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【 CASE REPORT 】

Biodistribution of Insulin Following Massive Insulin Subcutaneous Injection

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Abstract:

A man in his 30s injected insulin several times into his abdomen and was found dead several hours later. Micropathological findings showed alveolar injury with hemorrhaging and cerebral parietal lobe nerve cell edema. Biochemical examinations showed that the blood insulin level was high, significantly so at the insulin injection sites. The blood glucose and C-peptide levels were low. The insulin level in the kidneys was low. In forensic medicine, a postmortem diagnosis of insulin subcutaneous injection is often difficult. When insulin injection is suspected, particularly high insulin levels can be expected at the insulin injection site, rather than in the blood.

Key words: insulin injection, blood concentration, tissue concentration, glucose, pulmonary edema

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Introduction

Insulin is an endocrine hormone released by pancreatic β-cells and the only hormone in the body that decreases blood glucose levels. Insulin is widely used for treating diabetes mellitus (DM). Administration of an insulin overdose leads to hypoglycemia (1-3), which can be lethal and is occasionally seen in forensic autopsy cases. Hypoglycemia is also a risk factor for several other conditions, such as cardiovascular, endocrine, and central nervous system diseases (4-7). Severe hypoglycemia is a fatal condition and requires immediate medical intervention, such as intravenous glucose injection. In forensic medicine, a postmortem diagnosis of subcutaneous insulin injection is often difficult to make in cases of homicide, suicide, and accidental death (8-11) because the pathophysiology underlying the metabolism of massive insulin administered by subcutaneous injection is complex (12, 13).

We herein report results of an autopsy of a person who died because of a self-inflicted massive dose of insulin. The pathological and biochemical findings and the biodistribution of insulin were also examined.

Case Report

A man in his 30s who had had DM for several years (details unknown) was visited by his girlfriend one day at approximately 10:00 p.m. She had suicidal tendencies. The next day, she said to him that she wanted to commit suicide. He then suggested that together they each inject insulin into their bodies three times. At 1:30 p.m., they both injected insulin (Novolin 30R Flex Pen®) several times into their abdomens and then went to sleep. His girlfriend regained consciousness at approximately 6:00 p.m. She ate noodles and a cup of rice and then went to sleep again. Around 10:50 p.m. (approximately 9 hours after injection), she woke up again and checked on him. His body was cold, so she called an ambulance. However, the ambulance crew did not take him to the hospital, as cadaveric rigidity had already set in. An autopsy was performed one day later.

A search found six empty insulin preparations in the trash can, two insulin preparations on the table, and one insulin preparation in the refrigerator. The actual amount of insulin injected into each person was unclear.

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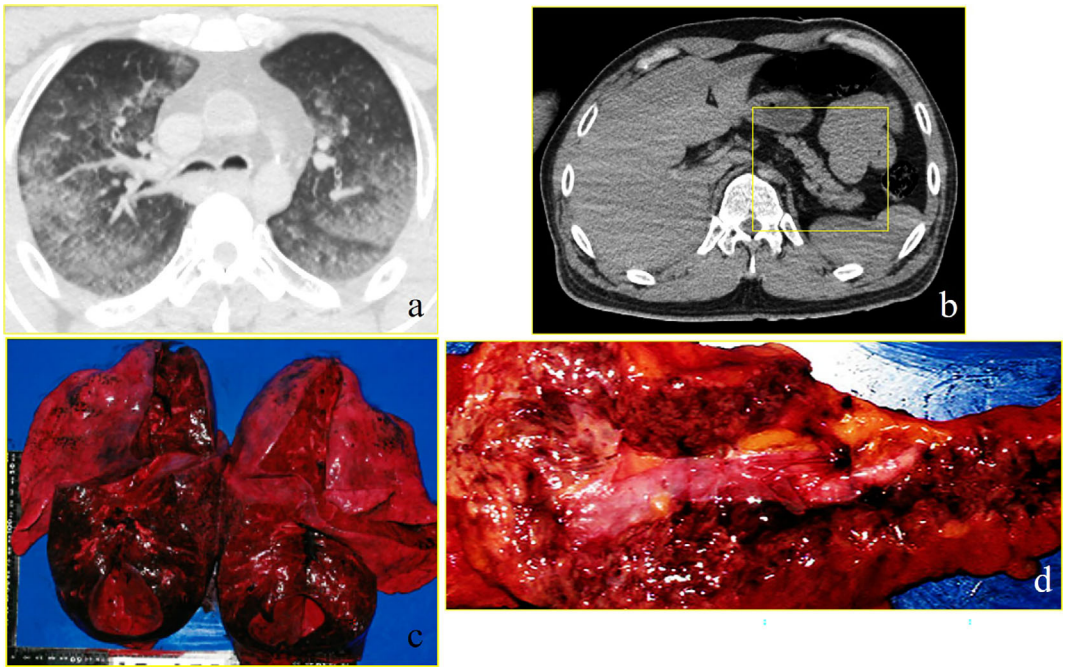

Figure 1. **(a) Chest morphology on postmortem CT (tracheal bifurcation level) frosted glass-formed high-absorption-range image indicating lung congestion and hypostasis of the blood. (b) Abdominal CT image showing a punctiform high-density area of the pancreas due to hemorrhaging. (c) Section of the lungs showing hemorrhaging and edema. (d) Internal hemorrhaging in the pancreas. CT: computed tomography**

Postmortem imaging examinations

Computed tomography (CT) showed lung edema (right and left lateral ventricle sites). Chest CT showed an increased vascular shadow on both sides of the lungs (Fig. 1a). A frosted glass-formed high-absorption-range image showed congestion of the lungs and hypostasis of the blood (tracheal bifurcation site; Fig. 1a). Abdominal CT showed hemorrhaging in the pancreas (Fig. 1b), and the fusion findings were unclear.

Macropathology

The case subject's height was 170 cm, and his weight was 76.5 kg. He had a dark reddish-purple hypostasis on his back and petechial hemorrhaging. His palpebral conjunctivae were edematous, with moderate to severe petechiae. There were 10 injection marks within an 8.5×4.5-cm area on the lower abdomen, with hemorrhaging in the subcutaneous fatty tissue (Fig. 2a-c).

The heart weighed 395 g, without petechial hemorrhaging on the surface. The left and right chambers were enlarged and filled with a large quantity of blood with soft clots. The heart cavity was expanded, and 350 mL of blood had pooled. In the endocardium, there were no petechiae. The lungs (left: 725 g; right: 605 g) were congested and edematous, showing multiple hemorrhaging on the cut surface, with no pleural effusion (Fig. 1c). The pancreas (115 g) was congested, with edema, subcapsular hemorrhaging, and internal stromal hemorrhaging in the cut section (Fig. 1d).

All other organs were edematous; the liver and brain weighed 2,630 and 1,410 g, respectively. No evidence of other types of pathology or trauma was found.

Micropathology

The subcutaneous fatty tissue hemorrhaging showed no inflammatory cells on hematoxylin and eosin (H&E) staining (Fig. 2d), and Berlin blue staining was negative. The pancreatic parenchyma showed diffuse findings of necrosis without inflammatory cells (Fig. 3a-i), and the pancreatic interstitial tissue was replaced with fatty tissue. The lungs showed moderate to severe alveolar injury and alveolar hemorrhaging (Fig. 3b-i). In addition, nerve cells in the cerebral parietal lobe were edematous (Fig. 3c-i). No other tissues showed any specific findings, except for congestion and edema (Fig. 3d-i, 3e-i, and 3f-i).

Immunohistochemical staining

Immunohistochemistry (IHC) for insulin was performed at the insulin injection site (Fig. 2e). Glucagon staining was negative (Fig. 2f). An examination of the islets of Langerhans demonstrated insulin and glucagon secretion. Anti-insulin and anti-glucagon antibody testing showed a paucity of islets of Langerhans. However, abundant glucagon-positive cells and scattered insulin positivity were seen (Fig. 3a-ii). There was insulin positivity in the alveolar cells in the lungs (Fig. 3b-ii), in the nerve cells in the cerebral parietal lobe (Fig. 3c-ii), in the proximal tubular cells in the kidneys (Fig. 3d-ii), and in the acidophiles in the adenohypophysis (Fig. 3e-ii), as well as partial insulin-positive findings in the liver (Fig. 3f-ii).

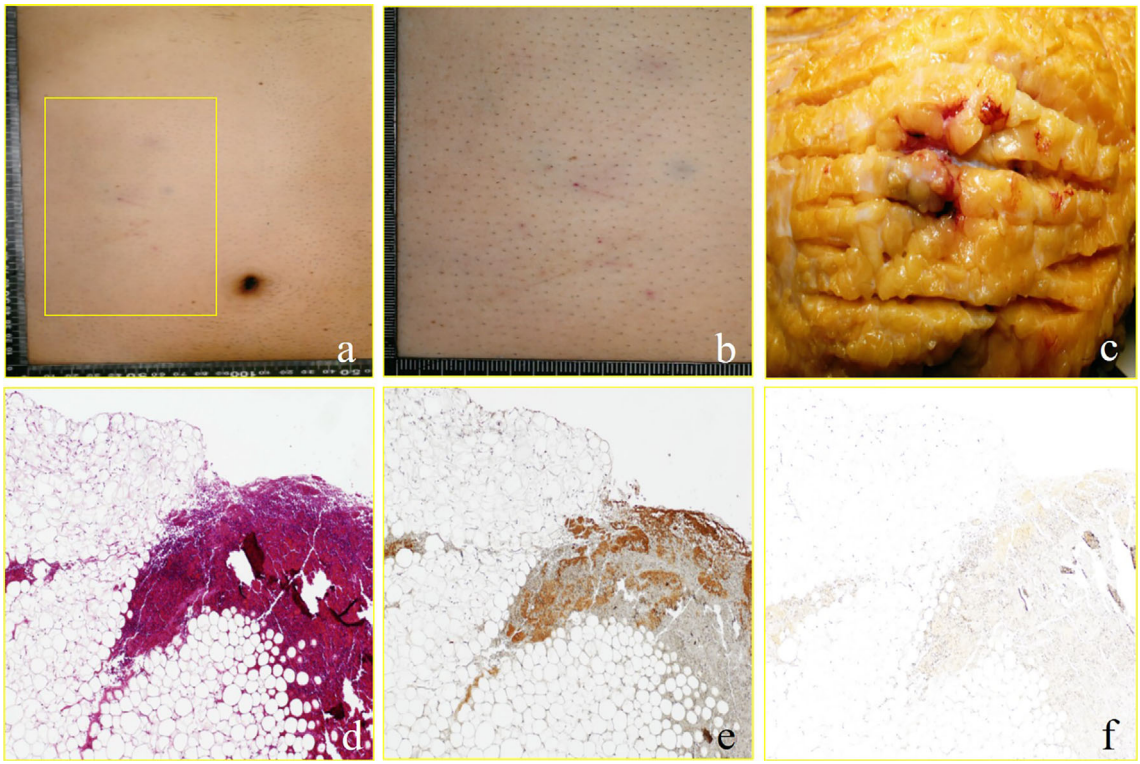

Figure 2. **(a) Abdominal insulin injection marks. (b) Magnified image of the abdominal injection insulin sites. (c) The subcutaneous tissue of an abdominal injection site. Histopathological and IHC findings show hemorrhaging in the subcutaneous fatty tissue due to abdominal injection. (d) Hematoxylin and Eosin staining, (e) insulin immunostaining, and (f) glucagon immunostaining (magnification ×100). IHC: immunohistochemistry**

Biochemical findings

Insulin measurement

In the present case, insulin was measured using a chemiluminescent enzyme immunoassay. In brief, 50 mg of tissue were lysed using T-PER Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, USA) and Protease Inhibitor Cocktail Set V (Wako Pure Chemical, Osaka, Japan) for the assay. The blood insulin level was high in the abdominal subcutaneous fatty tissue at the insulin injection site (200,000 μIU/mL) and in the pancreatic tissue (13,300 μIU/mL). In addition, the right heart blood showed a slightly higher insulin level (374 μIU/mL) than the left heart blood (163 μIU/mL), and the insulin level in the iliac vein blood was the highest (5,640 μIU/mL). The insulin level was also high in the vitreous humor (right: 44.7 μIU/mL) and cerebrospinal fluid (CSF; 41.2 μIU/mL). In other body fluids and tissues, the insulin level ranged from 0.57 μIU/mL (bile) to 37.9 μIU/mL (pericardial fluid). Table 1 shows the details of the insulin levels in the present case.

Cases of acute psychotropic drug intoxication and asphyxia were also examined as controls. The left heart blood, right heart blood, and iliac vein blood insulin levels were 0.3-2.13 μIU/mL. In the controls, pancreatic tissue showed a low insulin level (acute psychotropic drug: 5.4 μIU/mL; asphyxia: 5.25 μIU/mL) compared to the present case. The CSF (acute psychotropic drug: 1.17 μIU/mL; asphyxia: 0.7 μIU/mL) and vitreous humor (acute psychotropic drug: 0.3 μIU/mL; asphyxia: 0.3 μIU/mL) also showed low insulin levels compared to the present case.

The insulin level in the abdominal subcutaneous fatty tissue was not measured. The insulin levels in the controls were as follows: left heart blood, 0.3 μIU/mL; pericardial fluid, 0.3 μIU/mL; and vitreous humor, 0.3 μIU/mL. In the acute psychotropic drug case, urine showed a high insulin level (21.2 μIU/mL). In the asphyxia case, heart septal tissue showed a high insulin level (64.6 μIU/mL). Table 2 shows the details of the insulin levels in the control cases.

Other measurements

Glucose levels in the left heart blood (2 mg/dL), right heart blood (1 mg/dL), and right vitreous humor [<20 mg/dL (less than the measurement limit)] were low compared to forensic reference values (14-17). The left and right heart plasma C-peptide levels were extremely low (left: 0.06 ng/mL; right: 0.12 ng/mL). The right heart blood HbA1c level was 6.9%. The right heart blood acetoacetate and 3-hydroxybutyric acid levels were <2 and 178 μmol/L, respectively, and total ketone bodies (178 μmol/L) did not show ketosis levels. Plasma levels of autoantibodies associated with the islets of Langerhans, specifically anti-glutamic acid decarboxylase (GAD) antibody and anti-insulinoma antigen 2 antibody, were 10.0 U/mL (clinically normal range <1.5 U/mL) and <0.4 U/mL (clinically normal range <0.4 U/mL), respectively.

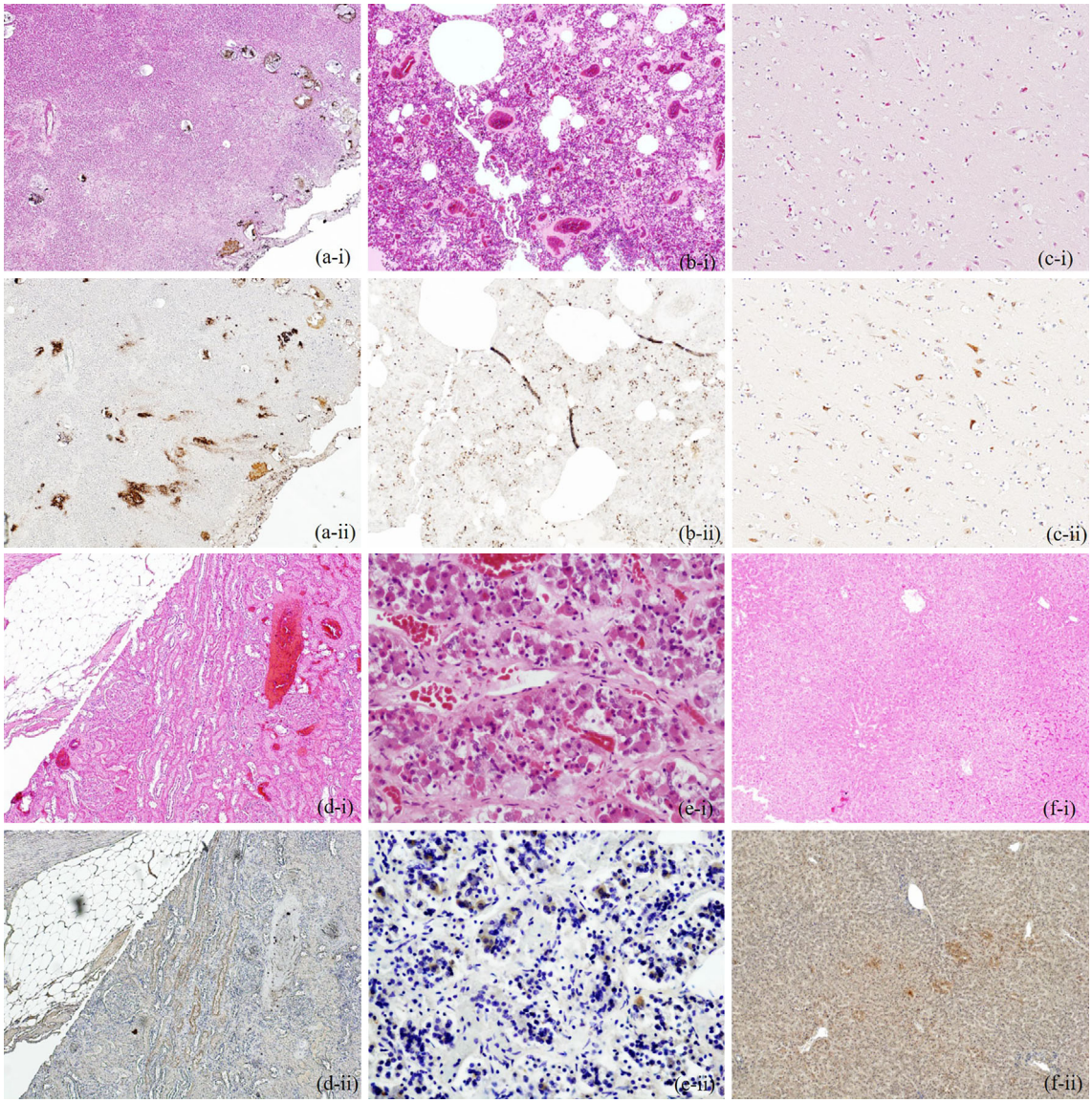

Figure 3. **(a) (i) Fusion findings of pancreatic tissue [Hematoxylin and Eosin (H&E) staining; magnification ×40]. (ii) Insulin-positive immunostaining in the pancreas. The islets of Langerhans do not show any insulin-positive findings (magnification ×40). (b) (i) Congestion, edema, and alveolar injury in the lung tissue (H&E staining; magnification ×40). (ii) Insulin-positive immunostaining in alveolar cells (magnification ×40). (c) (i) Acidophilic changes and edema in nerve cells in the cerebral parietal lobe (H&E staining; magnification ×100). (ii) Insulin-positive immunostaining in nerve cells in the cerebral parietal lobe (magnification ×100). (d) (i) Congestion in kidney tissue (H&E staining; magnification ×40). (ii) Insulin-positive immunostaining in the proximal tubular cells of the kidney (magnification ×40). (e) (i) Adenohypophysis acidophiles (H&E staining; magnification ×100). (ii) Insulin-positive immunostaining in the adenohypophysis (magnification ×100). (f) (i) Sinusoidal congestion in the liver tissue (H&E staining; magnification ×40). (ii) Partly insulin-positive hepatocellular findings (magnification ×40).**

The right heart blood C-reactive protein level was 0.51 mg/mL (forensic cut-off value <2.0 mg/mL), and the neopterin level as a marker of systemic inflammation was 478 pmol/mL (forensic cut-off value <200 pmol/mL). Allergy-related biochemical examinations showed no abnormalities (right heart blood: immunoglobulin E, 82.4 IU/mL; histamine, 107.0 ng/mL; tryptase, 5.60 μg/L).

There was no renal or liver dysfunction.

Postmortem microbiology

A mitochondrial DNA 3243 mutation [AAGATGGCAG (A/G) GCCCGGTAAT] (18) was detected in whole blood.

Toxicology

Alcohol was detected in several body fluids, as follows: left heart blood, 0.60 mg/mL; right heart blood, 0.60 mg/mL; urine, 1.36 mg/mL; and gastric contents, 0.84 mg/mL.

Table 1. Insulin Levels in the Body Fluids and Tissues in the Subject Case.

Insulin concentration (tissue)		Insulin concentration (body fluid)	
Sample	Insulin (μIU/mg)	Sample	Insulin (μIU/mL)
Abdominal fat tissue	10,000	Iliac vein blood	5,640
Pancreas	665	Right heart blood	374
Kidney (right)	1.01	Left heart blood	163
Kidney (left)	0.89	Vitreous humor (right)	44.7
Pituitary gland	0.81	Cerebrospinal fluid	41.2
Lung (left upper lobe)	0.64	Pericardial fluid	37.9
Right atrium	0.55	Urine	2.59
Left atrium	0.51	Bile	0.57
Right ventricle	0.45	Gastric contents	0.46
Lung (right upper lobe)	0.43		
Ventricular septum	0.42		
Liver	0.38		
Femoral muscle (right)	0.17		
Left ventricle	0.17		
Femoral muscle (left)	0.14		
Hippocampus	0.08		
Midbrain (substantia nigra)	0.08		
Cerebrum (parietal lobe)	0.07		

Table 2. Insulin Levels in the Body Fluids and Tissues of Acute Psychotropic Drug Intoxication and Asphyxia Cases (Control Cases).

Acute drug intoxication				Asphyxia			
Insulin concentration (organs)		Insulin concentration (body fluid)		Insulin concentration (organds)		Insulin concentration (body fluid)	
Sample	Insulin (μIU/mg)	Sample	Insulin (μIU/mL)	Sample	Insulin (μIU/mg)	Sample	Insulin (μIU/mL)
Abdominal fat tissue	-	Urine	21.2	Abdominal fat tissue	-	Right heart blood	2.13
Kidney (left)	0.92	Bile	6.47	Ventricular septum	3.23	Bile	1.54
Kidney (right)	0.53	Cerebrospinal fluid	1.17	Left ventricle	2.36	Cerebrospinal fluid	0.70
Ventricular septum	0.38	Iliac vein blood	0.80	Kidney (right)	1.50	Iliac vein blood	0.57
Liver	0.36	Right heart blood	0.51	Kidney (left)	1.18	Urine	0.45
Right ventricle	0.28	Left heart blood	0.30	Left atrium	0.94	Left heart blood	0.30
Pancreas	0.27	Vitreous humor (right)	0.30	Lung (left upper lobe)	0.81	Vitreous humor (right)	0.30
Left ventricle	0.27	Pericardial fluid	0.30	Right ventricle	0.80	Pericardial fluid	0.30
Lung (right upper lobe)	0.22	Gastric contents	-	Lung (right upper lobe)	0.69	Gastric contents	-
Left atrium	0.21			Right atrium	0.36		
Lung (left upper lobe)	0.21			Liver	0.32		
Right atrium	0.18			Pancreas	0.26		
Pituitary gland	0.14			Midbrain (substantia nigra)	0.14		
Midbrain (substantia nigra)	0.12			Femoral muscle (left)	0.14		
Hippocampus	0.06			Cerebrum (parietal lobe)	0.14		
Femoral muscle (left)	0.05			Hippocampus	0.07		
Femoral muscle (right)	0.05			Femoral muscle (right)	0.04		
Cerebrum (parietal lobe)	0.03			Pituitary gland	Below the level of detection.		

Findings of drug screening, including blood screening for amphetamines and psychotropic drugs using immunoassay and gas chromatography-mass spectrometry, were negative (19).

Discussion

The present case subject had severe pulmonary edema and thus had likely been in a state of advanced, fatal circulatory failure at the time of death. His blood insulin levels were high compared to blood C-peptide levels (14, 20). Insulin

levels in the abdominal subcutaneous fatty tissue were high compared to those in other body fluids and tissues, and the blood and vitreous humor glucose levels were extremely low (21, 22). Therefore, the cause of death was determined to be circulatory failure associated with hypoglycemia due to subcutaneous injection of a massive dose of insulin into the abdominal wall. In the present case, heart blood included coagulated blood and a fibrin clot, which are findings of subacute or prolonged death on forensic pathological findings. Furthermore, on forensic pathology, there were no petechiae in the endocardium, which are seen with arrhythmia. Thus, the findings of death due to fatal arrythmia associated with hypoglycemia were not seen on the specialist forensic pathological examination. In the present case, the left and right heart plasma C-peptide levels were extremely low (left: 0.06 ng/mL; right: 0.12 ng/mL). Insulin secretion might have remained. One piece of evidence for this is that C-peptide did not dry up.

No findings suggested anaphylactic shock caused by a massive insulin dose. Because the ketone levels were also low, no findings suggested diabetic ketoacidosis (23, 24).

The initial police investigation found that the case subject had type 2 DM and was taking insulin. However, a biochemical examination of the blood during the autopsy confirmed positive anti-glutamic acid decarboxylase antibody and the mitochondrial DNA 3243 mutation (25, 26). DM due to the mitochondrial DNA 3243 mutation is reported to account for 0.5-1% of the general diabetic population in Japan (27, 28), although the present case did not have short stature or deafness. On immunostaining for insulin, the islets of Langerhans were negative. Therefore, the present case was classified as atypical type I DM. The present case of GAD antibody-positive type I DM with mitochondrial DNA 3243 mutation is rare (25).

Insulin levels in the body fluids and tissues were compared to those in control cases (acute psychotropic drug intoxication and asphyxia). The insulin level in the present case was highest in the abdominal subcutaneous fatty tissue at the insulin injection site and second highest in the pancreas. Visual pancreatic findings confirmed interstitial hemorrhaging, and the pancreatic parenchyma showed signs of liquefactive necrosis. It is known that insulin targets fat, muscle, and the liver. In addition, in the brain, insulin receptor substrate (IRS), which is the main substrate of insulin receptors, is present, along with insulin receptors (29). Important targets of insulin distribution are fat, muscle, the liver, the brain, and the pancreas (β cells), and these have all been identified to play an important role in blood sugar and energy metabolism (30). However, in the present case, the insulin levels in the liver, muscle, and brain were low, except for at the insulin injection site. It is not possible to determine why there was a high insulin level only in the pancreas, as insulin receptors and IRS-1 do not act to promote insulin secretion. It is unlikely that exogenous insulin promotes insulin secretion through them.

The reason for the high insulin level in iliac vein blood is unclear; it may be that insulin circulation did not have time to stabilize, as the insulin level was higher in the right heart blood than in the left heart blood. The subject likely died relatively quickly after injection. The high insulin level in the iliac vein blood was probably because of dispersion caused by subcutaneous injection of insulin into the abdominal wall. The higher insulin level in the pericardial fluid than in the myocardial tissue was probably because a specific insulin level was maintained in the venous system rather than due to insulin acting on the heart in the early stage, given the high insulin level in iliac vein blood compared to right heart blood. In the liver (31) and brain (32, 33), where insulin action is notable, the insulin level was low in the case subject, showing no notable difference from control cases. The high insulin level in the CSF was probably due to influx from the venous system in the choroid plexus (34). In the vitreous humor, in which diabetes-related factors such as glucose were relatively stable, the insulin level was high (35, 36). However, why the glucose and insulin levels were stable in the vitreous humor and the stage they represent are unclear.

The plasma half-life of insulin is short, generally 4-5 min (11, 37). However, in the case subject, there were extremely high insulin levels in the abdominal subcutaneous fatty tissue, pancreas, heart blood, and iliac vein blood. Insulin injected into fatty tissue is believed to have a decreased rate of metabolism, and the measurement of the tissue insulin level is believed to be useful, even if the blood insulin level is low. These results indicated that the case subject died either shortly after injecting insulin or after long-term continuous insulin administration. Generally, insulin is metabolized by the kidneys; therefore, high insulin levels in the kidneys and urine may be expected in acute psychotropic drug intoxication cases. However, the insulin levels in the pancreas, kidneys, and urine of the case subject indicated that he died shortly after injection of a massive dose of insulin rather than following continuous insulin administration.

The present case had findings of rigor mortis within about 9 hours after the insulin injection. Novorin 30R is a mixed preparation of fast-acting insulin and middle model insulin. The action of Novorin 30R takes approximately 30 minutes to develop. In addition, the duration of action is 2-8 hours. However, it is difficult to accurately estimate the postmortem period based only on the posthumous findings, as the findings of cadaveric rigidity can change due to various factors, such as the outside and internal temperature. Thus, there was no clear contradiction between the post-injection elapsed time and timing of rigor mortis in the present case.

Immunostaining for insulin in each type of tissue was related to the interstitial insulin levels, and there were positive findings in each tissue. Particularly in the exocrine pancreas, immunostaining was positive for insulin, suggesting insulin in the pancreas.

Hypoglycemia due to insulin administration has no specific findings. Cases in which hypoglycemia is suspected ac-

cording to the medical history or autopsy findings require biochemical and toxicological examinations. When insulin administration in fatty tissue is suspected, high insulin levels can be expected at the insulin injection site rather than in the blood.

Conclusion

Measuring insulin levels in tissues can aid in the diagnosis of insulin-induced hypoglycemia. In addition, measuring insulin levels in the body fluids and tissues can aid in determining the pathophysiology of insulin injection cases. When insulin administration in fatty tissue is suspected, particularly high insulin levels can be expected at the insulin injection site rather than in the blood.

The authors state that they have no Conflict of Interest (COI).

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