on the myocardium. In spite of certain precautions regarding patients under treatment with clozapine, such as prescription by specialists only, control of bone marrow function and weekly ECG after an increase in dose, sudden unexpected deaths may occasionally occur.

References

1. Idänpään-Heikkilä J, Alhava E, Olkinuora M, Palva IP (1977) Agranulocytosis during treatment with clozapine. Eur J Clin Pharmacol 11:193–198
2. Gerlach J, Jørgensen EO, Peacock L (1989) Long-term experience with clozapine in Denmark: research and clinical practice. Psychopharmacology (Berlin) 99:92–96
3. Christensen H, Steentoft A, Worm K (1985) Muscle as an autopsy material for evaluation of fatal cases of drug overdose. J Forensic Sci Soc 25:191–206
4. Sticht G, Käferstein H, Henßge C (1988) Befunde bei chronischer Einnahme und akuter Überdosierung von Leponex (R). Beitr Gerichtl Med 67:81–86
5. Vesterby A, Pedersen JH, Kæmpe B (1980) Sudden death during clozapine (Leponex(R)) therapy. Ugeskr Læger 142:170–171
6. Meeker JE, Herrmann PW, Som CW, Reynolds PC (1992) Clozapine tissue concentrations following an apparent suicidal overdose of Clozanil(R). J Anal Toxicol 16:54–56
7. Gossweiler B (1983) Vergiftungen mit Leponex®. Schweiz Apoth Ztg 121:366–368
8. Lapierre YD, Ghadirian A, St-Laurent J, Chandhry RP (1980) Clozapine in acute schizophrenia-efficacy and toxicity. Curr Ther Res 27:391–400