Therapeutic Time Window of Cannabidiol Treatment on Delayed Ischemic Damage *via* High-Mobility Group Box1-Inhibiting Mechanism

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Cannabidiol decreases cerebral infarction and high-mobility group box1 (HMGB1) in plasma in ischemic early phase. However, plasma HMGB1 levels in ischemic delayed phase reach higher concentration with the progressing brain injury. In this study, we investigated the therapeutic time window of cannabidiol on functional deficits, glial HMGB1 and plasma HMGB1 levels in a 4 h mouse middle cerebral artery (MCA) occlusion model. Cannabidiol-treated mice were divided into 3 groups as follows: group (a) treated from day 1, group (b) treated from day 3, group (c) treated from day 5 after MCA occlusion. Moreover, minocycline, microglia inhibitor, and fluorocitrate, an inhibitor of astroglial metabolism, were used to compare with cannabidiol-treated group. Repeated treatment with cannabidiol from 1 and 3 d at the latest after cerebral ischemia improved functional deficits and survival rates. However, cannabidiol from 5 d could not improve the ischemic damage as well as fluorocitrate-treated group. Moreover, both group (a), group (b) and minocycline but not group (c) and fluorocitrate-treated group had a decrease in the number of Iba1 expressing HMGB1 positive cells and HMGB1 levels in plasma. Cannabidiol may provide therapeutic possibilities for the progressing brain injury *via* HMGB1-inhibiting mechanism.

Key words cannabidiol; cerebral ischemia; therapeutic time window; high-mobility group box1

Cannabis contains about 60 different cannabinoids, including the psychoactive component, Δ^9-tetrahydrocannabinol as well as non-psychoactive components, which include cannabidiol, cannabinol and cannabigerol. Among these components, cannabidiol, a non-psychoactive constituent of cannabis, is known to exert potent anti-inflammatory, immunomodulatory and analgesic effects.[1,2] In addition, cannabidiol has been shown to be protective against global and focal ischemic injury.[3,4]

Although cannabidiol is generally known to have a very low affinity (in the micromolar range) for the cannabinoid CB_1 and CB_2 receptors, it has many pharmacological actions,[5] including anxiolytic actions and anti-inflammatory and a neuroprotective effect against ischemic injury. These actions are thought to be dependent on a new abnormal cannabidiol receptor, but not the non-CB_1 or non-CB_2 receptor.[6,7] We have also reported that cannabidiol has a cerebroprotective action *via* a cannabinoid receptor-independent mechanism.[8] In addition, cannabidiol has been shown to significantly prevent infarction and myeloperoxidase (MPO) activity after reperfusion *via* a CB_1 and CB_2 receptor-independent mechanism.[9] Recently, we have reported that cannabidiol decreased the cerebral infarction *via* MPO expressing high-mobility group box1 (HMGB1)-inhibiting mechanism. In addition, cannabidiol decreased the level of HMGB1 in plasma in ischemic early phase.[10]

HMGB1, a non-histone DNA-binding protein, is widely expressed in various tissues, including the brain. HMGB1 has been implicated in diverse intracellular functions, including the stabilization of nucleosomal structure and the facilitation of gene transcription.[11] HMGB1 is known to be massively released into the extracellular space by monocytes and macrophages or necrotic cells immediately after an ischemic insult, and induces expression of several genes related to progressive inflammation, leading to apoptosis in the post-ischemic brain.[12—16] In addition, we have reported that repeated treatment with minocycline, microglia inhibitor, for 14 d improved functional deficits, and decreased plasma levels of HMGB1 and the expression of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) positive cells at 14 d after cerebral ischemia,[17] suggesting that the extracellular HMGB1 level is of considerable importance for the treatment of post-ischemic injury.

Cannabidiol decreased cerebral infarction and HMGB1 in plasma in ischemic early phase. However, plasma HMGB1 levels in ischemic delayed phase reached higher concentration with the progressing brain injury.[10] Therefore, we examined whether cannabidiol can inhibit the progressive inflammation reaction related with HMGB1 and estimated the therapeutic time window of cannabidiol in ischemic delayed phase.

MATERIALS AND METHODS

Animals Male ddY mice (25—35 g, Kiwa Experimental Animal Laboratory, Wakayama, Japan) were kept under a 12-h light/dark cycle (lights on from 07:00 to 19:00 h) in an air-conditioned room (23±2 °C) with food (CE-2, Clea Japan, Tokyo, Japan) and water available *ad libitum*. All procedures regarding animal care and use were performed in compliance

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with the regulations established by the Experimental Animal Care and Use Committee of Fukuoka University.

Focal Cerebral Ischemia Focal cerebral ischemia was induced according to the method described in our previous study.[18] Mice were anesthetized with 2% halothane and maintained thereafter with 1% halothane (Flosen, Takeda Chemical Industries, Osaka, Japan). After a midline neck incision, the left common and external carotid arteries were isolated and ligated. A nylon monofilament (8-0; Ethilon, Johnson & Johnson, Tokyo, Japan) coated with silicon resin (Xantopren, Heleus Dental Material, Osaka, Japan) was introduced through a small incision into the common carotid artery, and advanced to a position 9 mm distal from the carotid bifurcation, for occlusion of the middle cerebral artery (MCA). Following occlusion, we stopped the 1% halothane anesthesia. We confirmed occlusion of the MCA by examining forelimb flexion after awakening from halothane anesthesia. Four hours after occlusion, the mice were re-anesthetized with halothane, and reperfusion was established by withdrawal of the filament.

Neurological Score The score was divided into 5 groups. 0; normal motor function, 1; flexion of torso and of contralateral forelimb upon lifting of the animal by the tail, 2; circling to the ipsilateral side but normal posture at rest, 3; circling to the ipsilateral side, 4; rolling to the ipsilateral side, 5; leaning to the ipsilateral side at rest (no spontaneous motor activity). Neurological score was measured at 1, 7, and 14 d after cerebral ischemia.

Rota-Rod Test in MCA-Occluded Mice Motor coordination was measured using the rota-rod test as described previously.[19] Mice were placed on a rotating rod (diameter: 3 cm; Neuroscience Inc., Tokyo, Japan) with a non-skid surface, and the latency to fall was measured for up to 2 min. The rotation speed was 10 rpm.

Fluorescent Immunostaining Mice ($n=5$ in each group) were sacrificed by decapitation after perfusion using saline and 4% paraformaldehyde at 1, 3, 7, and 14 d after MCA occlusion. The brains were removed of fat and water by an auto-degreasing unit (RH-12, Sakura Seiko Co., Tokyo, Japan) and then embedded in paraffin. Subsequently, 5-μm sections were mounted on slides and dried at 37 °C for 1 d. After deparaffinization and rehydration, these sections were rinsed twice for 1 min with phosphate buffered saline (PBS, pH 7.4). The sections were incubated overnight at 4 °C in a 1 : 200 dilution of rabbit polyclonal anti-MPO (myeloperoxidase) (DAKO Inc., Carpinteria, CA, U.S.A.), anti-Iba1 (ionized calcium-binding adapter molecule 1) (Wako Pure Chemical Industries Ltd., Osaka, Japan) and anti-GFAP (glial fibrillary acidic protein) (Ventana, Kanagawa, Japan) primary antibodies and a 1 : 200 dilution of goat polyclonal anti-HMGB1 primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.). Sections were then incubated in a 1 : 200 dilution of donkey anti-goat immunogobulin G (IgG)-FITC (fluorescein isothiocyanate) secondary antibody (Santa Cruz Biotechnology) and goat anti-rabbit IgG-Texas red secondary antibody (Santa Cruz Biotechnology) for 1 h. Coverslips were mounted and sections were analyzed by fluorescence microscopy.

TUNEL Staining After deparaffinization and rehydration, these sections were assayed for TUNEL using direct binding of fluorescein-conjugated dUTP (green fluorochrome) with anti-mouse NeuN (Chemicon International, Temecula, CA, U.S.A.) providing the red counterstain, and using the FITC-Apoptosis detection system (Promega, Madison, Wisconsin, U.S.A.). The coverslips were mounted then analyzed by fluorescence microscopy.

HMGB1 Measurements Plasma samples were fractionated by SDS-PAGE,[10] and HMGB1 levels were determined by immunoblotting using a standard curve for recombinant HMGB1 as a reference (Sigma-Aldrich, St. Louis, MO, U.S.A.).

Drug Preparation Cannabidiol (TOCRIS bioscience, Bristol, U.K.) was dissolved at 0.3 mg/ml in 1% Tween. Cannabidiol (0.3 mg/ml) was administered intraperitoneally (i.p. 0.1 ml/10 g) at 3 mg/kg on the following schedule depending on the group: group (a) treated daily for 14 d from day 1, group (b) treated daily for 12 d from day 3, group (c) treated daily for 10 d from day 5 after MCA occlusion. Minocycline (1 mg/ml, Sigma-Aldrich, St. Louis, MO, U.S.A.), microglia inhibitor, was administered intraperitoneally (i.p. 0.1 ml/10 g) from 5 d after cerebral ischemia. In addition, fluorocitrate (1 nmol/site, Sigma-Aldrich, St. Louis, MO, U.S.A.), an inhibitor of astroglial metabolism, was injected intracortically from 5 d after cerebral ischemia.

The fluorocitrate solution for intracortical injection was prepared as follows: 8 mg of D,L-fluorocitric acid, Ba, salt (Sigma-Aldrich, St. Louis, MO, U.S.A.) was dissolved in 1 ml of 0.1 M HCL. Two to three drops of 0.1 M Na_2SO_4 was added to precipitate Ba^{2+}. Two milliliters of 0.1 M Na_2HPO_4 was added, and the suspension was centrifuges at 1000 ***g*** for 5 min. The supernatant was diluted with 0.9% NaCl to the final concentration, and pH was adjusted to 7.4.

The fluorocitrate solution was microinjected stereotaxically into the sensory-motor cortex region (anterior: −0.22 mm; lateral: 2.5 mm from bregma; depth: 2 mm from the skull surface). The coordinates were based on the mouse brain atlas of Franklin and Paxinos. One microliter was injected continuously at a rate of 0.25 μl/min through a stainless steel cannula (28 gauge) connected to a 25-μl syringe driven by a slow-injection pump.

Statistical Analysis Results are expressed as the mean±S.E.M. Multiple comparisons were evaluated by Tukey–Kramer's test after one-way ANOVA. $p<0.05$ was considered to be statistically significant.

RESULTS

The Expression Changes of HMGB1 on Neutrophils, Microglia, and Astrocytes Following Cerebral Ischemia HMGB1 expressing MPO-positive cells were observed at 1 and 3 d after cerebral ischemia. Similarly, Iba1 expressing HMGB1 positive cells were observed at 1, 3, 7 and 14 d in the ischemic core including the striatum after cerebral ischemia. On the other hand, GFAP expressing HMGB1 positive cells were observed at 7 and 14 d in the ischemic penumbra. TUNEL positive cells increased in the ischemic core at a peak 7 d after cerebral ischemia, but they were rarely observed in the ischemic penumbra except for 7 d after cerebral ischemia (Fig. 1).

Effect of Cannabidiol on Neurological Impairment after Cerebral Ischemia The mice subjected to MCA occlusion had a significantly impaired neurological func-

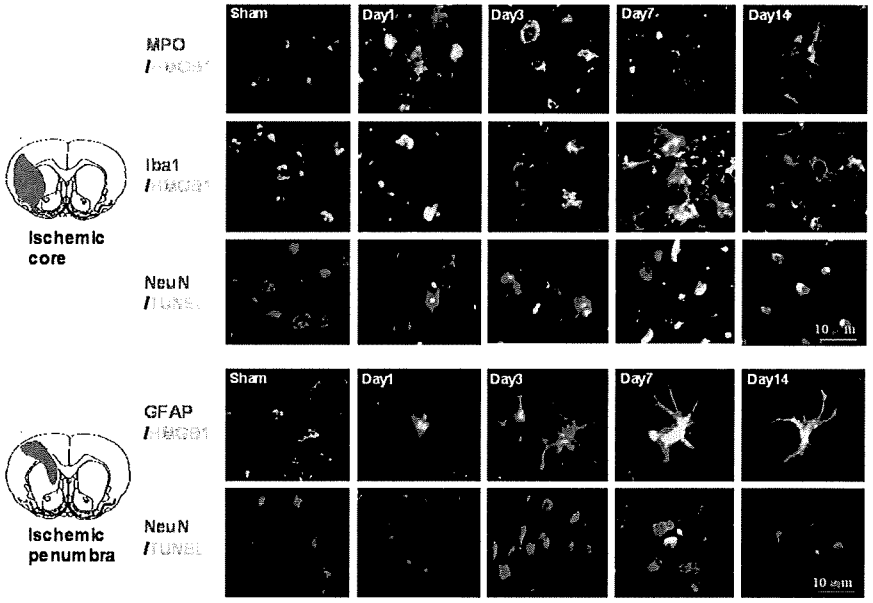

Fig. 1. Expression Changes in HMGB1 on Neutrophils, Microglia, and Astrocyte Following Cerebral Ischemia

MPO-positive cells in the ischemic core, including the striatum, significantly increased at 1 and 3 d after cerebral ischemia and HMGB1 expressing MPO-positive cells was observed at 1 and 3 d after cerebral ischemia. Similarly, Iba1 expressing HMGB1 positive cells were observed at 1, 3, 7 and 14 d in the ischemic core including striatum after cerebral ischemia. On the other hand, GFAP expressing HMGB1 positive cells were observed at 7 and 14 d in the ischemic penumbra after cerebral ischemia. TUNEL positive cells increased in the ischemic core at a peak 7 d after cerebral ischemia. On the other hand, they were rarely observed in the ischemic penumbra except at 7 d after cerebral ischemia ($n=5$). Scale bar; 10 μm. MPO, Iba1, GFAP, and NeuN, positive cells: red, HMGB1 and TUNEL, positive cells: green, merge: yellow.

tion. Cannabidiol-treated group (a) and group (b) had an improved neurological score 14 d after cerebral ischemia compared with the vehicle treated group. On the other hand, group (c) did not have an improved neurological score [$F(4,20)=11.767$, $p<0.01$, one-way ANOVA; vehicle treated group, group (a), group (b), group (c), $^{**}p<0.01$ compared with sham, 1 d after cerebral ischemia. $F(4,20)=8.463$, $p<0.01$, one-way ANOVA; vehicle treated group, group (a), group (b), group (c), $^{**}p<0.01$ compared with sham, 7 d after cerebral ischemia. $F(4,20)=14.200$, $p<0.01$, one-way ANOVA; vehicle treated group, group (a), group (c), $^{**}p<0.01$ compared with sham. Group (a), group (b), $^{**}p<0.01$ compared with vehicle treated group, 14 d after cerebral ischemia, $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared with vehicle treated group, Tukey–Kramer's test, Fig. 2B].

Effect of Cannabidiol on Motor Coordination after Cerebral Ischemia The mice subjected to MCA occlusion had a significantly impaired motor coordination. Cannabidiol-treated group (a) and group (b) showed improved motor coordination on a rota-rod test compared with the vehicle treated group. On the other hand, group (c) did not show improve motor coordination [$F(4,20)=86.283$, $p<0.01$, one-way ANOVA; vehicle treated group, group (a), group (b), group (c), $^{**}p<0.01$ compared with sham, 1 d after cerebral ischemia. $F(4,20)=19.092$, $p<0.01$, one-way ANOVA; vehicle treated group, group (a), group (b), group (c), $^{**}p<0.01$ compared with sham, 7 d after cerebral ischemia. $F(4,20)=12.775$, $p<0.01$, one-way ANOVA; vehicle treated group, group (c), $^{**}p<0.01$ compared with sham, group (a), $^{\#}p<0.05$ compared with vehicle treated group, 14 d after cerebral ischemia, Fig. 2C].

Effect of Cannabidiol on the Survival Rate after MCA Occlusion Cannabidiol-treated group (a) and group (b) had an improved survival rate compared with the vehicle treated group. On the other hand, group (c) did not show an improved survival rate (Fig. 2D).

Effect of Microglia Inhibitor, Minocycline, and Gliotoxin, Fluorocitrate, on Neurological Impairment, Motor Coordination, and Survival Rate in MCA Occluded Mice It is shown the schedule of minocycline 10 mg/kg and fluorocitrate 1 nmol/site after MCA occlusion (Fig. 3A). Minocycline-treated group but not fluorocitrate-treated group showed improved neurological score, motor coordination and survival rates. [Neurological score: $F(3,22)=14.083$, $p<0.01$, one-way ANOVA; vehicle treated group, fluorocitrate-treated group, $^{**}p<0.01$, $^{*}p<0.05$ compared with sham, 14 d after cerebral ischemia (Fig. 3B). Motor coordination: $F(3,22)=240.010$, $p<0.01$, one-way ANOVA; vehicle treated group, minocycline-treated group, fluorocitrate-treated group, $^{**}p<0.01$ compared with sham. Minocycline-treated group, $^{\#\#}p<0.01$ compared with vehicle-treated group, 14 d after cerebral ischemia (Fig. 3C)].

Effect of Cannabidiol on Microglia- and Astrocyte-Expressing HMGB1 and TUNEL Positive Cells Cannabidiol-treated group (a) and group (b) had a decrease in the number of Iba1 expressing HMGB1 positive cells and TUNEL positive cells in the ischemic core. On the other hand, group (c) did not show a decrease in these cells. Group (b) showed no changes in the GFAP expressing HMGB1 positive cells and TUNEL positive cells in the ischemic penumbra. GFAP expressing HMGB1 positive cells from group (a) displayed the morphology of fibrous astrocytes. In addition, TUNEL positive cells were rarely observed. On the other hand, treatment of group (c) and fluorocitrate resulted in a disruption in the morphology of the astrocytes and the number of TUNEL positive cells did not decrease in the ischemic penumbra 14 d after cerebral ischemia (Fig. 4A). The amount of HMGB1 released into the plasma was significantly increased at 14 d after cerebral ischemia. Group (a), group (b) and minocycline-treated group did not

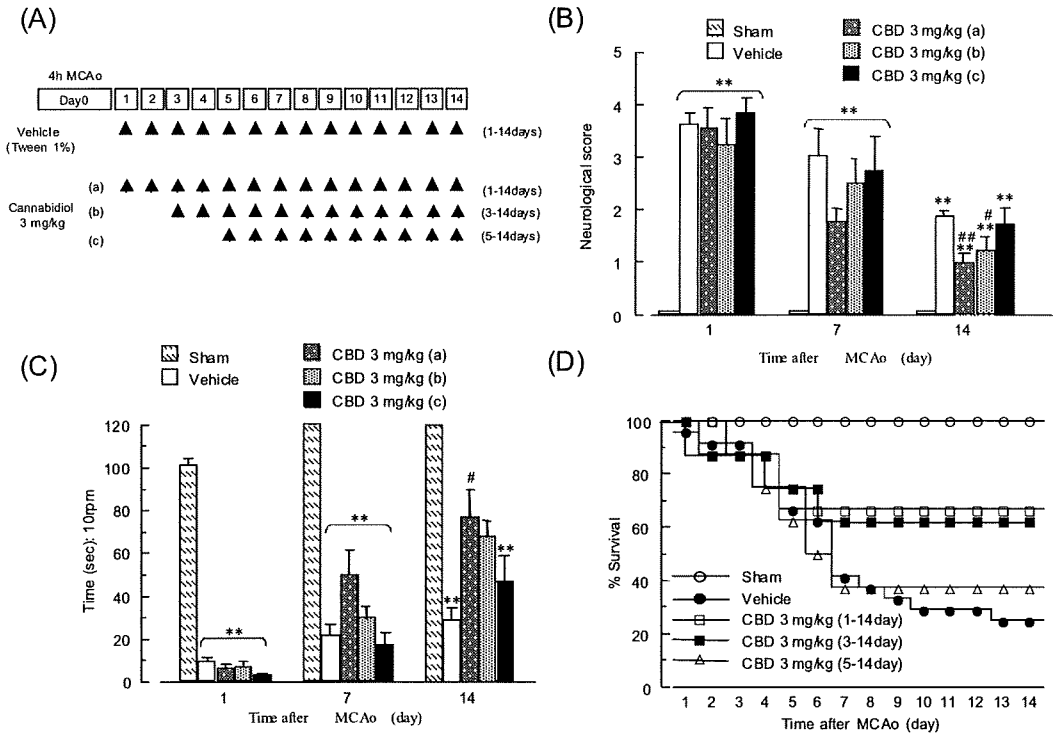

Fig. 2. Effect of Cannabidiol on Neurological Impairment, Motor Coordination, and Survival Rate in MCA Occluded Mice

It is shown the schedule of repeated treatment with cannabidiol (3 mg/kg, i.p.) after MCA occlusion (A). MCA occluded mice exhibited a significantly impaired neurological function and motor coordination, and decreased survival rate. Cannabidiol-treated group (a) and group (b) showed improved neurological score (B), motor coordination (C) and survival rates (D). On the other hand, group (c) did not show improved functional deficits and survival rate. Values are expressed as the mean±S.E.M. (B) $^{**}p<0.01$ compared with sham group, $^{\#\#}p<0.01$ compared with 14d vehicle treated group. (C) $^{**}p<0.01$ compared with sham group, $^{\#}p<0.05$ compared with 14 d vehicle treated group ($n=6$—8). (Tukey–Kramer's test).

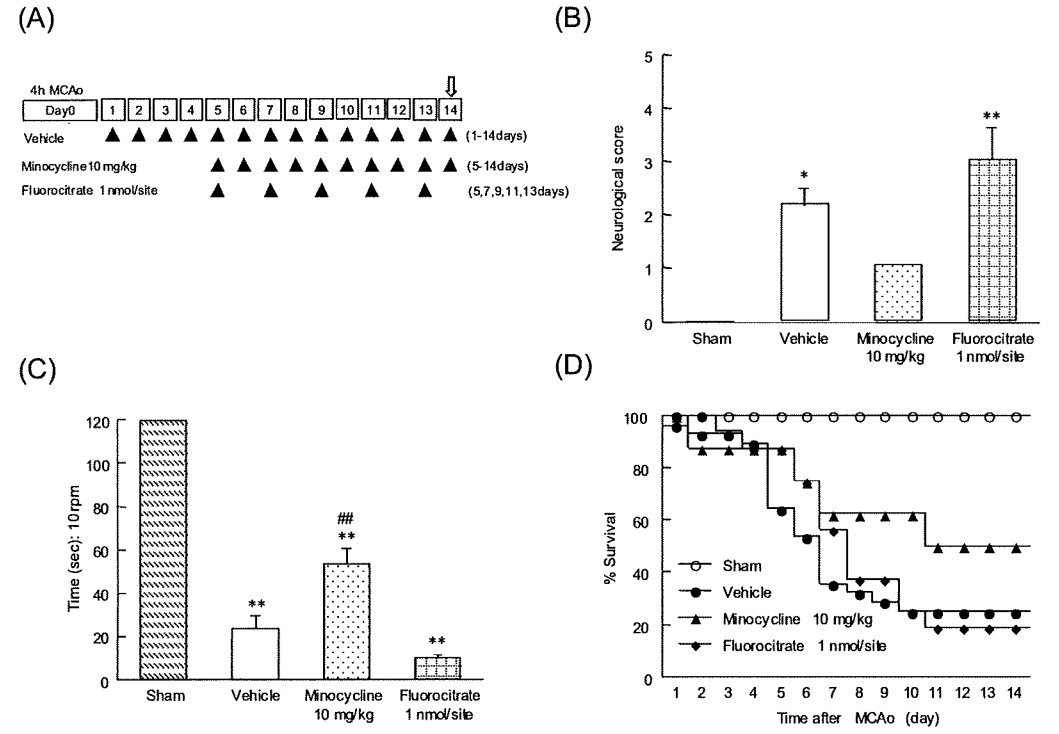

Fig. 3. Effect of Microglia Inhibitor, Minocycline, and Gliotoxin, Fluorocitrate, on Neurological Impairment, Motor Coordination, and Survival Rate in MCA Occluded Mice

It is shown the schedule of minocycline 10 mg/kg and fluorocitrate 1 nmol/site after MCA occlusion (A). Minocycline-treated group but not fluorocitrate-treated group showed improved neurological score (B), motor coordination (C) and survival rates (D). Values are expressed as the mean±S.E.M. (B) $^{**}p<0.01$ compared with sham group. (C) $^{**}p<0.01$ compared with sham group, $^{\#\#}p<0.01$ compared with 14 d vehicle treated group ($n=6$—8) (Tukey–Kramer's test).

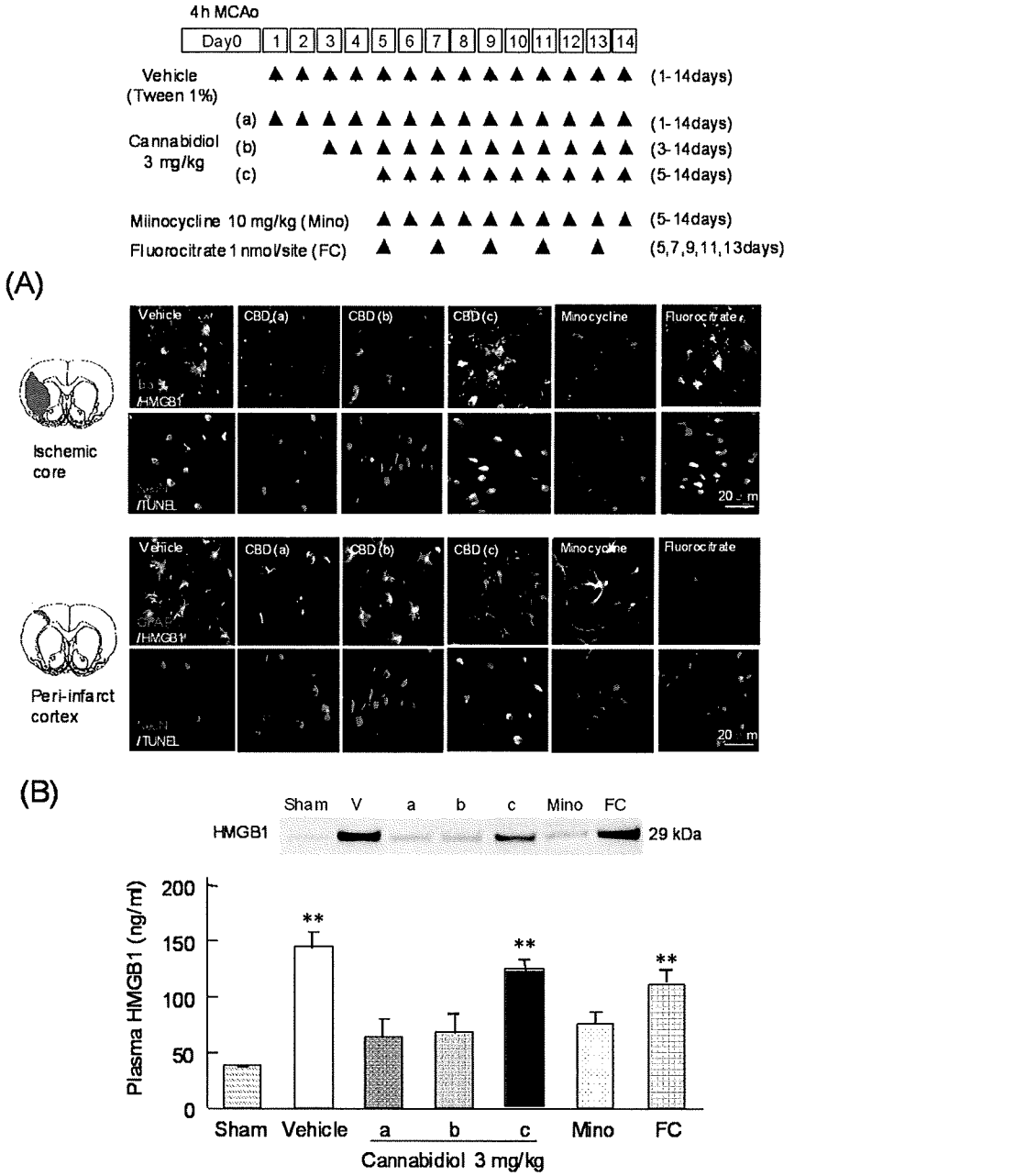

Fig. 4. Effect of Cannabidiol on Microglia- and Astrocyte-Expressing HMGB1 and TUNEL Positive Cells

Cannabidiol-treated group (a) and group (b) decreased the number of Iba1 expressing HMGB1 positive cells and TUNEL positive cells in the ischemic core. On the other hand, group (c) did not show a decrease in these cells (A) (n=5). Scale bar; 20 μm. Iba1, GFAP, and NeuN positive cells: red, HMGB1 and TUNEL positive cells: green, merge: yellow. The amount of HMGB1 released into the plasma was significantly increased at 14 d after cerebral ischemia. Group (a), group (b) and minocycline-treated group but not both group (c) and fluorocitrate-treated group had a significantly decreased level of HMGB1 in plasma (B). Values are expressed as the mean±S.E.M. $**p<0.01$ compared with sham group (n=3—5) (Tukey–Kramer's test).

increase the HMGB1 level in plasma [$F(6,21)=6.380$, $p<0.01$, one-way ANOVA; vehicle treated group and group (c), fluorocitrate-treated group, $**p<0.01$ compared with the sham-operated group, Fig. 4B].

DISCUSSION

Repeated treatment with cannabidiol (3 mg/kg, i.p.) from 1 d or 3 d after cerebral ischemia improved the functional deficits, survival rates and decreased the HMGB1 level in plasma and the number of Iba1 expressing HMGB1 positive cells. Additionally, both groups decreased TUNEL positive cells in ischemic core after cerebral ischemia. On the other hand, cannabidiol from 5 d but not minocycline from 5 d after cerebral ischemia did not improve the functional deficits as well as fluorocitrate-treated group. Our results suggest that cannabidiol will open the therapeutic possibilities for treatment of post-ischemic injury *via* modulating glial HMGB1.

In this study, Iba1 expressing HMGB1 and TUNEL positive cells increased during 1—14 d after cerebral ischemia in the ischemic core with the impairment of neurological functions. On the other hand, astrocyte expressing HMGB1 positive cells were observed during 7—14 d after cerebral ischemia in the penumbra region, and TUNEL positive cells were rarely observed in this region compared with ischemic core region. These results suggest that the role of microglia

expressing HMGB1 would be different from that of astrocyte expressing HMGB1 because the number of TUNEL positive cells was different in each of the ischemic regions.

Repeated treatment with cannabidiol from 1 d or 3 d after cerebral ischemia improved the functional deficits, such as neurological score and motor coordination, and survival rates. In addition, both groups did not increase the HMGB1 level in plasma, and decreased the number of Iba1 expressing HMGB1 positive cells and TUNEL positive cells. However, treatment with cannabidiol from 5 d after cerebral ischemia did not improve them. From these results it was determined that the therapeutic time window of cannabidiol in ischemic delayed phase is at the latest 3 d after ischemic insult through an activated microglia-expressing HMGB1 inhibiting mechanism.

In previous study, cannabidiol produced a cerebroprotective effect that was mediated by inhibition of MPO activity after cerebral ischemia, *via* a CB_1 and CB_2 receptor-independent mechanism in ischemic acute phase.[9] In addition, although the cerebroprotective effect of cannabidiol was partly inhibited by 5-HT1A receptor antagonist, WAY100135,[18] but not the MPO inhibition of cannabidiol. Therefore, the effect of cannabidiol would depend on antioxidant effect or other effects. Recently, it has been reported that cannabidiol inhibits microglial cell migration,[20] and prevents astroglial activation *via* inhibiting the expression of GFAP mRNA and protein.[21] Moreover, it has also been reported that cannabidiol has the ability to enhance adenosine signaling through inhibition of uptake,[25] suggesting that it leads to a decrease of ATP induction. Taken together, cannabidiol might inhibit glial activation both microglia and astrocyte *via* a decrease of ATP induction, and then cannabidiol might inhibit the expression of HMGB1 in both glial cells.

We have reported that repeated treatment with minocycline, a microglia inhibitor, for 14 d from 1 d after cerebral ischemia decreased activated microglia expressing HMGB1 within the brain, and also decreased plasma HMGB1 at 14 d after MCA occlusion. Additionally, minocycline significantly decreased the number of TUNEL positive cells at 14 d after cerebral ischemia.[17] However, minocycline did not affect reactive astrocytes expressing HMGB1 on the same time frame. Next, to inhibit reactive astrocytes induced in ischemic delayed phase, we used fluorocitrate, an inhibitor of astroglial metabolism.

Fluorocitrate (1 nmol/site) temporary inhibits astroglial metabolism but not neuron without destruction of the astroglial cells, and 24 h after its injection, the astroglial cells appear to have largely recovered.[22] As the results, repeated treatment with fluorocitrate at once per 2 d from 5 d inhibited reactive astrocytes expressing HMGB1 and caused neurological deterioration at 14 d after cerebral ischemia. In addition, plasma HMGB1 did not be affected by the injection of fluorocitrate. Taken together, these suggest that activated microglia expressing HMGB1 releases the large amount of HMGB1 into extracellular space, and it may be related to progressive inflammatory reaction in the ischemic core in ischemic early phase. In contrast, reactive astrocytes expressing HMGB1 partially release HMGB1 and influence the long-term recovery after brain injury, through neurite outgrowth, synaptic plasticity, or neuron regeneration.[23] Actually, stimulated astrocytes released HMGB1 protein and induced neuroblastoma cell differentiation.[26]

Recent evidence identifies HMGB1 as a cytokine-like mediator of delayed endotoxin lethality.[12,14] In addition, high serum levels of HMGB1 in patients with sepsis or hemorrhagic shock have been reported to be associated with increased mortality and disease severity.[14,24] In this study, plasma HMGB1 levels were correlated with the extent of brain injury, which suggests that plasma HMGB1 levels would be a marker of progressive brain injury after ischemic insult.

In conclusion, we are the first to demonstrate that repeated treatment with cannabidiol from 3 d after cerebral ischemia has cerebroprotective effect and attenuates microglia expressing HMGB1 without affecting reactive astrocytes expressing it. Our results suggest that cannabidiol will provide therapeutic possibilities for progressively expanding inflammatory responses after stroke *via* modulating microglial HMGB1.

Acknowledgments Part of this study was supported by a Grant-in-Aid for Scientific Research (No. 17590479) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Advanced Materials Institute of Fukuoka University and the Naito Foundation, Suzuken Memorial Foundation.

REFERENCES

1) Consroe P., Laguna J., Allender J., Snider S., Stern L., Sandyk R., Kennedy K., Schram K., *Pharmacol. Biochem. Behav.*, **40**, 701—708 (1991).
2) Cunha J. M., Carlini E. A., Pereira A. E., Ramos O. L., Pimentel C., Gafliardi R., Sanvito W. L., Lander N., Mechoulam R., *Pharmacology*, **21**, 175—185 (1980).
3) Hampson A. J., Grimaldi M., Axelrod J., Wink D., *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 8268—8273 (1998).
4) Hampson A. J., Grimaldi M., Lolic M., Wink D., Rosenthal R., Axelrod J., *Ann. N.Y. Acad. Sci.*, **899**, 274—282 (2000).
5) Wiley J. L., Martin B. R., *Chem. Phys. Lipids*, **121**, 57—63 (2002).
6) Baker D., Pryce G., Davies W. L., Hiley C. R., *Trends. Pharmacol. Sci.*, **27**, 1—4 (2006).
7) Begg M., Pacher P., Batkai S., Osei-Hyiaman D., Offertaler L., Mo F. M., Liu J., Kunos G., *Pharmacol. Ther.*, **106**, 133—145 (2005).
8) Hayakawa K., Mishima K., Abe K., Hasebe N., Takamatsu F., Yasuda H., Ikeda T., Inui K., Egashira N., Iwasaki K., Fujiwara M., *Neuroreport*, **15**, 2381—2385 (2004).
9) Hayakawa K., Mishima K., Nozako M., Hazekawa M., Irie K., Fujioka M., Orito K., Abe K., Hasebe N., Egashira N., Iwasaki K., Fujiwara M., *J. Neurochem.*, **102**, 1488—1496 (2007).
10) Hayakawa K., Mishima K., Irie K., Hazekawa M., Mishima S., Fujioka M., Orito K., Egashira N., Katsurabayashi S., Takasaki K., Iwasaki K., Fujiwara M., *Neuropharmacology*, **55**, 1280—1286 (2008).
11) Bustin M., *Mol. Cell. Biol.*, **304**, 133—155 (1999).
12) Abraham E., Arcaroli J., Carmody A., Wang H., Tracey K. J., *J. Immunol.*, **165**, 2950—2954 (2000).
13) Kim J. B., Sig Choi J., Yu Y. M., Nam K., Piao C. S., Kim S. W., Lee M. H., Han P. L., Park J. S., Lee J. K., *J. Neurosci.*, **26**, 6413—6421 (2006).
14) Wang H., Bloom O., Zhang M., Vishnubhakat J. M., Ombrellino M., Che J., Frazier A., Yang H., Ivanova S., Borovikova L., Manogue K. R., Faist E., Abraham E., Andersson J., Andersson U., Molina P. E., Abumrad N. N., Sama A., Tracey K. J., *Science*, **285**, 248—251 (1999).
15) Huttunen H. J., Rauvala H., *J. Intern. Med.*, **255**, 351—366 (2004).
16) Riuzzi F., Sorci G., Donato R., *J. Biol. Chem.*, **281**, 8242—8253 (2006).
17) Hayakawa K., Mishima K., Nozako M., Hazekawa M., Mishima S., Fujioka M., Orito K., Egashira N., Iwasaki K., Fujiwara M., *Stroke*,

39, 951—958 (2008).
18) Mishima K., Hayakawa K., Abe K., Ikeda T., Egashira N., Iwasaki K., Fujiwara M., *Stroke*, **36**, 1071—1076 (2005).
19) Egashira N., Iwasaki K., Takashima A., Watanabe T., Kawabe H., Matsuda T., Mishima K., Chidori S., Nishimura R., Fujiwara M., *Brain Res.*, **1059**, 7—12 (2005).
20) Walter L., Franklin A., Witting A., Wade C., Xie Y., Kunos G., Mackie K., Stella N., *J. Neurosci.*, **23**, 1398—405 (2003).
21) Esposito G., Scuderi C., Savani C., Steardo L. Jr., De Filippis D., Cottone P., Iuvone T., Steardo L., *Br. J. Pharmacol.*, **151**, 1272—1279 (2007).
22) Paulsen R. E., Contestabile A., Villani L., Fonnum F., *J. Neurochem.*, **48**, 1377—1385 (1987).
23) Chen Y., Swanson R. A., *J. Cereb. Blood Flow Metab.*, **23**, 137—149 (2003).
24) Ombrellino M., Wang H., Ajemian M. S., Talhouk A., Scher L. A., Friedman S. G., Tracey K. J., *Lancet*, **354**, 1446—1447 (1999).
25) Carrier E. J., Auchampach J. A., Hillard C. J., *Proc. Natl. Acad. Sci. U.S.A.*, **103**, 7895—7900 (2006).
26) Passalacqua M., Patrone M., Picotti G. B., Del Rio M., Sparatore B., Melloni E., Pontremoli S., *Neuroscience*, **82**, 1021—1028 (1998).

OBSTETRICS

Intrapartum fetal heart rate monitoring in cases of congenital heart disease

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OBJECTIVE: We evaluated the intrapartum fetal heart rate (FHR) patterns in fetuses with congenital heart disease (CHD).

STUDY DESIGN: One hundred sixteen cases of fetal CHD were identified at our institute between 2000-2007; 464 fetuses without CHD were used as controls. The incidences of abnormal FHR patterns and umbilical blood gases were compared.

RESULTS: More fetuses with CHD showed variant FHR than did control fetuses (46.6% vs 17.7%; $P < .01$). Cesarean section deliveries that were based on fetal indications were performed more frequently in fetuses with CHD than in control fetuses (12.9% vs 3.2%; $P < .01$). Isomerism and tetralogy of Fallot were observed frequently with variant FHR. When chromosomal abnormalities and intrauterine growth restriction were excluded, the fetuses with CHD showed more variant FHR than did the control fetuses.

CONCLUSION: Fetuses with CHD are more likely to show abnormal FHR patterns than are control fetuses. We suggest that cardiac abnormalities are associated with abnormalities in FHR patterns.

Key words: congenital heart disease, fetal heart rate monitoring, variant FHR pattern

Cite this article as: Ueda K, Ikeda T, Iwanaga N, et al. Intrapartum fetal heart rate monitoring in cases of congenital heart disease. Am J Obstet Gynecol 2009;201:64.e1-6.

Electronic fetal heart rate (FHR) patterns have been used in the antepartum and intrapartum treatment of pregnant women. Fetal oxygenation and acid-base status are evaluated with FHR patterns, which are produced in the autonomic nervous system and consist of the afferent nerve, the cardiovascular center in the brain stem, the efferent nerve, and the heart. This has given rise to the hypothesis that abnormalities in the brain or heart can cause aberrant FHR patterns without hypoxic and/or acidotic stress.

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Received Sept. 25, 2008; revised Jan. 4, 2009; accepted March 6, 2009.

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Authorship and contribution to the manuscript is limited to the 8 authors indicated. There was no outside funding or technical assistance with the production of this article.

0002-9378/$36.00

doi: 10.1016/j.ajog.2009.03.015

Many investigators have reported that congenital anomalies of the central nervous system are associated strongly with aberrant FHR patterns.[1-5] For example, Biale et al[1] showed that 71% of fetuses with brain anomalies exhibited nonreactive signs on nonstress tests. Terao et al[2] found characteristic FHR pattern abnormalities in anencephalic fetuses, the degree of which reflected the defect in the brain.

Conversely, there have been only a few reports about the relationship between congenital heart disease (CHD) and FHR patterns, although CHD is the most common congenital defect.[6-8] The pioneer work of Garite et al[6] identified this correlation in 27 patients with CHD. There are 3 important questions to be answered in addressing this issue. First, we must assess whether CHD causes real hypoxemia and/or acidosis or whether CHD causes FHR abnormalities without hypoxemia or acidosis. Second, is the high incidence of aberrant FHR associated with CHD per se or associated with the other major abnormalities that frequently accompany CHD, including chromosomal abnormalities and intrauterine growth restriction (IUGR)? Third, are there any special subtypes of CHD with which aberrant FHR is associated frequently? The answer to the last question should extend our understanding of the mechanism and pathophysiologic condition of FHR. To answer these questions, we analyzed the intrapartum FHR patterns of fetuses with CHD and compared them with those of matched control fetuses.

Materials and Methods

Between 2000-2007, 116 fetuses with CHD who were delivered at the National Cardiovascular Center were enrolled as the study subjects. Nineteen fetuses were delivered by elective cesarean section and were excluded from the study. Corresponding to each study subject, we selected 4 consecutive control fetuses (case/control ratio, 1/4), who were matched for gestational age and birthweight. Thus, we enrolled 464 fetuses as control subjects. Their medical charts were reviewed for antepartum and intrapartum risk factors, umbilical arterial gases, postnatal treatments, and outcomes.

Our protocol for detecting CHD was as follows: a fetal cardiac ultrasound examination was performed on the pregnant women at mid term. Cardiac abnormalities were detected by the

TABLE 1
Population characteristics

Characteristics	Study subjects (n = 116)	Control patients (n = 464)	*P* value
Birthweight (g)	2729 ± 553	2754 ± 531	NS
Gestational age (wk)	38 + 2	38 + 2	NS
Apgar score			
< 7 (1 min)	20 (17.2%)	28 (6.0%)	< .05
< 7 (5 min)	11 (9.5%)	5 (1.0%)	< .05
Sex			
Male	63 (54.3%)	245 (52.8%)	NS
Female	53 (45.7%)	219 (47.2%)	NS
Delivery			
Induction	55 (47.4%)	204 (44.0%)	NS
Operative vaginal	18 (15.5%)	45 (9.7%)	NS
Emergency CS	29 (25.0%)	84 (18.1%)	NS
Due to variant FHR	15 (12.9%)	15 (3.2%)	< .05
Due to arrest of delivery	14 (12.1%)	69 (14.9%)	NS

No marked differences were observed between the 2 groups in gestational age, birthweight, or sex ratio; however, their Apgar scores did differ. The emergency cesarean section (CS) was performed often in study subjects because of fetal heart rate (FHR) pattern.
CS, cesarean section; *NS*, not significant.
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ultrasound radiologist and then analyzed by a fetal/pediatric cardiologist for diagnosis.

CHD was categorized into 12 subtypes: heart isomerism, univentricular heart (UVH), tetralogy of Fallot (TOF), transposition of the great arteries, double outlet of the right ventricle, hypoplastic left heart syndrome, common arteriovenous canal, ventricular septal defect, coarctation or interruption of the aortic arch (COA/IAA), aortic stenosis, pulmonary stenosis or atresia (PS/PA), and others. Dextrocardia, Ebstein anomaly, truncus arteriosus, heart tumor, and patent ductus arteriosus were categorized as "others," because they were each represented by < 5 cases.

Electronic FHR monitoring was performed continuously until delivery, once the woman had progressed into active labor (cervical dilation, > 3 cm; dilation rate, > 1 cm/hr). At least 1 obstetrician stayed at the bedside or at a central FHR monitor on a 24-hour basis. Electrical FHR monitoring was also performed until just before the start of any emergency cesarean delivery. FHR monitoring was recorded at a paper speed of 3 cm/min and interpreted according to the National Institute of Child Health and Human Development guidelines.[9] Variable decelerations were classified according to Kubli et al.[10] *Prolonged deceleration* was defined as those decelerations that lasted ≥ 2 minutes and decreased to < 110 beats/min and that occurred once during labor, except just before delivery. *Late deceleration* was defined as recurrent if the deceleration occurred during > 50% of the uterine contractions in a 20-minute segment.

FHR monitoring charts that were recorded < 3 hours during labor were excluded for the analysis. Baseline FHR, baseline variability, and decelerations were interpreted and recorded on an hourly basis. Each hour was divided into 20-minute intervals, and the temporal changes from baseline, baseline variability, and deceleration were analyzed. We classified the pattern that was observed in the last 3 hours before delivery, but not in the last 20 minutes before delivery, because decelerations (especially variable and prolonged decelerations) are observed frequently at that time, even in normal vaginal deliveries.[11] When different degrees of variable deceleration were recorded, the most severe incident was used for the analysis.[12] The FHR charts were reviewed by 1 investigator, who was blinded to the blood gas analysis, diagnosis of CHD, and infant outcome.

The incidence of FHR in the groups was compared with the use of a χ^2 test and Fisher exact test. Data are presented as mean ± SD. A probability value of < .05 was considered statistically significant. A retrospective power analysis was performed to confirm that the study design was adequate.

RESULTS

As shown in Table 1, birthweights, gestational ages at birth, and sex ratios did not differ significantly between the groups. The incidence of Apgar scores < 7 was significantly higher in infants with CHD than in the controls, at both 1 minute and 5 minutes after delivery.

The prevalence of each FHR deceleration is shown in Table 2. Thirty percent of patients with CHD showed severe variable deceleration during the intrapartum period, which was much higher than the incidence in the control subjects (30.2% vs 8.6%, respectively; $P < .01$). A significantly higher incidence of prolonged deceleration was observed in the patients with CHD than in the control subjects (9.5% vs 3.2%, respectively; $P < .01$). There was no difference in the incidence of recurrent late deceleration or lost/decreased baseline variability between the 2 groups. Variant patterns, which included the atypical FHR patterns described earlier, occurred in 46.6% of patients with CHD, which was significantly higher than the incidence of 17.7% in the control group ($P < .01$). However, no unusual types of FHR were seen in the fetuses with CHD.

Table 1 shows the modes of delivery in the CHD and control groups. Twenty-nine patients (25.0%) with CHD were delivered by emergency cesarean section, which was a higher proportion than the 84 patients (16.8%) in the control group ($P = .11$, not statistically significant).

The incidence of emergency cesarean section deliveries in response to a nonreassuring fetal status was significantly higher during the birth of the fetuses with CHD than during the deliveries of the control fetuses (12.9% vs 3.2%, respectively; $P < .01$). The incidence of emergency cesarean deliveries in response to other indications, such as failure to progress to delivery, was very similar in both groups (12.1% vs 15.1%, respectively). Delivery was induced for 55 fetuses (47.4%) with CHD and 204 (44.0%) control fetuses. Eighteen patients with CHD (17%) underwent instrumental vaginal deliveries, similar to the incidence in the control group (45 patients, 10%).

Umbilical arterial pH was compared between the patients with CHD and the control subjects (Table 3). The average umbilical arterial pH values were similar in both groups, and there was no significant difference between the 2 groups in the incidence of acidosis at any level.

In a comparison of the patients who were delivered by emergency cesarean section in the 2 groups, the average umbilical arterial pH was lower in the control patients than in the study subjects, but the difference was not statistically significant. Three subjects in the control group showed values of pH < 7.2 (20%), but there were no cases of pH < 7.1 in the patients with CHD (Table 4). FHR deceleration patterns were analyzed in the patients with CHD according to the 15 subtypes of CHD (Table 5). When compared with the control group, fetuses with TOF (10/12; 83.3%), UVH (6/8; 75%), aortic stenosis (4/7; 57.1%), isomerism (6/13; 46.2%), and COA/IAA (6/13; 46.2%) showed a statistically higher incidence of variant FHR patterns.

We excluded 44 patients from the CHD group who had IUGR, chromosomal abnormalities, or other major structural abnormalities that might have contributed to variant FHR patterns. The remaining 72 patients were included in a subanalysis in which the incidence of variant FHR patterns during labor was still significantly higher in the CHD group than in the normal control group (38.8% vs 17.7%, respectively; $P < .01$). The same findings were observed for the incidences of severe variable deceleration and prolonged deceleration (Table 6).

TABLE 2
Incidence of FHR deceleration and minimal baseline variability in patients with congenital heart disease

Characteristics	Study subjects (n = 116)	Control patients (n = 464)	*P* value
Severe variable	35 (30.2%)	40 (8.6%)	< .05
Prolonged	11 (9.5%)	15 (3.2%)	< .05
Recurrent late	4 (3.4%)	23 (5.0%)	NS
Loss/decreased variability	4 (3.4%)	4 (0.9%)	NS
None	62 (53.4%)	384 (82.3%)	< .05

The total number of patients with nonreassuring fetal heart rate (FHR) patterns was significantly higher in the group with congenital heart disease than in the control group. Severe variable deceleration and prolonged deceleration were more frequently seen in fetuses with congenital heart disease.
NS, not significant.
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COMMENT

In this study, we demonstrated that severe variable deceleration and prolonged deceleration occurred significantly more frequently during labor and delivery in fetuses with CHD than in the matched control group. This was associated with a higher incidence of emergency cesarean deliveries in response to nonreassuring fetal status in the CHD group, compared with that in the control group. However, there were no cases of fetal acidosis during the emergency cesarean deliveries in the CHD group. From these findings, we infer that abnormally developed hearts tend to elicit aberrant FHR patterns, including variable and prolonged decelerations, not through the common mechanism that is observed in normal fetuses but through an alternative mechanism.

The mechanisms by which FHR patterns are elicited have been investigated.[13] Variable deceleration is thought to occur through an autonomic nervous reflex, which involves the efferent parasympathetic nerve, the cardiovascular center in the medulla oblongata, the afferent vagal nerve, and the cardiac rhythmic system (ie, sinoatrial node and atrioventricular node). The stimulation is

TABLE 3
Comparison of umbilical arterial PH between the groups

pH	Study subjects (n = 116)	Control patients (n = 464)	*P* value
Average	7.290 ± 0.097	7.304 ± 0.076	NS
< 7.0	2 (1.7%)	2 (0.9%)	NS
7.0-7.1	2 (3.4%)	7 (1.9%)	NS
7.1-7.2	8 (10.3%)	26 (7.5%)	NS
> 7.2[a]	104	429	NS

No difference between the 2 groups was observed in the average umbilical arterial pH or the number of fetuses with pH < 7.2. The diagnoses of fetuses with pH < 7.2 were tetralogy of Fallot (TOF; n = 3), isomerism heart (n = 3), univentricular heart (UVH; n = 1), ventricular septal defect (VSD; n = 2), common arteriovenous canal (CAVC; n = 1), and hypoplastic aorta (n = 1). Seven fetuses had chromosomal abnormalities or intrauterine growth restriction. Ten fetuses had variant fetal heart rate patterns, 3 fetuses had severe variable deceleration, and 2 fetuses had prolonged deceleration. Loss/decreased variability and late deceleration were each observed in 2 fetuses, which were relatively high proportions in this subgroup compared with those fetuses in the whole study group (17.7% vs 3.4% for loss/decreased variability and 17.7% vs 3.4% for late deceleration, respectively).
NS, not significant.
[a] 7.2 > pH: TOF 3, isomerism 3, VSD 2, UVH 2, CAVC 1, hypoplastic aorta 1.
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TABLE 4
Comparison of umbilical arterial pH in emergency c-section cases between the groups

pH	Study subjects (n = 15)	Control patients (n = 15)	*P* value
Average	7.307 ± 0.041	7.249 ± 0.134	NS
< 7.0	0	1 (6.7%)	NS
7.0-7.1	0	1 (13.3%)	NS
7.1-7.2	0	1 (20.0%)	NS
> 7.2	15	12	NS

Three of the normal fetuses who were born by emergency cesarean section had pH < 7.2, but none of the fetuses with congenital heart disease who were born by emergency cesarean section had pH < 7.2.
NS, not significant.

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either umbilical compression, especially of the artery, which leads to sufficient arterial hypertension to stimulate the baroreflex, or fetal head compression, which also leads to an autonomic nervous reflex.[13] Therefore, it is readily comprehensible that an abnormality of the brain, the heart, or the nerve that connects the 2 organs is associated strongly with variant FHR patterns. This has been substantiated by several lines of clinical and experimental evidence. Garite et al[6] reported that fetuses with congenital anomalies, especially those of the brain or heart, more frequently showed aberrant FHR patterns. Biale et al[1] reported the same observation in 1985. Terao et al[2] demonstrated different atypical FHR patterns in anencephalic fetuses with different degrees of brain deficit.

We have inferred several possible mechanisms other than acidosis for abnormal FHR patterns: an evaluation of the CHD subtypes that are most susceptible to FHR abnormalities should increase our understanding of the pathophysiologic condition of the "heart anomaly and FHR" issue. In this study, isomerism and UVH produced the most frequent aberrant FHR patterns. This could have been caused by the structural characteristics of these 2 subsets of CHD that occur in the early stages of heart development, with aberrant laterality, which could interrupt or modify the electrical impulses. Isomerism and UVH are known to be associated with abnormalities of the conduction system in the heart, such as dual sinus or atrioventricular node, absent sinus node, or complete heart block.[14-18] Although we excluded fetuses in whom intrapartum FHR monitoring would be difficult (such as those with a complete atrioventricular block), it is likely that an inherited abnormality of the impulse conduction system is possibly associated with the occurrence of aberrant FHR patterns.

TABLE 5
Incidence of variant FHR patterns in subjects with CHD

Subjects with CHD (n)	Variant FHR	Severe VD	Prolonged D	Recurrent LD	Minimam variability	Normal FHR
Isomerism (n = 13)	6 (46.2%)[b]	1	3	0	2	7
UVH (n = 8)	6 (75.0%)[b]	3	3	0	0	2
TOF (n = 12)	10 (83.3%)[b]	7	1	1	1	2
DORV (n = 7)	1 (14.3%)	1	0	0	0	6
VSD (n = 8)	3 (37.5%)	3	0	0	0	5
HLHS (n = 7)	2 (28.6%)	1	0	1	0	5
TGA (n = 7)	2 (28.6%)	2	0	0	0	5
AS (n = 7)	4 (57.1%)[b]	4	0	0	0	3
CAVC (n = 7)	2 (28.6%)	1	0	1	0	5
PS/PA (n = 5)	2 (40.0%)	1	1	0	0	3
COA/IAA (n = 13)	6 (46.2%)[b]	4	0	1	1	7
Others (n = 22)[a]	10 (45.5%)	7	3	0	0	12
Total (n = 116)	54 (46.6%)	35	11	4	4	62

Tetralogy of Fallot (TOF), isomerism, univentricular heart (UVH), pulmonary stenosis or atresia (PS/PA), and coarctation or interruption of the aortic arch (COA/IAA) were the major heart abnormalities that were associated with aberrant fetal heart rate (FHR) patterns. Ebstein anomaly (n = 4), truncus arteriosus (n = 2), heart tumor (n = 4), and patent ductus arteriosus (n = 2) were categorized as "others," because they were each represented by < 5 cases.
AS, aortic stenosis; *CAVC*, common arteriovenous canal; *CHD*, congential heart disease; *D*, deceleration; *DORV*, double outlet right ventricle; *HLHS*, hypoplastic left heart syndrome; *LD*, late deceleration; *TGA*, transposition of grate aorta; *VD*, variable deceleration; *VSD*, ventricular septal defect.

[a] Others included Ebstein anomaly (n = 4), truncus of the arteriosus (n = 2), heart tumor (n = 4), and patent ductus arteriosus (n = 2); [b] Incidence of variant FHR patterns in this category was significantly higher than that of study subjects ($P < .05$).

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TABLE 6

Incidence of variant FHR patterns in subjects with CHD (excluded IUGR, chromosome abnormality, and other major anomalies)

Subjects with CHD (n)	Variant FHR	Severe VD	Prolonged D	Recurrent LD	Minimam variability	Normal FHR
Isomerism (n = 6)	3 (50.0%)	0	2	0	1	3
UVH (n = 3)	2 (66.7%)	1	1	0	0	1
TOF (n = 6)	4 (66.7%)[b]	4	0	0	0	2
DORV (n = 6)	0	0	0	0	0	6
VSD (n = 3)	1 (33.3%)	1	0	0	0	2
HLHS (n = 6)	1 (16.7%)	1	0	0	0	5
TGA (n = 6)	2 (33.3%)	2	0	0	0	4
AS (n = 3)	1 (33.3%)	1	0	0	0	2
CAVC (n = 5)	1 (20.0%)	1	0	0	0	4
PS/PA (n = 4)	2 (50.0%)	1	1	0	0	2
COA/IAA (n = 6)	2 (33.3%)	2	0	0	0	4
Others (n = 18)[a]	9 (50.0%)	7	1	0	0	9
Total (n = 72)	28 (38.9%)	21	6	0	1	44

Patients with tetralogy of Fallot (TOF), isomerism, univentricular heart (UVH), and pulmonary stenosis or atresia (PS/PA) showed more instances of nonreassuring fetal heart rate (FHR) than were observed in the control fetuses. However, the incidence of nonreassuring FHR patterns that were associated with coarctation or interruption of the aortic arch (COA/IAA) decreased when intrauterine growth restriction, chromosome abnormalities, and other major fetal abnormalities were excluded.

AS, aortic stenosis; *CAVC*, common arteriovenous canal; *CHD*, congential heart disease; *D*, deceleration; *DORV*, double outlet right ventricle; *HLHS*, hypoplastic left heart syndrome; *IUGR*, intrauterine growth restriction; *LD*, late deceleration; *TGA*, transposition of grate aorta; *VD*, variable deceleration; *VSD*, ventricular septal defect.

[a] Others included Ebstein anomaly (n = 4), truncus of the arteriosus (n = 2), heart tumor (n = 4), and patent ductus arteriosus (n = 2); [b] Incidence of variant FHR patterns in this category was significantly higher than that of study subjects ($P < .05$).

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Other explanations are required for the development of aberrant FHR patterns with TOF, PS/PA, and COA/IAA. One explanation is that the pressure overload on the right ventricle in patients with TOF or PS/PA is severe enough to elicit aberrant FHR patterns. In fact, right heart tumors (2/2) produced nonreassuring patterns; hypertrophy of the right ventricle of unknown cause (1/1), which was categorized as "other" in this study, also produced a nonreassuring pattern. We deduce from these data that right-ventricle hypertrophy could be a factor that affects variant FHR. However, it is unknown whether increased workloads on the right ventricle, its aberrant electrical conduction system, or other factors are responsible for these variant FHR patterns.

The predominant occurrence of severe variable and prolonged decelerations in the CHD group persisted even after we had excluded patients with IUGR, aneuploidy, or major congenital anomalies. TOF was still associated significantly with aberrant FHR patterns. Conversely, the high incidence of aberrant FHR that was observed with COA/IAA decreased after the exclusion of these confounding cases,[19-23] which suggests that the mechanism of variant FHR is based on an extracardiovascular complication in some subtypes of CHD. Further investigations are required to test our speculation.

It is unlikely that more parturient women with fetuses with CHD would decide to undergo a cesarean section delivery than women with normal fetuses, because the incidence of other indications for cesarean deliveries (such as failure to progress in labor) was equivalent in the 2 groups. However, we could not exclude the possibility that the higher incidence of cesarean deliveries in the study group that resulted from FHR abnormalities may have been attributable to the obstetrician's intolerance of abnormal FHR patterns in fetuses who were at risk of poor outcomes. Although the indications for cesarean section delivery are standardized at our institutes, the physician's decision to perform a cesarean section after consideration of the antenatal fetal diagnosis cannot be controlled.

We conclude that variant FHR patterns are detected more often in fetuses with CHD than in control fetuses. We speculate that cardiac abnormalities are associated with abnormalities in FHR patterns. The interpretation of FHR patterns in fetuses with CHD requires special care, because not all of them indicate fetal acidosis. ■

ACKNOWLEDGMENTS

We thank Dr J. T. Parer for providing professional advice.

REFERENCES

1. Biale Y, Brawer-Ostrovsky Y, Insler V. Fetal heart rate tracings in fetuses with congenital malformations. J Reprod Med 1985;30:43-7.

2. Terao T, Kawashima Y, Noto H, et al. Neurological control of fetal heart rate in 20 cases of anencephalic fetuses. Am J Obstet Gynecol 1984;149:201-8.

3. Trounce J, Fagan D, Young I, et al. Disorders of neuronal migration: sonographic features. Dev Med Child Neurol 1986;28:467-71.
4. Navot D, Mor-Yosef S, Granat M, et al. Antepartum fetal heart rate pattern associated with major congenital malformations. Obstet Gynecol 1984;63:414-7.
5. Chiswick VM, D'Souza SW, Occleshaw JV, et al. Computerized transverse axial tomography in the newborn. Early Hum Dev 1977;1: 171-80.
6. Garite TJ, Linzey M, Freeman RK, et al. Fetal heart rate and fetal distress in fetuses with congenital anomalies. Obstet Gynecol 1979;53: 716-20.
7. Wu RW, Chen CP, Wang KG. Implications of prolonged fetal heart rate deceleration during the second stage of labor. J Formos Med Assoc 1996;95:231-5.
8. Cedergen MI, Kallen BAJ. Obstetric outcome of 6346 pregnancies with infants affected by congenital heart defects. Eur J Obstet Gynecol 2006;125:211-6.
9. National Institute of Child Health and Human Development Research Planning Workshop. Electronic fetal heart rate monitoring: research guidelines for interpretation. Am J Obstet Gynecol 1997;177:1385-90.
10. Kubli FW, Hon EH, Khazin AF, Takemura H. Observation on heart rate and pH in the human fetus during labor. Am J Obstet Gynecol 1969;104:1190-206.
11. Onishi J, Ikeda T, Nada S, et al. Evolution of fetal heart rate patterns in the 60 min before vaginal delivery in low risk pregnancies. J Soc Gynecol Investig 2006;13:221.
12. Parer JT, Ikeda T. A framework for standardized management of intrapartum fetal heart rate patterns. Am J Obstet Gynecol 2007; 197:26.e1-6.
13. Parer JT. Fetal cardiorespiratory physiology. In: Parer JT, ed. Handbook of fetal heart rate monitoring. Philadelphia: Saunders; 1997: 47-79.
14. Wu MH, Wang JK, Lin JL, et al. Cardiac rhythm disturbances in patients with left atrial isomerism. Pacing Clin Electrophysiol 2001; 24:1631-8.
15. Berg C, Geipel A, Kamil D, et al. The syndrome of left isomerism: sonographic findings and outcome in prenatally diagnosed cases. J Ultrasound Med 2005;24:921-31.
16. Ferrero P, Massa R, Amellone C. "Sinus node" dysfunction associated with left atrial isomerism. J Cardiovasc Med 2008;9:953-6.
17. Icardo JM, Sanchez de Vega MJ. Spectrum of heart malformations in mice with situs solitus, situs inversus, and associated visceral heterotaxy. Circulation 1991;84:2547-58.
18. Morishima M, Ando M, Takao A. Visceroatrial heterotaxy syndrome in the NOD mouse with special reference to atrial situs. Teratology 1991;44:91-100.
19. Kaneko M, Sameshima H, Ikeda T, et al. Intrapartum fetal heart rate monitoring in cases of cytomegalovirus infection. Am J Obstet Gynecol 2004;191:1257-62.
20. Hanson G, Dawes GS, Redman CW. Characterization of the reduced heart rate variation in growth-retarded fetuses. BJOG 1984;91:751-5.
21. Visser GH, Bekedam DJ, Ribbert LS. Changes in antepartum heart rate patterns with progressive deterioration of the fetal condition. Int J Biomed Comput 1990;25:239-46.
22. Kariniemi V, Aula P. Heart rate patterns in trisomic fetuses. J Perinat Med 1982;10: 242-6.
23. Martines JM, Comas C, Ojuel J. Fetal heart rate patterns in pregnancies with chromosomal disorder or subsequent fetal loss. Obstet Gynecol 1996;87:118-21.

Early Human Development 85 (2009) 235–238

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Intrapartum fetal heart rate patterns in infants (≥34 weeks) with poor neurological outcome

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ARTICLE INFO

Article history:
Received 8 February 2008
Received in revised form 28 July 2008
Accepted 22 October 2008

Keywords:
Acidemia
Fetal heart rate monitoring
Cerebral palsy
Nonreassuring patterns
Population-based

ABSTRACT

Background: Cases suggestive of non-acidemia related cerebral palsy (CP) are likely misdiagnosed as acidemia related CP because of the presence of nonreasuring fetal heart rate (FHR) patterns.
Aims: Our purpose was to compare intrapartum FHR patterns between the cases of neurological damage and the cases without disability after severe metabolic acidemia and neonatal encephalopathy, and also to compare the FHR patterns between cases with CP due to asphyxia and cases with CP of other etiology in infants born after 34 weeks.
Study design: From 1998 to 2003, our peer review conferences determined 136 infants with high-risk factors for neurological impairment in the unselected 65,197 live births. High-risk infants were chosen according to our criteria. Among them 58 were eligible infants because they were born at ≥34 weeks of gestation and also had legible FHR traces.
Outcome measures: Incidence of nonreassuring FHR patterns.
Results: Fifteen infants were acidemia related and 43 were non-acidemia related high-risk infants. Ten of the 15 acidemia infants developed CP and all had shown bradycardia ≥13 min with a nadir <80 bpm. In the 43 non-acidemia infants, 35 had CP, mental retardation, epilepsy, or hearing loss and 74% (26/35) of them had shown nonreassuring FHR patterns. Incidence of severe bradycardia was significantly elevated in the acidemia related CP compared with acidotic infants without disability, and those with non-acidemia related CP.
Conclusions: Even in infants with non-acidemia related CNS impairments, who were born at ≥34 weeks of gestation, 74% had shown intrapartum nonreassuring FHR patterns.

1. Introduction

Recent studies suggested that only 10% of infants with cerebral palsy (CP) were caused by intrapartum asphyxia [1–3]. Abnormal intrapartum fetal heart rate (FHR) patterns were frequently associated with congenital anomalies [4] and cytomegalovirus (CMV) infected fetuses [5], which are also known to be high-risk for poor neurological outcomes. These results suggest that nonreassuring FHR patterns do not necessarily cause brain damage, but are associated with pre-existing central nervous system (CNS) abnormalities. However, this speculation has not been tested in an unselected population-based study.

MacLennan categorized 3 essential criteria to link acute intrapartum hypoxia to a possible cause of CP, and also listed other factors suggesting that CP was caused by other than acute intrapartum hypoxia [6]. In 2003, the Neonatal Encephalopathy Committee Opinion [7] used the MacLennan's Criteria [6] and American College of Obstetricians and Gynecologists (ACOG) 163 criteria [8] to form the foundation of a new approach. However, cases suggestive of non-acidemia related CP are likely misdiagnosed as acidemia related CP. In this unselected population-based study, we used the modified criteria of International Consensus and the Neonatal Encephalopathy Committee Opinion to distinguish non-acidemia related infants from those which were acidemia related, and evaluated the incidence of nonreassuring FHR patterns in each group. We also sought to find if there were any specific patterns associated with acidemia related CP and non-acidemia related CP.

2. Methods

We have 5 secondary-level and 1 tertiary-level perinatal medical centers in Miyazaki, which cover nearly 11,000 deliveries per year [9]. Approximately 80% of them give birth in the private clinics, and the remaining 20% in the secondary and tertiary centers. High-risk pregnancies and newborns that need to refer to the regional secondary centers are listed in Table 1. Those who have the above-mentioned high-risk factors are basically treated in the secondary or tertiary centers.

We started the peer review audit conference to evaluate possible causes of perinatal death and neurological damage in 1998, where

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doi:10.1016/j.earlhumdev.2008.10.007

Table 1
Referral indications

1. High risk pregnancy
1) Multiple pregnancy
2) Intrauterine growth restriction
3) Fetal anomalies
4) Polyhydroamnios, oligohydroamnios
5) Placenta previa
6) Pregnancy complicated endocrine disorders
7) others
2. Emergency maternal transfer
1) Fetal indication
a. Prematurity
b. Nonreassuring fetal status
2) Fetal and maternal indications
a. Pregnancy induced hypertension
b. Placental abruption
3) Maternal indications
a. Obstetric bleeding
b. Shock
c. Sepsis
3. Neonatal transfer
1) Low birth weight infant <2000 g
2) Gestational age <34 weeks
3) Respiratory, cardiovascular, renal disorders
4) Digestive problem ex. Abdominal distention, bloody stool etc.
5) Severe hyperbilirubinemia
6) Asphyxia
7) Anomalies
8) Neurological problem
9) Not doing well

obstetricians and neonatologists from all the 6 perinatal centers participated twice a year. We examined all the perinatal and neonatal deaths. Infants with neurological damage or high-risk for neurological damage at the time of the conference were also investigated. The inclusion criteria for neurological high-risk infants are listed in Table 2.

The conference subclassified the neurologic high-risk infants into two groups, acidemia related and non-acidemia related infants according to the International Consensus Criteria [6]. We modified them such that high-risk infants for acidemia-related were defined if they had both 1) evidence of a metabolic acidosis (umbilical arterial pH<7.0 and base deficit ≥12 mmol/l) and 2) early onset of severe or moderate neonatal encephalopathy as shown in Table 3. Since the infants' long-term outcomes were not known at the time of the conference, subtypes of CP were not used for the criteria. The other neurological high-risk infants were classified as non-acidemia related. These categories were applied to the infants born at ≥34 weeks of gestation.

Intrapartum FHR charts were interpreted according to the guidelines of National Institute for Child and Human Development (NICHD) [10]. Reassuring FHR was defined when there were normal FHR

Table 2
Inclusion criteria of the neurological high-risk infants

1. Umbilical arterial pH<7.0 or base deficit ≥12 mmol/l.
2. Abnormal neurological findings during the neonatal period.
a. Seizure activity
b. Hypertonia or hypotonia
c. Abnormal reflex.
d. Irritability or hyperexcitability
e. Poor sucking and swallowing reflexes
f. Shallow, irregular respirations.
g. Apnea (not caused by prematurity)
3. Abnormal neurological images during the neonatal period.
a. Intraventricular hemorrhage (grades 3–4)
b. Periventricular leukomalacia
c. Hydrocephalus
d. Congenital CNS anomalies
4. Congenital infection which may cause neurological damage.
5. Severe IUGR (≥3SD)

Table 3
Criteria to define acidemia related and non-acidemia related high-risk infants

1. Acidemia related
1) Metabolic acidosis (pH<7.0 and base deficit ≥12 mmol/l).
2) Early onset of severe to moderate neonatal encephalopathy.
2. Non-acidemia related
1) Umbilical arterial pH≥7.0 or base deficit <12 mmol/l.
2) Congenital major or multiple anomalies.
3) Central nervous system infection or sepsis
4) Central nervous system anomalies
5) Intrauterine growth restriction
6) Decreased variability from the onset of labor
7) Microcephaly at birth
8) Extensive chorioamnionitis
9) Congenital coagulation disorders
10) Multifetal pregnancy
11) Major postnatal risk factors of cerebral palsy

baselines, moderate baseline variability, with accelerations, without late, variable, or prolonged decelerations. Nonreassuring FHR patterns were defined as those except for the reassuring patterns. Variable decelerations were classified into mild, moderate, and severe by the classification of Kubli et al. [11]. Prolonged deceleration were defined as those decelerations lasting ≥2 min with a decrease to <100 beats/min. Late decelerations were defined as recurrent if they occurred during more than 50% of the uterine contractions in a 1-h segment. The FHR monitoring charts at least 1 h before delivery were used for analysis. Baseline FHR, baseline variability, acceleration, and decelerations were interpreted and recorded on an hourly basis. According to our previous study [12], we classified the pattern by the worst pattern immediately preceding delivery. If ≥1 prolonged decelerations occurred, we defined the pattern as prolonged deceleration. When varying degrees of variable decelerations were present, the most severe incident was used. The FHR monitoring charts were reviewed by four investigators, and determined by majority. The investigators were blinded to the blood gas analysis and neurological outcomes.

Pediatric neurologists, independent of the current study, made a diagnosis of infant with CP, mental retardation (MR), epilepsy and hearing loss at age one year or older.

Severely handicapped individuals are independently registered by the Miyazaki Prefectural Health Service Centers, in which 142 infants were registered by two years old during 1998–2003. In this registration system, individual information is protected and only collective numbers are revealed.

3. Results

In a total of 65,197 live births from 1998 through 2003, we had 190 (0.29%) stillbirths, 115 (0.18%) neonatal deaths, and 136 (0.21%) infants with the neurological damage or high-risk factors.

As shown in Fig. 1, 17 infants had acidemia, and 119 had non-acidemia. The inclusion criteria (Table 2) of these infants were abnormal neurologic signs ($n=52$), abnormal CNS images ($n=68$), infectious disease ($n=10$), and severe intrauterine growth restriction (IUGR; $n=18$).

Among them, 66 infants were ≥34 weeks of gestation. Fifteen (23%) of them were classified as acidemia, and the remaining 51 (77%) were non-acidemia according to the umbilical arterial blood gas analysis. In acidemia group, the umbilical arterial blood could not be obtained in two cases, and very early neonatal blood was used following the recommendation of the International Consensus [6]. The FHR traces of the 8 infants were either missing or illegible. The remaining 58 infants were the study subjects, in which 15 were acidemia and 43 were non-acidemia. After at least 1-year follow-up, 11 acidemia infants and 35 non-acidemia infants were diagnosed as having CP, MR, epilepsy, or hearing loss.

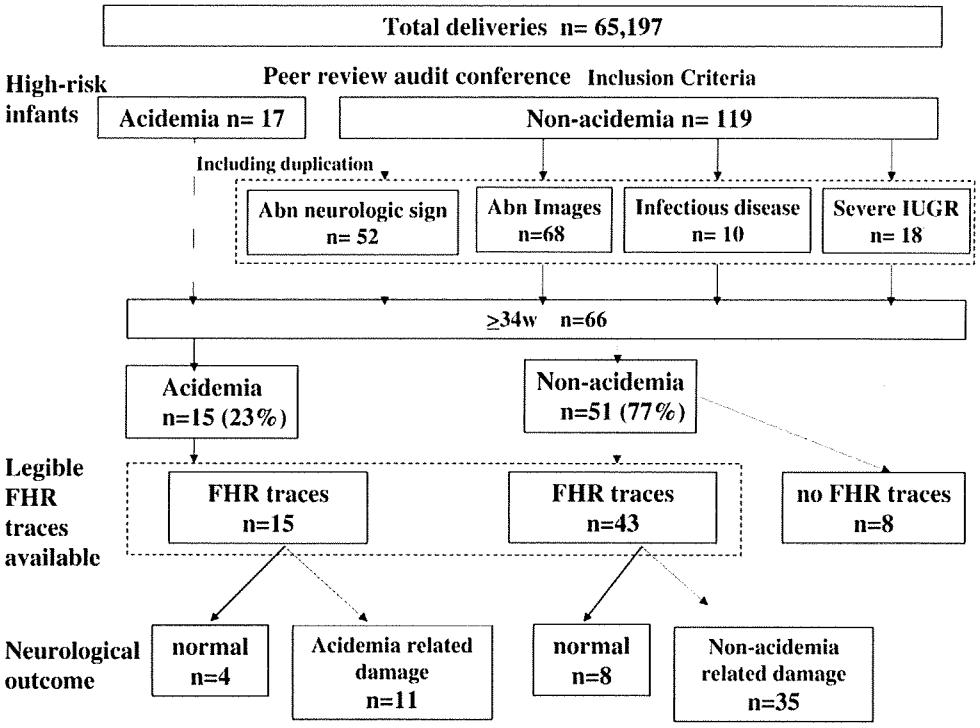

Fig. 1. Flow diagram of the current study. Abn; abnormal, IUGR; intrauterine growth restriction, FHR; fetal heart rate.

First, we compare the FHR patterns between the 10 cases with CP and the 4 cases without disability after severe metabolic acidemia and neonatal encephalopathy (Table 4). Ten cases with CP showed bradycardia with a nadir <80 bpm and duration ≥13 min. On the other hand, none of the 4 cases without disability showed such severe bradycardia as mentioned before (Table 4). The incidence of severe bradycardia (<80 bpm, ≥13 min) was significantly elevated in acidemia related CP (10/10 versus 0/4, $p<0.001$, Fisher test).

Table 4
Neurological outcomes and their intrapartum FHR patterns

	Acidosis		Non-acidosis	
	(n=15)		(n=43)	
Neurological outcomes	Normal long-term follow up[a]	Damaged	Normal long-term follow up[a]	Damaged
	(n=4)	(n=11)	(n=8)	(n=35)
Normal[a]	4		8	
Cerebral palsy		10[b]		23
Mental retardation				7
Epilepsy		1		2
Hearing loss				3
FHR pattern				
Bradycardia	1	11	1	2
Prolonged decerelation	1		1	9
Severe variable deceleration			1	3
Recurrent late deceleration			1	10
Recurrent late deceleration+LOV	2			
LOV				2
Reassuring			4	9

FHR; fetal heart rate, LOV; loss of variability.
[a] Means normal long-term follow up at one year or later.
[b] All infants with cerebral palsy showed bradycardia <80 bpm for ≥13 min.

Thirty-five of the non-acidemia related neurological damages were associated with congenital abnormalities (n=17), congenital CMV infection (n=6), IUGR (1=4), seizures of unknown causes (n=2), bacterial infection (n=2), hypotonia of unknown causes (n=1), and others (n=3). Even though they were categorized as non-acidemia, 74% (26/35) of them had shown nonreassuring FHR patterns, including late deceleration (n=10), prolonged deceleration (n=9), severe variable deceleration (n=3), bradycardia (n=2) and decreased baseline variability (n=2) (Table 4). The remaining 9 infants had reassuring FHR patterns and were associated with congenital abnormalities (n=6), congenital CMV infection (n=1), and hypotonia and seizure of unknown causes (n=2).

We also compared the FHR patterns between cases with CP due to acidemia and cases with CP of other etiology (non-acidemia). For this purpose, we excluded 3 cases with asphyxia with a moderate acidemia (pH 7.01–7.10, n=3) from non-acidemia related, since the pathogenetical mechanism in these cases was likely to be the same as in the acidemia related group. Incidence of bradycardia (<80 bpm, ≥13 min) was significantly higher ($p<0.001$, Fisher test) in acidemia related CP (10/10) compared with that in non-acidemia related CP (0/32). In non-acidemia, the most frequent pattern was recurrent late deceleration (n=10), followed by prolonged decelerations (n=9), severe variable decelerations (n=3), bradycardia (n=2) and loss of variability (n=2). Thus, FHR patterns between the two groups were totally different.

4. Comment

Several FHR patterns have been described that are consistent with pre-existing fetal brain damage, which are different from those with intact CNS [4,5,13–18]. For example, Garite et al. [4] showed a significant increase (51%) in FHR abnormalities in infants with congenital malformations, although there were no characteristic patterns that would specifically identify them. We previously reported that 40% of the CMV-infected fetuses showed either prolonged deceleration or recurrent late decelerations, which was significantly higher than the matched controls [5]. The FHR patterns of 20 anencephalic fetuses showed varying degrees of decreased variability, correlating to the severity of

brain defects [16]. Other reports also showed abnormal FHR patterns in 50 to 70% of congenital malformations [17,18].

In this unselected population-based study, 74% of the infants with non-acidemia related CNS impairments showed various types of nonreassuring FHR patterns. Taking it into consideration that 8 of the 51 non-acidemia infants were missing from evaluation (Fig. 1), nonreassuring FHR patterns were still present in at least 60% (26/43).

This observation delivers important message such that more than 70% of the infants with non-acidemia related CNS impairments may show nonreassuring FHR patterns as a result of pre-existing CNS disorders, but not as a causal relationship to damage their CNS. Otherwise, nonreassuring FHR patterns during the intrapartum period may lead to a misdiagnosis of acidemia related neurological impairment.

In this study, we found that fetal bradycardia with a nadir <80 bpm and duration ≥13 min was the specific pattern in acidemia related CP, compared with the cases without disability after severe acidosis. Our observation was consistent with the previous report showing that neonatal asphyxia or death occurred when more than 10 min elapsed after bradycardia (<90 bpm) occurred in cases with uterine rupture [19]. Similarly, we also found that severe bradycardia was predominantly observed in acidemia related CP compared with non-acidemia related CP which showed various patterns including prolonged deceleration and recurrent late deceleration.

MacLennan [6] defined 3 essential and 5 non-specific factors that suggest a cause of CP due to acute intrapartum hypoxia. However, we had difficulties in differentiating four asphyxiated infants according to the guidelines. They were cord prolapse ($n=2$) and dystocia related birth trauma ($n=2$), which showed bradycardia or prolonged deceleration but did not meet the criteria of severe acidosis (range: pH 7.025–7.100, base deficit 11.0–11.7 mmol/l). Three of them developed CP or epilepsy, and one developed normal. Our observation agreed with previous reports. The application of guidelines of severe acidosis (pH<7.00) would miss a high percentage of the babies who had asphyxial neonatal encephalopathy [20]. That is, a pH value <7.0 identifies a very high risk group of babies at risk for adverse outcome. On the other hand, only a small subset of the infants actually have adverse neurological outcome if the pH>7.0. This is due to the fact that so many infants are born with a normal pH and although percentage wise the majority of these infants do well, a small percent do not. Kirkendall and Phelan reported that severe acidosis at birth was manifested approximately 8% to 9% in neurologically damaged infants [21].

In a total of 65,197 live births, 46 infants of ≥34 weeks of gestation had poor neurological outcomes (11 for acidemia at birth and 35 with other perinatal high-risk factors), leading to a rough estimation of neurological impairment in 0.07% of all births. This estimation is compatible with some previous reports. For example, in the California Cerebral Palsy Project, 192 children with moderate to severe cerebral palsy gave a prevalence of 1.23/1000 survivors. Fifty-three percent of them had birthweight greater than or equal to 2500 g, which is 0.65/1000 (0.065%) [22].

One limitation of this study was that we did not follow all of the 65,197 babies to search for neurological sequalae. Although almost all of the high-risk pregnancies and newborns were transferred to the secondary or tertiary centers in our district, a few of them might not be registered in our peer review audit conferences. Even though some neonates seem normal at birth, they may develop CP later [23]. In order not to miss these cases, we needed to cooperate with our public health service centers. Registration of severely handicapped infants ($n=142$) during the study period of 1998 through 2003 also supports our assumption that we were able to enroll most of the high-risk infants for neurological damages (registered cases were136 in this study).

Despite this limitation, we categorized acidemia and non-acidemia related high-risk infants with the best clinically available materials, and evaluated their FHR monitoring records in an unselected, population-based setting. To our best knowledge, this is one of the few unselected, population-based studies to show that nonreassuring FHR patterns are present in 70% of infants (≥34 weeks of gestation) who subsequently develop CNS sequalae with other causes than intrapartum asphyxia. We also found that bradycardia (<80 bpm, ≥13 min) was the specific pattern in acidemia related CP. Further investigation and reassessment should proceed to support our observation.

References

[1] Blair E, Stanley FJ. Intrapartum asphyxia: a rare cause of cerebral palsy. J Pediatr 1988;112:515–9.
[2] Nelson KB. What proportion of cerebral palsy is related to birth asphyxia? J Pediatr 1988;112:572–4.
[3] Paneth N, Keily J. The frequency of cerebral palsy: a review of population studies in industrial nations since 1950. In: Stanley FJ, Alberman E, editors. The epidemiology of the cerebral palsies. Philadelphia: Lippincott; 1984. p. 46–56.
[4] Garite TJ, Linzey EM, Freeman RK, Dorchester W. Fetal heart rate patterns and fetal distress in fetuses with congenital anomalies. Obstet Gynecol 1979;53:716–20.
[5] Kaneko M, Sameshima H, Ikeda T, Ikenoue T, Minematsu T. Intrapartum fetal heart rate monitoring in cases of cytomegalovirus infection. Am J Obstet Gynecol 2004;191:1257–62.
[6] MacLennan A. A template for defining a causal relation between acute intrapartum events and cerebral palsy: International Consensus Statement. BMJ 1999;319:1054–9.
[7] Neonatal Encephalopathy Committee Opinion-2003. Washington, DC: American College of Obstetricians and Gynecologists and The American Academy of Pediatrics; 2003.
[8] American College of Obstetricians and Gynecologists. Fetal and neonatal neurologic injury. Technical Bulletin #163. Washington, DC: American College of Obstetricians and Gynecologists; 1992.
[9] Kodama Y, Sameshima H, Ikenoue T. Regional population-based study on pregnancy outcomes in women with diabetes mellitus in Japan. J Obstet Gynaecol Res 2007;33(1):45–8.
[10] National Institute for Child and Human Development Research Planning Workshop. Electronic fetal heart rate monitoring: research guidelines for interpretation. Am J Obstet Gynecol 1997;177:1385–90.
[11] Kubli FW, Hon EH, Khazin AF, Takemura H. Observation on heart rate and pH in the human fetus during labor. Am J Obstet Gynecol 1969;104:1190–206.
[12] Sameshima H, Ikenoue T, Ikeda T, Kamitomo M, Ibara S. Unselected low-risk pregnancies and the effect of continuous intrapartum fetal heart rate monitoring on umbilical blood gases and cerebral palsy. Am J Obstet Gynecol 2004;190:118–23.
[13] Paul R, Yonekura L, Cantrell C, Turkel S, Pavlova Z, Sipos L. Fetal injury prior to labor: does it happen? Am J Obstet Gynecol 1986;154:1187–93.
[14] Karp LE, Meis PJ. Trisomy-18 and antenatal fetal distress. J Reprod Med 1977;19:345–7.
[15] Powell Phillips WD, Towel ME. Abnormal fetal heart rate associated with congenital abnormalities. Br J Obstet Gynaecol 1980;87:270–4.
[16] Terao T, Kawashima Y, Noto H, Inamoto Y, Lin TY, Sumimoto K, et al. Neurological control of fetal heart rate in 20 cases of anencephalic fetuses. Am J Obstet Gynecol 1984;149:201–8.
[17] Navot D, Mor-Yosef S, Granat M, Sadovsky E. Antenatal fetal heart rate pattern associated with major congenital malformations. Obstet Gynecol 1983;63:414–7.
[18] Biale Y, Brawer-Ostrovsky F, Insler V. Fetal heart rate tracings in fetuses with congenital malformations. J Reprod Med 1985;30(1):43–7.
[19] Leung AS, Leung UK, Paul RH. Uterine rupture after previous cesarean delivery: maternal and fetal consequences. Am J Obstet Gynecol 1993;169:945–50.
[20] Phelan JP, Martin GI, Korst LM. Birth asphyxia and cerebral palsy. Clin Perinatol 2005;32:61–76.
[21] Kirkendall C, Phelan JP. Severe acidosis at birth and normal neurologic outcome. Prenat Neonatal Med 2001;6:267–70.
[22] Grether JK, Cummins SK, Nelson KB. The California Cerebral Palsy Project. Paediatr Perinat Epidemiol 1992;6(3):339–51.
[23] Grant A, O'Brien N, Joy MT, Hennessy E, MacDonald D. Cerebral palsy among children born during the Dublin randomized trial of intrapartum monitoring. Lancet 1989;2(8674):1233–6.

Current Commentary

The 2008 National Institute of Child Health and Human Development Report on Fetal Heart Rate Monitoring

Julian T. Parer, MD, PhD, Tomoaki Ikeda, MD, PhD, and Tekoa L. King, CNM, MPH

Standardization of fetal heart rate (FHR) interpretation and management guidelines has been elusive, and no system is currently widely accepted in the United States. The recently summarized 2008 *Eunice Kennedy Shriver* National Institute of Child Health and Human Development workshop proposed a three-tier system of interpretation of FHR patterns, but left management recommendations to the professional associations. The middle tier, called indeterminate Category II, which contains the variant FHR patterns seen most frequently, is vast and heterogeneous. We propose that this category can be subcategorized at least tentatively, based on evidence available from previously published studies. Such subcategorization will allow the organizations proposing management recommendations to more readily set up guidelines for graded interventions and clinical responses to the spectrum of FHR patterns, with the aim of minimizing fetal acidemia without excessive obstetric intervention. Such management algorithms will need to be tested by appropriately designed clinical studies.
(Obstet Gynecol 2009;114:136–8)

The recent *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Workshop Report on Electronic Fetal Monitoring,[1] in partnership with the American College of Obstetricians and Gynecologists (ACOG) and Society for Maternal Fetal Medicine, was planned as an update on the last workshop, published in 1997,[2,3] with the aim of revisiting the definitions of the fetal heart rate (FHR) patterns, and updating pattern interpretation. We attended the meeting and had an opportunity for input. However, the final consensus document could not contain all of the proposals and minority opinions raised by the committee participants. For that reason we are suggesting proposals for future consensus meetings on FHR monitoring, particularly with regard to management of FHR patterns.

THE THREE-TIER SYSTEM OF INTERPRETATION

Four possible approaches to classification and interpretation were presented at the conference, the three-tier Canadian,[4] three-tier British,[5] three-tier Miller (United States), and five-tier U.S. and Japanese Framework[6] classifications. The consensus was to accept a three-tier approach, because of its simplicity and ease of teaching.[7] This results in a classification virtually identical to that stated in 1997. The "normal" (normal rate, normal variability, absence of decelerations) and the "abnormal" (absent variability with decelerations or bradycardia) tracings were agreed upon, but consensus could not be reached in the intermediate group, termed the "indeterminate" category, or Category II, in 2008. This category (II) is a hugely heterogeneous group of patterns, with the possibility of differences in baseline rate, variability, decelerations of different type and severity, different evolutions, and based on the best available evidence, differences in fetal risk.

EVIDENCE OF VARIABLE RISK WITHIN THE INDETERMINATE CATEGORY

Early work going back 40 years that attempted to determine the clinical significance of FHR patterns strongly suggested that the degree of fetal acidemia was related to depth of decelerations, whether they be

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Financial Disclosure
The authors did not report any potential conflicts of interest.

ISSN: 0029-7844/09

late or variable decelerations.[8-10] Similarly, the relationship between decreased or absent FHR variability and fetal acidemia was convincingly established, although admittedly in observational studies rather than randomized controlled trials.[9-11] Some of this work was not included in the most recent ACOG Practice Bulletin of 2005.[12] Such observational studies gave rise to the clinical practice of moving toward delivery when FHR variability was decreasing, or was lost in the presence of recurrent decelerations, to such an extent that it is now impossible to study outcomes in such cases in a randomized fashion, because the tracings do not continue due to the intervention. In other words, with such patterns the older data are all we have, and we are unlikely to have any more except fortuitously collected ones. For example, the unique data presented by Paul, Hon, and coauthors in 1975[9] related fetal pH to three groups of late decelerations (mild, moderate, and severe) and was also dichotomized by FHR variability (average or reduced). These data show the striking relationship of acidemia to severity of decelerations, and also the ameliorating influence of FHR variability. There were 28 fetuses in the most acidemic group of severe late decelerations with reduced variability, and it would be ethically unacceptable to prospectively collect such data now. We believe that the heterogeneity of the Category II patterns is such that meaningful studies cannot be done without creating subcategories, preferably by creating at least a five-tier system.

Five-tier systems are very common in medicine and other fields and are not generally regarded as excessively complex. For example, quality of evidence and classification of recommendations (Canadian Task Force on the Periodic Health Examination), risks of drugs in pregnancy (U.S. Food and Drug Administration), adverse event grading scale for patients in experimental studies (used by committees on human research) all use at least five gradations. The Plymouth FHR interpretation and decision support system[13] uses five tiers, as does the Framework approach.[6]

COMPUTER-ASSISTED FETAL HEART RATE INTERPRETATION

Computer-assist devices have been agonizingly slow in coming into clinical usage in management of FHR patterns, although they have been discussed for decades and in fact were considered imminent during the NICHD workshops on FHR in the 1990s. This expectation was probably largely responsible for avoiding the terminology of mild, moderate, and severe categories of decelerations in the 1997 NICHD report, because it was felt that computer analysis would make such visual distinctions obsolete. We are now of the opinion that this was a disservice to the subject, because the computer analyses did not become available, and the absence of such subcategorizations probably discouraged acceptance of the rich literature on observational studies of the past that correlated depth and duration of decelerations, and the importance of FHR variability, to the risk of fetal acidemia. This in turn, we believe, has impeded progress toward developing management guidelines. Although we now have devices that are approved by the U.S. Food and Drug Administration for FHR pattern interpretation, they are not yet diagnostic devices, lack definitive evidence of efficacy, and are unlikely to be widely used very soon in clinical practice.

RESEARCH RECOMMENDATIONS FOR CATEGORY II

The 2008 NICHD document appropriately focuses on the need for further studies, particularly in Category II, where there occur most deficits in our knowledge about the risk of fetal acidemia and about how these patterns evolve into more serious patterns. Because most FHR patterns fall into this category, inconsistency in management by clinicians persists. We believe that subcategorization of this group, based on evidence (albeit limited) already available, would be a stimulus for individual research projects to determine best practices in specific settings and with specific populations. We believe that such categorization should not be limited by fears of medical–legal consequences, because such fears may impede future progress in appropriate studies of FHR monitoring and management of patterns.

We have presented one such approach based on a five-tier color-coded approach.[6] This framework classified all possible FHR patterns (134), based on baseline rate (five categories), baseline variability (three categories), and decelerations (11 categories). The patterns were then color coded based on two factors 1) risk of acidemia and 2) risk of evolution to a more serious pattern. The pattern with least risk, green, corresponds to the 2008 NICHD Category I, and that with the greatest risk, red, includes all of the 2008 NICHD Category III. Three intermediate categories, blue, yellow, and orange, are an attempt to subclassify patterns based on increasing risk to the fetus. These three colors roughly correspond to 2008 NICHD Category II, the Indeterminate group, although we also include more patterns in the red zone than specified in the NICHD Category III. Justification for the subcategorization is given in the original article[6]

and in the references cited above. Tentative management recommendations are also given. We continue to stress that this is a proposal not yet tested fully, although some preliminary validation is emerging (unpublished).

THE THIN LINE BETWEEN INTERPRETATION AND MANAGEMENT

A stated aim of the 2008 Workshop was to recommend a system of interpreting FHR patterns for use in the United States, based on existing classification systems. The aim was not to develop management algorithms, which was considered to be a function of professional specialty entities, although some general management principles were agreed upon for the three categories.

It is our opinion that there is such a thin line between "interpretation" and "management" that it is virtually impossible to separate them in such a document as that produced from the Workshop. This segue to management is seen for Category I tracings, which may be "followed in a routine manner, and no specific action is required." Category III tracings, although they "are predictive of abnormal fetal acid–base state at the time of observation," are followed by the recommendation for "prompt evaluation" and efforts to ameliorate the pattern by conservative techniques.

These clearly are management suggestions, but the second recommendation is incomplete. A pattern which has evolved to Category III, with absent FHR variability and recurrent decelerations or bradycardia, should, we believe, be managed initially by rapid preparation for delivery.[6] This will probably be required for most cases in this category, and we believe that conservative ameliorating techniques, which in most cases will already have been tried, are unlikely to abolish the pattern.

SUMMARY

We recommend that when the specialty organizations produce tentative and testable recommendations for pattern management, they will recognize the heterogeneity of the indeterminate group and include subcategorization of these patterns. This can be done on evidence from already available observational studies, some going back many decades. Such a categorization will expedite studies of recommendations for research made in the NICHD document of 1997, and reiterated in 2008, and will serve as a beginning approach to standardization of FHR pattern management. Such recommendations will then be able to be tested by appropriate studies, to see if fetal metabolic acidemia can be minimized without excessive obstetric intervention.

REFERENCES

1. Macones GA, Hankins GD, Spong CY, Hauth J, Moore T. The 2008 National Institute of Child Health and Human Development workshop report on electronic fetal monitoring. Update on definitions, interpretation, and research guidelines. Obstet Gynecol 2008;112:661–6.
2. Electronic fetal heart rate monitoring: research guidelines for interpretation. The National Institute of Child Health and Human Development Research Planning Workshop. Am J Obstet Gynecol 1997;177:1385–90.
3. Electronic fetal heart rate monitoring: research guidelines for interpretation. The National Institute of Child Health and Human Development Research Planning Workshop. J Obstet Gynecol Neonatal Nurs 1997;26:635–40.
4. Liston R, Sawchuck D, Young D. Society of Obstetrics and Gynaecologists of Canada, British Columbia Perinatal Health Program. Fetal health surveillance: antepartum and intrapartum consensus guideline [published erratum appears in J Obstet Gynaecol Can 2007;29:909]. J Obstet Gynaecol Can 2007;29 suppl S3–56.
5. Royal College of Obstetricians and Gynecologists. Clinical Effectiveness Support Unit. The use of electronic fetal monitoring: the use and interpretation of cardiotocography in intrapartum fetal surveillance. Evidence-based clinical guideline No. 8. London (UK): RCOG Press; 2001.
6. Parer JT, Ikeda T. A framework for standardized management of intrapartum fetal heart rate patterns. Am J Obstet Gynecol 2007;197:26.e1–6.
7. Spong CY. Electronic fetal heart rate monitoring: another look [editorial]. Obstet Gynecol 2008;112:506–7.
8. Kubli FW, Hon EH, Khazin AF, Takemura H. Observations on heart rate and pH in the human fetus during labor. Am J Obstet Gynecol 1969;104:1190–206.
9. Paul RH, Suidan AK, Yeh S, Schifrin BS, Hon EH. Clinical fetal monitoring. VII. The evaluation and significance of intrapartum baseline FHR variability. Am J Obstet Gynecol 1975;123:206–10.
10. Parer JT, King T, Flanders S, Fox M, Kilpatrick SJ. Fetal acidemia and electronic fetal heart rate patterns: is there evidence of an association? J Matern Fetal Neonatal Med 2006;19:289–94.
11. Williams KP, Galerneau F. Intrapartum fetal heart rate patterns in the prediction of neonatal acidemia. Am J Obstet Gynecol 2003;188:820–3.
12. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin. Clinical Management Guidelines for Obstetrician-Gynecologists, Number 70, December 2005 (Replaces Practice Bulletin Number 62, May 2005). Intrapartum fetal heart rate monitoring. Obstet Gynecol 2005;106:1453–60.
13. Keith RD, Beckley S, Garibaldi JM, Westgate JA, Ifeachor EC, Greene KR. A multicenter comparative study of 17 experts and an intelligent computer system for managing labour using the cardiotocogram. Br J Obstet Gynaecol 1995;102:688–700.