A Stable *in Vitro* Method for Assessing the Toxicity of Potassium Cyanide and Its Antidote

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Background: Hydrogen cyanide possesses a high acid-dissociation constant of 9.14, favoring its vaporization and depletion from the culture media at physiological pH, which may cause the cyanide toxicity unstable *in vitro*.
Objective: We investigated whether adjustment of culture medium pH stabilizes cyanide concentration and decreases the effective concentration of potassium cyanide (KCN).
Methods: Murine fibroblast cells were exposed to different concentrations of KCN in media maintained at pH 7.4 or 9.2, in the presence or absence of hydroxocobalamin. After incubation for 1 h, we evaluated medium pH, cyanide concentration, cytochrome activity, and cell viability.
Results: Cyanide concentration decreased to 18.8% in pH 7.4 medium compared to 83.2% in pH 9.2 medium. A significant decrease in cytochrome activity was observed at 40 mM and 1.25 mM KCN in pH 7.4 and pH 9.2 media, respectively. In pH 9.2 medium, dose-dependent cytotoxicity of KCN and antidotal effects of hydroxocobalamin were observed.
Conclusion: Adjustment of culture medium pH to 9.2 could stabilize cyanide concentration and decrease the effective concentration of KCN, allowing stable evaluation of KCN toxicity and antidotal efficacy.

Key words: Cyanide poisoning, antidote, pH, hydrogen cyanide, hydroxocobalamin

INTRODUCTION

Cyanide is widely used for industrial purposes, but may also be exploited as a suicidal, homicidal, and chemical warfare agent [1, 2]. Acute cyanide poisoning induces rapid and lethal neurological symptoms that require immediate and vigorous medical treatment [3, 4].

Many researchers have studied the toxicity profile of cyanide in vitro by using cultured cells and in vivo in experimental animals [5–7]. Consideration of animal welfare has led to efforts focused on determining whether in vitro data can be extrapolated to assess acute toxicity in experimental animals and humans [8–13]. However, a weak correlation was found between the cytotoxicity of potassium cyanide (KCN) in vitro and its acute toxicity in vivo [14–16], because the ratio of in vitro median effective concentration (EC_{50}) to human lethal dose low (LD_{Lo}) for KCN is much higher than that of other toxic chemical compounds [16]. This discrepancy could result from the existence of in vitro conditions that do not resemble specific mechanisms of neurotoxicity in vivo [11]. Thus, the pheochromocytoma cell line (PC12) has been frequently used to evaluate in vitro neurotoxicity of KCN, because the cells are similar to sympathetic neurons [5, 7, 17, 18]. However, in undifferentiated PC12 cells exposed to KCN for 1 h, the EC_{50} was 2.5 mM [5], which is higher than the lethal blood concentration in humans. On the other hand, in differentiated PC12 cells treated with nerve growth factor, the EC_{50} of KCN was 0.1 mM following 24-h exposure [18]. These findings suggest that the in vitro assay itself may be one of reasons for a weak correlation between in vitro cytotoxicity and in vivo acute toxicity, in addition to the specific neurotoxicity of KCN in neurons.

In vitro toxicity of KCN can be affected by many factors including medium composition, pH of the medium, container forms, and incubation temperature [19, 20]. Since the acid dissociation constant of hydrogen cyanide (HCN) is 9.14 at 25℃, physiological pH accelerates vaporization of HCN from the culture medium and drastically decreases cyanide concentration. Arun P *et al.* reported that the pH of the culture medium is an important determinant, among various factors, of cyanide removal from the medium [19].

The objective of the present study was to investigate whether adjustment of pH from 7.4 to higher than 9.14 could stabilize the concentration of cyanide in the culture medium and decrease the effective concentration of KCN. Furthermore, we evaluated the antidotal effect of hydroxocobalamin (OHCbl) against KCN-induced toxicity under these conditions.

MATERIALS AND METHODS

Reagents

KCN and trypan blue were purchased from Wako (Japan). Fetal bovine serum (FBS), alpha-minimum essential medium (α-MEM), minimum essential medium (MEM) and Roswell Park Memorial Institute (RPMI)-1640 were purchased from Gibco-Invitrogen (Grand Island, NY, USA). HEPES and 3-(4, 5-dimethyl-2-thiaz-

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olyl)-2, 5-diphenyl-2H- tetrazolium bromide reagent (MTT) were from Dojindo (Japan), and OHCbl was purchased from Merck Serono (Tokyo, Japan).

Cell Culture and Media

NIH-3T3 murine fibroblast cells were obtained from Cell Bank (RIKEN, Japan). The cells were seeded onto a 96-well microplate at a density of 1×10^4, and grown in α MEM containing 2200 mg·L^{-1} of sodium bicarbonate ($NaHCO_3$), 1000 mg·L^{-1} of glucose 100 U·mL^{-1} penicillin, 100 U·mL^{-1} streptomycin, and 250 U·mL^{-1} amphotericin. The cells were maintained at 37℃ in a humid atmosphere of 5% CO_2 and 95% air.

KCN and OHCbl were diluted using three types of media including the complete medium with $NaHCO_3$ (described above), the complete medium with HEPES, or the glucose-serum-free medium. The complete medium with HEPES was composed of MEM, supplemented with 10 mM HEPES, 20% FBS, 1% glutamine, 1000 mg·L^{-1} glucose, and maintained at 37 ℃ incubator with 100% air. The glucose-serum free medium was composed with RPMI-1640 without glucose, supplemented with 10 mM HEPES, and maintained at 37 ℃ incubator with 100% air.

To adjust the pH of the culture media, 1 N hydrochloric acid and 1 N sodium hydroxide were added to the media immediately before culture media replacement.

MTT Assay

Cytochrome activity was assessed using the MTT assay, as reported by Mosmann [21]. Briefly, cells plated in a 96-well microplate were washed twice using phosphate-buffered saline (Invitrogen), after which 100 μL of 0.5 mg·mL^{-1} of MTT was added to each well and incubated for 5 h. Approximately 100 μL of 20% sodium dodecyl sulfate was then added and incubated for 12 h. Finally, the absorbance was measured at a wavelength of 570 nm using a spectrophotometer (Hitachi, Japan); the readings were recorded as $\Delta OD^{570}/1 \times 10^4$ cells.

Cell Viability Assay

Cell viability was determined using the trypan blue dye exclusion (TBDE) assay. Adherent cells cultured in a 96-well plate were collected by trypsinization. An aliquot of the cell suspension was diluted 1 : 1 with 0.4% trypan blue solution and the cells were counted using a hemocytometer (Burker-turk line, Erma, Japan). Cell viability was expressed as the percentage of trypan blue-negative cells.

Measurement of Cyanide Concentration

Cyanide concentrations in the media were measured using headspace gas chromatography mass spectrometry (HS-GC-MS) as reported by Seto [22]. Briefly, 100 μL of the KCN solution was added to a 4-mL clear vial (Sigma-Aldrich, St. Luis, MO, USA) and immediately sealed. After heating for 30 min at 70℃, the evaporated sample was extracted and injected into a gas chromatograph equipped with a nitrogen phosphorus detector (Agilent 6890, Agilent Technologies, USA), and the peak area of HCN was measured using HS-GC-MS. A standard curve was constructed by plotting the HCN peak areas against the respective concentrations of the KCN solution (0.625–5.0 mM). Cyanide concentrations in the media were calculated based on the HCN areas obtained.

Statistical Analysis

Statistical analysis was performed using SPSS II for Windows 21.0.1J (SPSS Inc., Tokyo, Japan). The data were subjected to the Mann–Whitney *U* test unless otherwise stated. A p value less than 0.05 was considered statistically significant.

RESULTS

Influence of pH on Cytochrome Activity

After removing the complete medium with $NaHCO_3$, the cells were treated with the complete medium with HEPES, with its pH adjusted from pH 4 to 10, and subjected to the MTT assay. A significant decrease in cytochrome activity was observed in cells with pH levels below 5 and above 9.6, compared to that observed at pH 7 (Fig.1A).

Additionally, the cells were treated with the complete medium with HEPES adjusted to pH 9.2 for various periods between 1 and 4 h and then subjected to the MTT assay. Although no significant decrease was observed when the cells were cultured in the complete medium with HEPES (pH 9.2) for 1 h, culture for periods longer than 2 h resulted in a significant decrease in cytochrome activity compared to that in the non-cultured cells (Fig. 1B).

Stability of the pH of the Culture Media

Culture medium pH was measured using a pH meter (TOADK, Tokyo, Japan) immediately after preparing different concentrations (0−40 mM) of KCN by using the complete medium with HEPES or the complete medium with $NaHCO_3$. The pH of the medium increased in response to KCN in a dose-dependent manner in both media tested (Fig. 2A).

Next, time-dependent pH change was compared between the complete medium with $NaHCO_3$ and the complete medium with HEPES. The pH of each medium containing 0, 5, or 10 mM of KCN was adjusted to 7.40 ± 0.05 or 9.20 ± 0.05, and the medium was incubated for 1 h. The pH of the KCN-containing complete medium with $NaHCO_3$ (pH 7.4) increased to approximately 7.8 after incubation for 1 h (Fig. 2B). Conversely, the pH of the KCN-containing complete medium with HEPES (pH 9.2) remained at approximately 9.2 for the entire incubation period (Fig. 2C).

Changes in Cyanide Concentration

To compare cyanide concentrations in the two types of media, 200 μL of complete medium with $NaHCO_3$ (pH 7.4) or complete medium with HEPES (pH 9.2) containing 5 mM of KCN was added to each well of a 96-well microplate and incubated for 1 h. Cyanide concentrations decreased to 0.94 ± 0.55 mmol·L^{-1} (18.8% of the initial concentration) in complete medium with NaHCO (pH 7.4), whereas it decreased to 4.16 ± 0.47 mmol·L^{-1} (83.2 % of the initial concentration) in the complete medium with HEPES (pH 9.2) (Fig. 3).

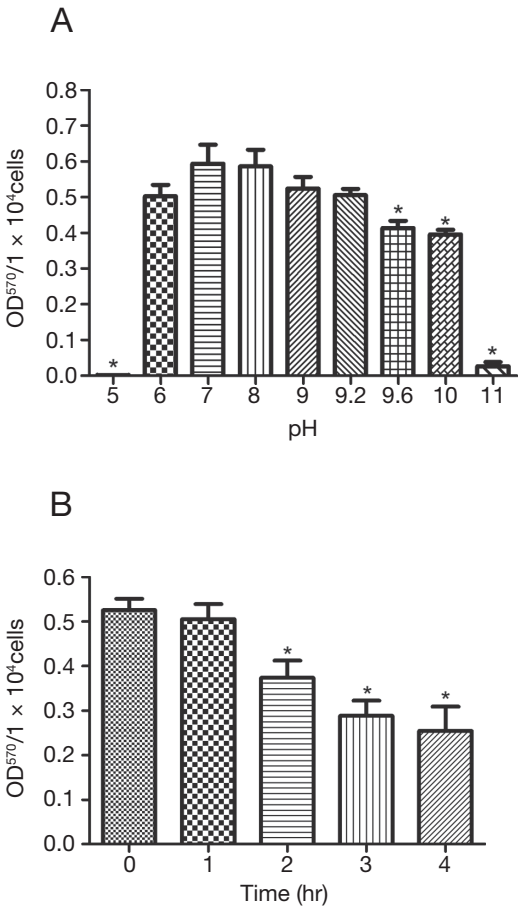

Fig.1 Influence of pH on Cytochrome Activity
A) Cells were cultured in 96-well plates using the complete media with HEPES adjusted to various pH levels (n = 5) for 1 h. *Significant at $p < 0.05$ compared to pH 7.
B) Cells were cultured for various periods in 96-well plates in the complete medium with HEPES with pH adjusted to 9.2 (n = 5). *Significant at $p < 0.05$ compared to 0 h incubation.

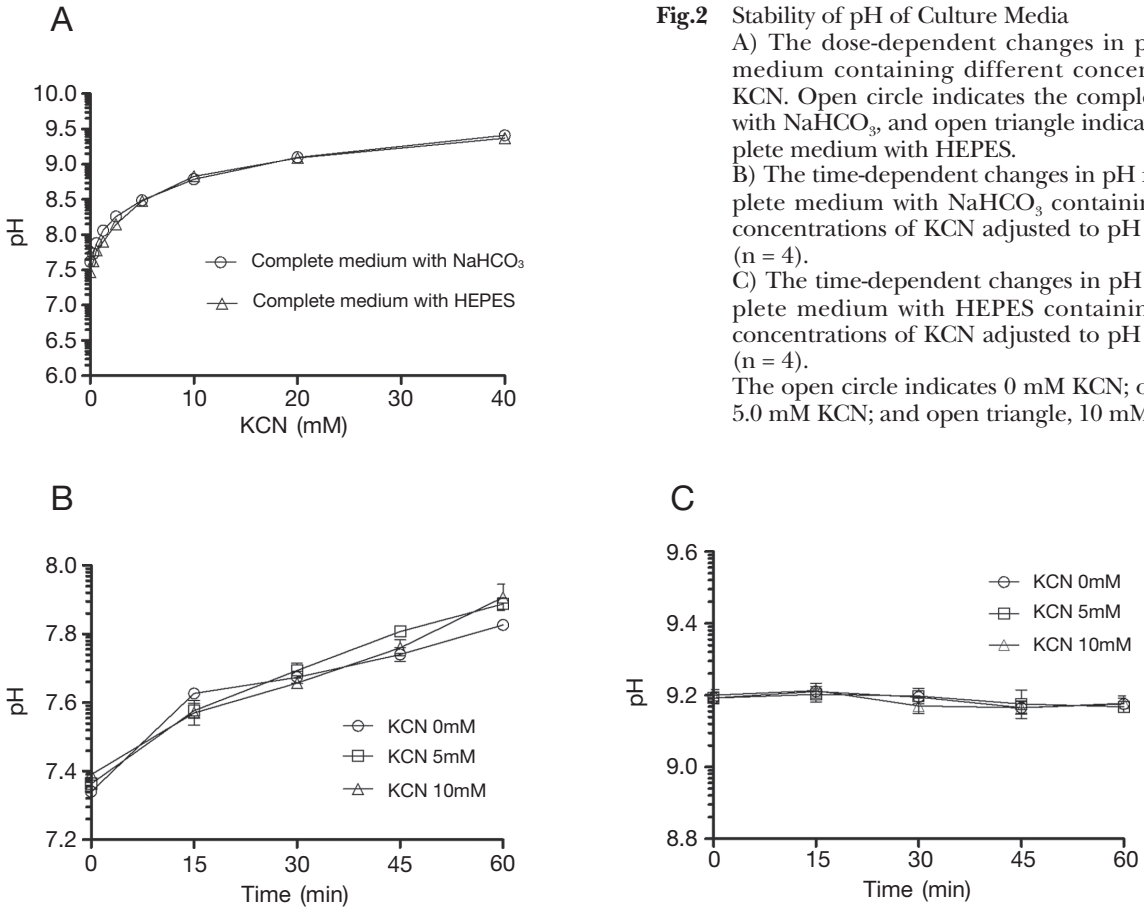

Fig.2 Stability of pH of Culture Media
A) The dose-dependent changes in pH for each medium containing different concentrations of KCN. Open circle indicates the complete medium with $NaHCO_3$, and open triangle indicates the complete medium with HEPES.
B) The time-dependent changes in pH for the complete medium with $NaHCO_3$ containing different concentrations of KCN adjusted to pH 7.40 ± 0.05 (n = 4).
C) The time-dependent changes in pH of the complete medium with HEPES containing different concentrations of KCN adjusted to pH 9.20 ± 0.05 (n = 4).
The open circle indicates 0 mM KCN; open square, 5.0 mM KCN; and open triangle, 10 mM KCN.

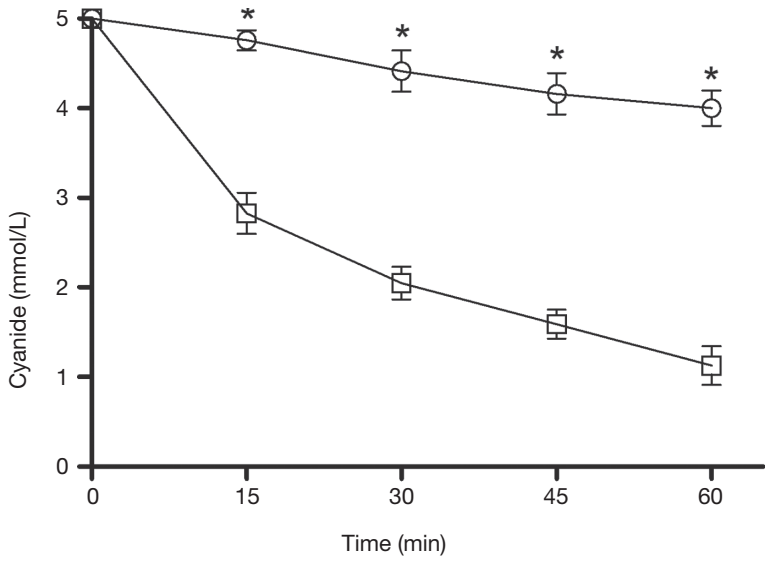

Fig.3 Changes in Cyanide Concentration
The open circle indicates the cyanide concentrations in the complete medium with HEPES containing 5.0 mM KCN at pH 9.2 (n = 4). The open rectangle represents the cyanide concentrations in the complete medium with $NaHCO_3$ containing 5.0 mM KCN at pH 7.4 (n = 4). *Significant at $p < 0.05$ compared to the complete medium (pH7.4).

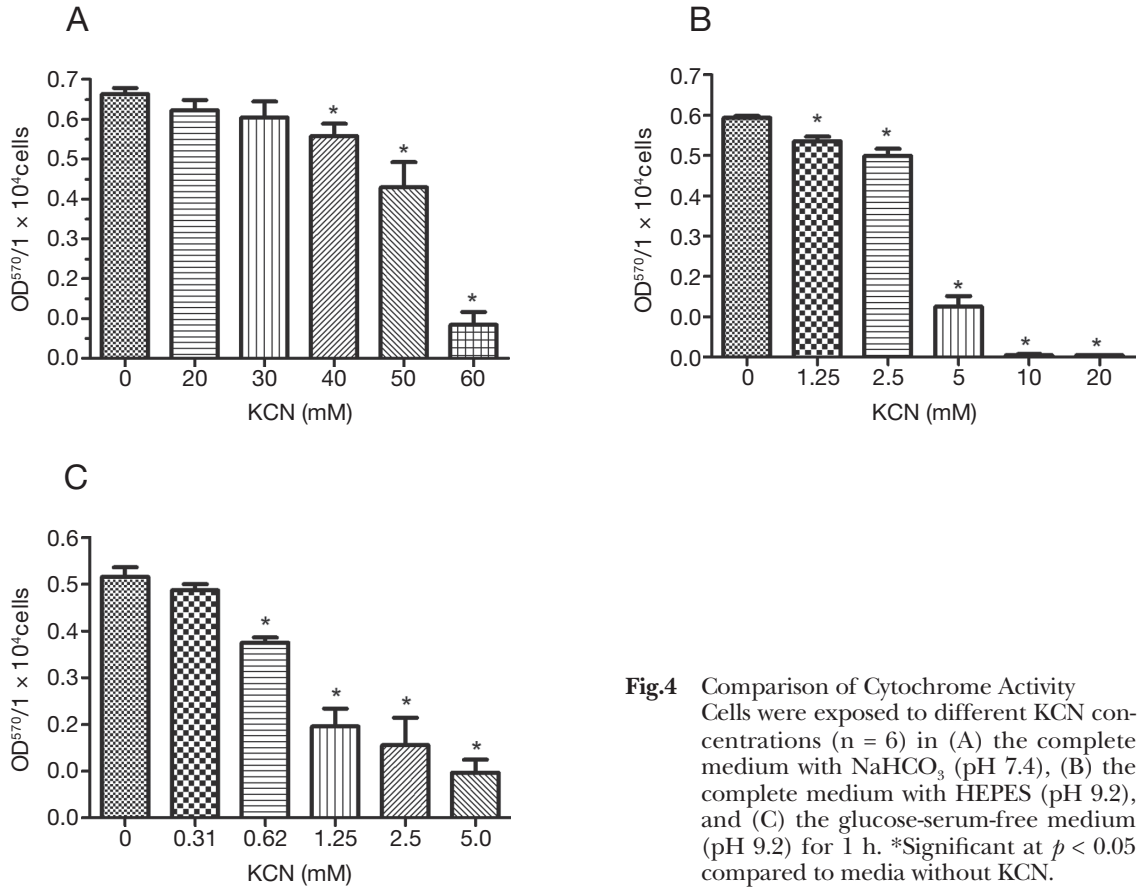

Fig.4 Comparison of Cytochrome Activity
Cells were exposed to different KCN concentrations (n = 6) in (A) the complete medium with $NaHCO_3$ (pH 7.4), (B) the complete medium with HEPES (pH 9.2), and (C) the glucose-serum-free medium (pH 9.2) for 1 h. *Significant at $p < 0.05$ compared to media without KCN.

Comparison of Cytochrome Activity

The toxicity of KCN on cytochrome activity was compared among the three culture mediums. Each medium containing various KCN concentrations were added to the cells and incubated for 1 h, then the cells were subjected to the MTT assay.

A significant decrease in cytochrome activity was observed in the cells exposed to 40 mM KCN in the complete medium with $NaHCO_3$ (pH 7.4), 1.25 mM KCN in the complete medium with HEPES (pH 9.2), and 0.62 mM KCN in the glucose-serum-free medium (pH 9.2) (Fig. 4A-C).

Antidotal effects of Hydroxocobalamin (OHCbl) on Cytochrome Activity

Various concentrations of hydroxocobalamin and KCN, prepared using the complete medium with HEPES (pH 9.2) or the glucose-serum-free medium (pH 9.2), were simultaneously added to the cells, and incubation was performed for 1 h. The cells were then subjected to the MTT assay. Cytochrome activity decreased to 0.037 ± 0.0073 in the cells exposed to 5 mM KCN alone prepared in the complete medium with HEPES (pH 9.2); however, this decrease reversed following the addition of OHCbl, in a dose-

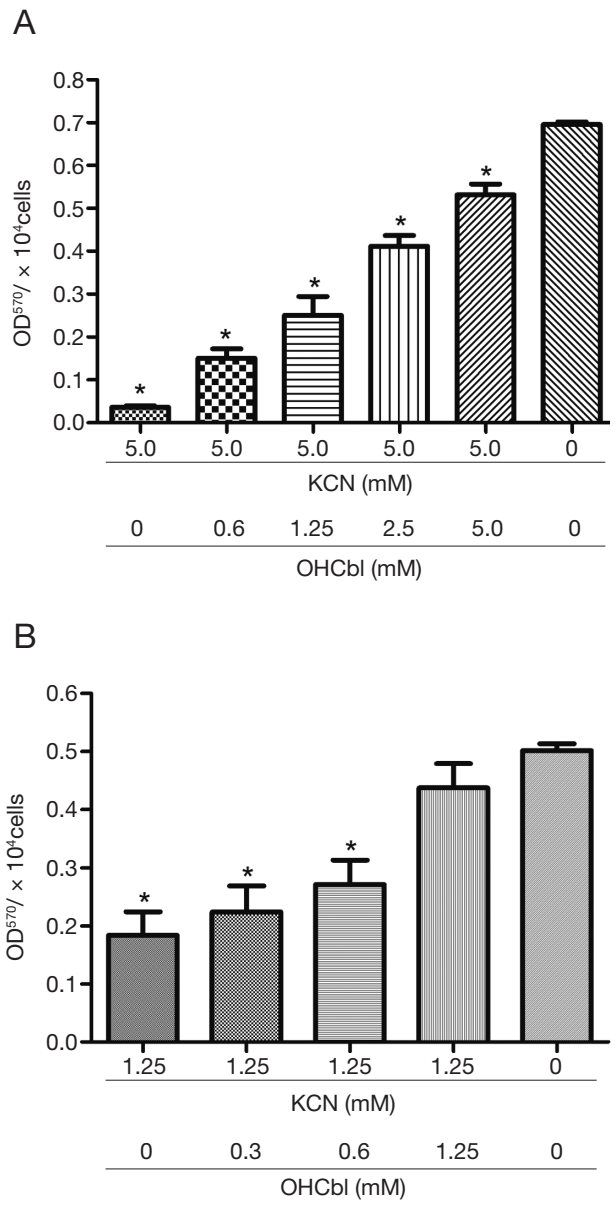

Fig.5 Antidotal Effects of Hydroxocobalamin (OHCbl) Measured by MTT Assay
Cells were treated with various concentrations of KCN for 1 h in the presence of various doses of OHCbl (A) in the complete medium with $NaHCO_3$ (pH 9.2) (n = 6), and (B) in the glucose-serum-free medium (pH 9.2) (n = 5). *Significant at $p < 0.05$ compared to media with no KCN or OHCbl.

dependent manner (Fig. 5A). Similarly, cytochrome activity decreased to 0.18 ± 0.090 in the cells exposed to 1.25 mM KCN alone prepared in the glucose-serum-free medium (pH 9.2), which also reversed upon the addition of OHCbl, in a dose-dependent manner. Equimolar addition of OHCbl (1.25 mM) caused no significant differences compared to control cells (Fig. 5B).

Antidotal effects of Hydroxocobalamin (OHCbl) on Cell Viability

To determine cell viability, various concentrations of OHCbl and KCN, prepared in complete medium with HEPES (pH 9.2) or glucose-serum free medium (pH9.2), were simultaneously added to the cells and incubated for 1 h. Then, the cells were subjected to the TBDE assay.

In the complete medium with HEPES (pH 9.2), cell viability reduced to 23.4% ± 10.2% after treatment with 10 mM KCN alone; however, it was restored upon OHCbl addition, in a dose-dependent manner (Fig. 6A). Equimolar addition of OHCbl (10 mM) resulted in 94.9% ± 7.2% cell viability, which was not significantly different from that observed in the medium alone (98.0% ± 2.0%, $p = 0.44$).

In the glucose-serum-free medium (pH 9.2), cell viability decreased to 23.7% ± 6.5% in the cells exposed to 5 mM KCN alone, and was restored upon OHCbl addition, in a dose-dependent manner (Fig. 6B).

DISCUSSION

In vitro cyanide toxicity is extremely variable [23] because various factors such as medium pH [19], type of culture container used [19], carbon dioxide pressure [24], and incubation temperature [20], can influence cyanide concentration in the culture medium by inducing vaporization of HCN. Our results showed that the complete medium with a pH of 9.2 maintains cyanide concentration significantly higher (Fig. 3), and lowers the effective concentration of KCN to a greater degree compared to that by the complete medium with a pH of 7.4 (Fig. 4). These findings indicate that the nominal effective concentration of KCN reported previously may have been measured at a higher value when the medium containing KCN was adjusted to pH 7.4.

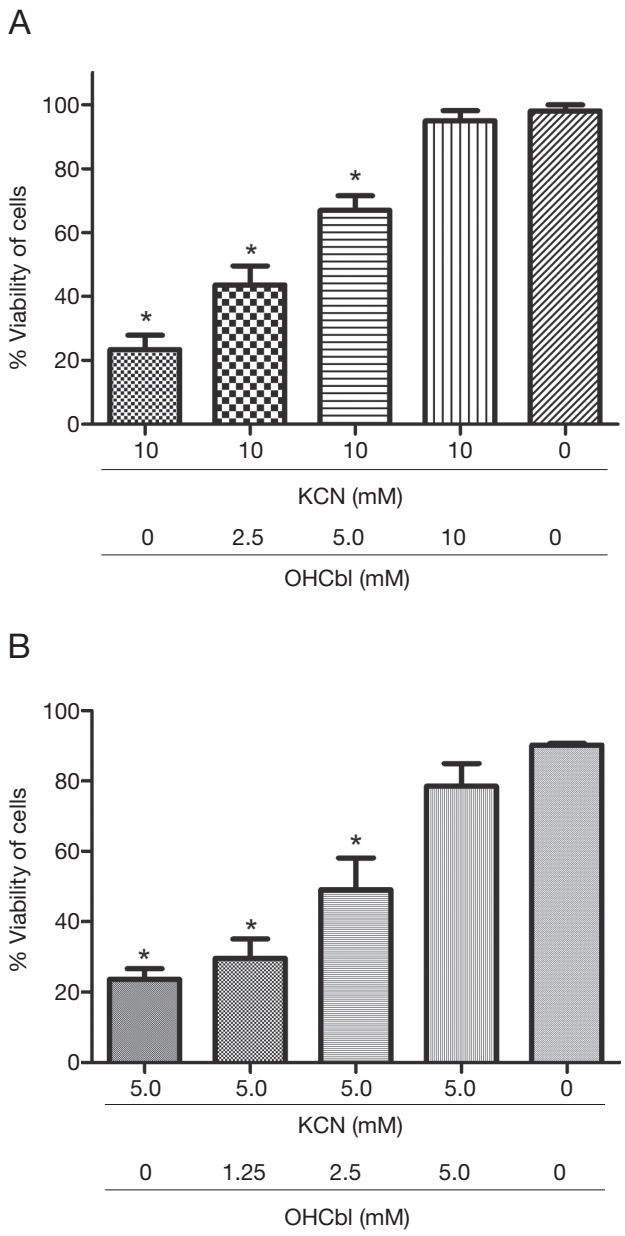

Fig.6 Antidotal Effects of Hydroxocobalamin (OHCbl) Measured by TBDE Assay
Cells were treated with various concentrations of KCN for 1 h in the presence of various doses of OHCbl (A) in the complete medium with $NaHCO_3$ (pH 9.2) (n = 5), and (B) the glucose-serum-free medium (pH 9.2) (n = 5). *Significant at $p < 0.05$ compared to the media with no KCN or OHCbl.

The pH of the culture medium increased in response to KCN in a dose-dependent manner (Fig. 2A). However, most previous in vitro studies [5, 6, 9, 16, 18, 25–30] did not state the exact pH of the culture medium containing KCN or NaCN, and the reported effective concentration of KCN widely varied, from 0.1 to 10 mM, in cytotoxicity tests (Table). This variability may result not only from differences in cellular sensitivity, but also from the instability of cyanide concentration induced by disparate pH of the culture medium.

In addition to medium pH, the constituents of the medium, including glucose [19, 27], serum [31], protein [9, 30], and amino acids [19], are known to be the important factors that affect in vitro KCN cytotoxicity. The reaction of cyanide with glucose involves the addition of cyanide to the terminal aldehyde group of glucose to form a cyanohydrin, which inactivates the cyanide ion in the culture medium [19, 32–34]. Consistent with this, Bhattacharya *et al.* reported that the cytotoxicity of 10 mM KCN alone, measured using the MTT assay, significantly reduced when an equimolar dose of glucose was added to rat thymocytes 10 min prior to application of KCN [27]. Additionally, it is generally recognized that binding of cyanide to proteins and serum may affect the bioavailability of chemicals including KCN. For example, Dierickx PJ *et al.* demonstrated that the EC_{50} of KCN in Fa32 cells derived from rat hepatoma cells was 9.7 mM in a normal culture medium containing 10% fetal calf serum in contrast to 2.9 mM in a protein-serum-free medium [9]. We also evaluated KCN cytotoxicity in the absence of these factors by using the glucose-serum-free medium. The EC of KCN was 0.62 mM in the glucose-serum-free medium at pH 9.2, which was lower than that in the other complete medium at the same pH (Fig. 4).

Furthermore, we evaluated the antidotal effect of OHCbl, which is a clinically-used chelating agent for acute cyanide poisoning. OHCbl binds cyanide at a 1:1 ratio and forms cyanocobalamin (vitamin B12), which is excreted in the urine [35]. OHCbl antagonized the toxicity of KCN in a dose-dependent manner, both

Table Review of previous studies

Ref.		Cells	Cyanide	EC (mM)	Exp (h)	pH#	Medium	Glu. (mM)	Serum	Evaluation
5	Satpute RM 2008	Rat PC12	KCN	2.5	0.5	-	CaKRS	0	0	Peroxide level
6	Bhattacharya R 2001	Rat Thymocytes	KCN	5	0.5	-	KHB	15	0	Peroxide level
7	Borowitz JL 1993	Rat PC12	KCN	1	0.08	7.4	KRB	6	0	Peroxide level
28	Maharaj DS	Rat brain homogenates	KCN	1	1	-	PBS	0	0	MDA level
25	Hariharakrishnan J	LLC-MK2	KCN	1.25	4	-	CaKRS	0	0	MDA level
5	Satpute RM 2008	Rat PC12	KCN	5	0.5	-	CaKRS	0	0	Intracellular Ca^{2+} level
7	Borowitz JL 1993	Rat PC12	KCN	5	0.08	7.4	KRB	6	5%FBS	Intracellular Ca^{2+} level
10	Prabhakaran K,	Rat primary cortical	KCN	0.3	24	-	DMEM	22	10% HS	TUNEL staining
18	Mills EM 1996	Rat PC12	KCN	0.1	24	-	RPMI-1640	10	5%FBS + 10%HS	TUNEL staining
29	Prabhakaran K,	Rat primary cortical	KCN	0.2	24	-	DMEM	22	10% HS	Caspase-3 activity
25	Hariharakrishnan J	LLC-MK2	KCN	1.25	24	-	CaKRS	0	0	Caspase-3 activity
29	Prabhakaran K,	Rat primary cortical	KCN	0.2	24	-	DMEM	22	10% HS	Caspase-3 activity
5	Satpute RM 2008	Rat PC12	KCN	0.5	1	-	CaKRS	0	0	ATP assay
25	Hariharakrishnan J	LLC-MK2	KCN	0.31	0.17	-	CaKRS	0	0	ATP assay
30	Seibert H 2002	Holstein semen	KCN	0.47*	1	-	HBSS	2	0	ATP assay
18	Mills EM 1996	Rat PC12	KCN	0.1*	24	-	RPMI-1640	10	5%FBS + 10%HS	LDH leakage
27	Bhattacharya R 2008	Rat thymocytes	KCN	5	2	-	RPMI-1640	15	0	LDH leakage
5	Satpute RM 2008	Rat PC12	KCN	2.5*	1	-	CaKRS	0	0	TBDE
6	Bhattacharya R 2001	Rat Thymocytes	KCN	5	6	-	KHB	15	0	Eosin Y exclusion
9	Dierickx PJ	Rat hepatoma (Fa32)	KCN	9.7*	24	-	DMEM	-	10%FCS	Neutral red uptake
9	Dierickx PJ	Rat hepatoma (Fa32)	KCN	2.9*	24	-	PFHM II	-	0	Neutral red uptake
16	Lestari F. 2005	Human skin fibroblast	KCN	5.8*	4	-	DMEM/F12	17.5	0	MTS assay
16	Lestari F. 2005	HepG2	KCN	4.7*	4	-	DMEM/F12	17.5	0	MTS assay
16	Lestari F. 2005	A549	KCN	4.27*	4	-	DMEM/F12	17.5	0	MTS assay
9	Dierickx PJ.	Rat hepatoma (Fa32)	KCN	5.8*	0.5	-	DMEM	-	10% FCS	MTT assay
25	Hariharakrishnan J	LLC-MK2	KCN	2.5	4	-	CaKRS	0	0	MTT assay
19	Arun P 2005	Human Neuroblastoma	NaCN	40*	4	-	DMEM	17.5	10%FBS	MTT assay
26	Bhattacharya R 1997	Rat Thymocytes	KCN	5	6	-	KHB	15	0	MTT assay
	Current study	NIH-3T3	KCN	0.62	1	9.2	RPMI-1640	0	0	MTT assay
	Current study	NIH-3T3	KCN	1.25	1	9.2	MEM	5.5	20% FBS	MTT assay
	Current study	NIH-3T3	KCN	40	1	7.4	MEM	5.5	20% FBS	MTT assay

PC12: Cell line derived from rat pheochromocytoma , LLC-MK1: Rhesus monkey kidney epithelial cells, HepG2: Human hepatocarcinoma cells
A549: Human alveolar epithelial lung cells, CaKRS: Calcium Kreb's Ringer solution, KHB: Kreb's Hensleit Buffer, KRB: Kreb's-Ringer Bicarbonate, PBS: Phosphate buffered saline,
DMEM: Dulbecco's Modified Eagle Medium, RPMI: Roswell Park Memorial Institute, HBSS: Hank's Balanced Salt Solution, PFHM: Protein-Free Hybridoma Medium
#pH: Value immediately after dilution of cyanide salt, Exp: Exposure time of cyanide, FBS: Fetal bovine serum, FCS: Fetal calf serum, HS: Horse serum, Glu.: Glucose concentration in the culture medium
TBDE：Trypan Blue Dye Exclusion test, °EC: Effective concentration, *Effective concentration 50 (EC_{50})

in terms of cytochrome activity and cell viability, and completely detoxified KCN toxicity upon addition of equimolar OHCbl to the glucose-serum-free medium (pH 9.2) (Figs. 5 and 6). Although the antidotal effect of OHCbl was apparent, no previous in vitro data are available for comparison. Thus, it would be interesting to comparatively assess the efficacy of other antidotes for cyanide, including α-ketoglutarate [36] and N-acetyl-cysteine [37], and the novel antidote [38, 39], by using this method.

While an alkalized culture medium itself could affect the viability of cultured cells, the complete medium with a pH of 9.2 did not show a significant decrease in the cytochrome activity of NIH-3T3 cells within 1 h of incubation, compared to the complete medium with a pH of 7.4 (Fig. 1A, B). However, it is possible that a combined effect of KCN and alkalized medium could have affected the toxicity of KCN in either an additive or a synergistic manner in this study.

In this study, the cytotoxicity of KCN was evaluated using murine NIH-3T3 cells because of ease of maintenance and tolerance for alkaline culture media. The brain is a major target for cyanide [40, 41], and vulnerability of neuronal cells to cyanide toxicity because of limited anaerobic metabolism, high energy dependence, and low energy reserve has long been recognized [42]. Therefore, further study is necessary to investigate whether differentiated PC12 cells or human neuroblastoma cells (SH-SY5Y) could be used in toxicity tests in the culture medium with pH of 9.2 to develop a more sensitive assay system for KCN.

The primary biochemical action of cyanide is inhibition of cytochrome c oxidase, the terminal enzyme of the electron transport chain [43], resulting in the loss of ionic homeostasis and excitotoxicity along with free radical-induced damage [41, 44]. Cyanide-impaired mitochondrial energy metabolism is followed by decreased cellular ATP content [45, 46], elevated cytosolic Ca^{2+} levels [47, 48], and lipid peroxidation [44]. Cyanide is known to cause an apoptotic mechanism of cell death induced by intracellular oxidative stress in differentiated PC12 cells [8, 17], in addition to necrotic cell death induced by inhibition of cytochrome enzymes. Since the cytotoxicity of KCN was evaluated by only using the MTT assay and TBDE in this study, further studies should address not only cytotoxicity but also biochemical actions of KCN in various cells.

In conclusion, adjustment of culture medium pH to 9.2 could stabilize the cyanide concentration and decrease the effective concentration of KCN, leading to a stable evaluation of KCN cytotoxicity and antidotal efficacy.

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