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Forensic aspects of homicides by insulin overdose

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ABSTRACT

Analysis of homicidal insulin overdose is a challenging task in forensic practice because of the difficulties in toxicological analysis as well as the elusive pathologic changes. We performed a detailed histopathologic examination on four autopsy cases involving insulin homicide, using H&E, immunohistochemistry (IHC) and immunofluorescence assays. Severe reactive astrocyte proliferation was obvious in the white matter of the cerebrum, corpus callosum, cerebellum and brain stem, especially in subcortical regions. We found a statistically significant increase in the number and total area of reactive astrocytes compared with controls ($p < 0.001$). Insulin was detected at the injection sites of subcutaneous soft tissues by using IHC, luminescence immunoassay and immunofluorescence. Most insulin deposits were located in the gaps between adipocytes, and a few deposits were located in peripheral nerves and inflammatory cells. We also detected protamine in the skin tissues in two of the four cases. Our study revealed that the presence of insulin and/or protamine at the injection sites, along with severe reactive astrocyte proliferation, could help diagnose insulin overdose.

1. Introduction

Insulin is a hormone which regulates glucose concentration. It consists of two peptide chains (A and B chains) which are connected by a disulfide bond. The molecular weight of insulin is 5807.69. The plasma half-life of insulin is short, approximately 4–6 min [1–4]. Proinsulin molecules also consist of two chains along with a connecting peptide (C-peptide) before secretion. Equimolar amounts of C-peptide and insulin will be released into circulation when insulin is secreted following hyperglycemia [5–8]. The plasma half-life of C-peptide is about 10–20 min [4]. Exogenous insulin administration can suppress the secretion of C-peptide due to a negative feedback system [9]. Subsequently, the C-peptide/insulin ratio in blood will decrease. According to Uezono et al., analysis of serum C-peptide concentration is necessary to diagnose hypoglycemia induced by exogenous insulin [10]. Cases of insulin overdose mostly occur in clinical practice and fatal cases are rarely reported [11–16].

In modern clinical practice, insulin is widely used to treat diabetes, but its administration should be carefully controlled since it could give rise to adverse results, such as severe hypoglycemia, coma and death. In the 1930s, insulin coma therapy (ICT) was thought to be a revolutionary treatment for schizophrenia [17], but in the 1950s, ICT was questioned [18,19] and gradually abolished in treating mental diseases.

Insulin overdose could be exploited as a murder weapon [17]. The first insulin homicide was reported in 1958 by Birkinshaw [20]. Some fictional writers have described insulin as the perfect murder weapon because of the elusive pathologic changes and the difficulties in toxicological screening [12]. In cases of fatal insulin overdose, accidental overdose is the most common manner of death, followed by suicide, whereas homicide is extremely rare [6,21]. In recent times, several cases of homicide due to insulin overdose have been reported in mainland China and such cases now tend to appear more frequently [22–25].

The purpose of this study is to investigate the pathologic changes resulting from insulin overdose in a series of established homicidal insulin overdose cases using H&E, immunohistochemistry (IHC), and immunofluorescence assays.

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2. Materials and methods

2.1. Victim information and preparation of human samples

The samples from four victims murdered by insulin overdose and used in this study were collected at Tongji Medical College, Huazhong University of Science and Technology (HUST). Information regarding the four cases is listed in Tables 1–3. The negative controls were chosen from three cases of sudden natural death independent of insulin. Tissue samples of the brain, heart, liver, kidney and skin at the injection sites were fixed in 10% buffered formalin solution for a week. All tissue blocks were dehydrated and embedded in paraffin. Serial sections of 4 μm thickness were used for H&E, IHC and immunofluorescence assays. In the control group, the tissues were sampled and tested in the same way. All experimental protocols involving human samples were approved by the victims' relatives and the Human Ethical Commission at Tongji Medical College, HUST.

2.2. Histopathology and immunohistochemistry

Sections of the tissues from the brain, heart, liver, kidney and skin at the injection sites were routinely stained with H&E. After deparaffinization, sections were dipped into 3% hydrogen peroxide solution for 10 min to quench endogenous peroxidase. Microwave-induced epitope retrieval was performed in citrate buffer (pH 6.0) at 100 °C for 30 min (skin specimens for 5 min). After incubation for 30 min with 5% bovine serum albumin to block nonspecific binding, the sections were incubated with insulin primary antibody (1:600, Boster, China), protamine primary antibody (1:2000, Boster, China) or GFAP primary antibody (1:5000, Abcam, UK) in phosphate-buffered saline at 4 °C overnight. Sections were incubated for 50 min at 37 °C with biotinylated secondary antibody and horseradish peroxidase–conjugated streptavidin (Biogood, China), respectively. Staining was visualized with 3, 30-diaminobenzidine chromogen, and counterstained with hematoxylin. Finally, sections were dehydrated through increasing concentrations of ethanol and xylene. Negative control sections were incubated with non-immune serum of the species from which the primary antibodies were derived. All the sections were examined under a Nikon Eclipse 90i microscope (Tokyo, Japan). A semi-quantitative evaluation of the reactive astrocytes was made by two different investigators without prior knowledge of the IHC results. The degree of pathologic change was graded as follows: (−) none; (+) mild; (+) moderate; (++) severe. All measurements were obtained using the same magnification of

Table 1
Basic information of the four cases in the study.

Case no.	Age/ gender	Positions of injection sites	Total injection volume	Perpetrators	Survival interval	Brief presentation
1	27 Male	Left upper arm, left opisthenar	800 IU/10 ml	Lover and ex-girlfriend (nurse)	<2 h	The woman and the victim were having an affair, and she wanted to end the relationship. One evening, she injected 200 IU insulin into the man's left upper arm, but lied that it was vaccine. After he lost consciousness, she injected the remainder of the insulin intravenously at the left wrist. She then dumped the body into a lake after confirming he was dead.
2	27 Male	Right abdomen, left elbow	400 IU/10 ml	Girlfriend (nurse)	<10 h	The woman and the victim were going to marry in several months, but marriage plans were cancelled after some trials and tribulations, leading her to take revenge on the male victim. The woman deceived him into drinking a cup of water laced with clonazepam. Once he was asleep, she injected him with insulin.
3	55 Male	Left arm, middle abdomen	>1500 IU	Ex-wife (housewife)	<8 h	A woman and the victim had divorced because the man had had an affair, but they still cohabited and had some disputes over property. The woman was an IDDM patient with access to insulin, and after deceiving the victim into taking a drink laced with 2 pills of clonazepam, she injected him with insulin.
4	86 Female	Abdomen	1200 IU/20 ml	Daughter (housewife)	<6 h	The woman and her mother, the victim, were having difficulties. The mother was an IDDM patient and her daughter regularly injected insulin for her. With an intent to finish a painful life, she injected an overdose of insulin into her mother. She then attempted suicide but failed.

Table 2
Information about the autopsy and scene investigation.

Case no.	Postmortem interval before autopsy	Scene investigation	Type of insulin	Degree of decomposition
1	15 days (in water for 3 days and frozen for 12 days)	2 empty insulin bottles found in the victim's home, the primary scene.	Gansulin (Mixed Protamine Zinc Recombinant Human Insulin Injection)	Moderate
2	About 12 h	2 empty insulin bottles were found	Humulin (Recombinant Human Insulin Injection)	No
3	Frozen for 4 days	>10 empty insulin bottles were found	Novolin (Recombinant Human Insulin Injection)	No
4	Frozen for 10 days	4 empty insulin bottles were found	Insulin Aspart (Mixed Protamine Zinc Recombinant Human Insulin Injection)	No

Table 3
Results of postmortem biochemistry and detection of clonazepam.

Case no.	Glucose concentration of blood (mmol/ L)[a]	Glucose concentration of vitreous humor (mmol/ L)[b]	Insulin concentration of blood (mU/ L)[c]	Clonazepam of blood
1	12.16	/	2.14	(−)
2	9.08	0.85	0.2	(+)
3	/	/	0.2	(+)
4	11.4 mmol/L	0.9	2.5	(+)

[a] Reference values: 3.9–6.1 mmol/L.
[b] Reference values [9]: approximate 0.2 mmol/L.
[c] Reference values: approximate 5.0–18.0 mU/L.