Journal of Radiation Research, 2013, 54, 277–284
doi: 10.1093/jrr/rrs107 Advance Access Publication 21 December 2012

Involvement of placental/umbilical cord blood acid–base status and gas values on the radiosensitivity of human fetal/neonatal hematopoietic stem/progenitor cells

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(Received 9 June 2012; revised 15 October 2012; accepted 16 October 2012)

Arterial cord blood (CB) acid–base status and gas values, such as pH, PCO_2, PO_2, HCO_3^- and base excess, provide useful information on the fetal and neonatal condition. However, it remains unknown whether these values affect the radiosensitivity of fetal/neonatal hematopoiesis. The present study evaluated the relationship between arterial CB acid–base status, gas values, and the radiosensitivity of CB hematopoietic stem/progenitor cells (HSPCs). A total of 25 CB units were collected. The arterial CB acid–base status and gas values were measured within 30 min of delivery. The $CD34^+$ HSPCs obtained from CB were exposed to 2 Gy X-irradiation, and then assayed for colony-forming unit-granulocyte-macrophage, burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte erythroid, macrophage and megakaryocyte cells. Acid–base status and gas values for PCO_2 and HCO_3^- showed a statistically significant negative correlation with the surviving fraction of BFU-E. In addition, a significant positive correlation was observed between gestational age and PCO_2. Moreover, the surviving fraction of BFU-E showed a significant negative correlation with gestational age. Thus, HSPCs obtained from CB with high PCO_2/HCO_3^- levels were sensitive to X-irradiation, which suggests that the status of arterial PCO_2/HCO_3^- influences the radiosensitivity of fetal/neonatal hematopoiesis, especially erythropoiesis.

Keywords: umbilical cord blood; acid–base status; gas values; radiosensitivity; hematopoietic stem and progenitor cells

INTRODUCTION

Hematopoietic stem/progenitor cells (HSPCs) can self-renew and differentiate into all hematopoietic lineages throughout the lifetime of an organism [1–4]. Owing to their high proliferative potential, HSPCs are extremely sensitive to extracellular oxidative stresses such as radiation or chemotherapeutic agents [5–11]. Damage to the hematopoietic system caused by ionizing radiation remarkably suppresses the production of mature blood cells in a dose-dependent manner [3–6, 12]. HSPCs are abundantly contained in not only bone marrow but also placental/umbilical cord blood (CB). CB is the fetal peripheral blood that plays a key role in the exchange of nutrients and gases with fetal capillary blood within the connective tissue of the villous core, and the fetus is grown and developed continuously in the maternal environment. In cases of maternal exposure caused by nuclear accidents or nuclear attacks, the survival of infants must be considered because they are totally dependent on the maternal environment during gestation. These events may indirectly cause radiation-induced damage to fetal CB [10, 11, 13].

It is known that radiosensitivity varies among individuals. Our previous studies have shown that the individual radiosensitivity of HSPCs depends on the expression of the antioxidant gene [14], and also that maternal/neonatal obstetric factors, such as the season of birth and neonatal gender, influence the radiosensitivity of fetal/neonatal HSPCs [15]. The arterial CB acid–base status and gas values provide useful information on the condition of the fetus or newborn, such as gas exchange, acid–base balance, and the metabolic state of the fetus [16, 17], and are clinically available as indicators of hypoxia stress [18]. On the other hand, it is also reported that arterial CB acid–base

status and gas values are involved in the hematopoietic system of the fetus. Juutistenaho *et al*. concluded that stress-related perinatal factors, particularly umbilical arterial pH, are associated with the number of HSPCs present in a CB sample [19]. Furthermore, we recently showed that CB mononuclear cell counts are correlated with arterial CB pH and PCO_2, suggesting the involvement of fetal hypoxia on the yield of mononuclear cells [20]. It is possible that the effect of arterial CB acid–base status and gas values indirectly influences the radiosensitivity of fetal/neonatal hematopoiesis. However, little information is currently available. In the present study, the relationships between arterial CB acid–base status and gas values and the radiosensitivity of human fetal/neonatal HSPCs were evaluated.

MATERIALS AND METHODS

Growth factors

Recombinant human interleukin-3 (IL-3) and human stem cell factor (SCF) were purchased from BioSource (Tokyo, Japan). Recombinant human granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO) were purchased from Sankyo Co. Ltd. (Tokyo, Japan). Recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) was purchased from PeproTech, Inc. (Rocky Hill, NJ, USA).

CB collection and maternal/neonatal obstetric factors

This study was approved by the Committee of Medical Ethics of Hirosaki University Graduate School of Medicine (Hirosaki, Japan). After obtaining informed consent from the mothers, CB was collected at the Hirosaki National Hospital (Hirosaki, Japan). The inclusion criteria included low-risk pregnancies, singleton gestations or vaginal deliveries, and newborns born without resuscitation or immediate rescue procedures. Immediately after delivery, a segment of the umbilical cord was double clamped, and blood was drawn from the umbilical artery into preheparinized plastic syringes for the determination of pH, base excess (BE), and arterial CB gas values. At the same time, CB units were collected before placental delivery (*in utero* collection) according to the guidelines of the Tokyo Cord Blood Bank. CB was collected into a sterile collection bag that contained citrate-phosphate-dextrose anticoagulant (CBC-20, Nipro, Osaka, Japan) until the flow ceased. Relevant perinatal data such as maternal age, gestational age, duration of labor, birth weight and birth height were obtained from the hospital medical records.

Separation and purification of $CD34^+$ cells

Within 24 hours after CB collection, the light-density mononuclear cells were separated by centrifugation on a Limphosepar I (1.077 g/ml; Immuno-Biological Laboratories, Takasaki, Japan) for 30 min at 400 *g* and washed three times with calcium- and magnesium-free phosphate-buffered saline (PBS (−); Sigma-Aldrich, Stockholm, Sweden) containing 5 mM ethylenediamine-N, N,N′,N′-tetraacetic acid (EDTA; Wako, Tokyo, Japan). The cells were then processed for $CD34^+$ cell enrichment using an Indirect CD34 MicroBead Kit. An autoMACS™ Pro Separator (Miltenyi Biotec GmbH, NRW, Bergisch Gladbach, Germany) was used for the positive selection of $CD34^+$ cells. The isolated $CD34^+$-enriched cell population is referred to as HSPCs in this study. At the end of the procedure, the recovery of $CD34^+$ cells was approximately 0.1–0.6%, and the purity of $CD34^+$ cells was 80–95% by flow cytometry.

Umbilical artery blood analysis

The umbilical artery was analyzed to determine pH, PCO_2, PO_2, HCO_3^- and BE levels as an indicator of arterial CB acid–base status and gas values using a portable blood analyzer (i-STAT300F, Abbott Point of Care Inc., IL, USA). This analysis was usually performed within 30 min of delivery, and in no case later than 60 min after delivery. PO_2 and PCO_2 are defined as the partial pressure of oxygen and carbon dioxide in the gas phase in equilibrium with blood, respectively. HCO_3^- is the bicarbonate ion concentration and BE indicates the deviation from the normal level of the amount of buffer base.

In vitro irradiation

Within 24 hours after isolation, the $CD34^+$ cells were exposed to X-rays (2 Gy, 150 kVp, 20 mA, 0.5-mm aluminum and 0.3-mm copper filters) from an X-ray generator (MBR-1520R; Hitachi Medical Co., Tokyo, Japan) at a distance of 45 cm between the focus and target at a dose rate of approximately 100 cGy/min, which was monitored with an ionization chamber.

Methylcellulose culture

Colony-forming cells (CFCs), including colony-forming unit-granulocyte macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E) and colony-forming unit-granulocyte erythroid, macrophage, megakaryocyte (CFU-Mix) cells, were assayed by methylcellulose culturing in MethoCult medium (StemCell Technologies Inc.). Irradiated cells were plated into the wells of 24-well culture plates (Falcon, Becton Dickinson Biosciences, Franklin Lakes, NJ) at 0.3 ml/well with a culture medium containing EPO (4 U/ml), G-CSF (10 ng/ml), GM-CSF (10 ng/ml), IL-3 (100 ng/ml), SCF (100 ng/ml), penicillin (100 U/ml) and streptomycin (100 U/ml). Each plate was incubated for 14 days at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. Colonies containing more than 50 cells were counted using an inverted microscope (Olympus, Tokyo, Japan).

Statistical analysis

Multivariate linear regression analysis was performed to test for associations between mutually adjusted maternal/neonatal obstetric factors and characteristics of CB samples including CB volume, total LD cells, total $CD34^+$ cells, and surviving fraction of each HSPC. Univariate analyses were subsequently performed using the Spearman rank correlation coefficient, depending on the distribution pattern of the data. The statistical analysis was performed using the software program Origin (Origin Lab, Northampton, MA, USA) for Windows. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Summary of the characteristics of CB and maternal/neonatal obstetric factors

A total of 25 CB units were collected at the end of the full-term deliveries. The median maternal age and gestational age were 28 years (range, 21–41) and 39 weeks, respectively. Gestational age ranged from 37 to 41, which is equivalent to full-term delivery (Table 1). The median placental and neonatal birth weights were 540 g (range, 385–690) and 3166 g (range, 2366–3620), respectively. The median net

Table 1. Placental/umbilical cord blood acid–base and gas assessments

Sample number	Gestational age (weeks)	Arterial CB acid–base status and gas values				
		pH	PCO_2 (mmHg)	PO_2 (mmHg)	BE (mmol/l)	HCO_3^- (mmol/l)
1	39	7.3	41.0	15.0	–4.0	21.7
2	39	7.3	50.4	24.0	–5.0	22.4
3	37	7.4	34.8	19.0	–4.0	23.0
4	38	7.4	38.9	10.0	–2.0	22.8
5	38	7.3	51.1	15.0	1.0	27.2
6	39	7.2	49.8	21.0	–7.0	20.7
7	39	7.2	49.8	21.0	–7.0	20.7
8	40	7.3	50.6	9.0	–2.0	24.4
9	37	7.4	35.5	8.0	–2.0	22.6
10	41	7.2	61.9	11.0	–1.0	26.4
11	40	7.5	24.2	43.0	–6.0	17.5
12	40	7.3	48.3	18.0	–3.0	23.3
13	39	7.2	55.3	19.0	–5.0	22.8
14	39	7.3	54.1	11.0	–3.0	23.8
15	37	7.4	30.5	17.0	–6.0	18.6
16	39	7.3	54.6	15.0	–2.0	25.1
17	39	7.2	50.9	21.0	–5.0	22.2
18	40	7.2	46.3	17.0	–9.0	18.7
19	41	7.3	41.4	24.0	–4.0	22.1
20	40	7.3	56.6	13.0	–1.0	26.2
21	39	7.3	48.9	14.0	–1.0	25.3
22	38	7.4	42.5	13.0	–1.0	24.5
23	41	7.3	54.5	14.0	–3.0	24.2
24	41	7.3	57.4	12.0	–2.0	25.4
25	40	7.2	62.1	16.0	–4.0	24.4
Median	39	7.3	49.8	15.0	–3.0	23.0
Range	37–41	7.20–7.47	24.2–62.1	8.0–43	–9.0–1.0	17.5–27.2

weight of CB and total duration of labor were 65 g (range, 27–91) and 458 min (range, 174–1613), respectively.

Assessment of CB acid–base status and gas values

Assessments of arterial CB acid–base status and gas values were performed because they provide useful information on fetal and neonatal condition. In the present study, arterial CB pH, PCO_2, PO_2, HCO_3^- and BE were evaluated (Table 1). The median pH, PCO_2, and PO_2 were 7.27 mmHg, 49.8 mmHg and 15.0 mmHg, respectively. The median BE and HCO_3^- were −3.0 mmol/ l and 23 mmol/l, respectively. There was wide variation between individual values, in particular, in PCO_2, PO_2, HCO_3^- and BE. Since these values in the umbilical artery are usually pH 7.27, PCO_2 50 mmHg, PO_2 17 mmHg, BE −2.7 mmol/l, and HCO_3^- 24 mmol/l [21], these measurements were considered to be near normal values.

Characteristics and radiosensitivity of HSPCs

To assess the radiosensitivity of HSPCs prepared from each individual, the number of each progenitor cells was evaluated in both non-irradiated and 2-Gy irradiated $CD34^+$ cells by a methylcellulose culture supplemented with appropriate cytokines. The number of CFU-GM, BFU-E, CFU-Mix and CFCs detected among non-irradiated 1×10^3 $CD34^+$ HSPCs was 98 ± 34, 46 ± 37, 25 ± 20, and 168 ± 61, respectively (Table 2), showing wide variation between individual samples. As a result of the irradiation to $CD34^+$ HSPCs, the

Table 2. Number of hematopoietic progenitor cells and their surviving fraction in each sample

Sample number	Progenitor cells/1 × 10^3 $CD34^+$ HSPCs				Surviving fraction (2 Gy)			
	CFU-GM	BFU-E	CFU-Mix	CFC	CFU-GM	BFU-E	CFU-Mix	CFC
1	116	42	30	188	0.13	0.22	0.24	0.17
2	126	56	34	216	0.17	0.21	0.34	0.20
3	66	36	12	114	0.27	0.75	0.50	0.45
4	64	10	24	98	0.33	0.25	0.19	0.29
5	70	10	8	88	0.26	0.15	0.19	0.24
6	90	12	19	121	0.10	0.25	0.06	0.11
7	76	10	16	102	0.27	0.50	0.13	0.27
8	81	36	17	133	0.32	0.16	0.59	0.27
9	98	14	16	128	0.23	0.54	0.16	0.26
10	56	28	2	86	0.37	0.16	1.25	0.32
11	86	24	4	114	0.23	0.35	0.50	0.26
12	102	62	16	180	0.29	0.23	0.16	0.26
13	150	8	8	166	0.28	0.31	0.06	0.27
14	66	12	8	86	0.32	0.25	0.25	0.30
15	147	22	51	220	0.18	0.70	0.19	0.23
16	102	36	51	189	0.17	0.35	0.27	0.23
17	162	47	49	258	0.20	0.44	0.16	0.24
18	94	106	28	228	0.30	0.26	0.29	0.28
19	82	58	42	182	0.26	0.36	0.15	0.27
20	70	104	4	178	0.21	0.29	0.25	0.26
21	88	144	50	282	0.20	0.16	0.29	0.20
22	186	24	84	294	0.31	0.73	0.32	0.35
23	58	104	10	172	0.05	0.19	0.00	0.13
24	84	64	4	152	0.24	0.20	0.50	0.23
25	120	76	28	224	0.23	0.30	0.14	0.24
Average	98	46	25	168	0.24	0.33	0.29	0.25
SD	34	37	20	61	0.08	0.18	0.25	0.07

average surviving fraction of CFU-GM, BFU-E, CFU-Mix and CFCs were 0.24, 0.33, 0.29 and 0.25, respectively.

Correlations between arterial CB acid–base status/gas values, maternal/neonatal obstetric factors and radiosensitivity of HSPCs

To clarify the effect of arterial CB acid–base status and gas values on the radiosensitivity of $CD34^+$ HSPCs, these relationships were assessed. A statistically significant negative correlation was observed between arterial PCO_2 and the surviving fraction of BFU-E ($r = -0.42$, $P < 0.05$; Fig. 1A). A similar correlation was found between arterial HCO_3^- and the surviving fraction of BFU-E ($r = -0.46$, $P < 0.05$; Fig. 1B). Neither arterial PCO_2 nor HCO_3^- showed a statistically significant correlation with the surviving fraction of CFU-GM (Fig. 2), CFU-Mix or CFCs (data not shown). In addition, the surviving fraction of each progenitor showed no significant correlation with arterial pH, PO_2 or BE (data not shown).

The relationship between the surviving fraction of BFU-E, the maternal/neonatal obstetric factors, arterial PCO_2 and HCO_3^- were estimated. A statistically significant positive correlation between gestational age and arterial PCO_2 was observed ($r = 0.49$, $P < 0.05$; Fig. 3A). Furthermore, gestational age showed a statistically significant negative correlation with the surviving fraction of BFU-E ($r = -0.42$, $P < 0.05$; Fig. 3B). In contrast, a significant positive correlation was observed between neonatal weight and arterial HCO_3^- ($r = 0.41$, $P < 0.05$; Fig. 3C), whereas a significant negative correlation was observed between neonatal weight and the surviving fraction of BFU-E ($r = -0.41$, $P < 0.05$; Fig. 3D). These results suggest that the arterial acid–base status and gas values for PCO_2 and HCO_3^- influence the radiosensitivity of fetal/neonatal hematopoiesis, especially erythropoiesis.

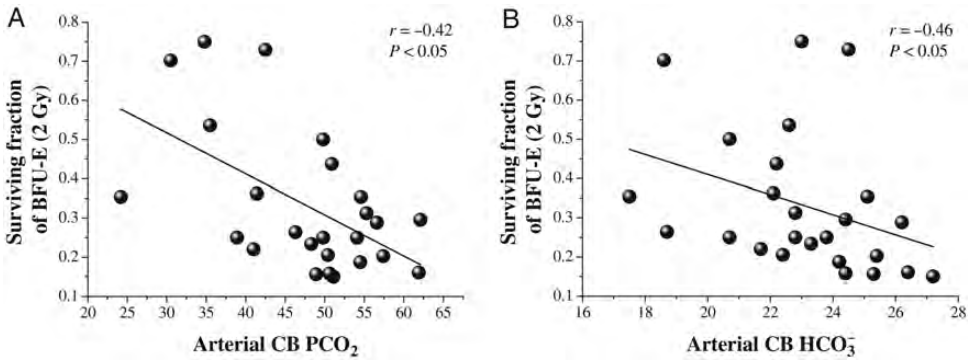

Fig. 1. Correlations between the arterial CB acid–base status and gas values and the surviving fraction of BFU-E. (A) Arterial CB PCO_2 showed a significant negative correlation with the surviving fraction of BFU-E ($n = 25$). (B) Arterial CB HCO_3^- showed a significant negative correlation with that of BFU-E. Spearman rank correlation coefficient: $^*P < 0.05$.

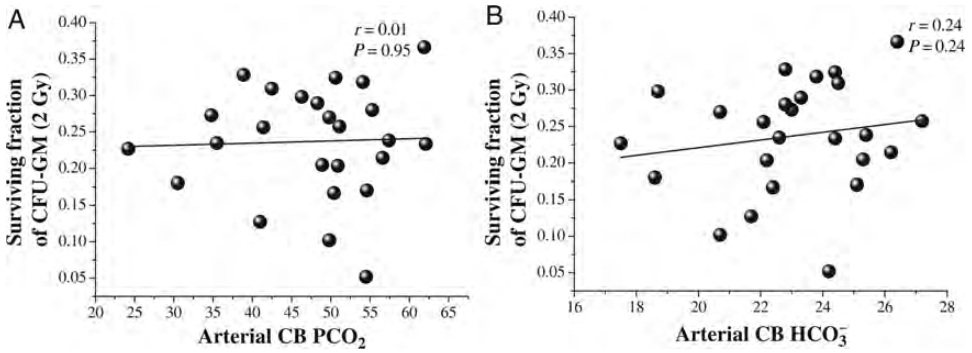

Fig. 2. Correlations between the arterial CB acid–base status and gas values and the surviving fraction of CFU-GM. (A) Arterial CB PCO_2 showed no correlation (not statistically significant) with the surviving fraction of CFU-GM ($n = 25$). (B) Arterial CB HCO_3^- showed no correlation (not statistically significant) with that of CFU-GM. Spearman rank correlation coefficient: $^*P < 0.05$.

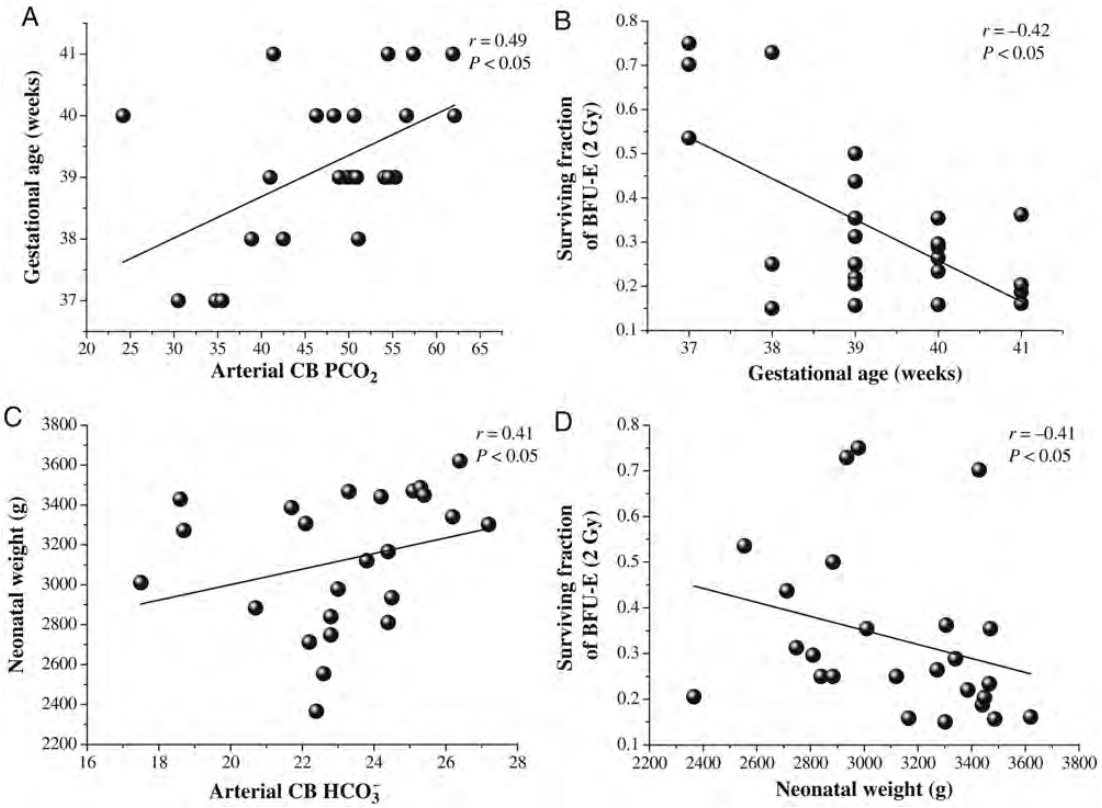

Fig. 3. Correlations between gestational age, arterial CB PCO_2, and the surviving fraction of BFU-E. (A) Gestational age showed a significant positive correlation with arterial CB PCO_2. (B) The surviving fraction of BFU-E showed a significant negative correlation with gestational age. Spearman rank correlation coefficient: $^*P < 0.05$. (C) There is a significant positive correlation between neonatal weight and arterial CB HCO_3^-. (D) There is a significant negative correlation between the surviving fraction of BFU-E and neonatal weight. Spearman rank correlation coefficient: $^*P < 0.05$.

DISCUSSION

The results of the present study reveal statistically significant negative correlations between arterial PCO_2 and the surviving fraction of BFU-E, and between arterial HCO_3^- and the surviving fraction of BFU-E.

Acid–base homeostasis is part of human homeostasis concerning the proper balance between acids and bases. The body is very sensitive to variations in pH level; therefore, the balance is tightly regulated [22]. The bicarbonate buffering system is especially important because CO_2 can be converted to HCO_3^- via a compensatory mechanism to maintain the balance if the concentration of CO_2 in the blood is increased. The change in PCO_2 level is proportional to the amount of CO_2 ventilation in the placenta, leading to a close relationship between PCO_2 and HCO_3^- [17, 23–27]. The primary alteration in acid–base balance consists of an increase in PCO_2 and HCO_3^- levels, and these changes affect the amount of circulating hematopoietic factor EPO [28], which promotes the maturation and differentiation of HSPCs into erythrocytes [29]. In the fetus, EPO is produced by the liver, which is equipped with oxygen sensors [30, 31]. Generally, PO_2 reduction in the blood is the predominant stimulus for up-regulation of EPO gene expression [29, 32]. However, the increase in PCO_2, which is caused by an increase in the CO_2 load due to the growing fetus [33], diminishes the oxygen affinity of hemoglobin by the Bohr effect, and this results in increasing peripheral oxygenation, thereby reducing the signal for EPO formation [28, 34–37]. Since EPO is required for the survival and radioprotection of committed erythrocyte progenitor cells [38, 39], it is speculated that the radiosensitivity of BFU-E is dependent on the increase in fetal PCO_2 and HCO_3^- levels, which involve fetal EPO levels.

A significant positive correlation was observed between arterial PCO_2 and gestational age (Fig. 3), and a significant negative correlation was found between gestational age and the surviving fraction of BFU-E. These results suggest that obstetric factors contribute to the rise in arterial PCO_2. Ostlund *et al.* have shown that EPO is correlated with birth weight at delivery, and that children with low birth weight have the highest EPO levels [40]. Conversely, Jazayeri *et al.* have reported a significant positive correlation between gestational age and umbilical arterial EPO [41].

Although the precise reason for this inconsistency is unclear, some possible explanations are the size of the study population, gestational age, type of delivery, and infant size. The two above-mentioned reports used 28 CB samples of between 29 and 40 weeks of gestation, and 28 CB samples between 27 and 43 weeks of gestation, respectively. In this study, we analyzed 25 CB samples with full-term delivery from 37 to 41 weeks of gestation. Although further studies will be required to assess EPO levels in arterial CB, these findings suggest that either gestational age or neonatal weight affect the radiosensitivity of erythrocyte progenitor cells.

The results of the present study suggest that acid–base balance-related factors can be an indicator of fetal/neonatal radiosensitivity. Further studies will be necessary to evaluate whether the influences of arterial CB PCO_2 and HCO_3^- levels on fetal/neonatal radiosensitivity are temporary or permanent events, and whether this event leads to other oxidative stress or diseases in the fetus and neonate. We have previously described the relationship between the initial expression of target genes of NEF2-related factor 2 (Nrf2), a key protein in the coordinated transcriptional induction of expression of various antioxidant genes, in non-irradiated hematopoietic stem cells and the surviving fraction of progenitor cells [14]. Kinalski *et al.* described the lipid peroxidation products and scavenging enzyme activity in placenta and CB, and also estimated the acid–base status and blood gases in pregestational diabetes mellitus, revealing that malondialdehyde levels and glutathione content increased significantly, and that newborns had higher PCO_2 than healthy controls [42]. Although we did not measure the initial expression of Nrf2 target genes in $CD34^+$ cells in the present study, it is possible that arterial CB PCO_2/HCO_3^- levels affect the radiosensitivity of progenitor cells through the Nrf2-dependent antioxidant system in CB HSPCs. To clarify the mechanism in more detail, additional research is being carried out in our laboratory using a larger number of CB samples.

FUNDING

This work was supported by a KAKENHI, Grant-in-Aid for Scientific Research (B; No. 21390336 IK), and a Grant for Co-medical Education Program in Radiation Emergency Medicine by the Ministry of Education, Culture, Sports, Science and Technology, Japan (2011). This work also received support from a Grant from Hirosaki University Institutional Research (2011).

ACKNOWLEDGEMENTS

We are indebted to the staff at the obstetrics and gynecology department of Hirosaki National Hospital for collecting the CB samples.

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