Toxicology Reports 12 (2024) 542–545

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Insulin overdose with fatal outcome?: Two forensic cases

Alaa Embaby [a,*], Marjorie Balai [b], Eric J.F. Franssen [c]

[a] Department of Medical Oncology and Clinical Pharmacology, The Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands
[b] Forensics Medicine, Public Health Services GGD Amsterdam, Amsterdam, the Netherlands
[c] Department of Clinical Pharmacy, OLVG Hospital, Amsterdam, the Netherlands

ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:
Toxicology
Toxicology screen
Insulin
Glucose
Antidiabetics
Postmortem

ABSTRACT

Intentional overdose with insulin preparations is rare. However, fatal consequences due to severe hypoglycemia could occur. Postmortem toxicology screening of insulin is a challenge, given the chemical characteristics of this protein and the difficulty of distinguishing between endogenous and exogenous insulin in blood. Here, we describe two cases of patients with diabetes using insulin and oral anti-diabetics. The main question in both cases was whether or not disturbance in glucose metabolism contributed to death. In case A, there was strong evidence that self-poisoning with insulin and subsequent hypoglycemia caused the death. However, this could not be confirmed due to lack of adequate forensic toxicology tests. In case B, no hypoglycemia was observed. Though, compared with case A, additional forensic examination was performed to investigate whether glycemic disturbances could have contributed to the death. In this report, we focus on the most appropriate analytical methods for the detection of exogenous insulin in the human body and give recommendations for toxicology testing of glucose levels and insulin in postmortem specimens.

1. Introduction

Since its discovery, insulin became indispensable in the treatment of diabetes mellitus [1]. According to the World Health Organization, there are approximately 9 million cases with insulin-dependent diabetes (type 1) worldwide [2]. Several insulin preparations are available for these patients, providing various options in accordance to the needs of the patient [3]. However, partly due to its narrow therapeutic window and risk of severe and even life-threatening hypoglycemia, exogenous insulins are not harmless [4]. Although rarely reported compared to other pharmaceutical agents, one has to be aware of deliberate overdosage in patients with suspicious symptoms [5]. In a retrospective study conducted by von Mach et al., it was found that roughly 90 % of all insulin overdoses registered in a regional poisons unit concerned suicidal attempts, raising the awareness for this issue [6]. This knowledge underscores the importance of postmortem toxicology screening of insulin. Here, we present two forensic cases in which hypoglycemia due to insulin overdose was considered as cause of death and discuss the designated toxicology tests for the determination of postmortem insulin and glucose levels.

2. Case A

2.1. Case description

A 70 years old man with a history of diabetes was discovered dead in his bedroom. His body was found by his neighbor. The general practitioner, who was warned by the neighbor, found five and one half empty insulin syringes (Novomix 30, 100 E/ml IU insulin, total volume approximately 16 ml) without a needle and one full syringe with a needle in the kitchen. The apartment was cluttered. An examination by a forensic physician was requested because there was a suspicion of suicide by insulin overdose. Apart from diabetes, the deceased had a medical history of heart failure, chronic obstructive pulmonary disease, alcohol abuse and borderline personality disorder. His prescribed home medication consisted of metformin, insulin (Novomix 30), inhalers, omeprazole, paracetamol and naproxen. At time of death inspection, the deceased was known to be sick for a few days and had been seen alive a day before.

2.2. Examination of the body

The deceased was wearing a pyama, lay on his left side and had scars

* Corresponding author.
E-mail address: a.embaby@nki.nl (A. Embaby).

https://doi.org/10.1016/j.toxrep.2024.05.004
Received 6 March 2024; Received in revised form 23 April 2024; Accepted 8 May 2024
Available online 11 May 2024
2214-7500/© 2024 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

on his forearms suggesting self-mutilation. There were no signs of bone fractures or other signs of physical violence. His body temperature was 34.0 degrees Celsius. The room temperature was 21.5 degrees Celsius. There was full body stiffness. The estimated postmortem interval was 6–8 h [7]. Femoral blood and urine were collected for toxicology screening. According to the investigation by the police there was no evidence for a crime. No autopsy was performed, since this is not standard procedure in the Netherlands [8]. It was assumed that a self-inflicted insulin overdose or auto-intoxication with insulin contributed to the cause of death. The case was evaluated with the prosecutor who did not order an autopsy but released the body to the family for burial.

2.3. Toxicology tests

A comprehensive toxicology screening was carried out to exclude poisoning with other foreign substances. Blood and urine were tested at the pharmacy laboratory using a qualitative analytical method based on Liquid Chromatography Iontrap Mass Spectrometry (LC-MS^n, Toxtyper®, Bruker, Germany) and a frequently updated library for foreign substances with low molecular weight (<1000 Daltons). Insulin, as a macromolecule, has a molecular weight of 5808 Daltons [9]. Consequently, determination of insulin levels in the available samples was not possible. There were no other detectable foreign substances (e.g. acetone, ethanol, methanol, benzodiazepines, amphetamines, opiates, barbiturates, cannabis, cocaine) in the tested blood- and urine samples. Glucose and lactate levels could not be measured since there was no vitreous humor available.

3. Case B

3.1. Case description

A 70 year old man with a history of diabetes was found dead on the floor in his living room. His neighbours discovered the body. The deceased was seen alive two days before. The last messages on his phone were also from two days before. The forensic physician was requested because the deceased had head injury. Additional medical information mentioned a medical history of depression, hypercholesterolemia and a lymphoma which was cured for five years. His medication consisted of insulin (Novomix 30), gliclazide, omeprazole, simvastatine, citalopram and pregabalin. At time of death inspection, there were many ashtrays filled with cigarette butts and a closet filled with liquor.

3.2. Examination of the body

The deceased was fully dressed, lay on his back on the floor in the living room, next to the vacuum cleaner with his head against the wall. There was a pool of blood on the floor next to the head of the deceased and a wound on his scalp was observed. There were no signs of bone fractures or other signs of physical violence. The corpse stains were fixated, the body was cold and the body stiffness was complete. The body temperature was not measured. Femoral blood and urine samples were collected for toxicological screening at time of death inspection. The urine rapid test was positive for amphetamine, but this was not confirmed at the laboratory test in heart blood and femoral blood. The body was seizured to the forensic laboratory for autopsy. Toxicology tests were thereafter performed on diverse specimens including vitreous humor.

3.3. Toxicology tests

A complete toxicology screening was performed to exclude poisoning with foreign substances. There were no detectable foreign substances; the ethanol concentration was below the limit of determination of 0.20 mg/L. The GHB concentration was 22 mg/L; this could fully be explained by the presence of endogenous GHB and postmortem formation of GHB[10]. Opioids, cocaine and metabolites, amfetamine, cannaboids, benzodiazepines, antidepressants, antipsychotic drugs, citalopram and gliclazide were tested among others. Glucose and lactate levels were measured in vitreous humor and HBA1c was determined in heart blood. The outcome of the measured concentrations is listed in Table 1. There was evidence that the deceased had hyperglycemia prior to death, which could have contributed to death.

Table 2 gives an overview of the similarities and differences between the two cases.

4. Discussion

Fatal insulin poisoning is an underestimated and underreported issue, partly due to limitations in the detection of exogenous insulin in postmortem blood samples [17]. We presented two forensic cases in which hypoglycemia due to insulin poisoning was considered as cause of death. In Case A there was strong evidence for insulin overdose whereas in Case B it was unknown in what extend insulin contributed to the sudden death. Unfortunately in none of the cases the laboratory test could present a certain cause of death. Yet, the approaches to the cases were interestingly different. In Case A, only femoral blood and urine samples were collected and no autopsy performed whereas in Case B, heart blood and vitreous humor had been obtained too and an autopsy was performed. In both cases a validated LC-MS/MS laboratory method was used for the detection of foreign substances. There is evidence that the calculation of an Insulin: C-peptide (I:C) ratio could be helpful to confirm the use of exogenous insulin; one would expect the I:C ratio to be >1.0 when pharmaceutical insulin is administered [18]. However, the I:C ratio is not reliable when the time after death (postmortem interval) is >24 h, as is probably the case in Case B [19]. Moreover, determination of glucose, C-peptide and insulin concentrations in

Table 1
Concentrations of the tested analytes in diverse body fluids. Interpretation of the results: Citalopram concentrations between 0.07 mg/L – 0.15 mg/L are considered therapeutic. Median postmortem citalopram and desmethylcitalopram concentrations of 40 mg/L and 20 mg/L have been reported, respectively [11]. Gliclazide concentrations between 1.0 – 4.0 mg/L are considered normal [12]. A glucose concentration of >13 mmol/L in vitreous humor could be an indication for hyperglycemia, as well as a combined glucose + lactate concentration of > 23.7 mmol/L [13,14] (Glucose can be transformed into lactate after death. Calculation is according to the formula described in Palmiere, 2015 [15]). The combined glucose + lactate concentration was 44.5 mmol/L, indicating that hyperglycemia was present at time of death. The high concentration of HbA1c indicates that there was poor antidiabetic control over the last few months before death. The determined GHB concentration could be explained by the presence of endogenous GHB.[16].

Analyte	Used specimens	Result	Reference concentrations
Citalopram	Femoral blood Heart blood	0.20 mg/L 0.93 mg/L	0.07 – 0.15 mg/L (therapeutic), median postmortem concentration in femoral blood 0.40 mg/L
Desmethylcitalopram	Femoral blood Heart blood	0.074 mg/L 0.31 mg/L	Median postmortem concentration in femoral blood 0.21 mg/L
Gliclazide	Femoral blood Heart blood	0.42 mg/L 3.1 mg/L	1.0 – 4.0 mg/L
Glucose	Vitreous humor	2.5 mmol/L	< 13 mmol/L
Lactate	Vitreous humor	84 mmol/L	No separate threshold is given.
HbA1c	Heart blood	81 mmol/mol	20 – 40 mmol/mol
GHB	Heart blood	22 mg/L	Proposed cut-off of 50 mg/L but higher concentrations are also reported

Table 2
Clinical similarities and differences between the two cases.

	Case 1	Case 2
Diabetes Mellitus	Yes	Yes
Oral antidiabetics	Yes	Yes
Insulin	Yes	Yes
Psychiatric disease	Yes	Yes
Last seen alive (hours before forensic examination)	24 hrs	48 hrs
Autopsy	No	Yes
Collected samples		
Urine	Yes	Yes
Femoral blood	Yes	Yes
Heart blood	No	Yes
Vitreous humor sample	No	Yes
LC-MS/MS laboratory method	Yes	Yes

postmortem blood could not be offered by our toxicology laboratory for several reasons. As mentioned earlier, insulin detection by our Toxtyper toxicology screen was not possible due to the large molecular weight of insulin [9]. Also, due to its decomposition, the obtained postmortem blood was not suitable for glucose tests or other analytical methods for insulin and C-peptide detection such as enzyme-linked immunosorbent essay (ELISA) [18,20]. Distinguishing endogenous insulin from synthetic insulin analogues is still a challenging issue in toxicology. Even difficulties in the differentiation between diverse pharmaceutical insulin preparations as a result of cross-reactivity in the available immunoassays are reported [21–23]. Several assays based on LC-MS/MS have been developed to overcome this concern [24–26]. This analytical method is more reliable in the identification and quantification of different substances. Nevertheless, the question is whether determination of insulin levels in blood is the most appropriate approach in forensic cases. As mentioned previously, the suitability of postmortem blood for toxicology tests is dependent on a list of factors, including chemical instability [18]. Studies have shown that complete degradation of insulin occurs within five days at temperatures around 20 degrees Celsius [18]. This knowledge indicates that, if not immediately collected and stored under cold conditions, the obtained specimens become obsolete. LC-MS/MS analysis could therefore be the preferred technique to determine insulin levels on the occasion that fresh frozen or refrigerated blood is available. Unfortunately, in practice, the time interval between death and forensic investigation is often unknown and the deceased could be found multiple days after death. In this regard, one should preferably use another matrix with less vulnerability to postmortem changes. A considerable alternative is vitreous humor, a well-protected matrix that is known to be less altered by contamination and putrefaction [27]. It has for instance been shown that vitreous humor is suitable for the estimation of the postmortem interval by measuring vitreous fluid concentrations of certain biochemical markers such as electrolytes (e.g. sodium, potassium and chloride) [28]. It is also used for the measurement of drugs, although limited by the difficulty of interpretation of the results in some cases [29]. Various studies reported about the usefulness of vitreous humor in the investigation of insulin poisoning [30–32]. Mainly mass spectrometry based analytical methods were tested and validated for the determination of insulin in vitreous humor. Hess et al. also discussed the measurement of glucose and lactate levels in vitreous fluid as indicators for severe hypoglycemia [32]. In nearly all studies investigating glucose metabolism in vitreous humor, sample collection was performed within 72 h after death [33]. Beckett et al. stated that if properly collected and stored between −20°C and −80°C, vitreous humor samples are suitable for at least up to three months for the detection of insulin analogues [20].

4.1. Clinical relevance

Despite its obvious relevance in forensic toxicology, vitreous humor is barely collected and analyzed in clinical practice [33]. This could be due to lack of experience with this matrix for post-mortem biochemistry. Nonetheless, sampling of vitreous fluid may be of added value in case of suspected insulin poisoning. In Case A, no vitreous fluid was available for bioanalysis, whereas in Case B vitreous fluid was available for glucose and lactate measurements. The European Council of Legal Medicine approved guidelines for the collection and preservation of biological samples for postmortem analysis, including vitreous humor [27]. Forensics are encouraged to include vitreous fluid sampling in their standard operating procedure to support the hypothesis of (auto-) intoxication with insulin or other compounds that are hard to analyze in blood or other body fluids. Moreover, standard sampling of vitreous fluid at time of forensic examination is expected to lead to higher supply of this matrix, which could promote the development of more accurate analytical methods at regional toxicology laboratories (e.g. immunoassays) [18,20].

5. Conclusions

Insulin poisoning is often a diagnosis per exclusionem due to lack of quantitative evidence in biological samples. In Case A, hypoglycemia due to auto-intoxication with insulin was a plausible cause of death, but this could not be confirmed in the obtained blood samples. Both the biochemical composition of this matrix in the postmortem setting and the lack of reliable analytical methods for the determination of insulin and insulin analogues were obstacles. Although insulin poisoning was unlikely in Case B (hyperglycemia was detected instead of hypoglycemia), this case was worth mentioning because of the availability of vitreous humor. The glucose and lactate levels measured in this matrix made it possible to exclude insulin overdose as cause of death. Collection of vitreous humor in similar forensic cases is highly recommended, as well as the improvement of the accessibility of useful analytical tools at regional toxicology laboratories.

CRediT authorship contribution statement

Eric J.F. Franssen: Writing – review & editing, Conceptualization. **Marjorie Balai:** Writing – review & editing, Data curation. **Alaa Embaby:** Writing – original draft, Project administration, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

References

[1] 100 Years of insulin - FDA [Available from: ⟨https://www.fda.gov/about-fda/fda-history-exhibits/100-years-insulin#:~:text=signed%20an%20agreement%20with%20Genentechthat%20derived%20from%20this%20technology⟩.
[2] World Health Organization - Diabetes [Available from: ⟨https://www.who.int/news-room/fact-sheets/detail/diabetes⟩.
[3] General principles of insulin therapy in diabetes mellitus [Available from: ⟨https://www.uptodate.com/contents/general-principles-of-insulin-therapy-in-diabetes-mellitus?search=insulin&source=search_result&selectedTitle=2-143&usage_type=default&display_rank=1#H1701819734⟩.
[4] Summary of Product Characteristics - Insulin Novomix EMA [Available from: ⟨https://www.ema.europa.eu/en/documents/product-information/novomix-epar-product-information_en.pdf⟩.
[5] D.D. Gummin, J.B. Mowry, M.C. Beuhler, D.A. Spyker, D.E. Brooks, K.W. Dibert, et al., 2019 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 37th Annual Report, Clin. Toxicol. (Philadelphia, Pa) 58 (12) (2020) 1360–1541.

[6] M.A. von Mach, S. Meyer, B. Omogbehin, P.H. Kann, L.S. Weilemann, Epidemiological assessment of 160 cases of insulin overdose recorded in a regional poisons unit, Int. J. Clin. Pharmacol. Ther. 42 (5) (2004) 277–280.
[7] Estimation of time of death, Method of Henssge [Available from: 〈https://www.swisswuff.ch/calculators/todeszeit.php〉.
[8] Duijst-Heesters. De lijkschouw en sectie beschouwd. 2016.
[9] PubChem - Compound summary of Insulin [Available from: 〈https://pubchem.ncbi.nlm.nih.gov/compound/126843196〉.
[10] M. Schulz, S. Iwersen-Bergmann, H. Andresen, A. Schmoldt, Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics, Crit. Care (London, England) 16 (4) (2012) R136.
[11] R.A. Ketola, I. Ojanperä, Summary statistics for drug concentrations in post-mortem femoral blood representing all causes of death, Drug Test. Anal. 11 (9) (2019) 1326–1337.
[12] Toxicology of gliclazide (article in Dutch) [Available from: 〈https://www.vergiftigingen.info/f?p=300:1210:12006569650839:::RP,1210,1040〉::.
[13] B. Zilg, K. Alkass, S. Berg, H. Druid, Postmortem identification of hyperglycemia, Forensic Sci. Int. 185 (1-3) (2009) 89–95.
[14] M.Z. Karlovsek, Diagnostic values of combined glucose and lactate values in cerebrospinal fluid and vitreous humour–our experiences. Forensic Sci. Int. 146 (Suppl) (2004) S19–S23.
[15] C. Palmiere, Postmortem diagnosis of diabetes mellitus and its complications, Croat. Med. J. 56 (3) (2015) 181–193.
[16] A.-S. Korb, G. Cooper, Endogenous Concentrations of GHB in Postmortem Blood from Deaths Unrelated to GHB Use, J. Anal. Toxicol. 38 (8) (2014) 582–588.
[17] L. Stephenson, C. van den Heuvel, M. Humphries, R.W. Byard, Characteristics of fatal insulin overdoses, Forensic Sci. Med. Pathol. 18 (4) (2022) 429–441.
[18] L.M. Labay, C.P. Bitting, K.M. Legg, B.K. Logan, The Determination of Insulin Overdose in Postmortem Investigations, Acad. Forensic Pathol. 6 (2) (2016) 174–183.
[19] Baselt R.C. Disposition of toxic drugs and chemicals in man. Twelfth ed.
[20] N. Beckett, R. Tidy, B. Douglas, C. Priddis, Detection of intact insulin analogues in post-mortem vitreous humour-Application to forensic toxicology casework, Drug Test. Anal. 13 (3) (2021) 604–613.
[21] C. Parfitt, D. Church, A. Armston, L. Couchman, C. Evans, G. Wark, T.J. McDonald, Commercial insulin immunoassays fail to detect commonly prescribed insulin analogues, Clin. Biochem. 48 (18) (2015) 1354–1357.
[22] K.D. Tinworth, P.C. Wynn, R.C. Boston, P.A. Harris, M.N. Sillence, M. Thevis, et al., Evaluation of commercially available assays for the measurement of equine insulin, Domest. Anim. Endocrinol. 41 (2) (2011) 81–90.
[23] A. Dayaldasani, M. Rodríguez Espinosa, P. Ocón Sánchez, V. Pérez Valero, Cross-reactivity of insulin analogues with three insulin assays, Ann. Clin. Biochem. 52 (Pt 3) (2015) 312–318.
[24] S.W. Taylor, N.J. Clarke, Z. Chen, M.J. McPhaul, A high-throughput mass spectrometry assay to simultaneously measure intact insulin and C-peptide, Clin. Chim. Acta 455 (2016) 202–208.
[25] N. Foulon, E. Goonatilleke, M.J. MacCoss, M.A. Emrick, A.N. Hoofnagle, Multiplexed quantification of insulin and C-peptide by LC-MS/MS without the use of antibodies, J. Mass Spectrom. Adv. Clin. Lab. 25 (2022) 19–26.
[26] Deepti Bhandarkar A.S., Vikas Trivedi, Tulsidas Mishra 2, Abhishek Gandhi 2, Swati Guttikar 2, Matsubara aT.. Development of bioanalytical method for determination of intact human insulin from plasma using LC/MS/MS 2017. Available from: 〈https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/technical/white_papers/13843/apo21765.pdf〉.
[27] R.J. Dinis-Oliveira, D.N. Vieira, T. Magalhães, Guidelines for collection of biological samples for clinical and forensic toxicological analysis, Forensic Sci. Res. 1 (1) (2016) 42–51.
[28] W. Li, Y. Chang, Z. Cheng, J. Ling, L. Han, X. Li, Y. Ding, Vitreous humor: a review of biochemical constituents in postmortem interval estimation, J. Forensic Sci. Med. 4 (2) (2018).
[29] Jones G.R. Postmortem Specimens. Encyclopedia of Forensic Sciences (Second Edition) 2013. p. pp. 270-274.
[30] K.M. Legg, L.M. Labay, S.S. Aiken, B.K. Logan, Validation of a fully automated immunoaffinity workflow for the detection and quantification of insulin analogs by LC–MS-MS in postmortem vitreous humor, J. Anal. Toxicol. 43 (7) (2019) 505–511.
[31] M. Thevis, A. Thomas, W. Schänzer, P. Ostman, I. Ojanperä, Measuring insulin in human vitreous humour using LC-MS/MS, Drug Test. Anal. 4 (1) (2012) 53–56.
[32] C. Hess, B. Madea, T. Daldrup, F. Musshoff, Determination of hypoglycaemia induced by insulin or its synthetic analogues post mortem, Drug Test. Anal. 5 (9-10) (2013) 802–807.
[33] C. Boulagnon, R. Garnotel, P. Fornes, P. Gillery, Post-mortem biochemistry of vitreous humor and glucose metabolism: an update, Clin. Chem. Lab. Med. 49 (8) (2011) 1265–1270.