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Quantitative RT-PCR assays of hypoxia-inducible factor-1α, erythropoietin and vascular endothelial growth factor mRNA transcripts in the kidneys with regard to the cause of death in medicolegal autopsy

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Abstract

Accumulating studies demonstrate that the expressions of hypoxia-inducible factor 1 (HIF-1), erythropoietin (EPO) and vascular endothelial growth factor (VEGF) depend on cellular oxygen tension, which is involved in the pathological process of tissue hypoxia and/or ischemia. The present study investigated hypoxia-inducible factor-1α (HIF-1α), EPO and VEGF mRNA expressions in the kidney with regard to the cause of death in medicolegal autopsy. Relative quantifications of HIF-1α, EPO and VEGF mRNAs, based on real-time TaqMan reverse transcription-polymerase chain reaction (RT-PCR), were performed on tissue specimens obtained from consistent sites of the bilateral renal cortices. The cases (total, $n = 245$, 6–48 h postmortem) included fatal blunt/sharp instrument injuries ($n = 53/31$), asphyxia ($n = 28$: aspiration, $n = 8$; strangulation/hanging, $n = 20$), drowning ($n = 27$), fire fatality ($n = 62$), acute myocardial infarction/ischemia (AMI, $n = 39$), and gastrointestinal hemorrhage ($n = 5$). Both HIF-1α and EPO mRNA levels were significantly lower in drowning cases. More characteristic findings were found for VEGF mRNA: it showed higher expression levels for AMI, acute blunt/sharp instrument injury, and aspiration, whereas it was lower for neck compression (strangulation/hanging), drowning, fire fatality with higher blood carboxyhemoglobin (COHb) levels ($> 60\%$), peracute blunt injury, and gastrointestinal hemorrhage. Quantitative assays of renal HIF-1α, EPO and VEGF mRNA transcripts are potentially useful for investigating the pathophysiology of death, and VEGF mRNA may be especially useful as an indication of acute circulatory failure.

Keywords: Hypoxia-inducible factor-1α; Erythropoietin; Vascular endothelial growth factor; Kidney; Messenger ribonucleic acid; Postmortem molecular biology; Cause of death

1. Introduction

Various extents of tissue hypoxia and/or ischemia are involved in the process of death. Quantitative evaluations of these pathophysiological changes, besides morphological observations, are expected for an evidence-based medicolegal diagnosis. Mammalian cells express multiple gene products in response to oxygen deficiency, such as hypoxia-inducible factor 1 (HIF-1), erythropoietin (EPO) and vascular endothelial growth factor (VEGF), which allow cellular metabolic adaptation to decreased oxygen availability [1–3]. The inductions of messenger ribonucleic acid (mRNA) transcripts of these three factors are closely related, although some differences between EPO and VEGF are known [4–9]. Previous immunohistochemical studies showed that normal or pathological expressions of EPO and VEGF were mainly found in proximal tubular cells and interstitial cells in human kidney [10,11]. These factors experimentally showed time-dependent expressions in response to tissue ischemia [9,12]. However, transcripts,

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as the forerunner of proteins, are anticipated to be more rapidly inducible than the latter. For the investigation of ischemic changes in the human myocardium, a previous study suggested the early expression of angiogenic factors (HIF-1 and VEGF) in acute myocardial ischemia and infarction [13]. To investigate systemic responses to circulatory failure and/or hypoxemia in acute deaths, combined analyses of these induced mRNAs in the kidneys, which play important roles in response to related pathophysiological changes [9,12], might be useful for medicolegal diagnosis of acute and even peracute deaths. However, practical data have not yet been established, possibly in part due to postmortem interference [14]. In previous studies, we suggested potential applications of quantitative mRNA assays in autopsy materials [15,16].

In the present study, quantitative RT-PCR assays of renal HIF-1α, EPO and VEGF mRNA transcripts were performed in serial medicolegal autopsy cases to examine their applicability in investigating the pathophysiology of death.

2. Materials and methods

2.1. Subjects and samples

Serial medicolegal autopsy cases within 48 h postmortem at our institute were examined: total, $n = 245$; 166 males and 79 females; 12–89 (median, 62.5) years of age (Table 1). Based on routine macromorphological, micropathological, biochemical and toxicological findings, the causes of death were classified as fatal blunt/sharp instrument injuries ($n = 53/31$), asphyxia ($n = 28$: aspiration, $n = 8$; strangulation/hanging, $n = 20$), drowning ($n = 27$), fire fatalities ($n = 62$) including cases with lower ($< 60\%$) and higher ($> 60\%$) blood carboxyhemoglobin (COHb) levels ($n = 35$ and 27, respectively), acute myocardial infarction/ischemia (AMI, $n = 39$), and gastrointestinal hemorrhage ($n = 5$). For these groups, clearly accountable cases were collected, excluding cases involving complications that may have influenced the process of death, and also cases having evident pathological renal disorders and/or blood urea nitrogen (BUN) more than 50 mg/dl [17–20]. The AMI group included cases without any morphological or toxicological evidence of the cause of death other than a cardiac attack [21,22].

Tissue specimens were taken from the consistent sites in the renal cortex on both the left and right kidneys during autopsy, then immediately submerged in 1 ml of RNA stabilization solution (RNAlaterTM, Ambion, Austin) and stored at 4 °C for less than 1 week until RNA extraction. Total RNA was isolated with ISOGEN (Nippon Gene, Toyama) according to the manufacturer's instructions, quantified spectrophotometrically, and stored at −80 °C until use.

2.2. TaqMan RT-PCR and relative quantification of mRNA transcripts

TaqMan RT-PCR was performed in a 50 μl reaction system with the use of the TaqMan EZ RT-PCR kit and TaqMan Gold RT-PCR kit, on an ABI PRISM 7700 Sequence Detector (Perkin–Elmer Applied Biosystems, Foster City). The contents of the amplification mix and the thermal cycling conditions were set according to the accessory protocols for the two TaqMan RT-PCR kits, respectively [16]. To normalize for differences in the amount of total RNA added to the reaction, amplification

Table 1
Case profiles ($n = 245$)

Cause of death	n	Male/female	Age (years)		Survival time (h)	PMI (h)		BUN (mg/dl)		Cr (mg/dl)	
			range	median		range	median	range	median	range	median
Asphyxia											
aspiration	8	6/2	12–78	62.5	< 0.5	16–42	24	8.3–24.5	14.8	0.3–4.7	1.5
strangulation/hanging	20	11/9	14–78	57.0	< 0.5	8–48	23	6.6–30.5	14.8	0.9–6.6	1.7
AMI	39	29/10	31–89	64.0	< 0.5–24	6–48	19	4.7–39.7	16.8	0.8–5.4	1.7
Drowning											
freshwater	21	12/9	42–88	62.0	< 0.5	8–48	20	6.5–44.6	13.8	0.1–9.6	1.0
saltwater	6	5/1	34–65	53.0	< 0.5	9–47	16	5.8–27.9	18.0	0.8–2.3	1.0
Fire fatalities											
COHb < 60%	35	22/13	32–89	72.0	< 0.5	6–35	15	2.7–47.9	15.0	0.7–5.8	1.6
COHb > 60%	27	18/9	32–87	65.0	< 0.5	6–40	12	7.0–38.7	13.8	0.4–6.6	1.3
Gastrointestinal hemorrhage	5	5/0	50–60	56.0	2–12	12–22	15	28.2–48.0	41.6	1.3–2.5	1.9
Blunt injury											
peracute death*	14	9/5	25–78	49.5	< 0.1	8–40	19	11.0–26.0	17.4	0.4–2.0	1.3
acute death	39	26/13	16–83	52.0	<0.5	8–39	17	5.7–27.8	13.9	0.2–3.0	1.3
Sharp instrument injury-acute death	31	23/8	19–81	46.0	< 0.5	6–47	15	0.3–36.7	10.9	0.5–5.8	1.2
Total	245	166/79	12–89	62.5	< 24	6–48	18	0.3–48.0	14.7	0.1–9.6	1.4

PMI, postmortem interval; AMI, acute myocardial infarction/ischemia; COHb, blood carboxyhemoglobin; BUN, right heart blood urea nitrogen; Cr, right heart blood creatinine; *, cases of multiple traumas involving severe brain and/or cardiac injury.