厚　生　科　学　研　究

（子ども家庭総合研究事業）

乳幼児死亡率改善の為の研究

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厚生科学研究費補助金（子ども家庭総合研究事業）
総括研究報告書

乳幼児死亡率改善の為の研究

主任研究者：澤口彰子（東京女子医科大学副学長・医学部法医学教室主任教授）

研究要旨：

本研究の目的は、乳幼児の死亡原因に大きな割合を占める乳幼児突然死症候群（SIDS）の研究を通じ、本邦の乳幼児死亡率を改善することにある。主要疾患 SIDS に対して、病因解明・予防・社会的対応・行政的対応の４側面から取り組み、同時に SIDS を含む乳幼児死亡率の動向について把握することを、具体的な目的とする。本年度は、SIDS の病因解明について、生理学的及び病理学的統合研究により覚醒不全説を支持する結果を得た。予防面では、３つの新しい方法論（多チャンネル圧センサーベッド・マイクロ波レーダー・Suckometer 機能を備えたおしゃぶり）を開発・検討した。社会的対応としては啓蒙の為に本研究班のホームページを作成し、“乳幼児突然死のための死亡状況調査に関する国際ガイドライン”を邦訳した。行政的対応としては、死体解剖保存法の改訂案の提唱・病理医に対する検案認定医資格取得の推奨・SIDS の診断に関する専門家間の見解の相違点の整理・日本の SIDS 関連裁判例の特徴の解析を施行した。この他、最近の疫学的データ（1995～1998 年の人口動態調査死亡票及び出生票）を用いて、乳幼児死亡率を検討した。現在の日本において、SIDS に対する行政的対応はキャンペーンによる啓蒙の推進のみであるが、現実には本研究報告書に示されるような法的整備をはじめとする具体的な施策に手をつける必要があることを提言する。

分担研究者：
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A．研究目的

本研究の研究目的は、乳幼児の死亡原因に大きな割合を占める乳幼児突然死症候群(SIDS)の研究を通じ、本邦の乳幼児死亡率を改善することにある。日本の乳幼児保健環境は既に整備されており、日本においてさらなる乳幼児死亡率の改善を期待することは主要疾患である SIDS の減少に頼る他はない。本研究では、主要疾患 SIDS に対して、病因解明・予防・社会的対応（啓蒙）・行政的対応の４側面から取組み、同時に、SIDS を含む乳幼児死亡率の疫学的動向について把握することを、具体的な目的とする。

B―１．研究方法総論

主要疾患 SIDS に次の四つの側面から取り組んだ。

＊病因解明：病理学的研究と生理学的研究の統合

＊予防：

1．現行のモニタリング法による SIDS 予防

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＊行政的対応：
1．死体解剖保存法第 8 条改訂の可能性の検討
2．乳幼児解剖率向上に際しての病理医導入の為の施策の検討
3．厚生省研究班による SIDS 診断の手引き・日本法医学会高津等による SIDS 診断の法医病理学的原則に関する提言・日本法医学会症例検討委員会による乳幼児突然死症例診断の手引きの３者間における見解の相違に関する検討
4．日本における SIDS 関連裁判事例の解析

更に、日本の乳幼児死亡率に関する疫学的動向全般に関して解析を行った。

Ｂ－２．研究方法各論

＊病因解明：

これまで SIDS の病因解明は、疫学的・生理学的・病理学的に個別に研究されてきたが、未だその病因は解明されていない。そこで、これらの各分野の統合を図り、各分野において提唱された病因仮説を別分野から再検証することが有効と思われる。本年度の本研究班においては、生理学的研究と病理学的研究の統合という視点から研究を展開した。

ベルギーブリュッセル自由大学付属小児病院のスリープユニットにおいて、ポリグラフィーを用い生理学的にプロスペクテイブに解析された SIDS 事例およびコントロール事例について、レトロスペクテイブに病理組織ブロックを収集した。生理学的データとして、睡眠中の閉塞性無呼吸の長さと頻度・睡眠中の中枢性無呼吸の長さと頻度を、病理学的データとして、低酸素状態の指標であるアポトーシス（TUNEL 法による）と神経系の可塑性（GAP43 の免疫組織化学による）及びセロトナージックニューロン数(tryptophan hydroxylase の免疫組織化学による）を、脳幹の覚醒経路において定量化した。各事例毎に、生理学的データと病理学的データをリンケージし、SIDS であるかないかを加味して、相関分析を施行した。

＊予防：

1．現行のモニタリング法による SIDS 予防の可能性の検討

米国の collaborative home infant monitoring evaluation (CHIME) で用いられたモニターとほぼ同様の機能を有する呼吸循環のモニター（Sensormedics 社 SomnoStar PT）を用いて、乳児患者 22 症例で記録解析を試みた。

2．非侵襲的な新しいモニタリング法の開発

患者にプローブを全く接触させずに行うモニターが真の非侵襲的モニターであるという観点から、多チャンネル圧センサーによる体位解析を行った。この方法は、圧センサー384 個を敷き詰めたベッドに対象者を寝かせるだけで呼吸の信号を検出しうる方法である。これと平行して、マイクロ波レーダーによる呼吸検知の方法も試みた。マイクロ波レーダーによる方法は、対象者より 50 cm ほど高い位置にレーダーの発信装置をおき反射波を検知することによって呼吸と心拍を検知しようというものである。

3．新生児の自律神経機能の検討

Non nutritive sucking (NNS)下で、心拍数の変動から自律神経系の動態を把握するシステムを確立し、日齢 1~3 の正常正期産新生

児を対象として NNS に対する RR 間隔反応の心拍調節における自律神経バランスの変化との関連を解析した。

4．睡眠時体位の検討

乳幼児突然死症候群（SIDS）の発生頻度は、うつ伏せ寝をやめることを中心とした予防キャンペーンによって、大幅に減少している。しかし、出生直後は分泌物が多いことより、児をうつ伏せ寝にして管理することが一般に行われている。母親と同室にするまでの間うつ伏せ寝にすることは、分娩後 1 日程度で退院することが一般化した欧米においては、その後の児の睡眠姿勢に影響を及ぼす可能性がある。それゆえ、出生直後であっても児を仰向けで管理する安全性について検討を行った。正常新生児 20 名を対象とし、生後 24 時間、パルスオキシメーターで酸素分圧および心拍数を連続記録し検討した。

＊社会的対応：

1．本研究班の 3 年間の実績の要旨を主体としたホームページを東京女子医科大学法医学教室のホームページ内に追加した。
2．“乳幼児突然死のための死亡状況調査に関する国際ガイドライン”を日本語訳した。

＊行政的対応：

1．死体解剖保存法第 8 条改訂の可能性の検討

監察医制度を全国化する場合、乳児死亡全例に対する解剖を義務づける場合、乳児異状死全例に対する解剖を義務づける場合の 3 者について、改訂案を検討した。

2．乳幼児解剖率向上に際しての病理医導入の為の施策の検討

病理医を導入した場合におろそかにされることが予測される死亡状況調査のありかたについて検討した。

3.厚生省研究班による SIDS 診断の手引き・日本法医学会高津等による SIDS 診断の法医病理学的原則に関する提言・日本法医学会症例検討委員会による乳幼児突然死症例診断の手引きの 3 者間における見解の相違に関する検討

3 者間の相違点全てを抽出した。

4．日本における SIDS 関連裁判事例の解析

判例データベース“判例マスター”“判例体系”を用いて抽出した 33 判例について、睡眠時体位・発生時期・剖検の有無・剖検診断・日本での定義設定が判決に及ぼした影響及び死因と判決の関係について解析した。

＊乳児死亡率の疫学的検討：

1．死因別乳児死亡率の出生体重による違い

1995~1998 年の人口動態調査死亡票及び出生票を用い、病死した乳児の内で出生体重が判明している 16327 人について、出生体重ごとの死因別・生存期間別の死亡状況を、累積死亡率と死亡率とを指標として整理した。

2．1990 年代におけるわが国の出生体重別乳児死亡率の改善

東北・東海・九州の 3 地域を対象とし、1989 年及び 1995~1998 年の 2 つの時期について、人口動態調査死亡票と出生票とをレコードリンケージし、同地域での乳児死亡率にどのような改善が認められたかを、出生体重との関連から解析した。

3．乳児期の病死と出生時要因との関連

1995~1998 年の 4 年間の人口動態調査死亡票及び出生票を用い、乳児期の病死に関するリスク要因（出生体重、単胎・多胎の別、妊娠週数、母の年齢、出生児数、死産経験等）を、単変量解析とともにポアソン回帰分析による多変量解析を用いて検討し

た。

C．結果

＊病因解明（病理学的研究と生理学的研究の統合）：

SIDS 児に特異的な２種類の正相関関係が見い出された。一つは、中脳の縫線核における Tryptophan hydroxylase 陽性ニューロン数と中枢性無呼吸の長さとの正の相関関係であり(p=0.027)、もう一つは、脚橋被蓋核における TUNEL 陽性グリア数と GAP43 陽性ニューロン数との正の相関関係である（P=0.041)。中脳の縫線核及び脚橋被蓋核は覚醒経路において重要な役割を果たす部位であり、これらの相関関係は SIDS 児の脳幹の覚醒経路において何らかの器質的変化がおこっていることを示し、SIDS における覚醒不全仮説を支持する。

＊予防：

1．現行のモニタリング法による SIDS 予防の可能性の検討

Somnostar PT によって、無呼吸の検出、その無呼吸の鑑別、無呼吸時の心拍や酸素飽和度といった情報が解析でき、有用であると思われた。

2．非侵襲的な新しいモニタリング法の開発

多チャンネル圧センサーベッドでは、51 症例全例で呼吸運動と体動の認識が可能であり、衣類や寝具が測定に影響することはなかった。

マイクロ波レーダーでは、小児患者 2 名で呼吸と心拍の検出を試みたところ、いずれの患者でも呼吸と心拍の検出が可能であった。しかし、呼吸と心拍を分離するためには、あらかじめ一定の値を外部から入力する必要があった。

3．新生児の自律神経機能の検討

心周期が NNS の周期に対して特徴的な応答を示すことがわかり、その変化は心臓迷走神経活動の消退と関与することが示唆された。

4．睡眠時体位の検討

全例に一時的な酸素飽和度の低下および 5 例（25％）に一時的な徐脈が認められたが、いずれも短期間であり自力で自然快復している。

以上より、正常で出生した児においては、出生直後からも仰向けで管理することは、医学的に問題がないと判断された。

＊社会的対応：作成したホームページは、当分の間東京女子医科大学法医学教室のホームページの一部として管理するが、将来は日本 SIDS 学会で管理するものとし、日本 SIDS 家族の会及び国立公衆衛生院のホームページとリンクするものとした。

＊行政的対応：

1．死体解剖保存法第 8 条改訂の可能性の検討

死体解剖保存法の第８条第３項として、”都道府県知事は、乳幼児における突然かつ予測不能な死亡の全例に対してその死因を判明させる為に解剖を施行させなければならない”とする法文を追加することを提唱する。この為に必要な年間推定解剖予算は 1500 万円から 5000 万円であり、予算計上可能な範囲にあると思われる。

2．乳幼児解剖率向上に際しての病理医導入の為の施策の検討

乳幼児突然死に対する剖検に病理医を導入する場合、病理医が日本法医学会の施行している検案認定医の資格をとるよう推奨すべきである。

3.厚生省研究班による SIDS 診断の手引き・日本法医学会高津等による SIDS 診断の法医病理学的原則に関する提言・日本法医学

会症例検討委員会による乳幼児突然死症例診断の手引きの３者間における見解の相違に関する検討

３者間における主な相違点は、（１）SIDSの診断に関する月齢及び年齢の取り扱い、（２）解剖をしていない症例の取り扱い、（３）うつぶせ寝が窒息の原因となり得るかどうかについての見解、（４）吐乳吸引に関する見解等であった。このような見解の相違は、SIDS 関連裁判の判決・鑑定等に影響し混乱を招くことが予想され、見解の統一の為、上記３者の報告者が直接に討議する機会を早い時期に設けることが必須である。

4．日本における SIDS 関連裁判事例の解析

刑事裁判例（業務上過失致死被告事件）は２例、他は民事裁判例（損害賠償請求事件）である。刑事裁判では、いずれも死因不明とみなされ、判決結果において被告は無罪とされている。１例は控訴審及び上告審でも争われており、原審・控訴審・上告審の全てで判決は SIDS とみなされ、請求・控訴・上告は全て棄却されている。別の１例では、同一事例が刑事裁判と民事裁判の双方において審議され、刑事裁判において死因不明の為無罪、民事裁判においては SIDS と判決され請求棄却とされている。睡眠時体位についてはうつぶせ寝の場合特に新生児でうつぶせ寝の場合に窒息とみなされやすい傾向がある。

SIDS は剖検による除外診断が義務づけられている為剖検が行われないと SIDS とは認定されにくい。剖検診断と死因に対する裁判所の判断が異なる事例も 8 例見受けられ、これらの事例については、その相違をもたらしたものが何かについて、詳細に検討する必要がある。

＊乳幼児死亡率の疫学的検討：

1．死因別乳児死亡率の出生体重による違い

出生体重 2500g 以上での「乳幼児突然死症候群」は、新生児期後の乳児期において「先天奇形、変形及び染色体異常」に匹敵する死因となっていたが、さらに出生体重が軽い児ほど死亡リスクが増大するとともに、低出生体重児ではその発生時期が遅い方向にずれていた。

2．1990 年代におけるわが国の出生体重別乳児死亡率の改善

出生体重分布の軽量化への変化によって減弱されてはいたが、出生体重別の死亡率改善に支えられてわが国の 1990 年代の乳児期の死亡率は改善した。しかも、2500g 以上の児での死亡率改善が、最もおおきな貢献を果たした。「周産期に発生した病態」および「先天奇形、変形および染色体異常」による死亡率は顕著に低下したが、乳幼児突然死症候群の明らかな増加がみられた。

3．乳児期の病死と出生時要因との関連

単産において、新生児死亡リスク及び新生児期後死亡リスクが高い特性は、低出生体重、古い年次、男児、世帯主の主な仕事が無職ないし不詳、短い妊娠期間、遅い出生順位、死産経験ありであり、母親の若年齢では新生児期後乳児死亡のリスクが増大していた。複産での乳児期の死亡リスク増大と関連する特性は、低出生体重、古い年次、世帯主の主な仕事が無職・不詳、短い妊娠期間であった。

4．死因別の乳児死亡率と出生時要因との関連

乳幼児突然死症候群については、低出生体重、10 代の母、遅い出生順位、男児、世帯主の主な仕事が無職・不詳、死産経験ありが死亡リスク増大と関連していた。

D．考察

＊病因解明：

SIDS に特異的な相関関係が抽出され、これらの相関関係は SIDS 児の脳幹の覚醒経路において何らかの器質的変化がおこっていることを示し、これまで確固とした証拠なくして提唱されてきた SIDS における覚醒不全仮説を、生理学的及び病理学的側面から支持するものと思われる。

＊予防：

本研究班で開発された３つの新しい方法論（多チャンネル圧センサーベッド・マイクロ波レーダー・NNS)はプロトタイプであるが有望であり、今後の研究の継続が望まれる。

＊行政的対応：

行政的対応として、まず法的整備が必要であると考え、本年度の研究によって死体解剖保存法改訂という具体案を示した。厚生省の行うキャンペーンによる啓蒙的活動のみでは、現実には限界があり、ここに示されたような具体的な行政施策の実施が必要となってきている。又、SIDS の診断に関する専門家間での公式見解の相違は、裁判における判決や鑑定に混乱を招く恐れがあり、公式な討議の場を複数回設定することが急務であると思われる。

E．結論：

現在の日本は高齢化社会であり、高齢人口を支える若年人口を確保することは国家的な課題である。この意味で、“乳幼児死亡率の改善”は必須の課題である。世界的にもトップクラスにある日本の乳幼児保健環境を更に改善するには、主要疾患である SIDS の減少に頼る他はなく、研究計画の継続が必須である。

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Health Science Research Grants (Research on Children and Families)
Report of Assigned Section

-Utilization between physiological study and pathological study on SIDS : Relationship among glial apoptosis, neuronal plasticity and sleep apnea in the arousal pathway

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Summary

Among 27,000 infants studied prospectively to characterize their sleep-wake behavior, 38 infants died suddenly and unexpectedly under 6 months of age (including 26 cases with sudden infant death syndrome (SIDS), 5 with congenital cardiac abnormalities, 2 from infected pulmonary dysplasia, 2 from septic shock with multiorgan failure, 1 with a prolonged seizure, and another with prolonged neonatal hypoxemia, 1 with meningitis and brain infarction). The frequency and duration of apneas recorded some 3 to 12 weeks prior to the infants' death were analyzed. The brain stem material was retrospectively collected from these 38 infants and studied in an attempt to elucidate the relationship between sleep apnea and neuronal pathological

changes in an arousal pathway. Immunohistochemical analyses were conducted on the following: tryptophan hydroxylase (TrypH) as a serotonin synthesizing enzyme and growth-associated phosphoprotein 43 (GAP43) as a marker of synaptic plasticity. The terminal-deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method was used to find the landmark of apoptosis. The positive pathological reactions were quantitatively analyzed.The pathological and physiological data were linked for each infant.

Statistically significant positive correlations were seen in the victims of SIDS. The number of TrypH-positive newrons in the dorsal raphe nucleus of the midbrain correlated with the duration of central apnea($p=0.027$). The number of TUNEL-positive glias in the pedunculopontine tegmental nucleus(TP) correlated with the average number of spines of GAP43-positive neurons in TP ($p = 0.041$).

The dorsal raphe nucleus of the midbrain and TP play important roles in the arousal pathway. The observed correlations suggest the possibility that the findings in the brainstems were related to the characteristics of the apneas. In addition, the correlation between apoptosis and neuronal plasticity in the arousal pathway might suggest the possibility of an imbalance in neuroinformational transfer leading to reprogramming of neuronal and glial cell death in the arousal pathway.

1. AIM

Several etiological hypotheses have been proposed for the sudden infant death syndrome (SIDS), including the role of apnea [1-3] or of arousal from sleep [4-9].

Neuropathological studies have suggested that the brain stems of infants who succumbed to SIDS were characterized by minute changes [10-19], such as gliosis or apoptosis (20,21), that were attributed to hypoxic insults. Gliosis was evaluated by the immunohistochemical detection of glial fibrillary acidic protein (GFAP)[15,16, 22-24] in the brainstem of SIDS victims, including the medullary reticular formation and the dorsal vagal nucleus [25].

An increase in apoptotic cells in the brains of SIDS victims was reported, in particular in watershed areas [21]. This finding was hypothesized to result from hypoxia [21,26,27]. Hypoxic insult could also favor changes in neurotransmitter synthesis, including serotonin or monoamines[27,28,29].

We hypothesized that hypoxic conditions induced apoptosis and change in the synthesis of neurotransmitter (Figure 1). An apoptotic cascade could then influence synaptic plasticity [32], and concentrations of neurotransmitters, such as serotonine [33,34,35], mainly in the raphe nuclei [36].

A recent report on the thalamic and hypothalamic centers [37] has suggested that the lack of a glial reaction does not support the hypothesis that a disturbed arousal causes sudden infant death(SID). Limited quantification of this state of the arousal response is possible by immunohistochemical analysis of. The pedunculopontine region of the upper brain stem is recognized as a critical modulator of activated behavioral states, such as wakefulness and rapid eye movement (REM) sleep [38]. There are major inputs to the pedunculopontine tegmental nucleus(TP) from the dorsal raphe and

the periaqueductal gray, which is considered to play an important role in the arousal process [39].

In the present study, we evaluated the presence of neuronal and glial apoptosis and the concentration of tryptophan hydroxylase (TrypH), a serotonin synthesizing enzyme in the dorsal raphe nucleus, periaqueductal gray matter of the midbrain, and TP. These structures were chosen as closely related to the arousal process. The pathological findings were then linked to the characteristics of apneas found in the polysomnography done prior to the deaths of the SIDS victims. , which was conducted prospectively.

B. Materials and Methods

B. 1. Physiological Analyses

Subjects analysed

The sleep characteristics of 38 apparently healthy infants were prospectively recorded some 3 to 12 weeks before they died. They had been selected from over 27,000 infants studied prospectively to determine normal sleep-wake characteristics over 20 years in various pediatric sleep laboratories. The families had been invited to join the study when leaving the maternity. The infants entered the study if they were born at term after a normal gestation, and if there was no family or personnal history of apnea, Apparent Life Thretening Event (ALTE) or SIDS. At the time of recording, the infants were aged between 2 and 27 weeks, were healthy, and received no medication.

Following the sleep recording, 38 infants died suddenly and unexpectedly. Following postmortem examinations and death scene investigations, 27 of the 38 infants were considered as SIDS victims[40]; 5 died from congenital cardiac abnormalities; 2 from infected pulmonary dysplasia; 2 from septic schock; 1 from a prolonged seizure; and 1 from prolonged neonatal hypoxemia. General characteristics of the dead infants are reported in Table I.The maximum delay between the estimated time of death and the postmortem examination was 24 hours.

Polygraphic monitoring

The 8-hour sleep studies were conducted overnight in a sleep laboratory, following standard recording techniques [41-44].The recordings were done in a quiet and darkened room, at an ambient temperature ranging between 20C and 23C. All infants slept supine, without restraints. Recording started around 21:00h. The infants were observed continuously during recording. They were fed on demand. Their behavior and any nursing intervention were charted. No infant had a pacifier during the record sessions. The following variables were recorded simultaneously: two scalp electroencephalogram with central and occipital leads, two electrooculograms and electrocardiogram. Thoracic respiratory movements were measured by impedance and airflow with thermistors taped under both nostrils and on the side of the mouth. Oxygen saturation was recorded continuously by a transcutaneous sensor (Nellcor, USA). Gross body movements were measured using an actigram placed on one arm. The data was collected on a computerized infant sleep recorder (Alice recording system III, Healthdyne, USA).

The method and the standard of analysing

Based on the polygraphic recordings, sleep stages and sleep apneas were scored

according to
standard definitions [41-46]. Apneas were scored when they lasted three seconds or longer.They were classified as central apnea when flat tracings were obtained simultaneously from the strain gauges and thermistors. Periodic breathing was defined as the succession of more than 2 central apneas separated from each other by less than 20 seconds. Obstructive apneas were defined as continuous deflections from the strain gauges, with a flat tracing recorded from the thermistors. A mixed apnea was defined as a central apnea directly followed by an obstructive episode. Mmixed apneas were scored together with the obstructrives episodes. The frequency of obstructive apneas was measured by dividing the total number of apnea by the total sleep time in minutes and multiplied by 60. The type, frequency (number per hour of sleep) and duration (in seconds) of sleep apneas were computed. The recordings were analyzed visually by two independent scorers without knowledge of subjects age or sex to ensure the reliabilitiy. Dicrepancies were discused and the agreed upon score was computed for analysis.

B. 2. Pathological Analyses

Subjects analysed

A total of 48 paraffin blocks were collected from the brain stems of the 38 infants who died unexpectedly: 7 blocks from the midbrain, 22 from the pons, and 19 from the medulla oblongata.

Standard histological brain examination

Hematoxyline-Eosin(HE) staining was carried out as the standard histological brain staining.

Immunohistochemical and nuclearhistochemical examination

The blocks were subjected to immunohistochemical and nuclearhistochemical analysis, using an anti-TrypH monoclonal antibody (Oncogene, USA; 1:100), an anti-GAP43 monoclonal antibody (YLEM, Italy; 1: 20), and the TUNEL method (In Situ Cell Death Detection Kit, Boehringer Mannheim, Germany) [47-49]. The 4 microm thick sections made from each block were preincubated with 1 mM of EDTA solution (pH, 8.0) using a microwave oven (Panasonic) at 800 W for 10 minutes to stain GAP43. After the blocking of intrinsic peroxidase by 3% hydrogen peroxide for 5 minutes and washing, the sections were incubated with antibody for three overnight for TrypH or for one overnight for GAP43 at 4C. Finally, TrypH and GAP43 were immunohistochemically visualized with the aid of a LSAB2 kit (Dako) and DAB reaction. Prior to the TUNEL method, the sections were pretreated by microwave irradiation in a 0.01M citric acid buffer (pH, 6.0) at 800 W for 5 minutes in the microwave device (Panasonic) and then TUNEL reaction was carried out in the same manner of the previous published papers[48,49].

Quantitization of immunohistochemistry and nuclear histochemistry

Measurements were made in the periaqueductal gray matter and dorsal raphe nucleus of the midbrain as well as the TP (compact part:TPc, dissipated parts:TPd, and both:TPt). The numbers of TrypH-positive neurons, spines per one GAP43-positive neuron, and TUNEL-positive glias were manually counted. Counting in an area 625x 102 microm2

was repeated 5 times in different overlapped sites and an average was recorded. Next, the density, which was measured as a percentage, of the number of reaction positive neurons or glias against the number of total neurons or glias. As to GAP43-positive dendritic spines, the average of the number per neuron was calculated and recorded.

The pathological measurements were done twice by the same pathologist and the quantitized data with large standard deviation were recounted or rejected.

B.3 Data analysis

Double blind analyses

The scorers of the sleep recordings as well as the pathologist did not known the causes of the infants' death.

Matching of physiological data and pathological data

For each infant, the data on apneas extracted from the sleep recordings were matched with those from the postmortem studies. Correlation analyses were conducted and Pearson's correlation coefficients with significant values were computed by SPSS ver. 8.0. In addition, inner-correlation analyses within the physiological data and pathological data were performed.

B.4. Confirmation of physiological charcteristics of SIDS group and non-SIDS group used in this study

One layout variant analyses were carried out using the general linear models procedure of SAS statistic analysis system release 6.12 to evaluate the contribution of each physiological parameter (frequency of central apnea, duration of central apnea, frequency of obstructive apnea, duration of obstructive apnea) with SIDS.

B.5. Ethical Issues

This study was approved by the Ethical Committee of the University Children's Hospital and was performed in accordance with the ethical standards prescribed by the 1964 Declaration of Helsinki.

C. Results

Results of standard histological brain examination

As the results of HE staining, in three cases specific abnormalities were identified. In one case diagnosed as SIDS, findings of meningitis and infarction were found. The infant was included in the control group. In two cases within the control group, a tumor with hemorrhagic infarct, and a polymicrogyria were found. These deaths were rediagnosed accordingly.

Results of matching between physiological data and pathological data

The results of correlation analyses and non-correlation tests for the three groups(total, SIDS, non-SIDS) were shown in Table 2-A,2-B and 2-C.

(I) Total cases;

As for the inner correlation of physiological data, a statistically significant positive correlation was found between the percentage of periodic breathing and the frequency of central apnea ($p < .001$).A statistically significant negative correlation between the frequency of central apnea and the frequency of obstructive apnea ($p = .024$).

Statistically significant positive correlations were found for the inner correlation of pathological data between (1) the number of spines and number of TUNEL-positive glias in the periaqueductal gray matter (p=.041); and (2) between the number of spines of GAP43-positive neurons in TP and the number of TUNEL-positive glias in TP (p=.041).

The following statistically significant correlations were found between the physiological and pathological data: (1) a negative correlation between the number of TrypH-positive neurons in the TPc and the duration of obstructive apnea (p=.048); and (2) a positive correlation between the number of Tunel-positive glias in the dorsal raphe nucleus and the duration of central apnea (p=.049).

(II) SIDS cases

As for the inner correlation of physiological data, the following statistically significant positive correlation were found: (1) between the frequency and the duration of central apnea (p=.025); (2) between the duration of central apnea and the percentage of periodic breathing (p=.012);(3) between the frequency of central apnea and the percentage of periodic breathing (p<.001).

As for the inner correlation of pathological data, the statistically significant positive correlation were found: (1) between the number of spines of Gap43-positive neurons in TP and the number of Tunel-positive glias in TP(p=0.041); (2) the number of Tunel-positive glias within TP (p<.001); and (3) the number of spines of Gap43-positive neurons within TP (p<.001).

As for the inter-correlation between physiological data and pathological data, the following statistical significant positive correlations were found between the duration of central apnea and the number of TrypH-positive neurons in dorsal raphe nucleus in the midbrain (p=.027) was recognized.

(III]Non-SIDS cases,

As for the inner correlation of physiological data, the following statistically significant correlation were found: negative correlation between the frequency of central apnea and the frequency of obstructive apnea (p=.009); and (2) a positive correlation between the percentage of periodic breathing and the frequency of central apnea (p=.001).

As for the inner correlation of pathological data, the statistical significant positive correlation were found for: (1) the number of spines of Gap43-positive neurons within the TP ??? (p<.001); and (2) between the number of TrypH-positive neurons in the TP and the number of spines of Gap43-positive neurons in the TP (p=.038).

As for the inter-correlation between physiological data and pathological data, the following signifiant correllations were seen:(1) a negative correlation between the frequency of central apnea and the number of spines of Gap43-positive neurons in TP (p=.006); and (2) a positive correlation between the frequency of obstructive apnea and the number of spines of GAP43-positive neurons in TP (p=.021).

(IV) Double common correlation among the three groups,

Double common significant correlations were shown in Table3-A and Table3-B.

(V)single correlation in SIDS groups:

The single correlations seen in the group of SIDS victims were as follows: (1) between the frequency and the duration of central apnea (p=.025); (2) between the duration of central

apnea and the percentage of periodic breathing (p=.012); (3) between the frequency of obstructive apnea and the percentage of periodic breathing (p=.049); (4) between the duration of central apnea and the number of TrypH-positive neurons in the dorsal raphe nucleus(p=.027); (5) as inter-correlation of Tunel-positive glias within TP(p<.001),

(6) between the number of spines of Gap43-positive neurons in TPd or TPt and the number of Tunel-positive glias in TPt or TPd(p=.041); (7) and an inter-correlation between the number of spines of Gap43-positive neurons in TPd and those in TPt (p<.001).

Physiological characteristics of SIDS group and non-SIDS groups:

As the results of variant analyses of the 38 dead infants, the following significant correlations were found with SIDS: the frequency of obstructive apnea (p=.001), duration of obstructive apnea (p=.026) and duration of central apneas (p=.049).

D. Discussion

Compared to the infants who died, the future SIDS victims were characterized by a longer duration of central apneas and a greater incidence of obstructive apneas during sleep. Similar findings were reported in infants who eventually died of SIDS, when compared to healthy control subjects [41,42].

The present postmortem findings in the brainstems of the SIDS victims were also reminiscent of previous reports, both for the presence of gliosis [15,16,22-24] and apoptosis [20,21] in the brain stems.

A recent report described a high incidence of apoptosis within the brain stem of SIDS victims, mainly within the gracile and cuneate nerves, spinal trigeminal tract nerves, tractus solitarius nerves, lateral reticular formation, and lateral cuneate nerve [20]. No apoptosis was reported however in the dorsal raphe nucleus or periaqueductal gray matter of the midbrain and the TP, structures that were examined in the present study. The authors hypothesized that hypoxia was responsible for the increase in the Tunel-positive cells in the brains of patients with SIDS, as both the increase in glial cells and the decrease in neurons can be attributed to hypoxic insults (20).

In the present study, apoptosis occurred mainly in glias, and not in neurons (Figure 3). Only nonspecific staining without chromatin condensation, considered to be typical in apoptosis, was seen in the neurons. However, once triggered, the neuronal apoptosis process can be fast and cells may disappear within 24 hours [20]. It can not be excluded that the so-called passive apoptosis detected by the TUNEL method [49] could include some postmortem changes. According to a previous report however, apoptosis in the human skin is relatively free of changes during the six days after death [49]. In the present study, the postmortem intervals between the supected time of death and postmortem examination were within 24 hours [50]. It is therefore suggested that the glial apoptosis could occur after the disappearance of neuronal apoptosis. Further investigations are required to determine whether the apoptotic findings within the dorsal raphe nucleus result from a direct result of hypoxic exposure. As in most developing systems, an excess of myelinating cells is generated during myelination and redundant oligodendrocytes undergo a type of degeneration known as

programmed cell death or apoptosis [51]. Significantly delayed myelination, hypomyelinsation, was reported in victims of SIDS [52,53]. Increasing of apoptosis of oligodendrocytes (oligodendroglias) may lead to hypomyelination in SIDS. The apoptosis of glias observed in the present study could be associated with these cellular changes.

Petito and Roberts speculated however, that apoptosis is a means of controlling the number of astrocyte cell during gliosis[28].The potential involvement of apoptosis in delayed neuronal death following ischemia are likewise undetermined [54]. The residual neurons from pontosubicular neuron necrosis (PNS) caused by hypoxic insults to the fetal or neonatal pontine nuclei and subiculum differ from typical ischemic necrosis, and resemble apoptosis [55]. Such findings can however be found in young children who succumbed suddenly [55].

In the present study significant correlation was found between the duration of central apnea and the number of TUNEL-positive glias of the dorsal raphe. The correlation was seen in the total patients group, but not in the SIDS subgroup, probably because of the number of subjects. Hypoxemic changes of glias (astrocytes) could be associated with the development of prolonged obstructive sleep apneas [15, 56].

Significant intercorrelations were measured between the number of spines of Gap43-positive neurons in the TP and the Tunel-positive glias in the TP of SIDS victims, as in the total group of subjects. Inner-correlation were found with apoptosis within the TP. TP is important not only for the expression of elements in REM sleep stage but also for arousal reaction [57]. Acetylcoline is found within TP and the malfunction of TP leads the imbalance of the choline/monoamine system in the brainstem [58,59]. The three diffuse projection systems arising in the brain stem, the noradrenergic projection originating in the locus coeruleus, the serotonergic projection from the dorsal raphe nucleus, and the cholinergic projection from neurons gathering in the laterodorsal tegmental nucleus are scattered in the TP function as controllers of sleep and wakefulness [60]. The TP is considered as the area of cholinergic projection, and the TPc coincides with the mesencephalic locomotor region [28]. The projections from the medial preoptic nucleus and nucleus accumbens to the mesencephalic locomotor region are mainly dopaminergic and the efferent projection from the hippocampus is serotonergic [61-66]. TrypH-positive neurons reflect the action of serotonergic neurons. In the SIDS victims, TrypH-positive neuronal cells were found in the periaqueductal gray matter, dorsal raphe nucleus, superior central nucleus, nucleus raphe magnus, and nucleus raphe obscurus. The percentage of TrypH-positive cells was reduced in the periaqueductal gray matter of the midbrain [67]. These findings lead to a hypothesis that a reduction in the number of TrypH-positive cells was related to a depression in serotonin synthesis, possibly resulting in a compromised arousal responses in the future SIDS infants.

The results of the present analysis confirmed a negative correlation between the duration of obstructive sleep apneas and the number of TrypH-positive cells in TPc. In the SIDS infants, the significant positive correlation was found between the duration of central apnea and the number of TrypH-positive neurons in dorsal raphe nucleus. The activity of TrypH was

shown to increase with chronic exposure of hypoxia but not with acute exposure [28]. This fact may explain the positive correlation between TrypH-positive neurons and apnea in the SIDS group.

"Plasticity of the brain" refers to the finding that functional changes induced by external or internal factors can be maintained after those factors disappear [68]. Plasticity of the brain is recognized not only in neurons but also in glias. Neuronal plasticity has been divided into two categories: one, plasticity of synaptic transmission; the other, plasticity of synaptic combinations. The former represents functional changes, not necessarily associated with morphometric changes. The latter requires observable morphometric changes (e.g., the number of synapses, the site of synapses, the number of dendric spines, the quality of receptors, and the number of receptors). These morphometric changes occur not only on the presynaptic but also on the postsynaptic site, such as dendritic spines [68]. GAP43 is a major protein of neuronal growth cones and presynaptic terminals [69-73]. It is a candidate for involvement in both axonal growth and synaptic plasticity. It has been shown that in several neuronal systems, the GAP43 expression is greater in the neurons that are extending axons, either during the development or the regeneration of injured axons found in intact adult neurons. GAP43, best characterized as growth-associated proteins, can inhibit phosphatidylinositol phosphate kinase. It can be phosphorylated by protein kinase C and affect neurotransmitter release.The present study indicated a positive correlations between the number of TrypH-positive neurons in TP and the number of spines of Gap43-positive neurons in Tp. The findings was seen in the non-SIDS group but in the SIDS group.

The so-called programmed cell death, which is often synonymous with apoptosis, participates significantly to brain plasticity, particularly in the process of growth and development. As the result of double mapping of apoptosis detected by the in situ labelling method and the detection of immature neurons by GAP43- positive staining, it was reported that apoptotic cell death occurs primarily among GAP43-positive neurons in the olfactory epithelium [74]. In the present study, the numbers of spines of GAP43-positive neurons in the TP positively correlated with the number of TUNEL-positive apoptotic glias also in the TP of the SIDS infants. The significant positive correlation between the number of spines of Gap43-positive neurons in TPd and the number of TUNEL-positive apoptotic glias in the TPd was confirmed in the SIDS group only. TP is an important part of the arousal pathway.

A physiologic role of apoptosis has been invoked to explain brain development[75]. Apoptosis may be a mechanism for maintaining cell numbers at a critical set point determined by a complex interaction of cellular and extracellular factors, including growth factors and other cellar biological factors[76]. Its role in reactive cellular changes in the brain is still an area of investigation [76]. The final correlation between apoptosis and plasticity in the arousal pathway may suggest a possible reprogramming of neuronal and glial cell death associated with an imbalance in neuro informational transfer in the arousal pathway.

E. Conculusion

The correlation found between the brain

stem changes and apnea suggest the possibility of characteristic changes on the arousal process and apnea. The neuropathological hypothesis shown in Figure 2 could be adopted for SIDS victims. Future studies should be conducted to confirm the relation between the observed changes in the brain stem of SIDS victims and a reduced propensity to arouse from sleep, and to diminish the frequency and duration of sleep apneas.

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F.Research Presentation

1. Presentation in academic meetings

(1) Toshiko Sawaguchi:The potential relationship between apneas, apoptosis & brainstem palticity. The sixth SIDS International Conference.2.8-11.2000, New Zealand.

1. Presentation by publications

(1) Toshiko Sawaguchi, Ineko Kato, Patricia Franco, Jose Groswasser, Martine Sottiaux, Hazim Kadhim, Satoru Shimizu, Sachio Takashima, Makio Kobayashi, Hajime Togari, Etsuko Nishizawa, Yukiko Tezuka, Rie Kurihara, Rika Ebata, Eri Okubo, Mie Hoshino, Hiroshi Nishida, Akiko Sawaguchi, Andre Kahn: Association between apnea and reactive astrocytes in brainstems of victims of post-neonatal deaths. Acta Crim Japon, 2001 in press.

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