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氣球血管形成術前及後之睡眠剝奪增加老鼠頸動脈損傷
後新生內膜增生-初步研究

**Sleep Deprivation Before and After Balloon Angioplasty
Significantly Augments Post-injury Neointimal
Proliferation in Carotid Arteries of Rats – Preliminary
Study**

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中文摘要

冠狀動脈疾病可以使用冠狀動脈氣球血管形成術(percutaneous transluminal coronary angioplasty)來治療。雖然此步驟可有效擴張狹窄的血管，但也會引發血管受傷造成內皮增生並使血管再度狹窄。睡眠剝奪已被報告過會對傷口造成不良之影響；也是發生心血管疾病的危險因子。然而，睡眠剝奪會使氣球血管形成術後引發之內膜增生造成何種程度之影響，目前並不清楚。

研究目的：

本研究想利用大白鼠睡眠剝奪模組與頸動脈氣球血管形成術模組來探討兩者之關聯，探討睡眠剝奪是否會造成氣球血管形成術後之血管新生內膜增生更明顯。

研究方法：

故將大白鼠分組為無睡眠剝奪(C組，即對照組)、睡眠剝奪後(A組)和睡眠剝奪前(B組)施行頸動脈氣球血管形成術，頸動脈氣球血管形成術前及後皆睡眠剝奪(AB組)，在休息13天後，取其頸動脈血管，橫切其管徑並在電子顯微鏡下觀察，比較經睡眠剝奪和正常生理作息之大白鼠之動脈血管管徑中之血管新生內膜。將切下之血管切片做蘇木紫& 伊紅(hematoxylin and eosin)染色並以形態測定方法計算血管新生內膜及中膜的表面面積比率，分析及評估新生內膜增生的變化是否會因睡眠剝奪而增加。

結果：

C 組有兩隻大白鼠，A 組有兩隻大白鼠，B 組有兩隻大白鼠，AB 組有兩隻大白鼠。血管新生內膜及中膜的表面面積比率：C 組為 0.900 ± 0.010 ，A 組為 1.195 ± 0.125 ，B 組為 1.435 ± 0.045 ，AB 組為 1.725 ± 0.021 。A 組、B 組、AB 組的血管內膜及中膜的表面面積比率皆比 C 組有顯著增加，分別各增加了 31.87%，58.24%，及 87.91%。且 A 組、B 組的血管新生內膜及中膜的表面面積比率皆比 C 組有顯著低。

結論：

無論是在睡眠剝奪之前或之後施行氣球血管形成術引起之新生內膜增生皆比無睡眠剝奪者明顯。而且施行氣球血管形成術前及後睡眠剝奪更引起新生內膜之增生。我們的結論是睡眠剝奪會增加氣球血管形成術後引發之新生內膜增生。



Background:

Coronary artery disease can be treated with percutaneous transluminal coronary angioplasty (PTCA). Although this procedure is efficacious in opening stenotic arteries it may also cause injury and inflammation to the vessel producing neointima formation and subsequently restenosis. Sleep deprivation has major effects on early inflammatory response and may produce numerous untoward effects on cardiovascular disease and wound healing. However, whether sleep deprivation may affect injury-induced neointimal proliferation of the vessel is unknown. This preliminary study is to investigate if sleep deprivation will augment balloon angioplasty induced neointimal proliferation in carotid arteries of rats.

Materials and Methods:

Rats were randomly assigned to the following four groups: Group C (control group): balloon angioplasty without sleep deprivation. Group A: balloon angioplasty after 24-hours sleep deprivation. Group B: balloon angioplasty before 24-hours sleep deprivation. Group AB: 24-hours sleep deprivation before and after balloon angioplasty. Twenty four hours sleep deprivation was performed by the disc-on-water method for the rats in Group A, Group B and Group AB. Balloon injury was performed with all rats anesthetized and afterwards subjected to an injury of the right carotid artery with a 2F-Fogarty

balloon catheter. The untouched left carotid artery was used as another (self) control. Thirteen days after the balloon injury, all of the rats were sacrificed and both carotid arteries were removed. The cross sections were later stained with hematoxylin and eosin (H&E) for morphometric analysis.

Results:

There were 2 rats in each group. The post injury neointima-to-media area ratio in Group C, Group A, Group B and Group AB were 0.90 ± 0.01 , 1.2 ± 0.13 , 1.44 ± 0.05 and 1.73 ± 0.02 , respectively. There were 31.87%, 58.24%, and 87.91 increase in post-injury neointima-to-media area ratio in Group A, Group B and Group AB, respectively, compared with Group C ($p < 0.05$). In addition, the post injury neointima-to-media area ratios in Group A and Group B were lower than that in Group AB ($p < 0.05$). There were no neointimal proliferations in the left carotid artery for all groups.

Conclusion:

This preliminary study shows that neointimal proliferation induced by balloon angioplasty is significantly increased whether the 24-hours sleep deprivation was before and/or after balloon angioplasty of rats. In addition, sleep deprivation before and after balloon angioplasty had significantly more neointimal proliferation than sleep deprivation before or after angioplasty alone. We concluded that sleep deprivation significantly augments post-injury neointimal proliferation in carotid artery angioplasty of rats.

關鍵字

睡眠剝奪(sleep deprivation)

氣球血管形成術(balloon angioplasty)

新生內膜增生(neointimal proliferation)



序言及致謝

醫學系畢業後，於林口長庚醫院見實習及其兒童醫院擔任小兒科住院醫師，完成三年住院醫師訓練後，即回到母校附設醫院擔任小兒科研究醫師，訓練急重症，胸腔及消化次專科。本想歷經各種大小考試之後，可以自此不會再有讀書與考試壓力，但環顧周遭同事，一個個皆繼續讀研究所，在這麼樣深造與進修的氣氛中，於是在畢業七年後又重拾學生身份。

因當時有篇論文由小兒胃腸科陳安琪主任推薦我請麻醉科吳世銓部長修改，在修改論文中感受到吳部長無私的熱心與體會到需自我精進研究之精神，加上對動物實驗有感興趣，於是懇請部長當我的指導教授。感謝吳部長願意指導我這位駑鈍又非麻醉科的學生。從吳部長的身上，不僅學到如何撰寫研究論文，也看到了源源不絕的活力與執著。這皆是學生我當學習之態度與效法之典範。

在當時的動物實驗，部長對剝奪睡眠與疼痛之關係著墨最深且有其獨到之見解，適逢又有藥學系主任吳介信教授的氣球擴張後內皮增生之研究經驗與成果，於是我們嘗試將剝奪睡眠與氣球擴張後內皮增生的研究相聯結，希望可以探討兩者之關係，並進一步了解其中之機轉。

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第一章 前言

第一節 研究背景

利用氣球動脈擴張術(PTCA)來治療冠狀動脈或其他血管狹窄造成之疾病如心肌梗塞、粥狀動脈硬化……等已是一種常見的的治療方法。在執行 PTCA 時，動脈壁會被擴張數次以增加管徑的大小，但這也使得往後的六個月內病人有 30-50%的機會發生再狹窄或再阻塞。雖然支架的問世，解決了一部份的血管再狹窄，但仍有 15%-30%的再狹窄會發生在使用支架的病人。

血管再狹窄主要是因為血管因受損引起新生內膜增生，終使血管再度狹窄。而新生內膜增生可以算是一種傷口癒合的過程，所以與發炎反應有關。

睡眠剝奪自 1980 年代起開始被廣泛的研究，並得知會造成免疫力減低、內分泌失調、認知與記憶力受損，且會影響傷口癒合並使心血管疾病發生機率增加。也有許多文獻顯示睡眠剝奪會影響發炎反應。

由上顯示，睡眠剝奪與新生內膜增生皆與發炎反應有關，然而，睡眠剝奪是否會對新生內皮增生產生何種程度的影響則未知。我們推測睡眠剝奪可能會增加新生內膜增生之現象。

第二節 研究目的

本計畫想來探討睡眠剝奪是否會增加氣球血管形成術後引發之新生內膜增生。以睡眠剝奪為平台，配合老鼠頸動脈氣球血管形成術造成新生內膜增生之模組，探討氣球血管形成術前及後之睡眠剝奪是否會增加老鼠頸動脈損傷後新生內膜增生。



第二章 文獻探討

第一節 冠狀動脈氣球血管形成術與新生內膜增生造成之血管再狹窄

冠狀動脈心臟病躍居國內死亡率第三名，傳統治療方式是施行氣球擴張術解決動脈阻塞。而冠狀動脈氣球擴張術即是臨床上最常使用的一種血管成形術，乃利用導管前端的氣球加壓將斑塊推擠在血管管壁上，以改善血管內血流，將阻塞狹窄的冠狀動脈打通。不過，這種手術方式約有三至五成的病患，在手術半年內血管再狹窄會再復發(Seruys PW. et al.

Circulation 1988)。血管再狹窄主要是因血管損傷後產生新生內膜增生；而新生內膜增生之機轉與血管受傷後產生平滑肌內膜移行(圖 1)及一連串的發炎反應有關(Farb A. et al. *Circulation* 1999)。



第二節 睡眠剝奪病理機轉

一個人每天所需的睡眠可能是由基因決定的平均 8 小時，如果減少他的睡眠時間會降低白天的警覺性。警覺度降低或嗜睡情形增加，所顯示出來的狀況是注意力不集中、記憶力減退、以及交通事故的危險增加。

許多臨床及動物實驗研究顯示：睡眠剝奪會造成免疫力、內分泌、認知、記憶··等不良之影響(Nisapel N. *Cell Mol Life Sci* 2007)，也會影響傷口癒合(Landis et al. *Research in Nursing and Health*. 1997)，甚至與發炎反應有關，換言之，睡眠剝奪會引起發炎反應(Malik SW. et al. *Prim Care Clin Office Pract* 2005)。



第三章 研究方法

第一節 研究材料

公的大白鼠(Sprague-Dawley rats)8 隻，年齡約 5-6 週大，體重約 300-400 公克。每隻大白鼠腦部皆植入腦波監視器，植入腦波監視器後休息 2 天使傷口癒合，。大白鼠飼養在長 60 (公分)× 寬 20(公分)×高 60 (公分) 的籠子，且鄰近有同樣飼養的大白鼠陪伴。



第二節 研究設計

將大白鼠分組為無睡眠剝奪(C組，即對照組)、睡眠剝奪後(A組)和睡眠剝奪前(B組)在右側頸動脈施行氣球血管形成術，另外則是頸動脈氣球血管形成術前及後皆睡眠剝奪(AB組)(圖2)。在休息13天後，犧牲大白鼠取其兩側頸動脈血管，橫切其管徑並在電子顯微鏡下觀察，比較有無施行氣球血管形成術及經睡眠剝奪和正常生理作息之大白鼠之頸動脈血管管徑中之血管新生內膜。將切下之血管切片做蘇木紫&伊紅(hematoxylin and eosin)染色並以形態測定方法計算血管新生內膜及中膜的表面面積比率，分析及評估新生內膜增生的變化是否會因睡眠剝奪而增加。

1. 頸動脈氣球血管形成術：

使用2F-Fogarty氣球導管，打氣至 $1.3\text{kg}/\text{cm}^2$ ，通過大白鼠右側之頸動脈血管3次(圖3)，且根據許多動物實驗之文獻報告，氣球血管形成術血管後產生之新生內膜增生在7-14天達最明顯之程度(圖4)，所以我們在施行頸動脈氣球血管形成術後第14天犧牲大白鼠，並取其左右頸動脈做切片染色。

2. 睡眠剝奪實驗模型：

雷赫特夏芬(Rechtschaffen)的大白鼠睡眠剝奪實驗設計(圖5及6)，不管是哪一組皆要在轉盤上適應5天。

當實驗組的老鼠想睡時，其腦波的睡眠波形會啟動動物籠的轉動。控制組的老鼠則可在不轉動的地板自由的睡覺。睡眠剝奪時間為24小時。

3. 形態測定方法

利用電腦色特殊軟體計算血管新生內膜及中膜的表面面積比率(圖 7)。



第三節 統計方法

新生內膜及中膜的表面面積比率以平均值及標準誤表示，並以 ANOVA 變異數分析各組之關係。 $p < 0.05$ 表示有統計學上之差異。



第四章 研究結果

第一節 描述性統計分析

C 組有兩隻大白鼠，A 組有兩隻大白鼠，B 組有兩隻大白鼠，AB 組有兩隻大白鼠。

1. 每組大白鼠其施行氣球血管形成術後之右側頸動脈血管皆有新生內膜之現象，而左側頸動脈血管則無新生內膜之現象(圖 8 及 9)。

2. 血管新生內膜及中膜的表面面積比率：C 組為 0.900 ± 0.010 ，A 組為

1.195 ± 0.125 ，B 組為 1.435 ± 0.045 ，AB 組為 1.725 ± 0.021 (表 1)。



第二節 推論性統計分析

A 組、B 組、AB 組的血管內膜及中膜的表面面積比率皆比 C 組有顯著增加，分別各增加了 31.87%, 58.24%, 及 87.91%。且 A 組、B 組的血管新生內膜及中膜的表面面積比率皆比 C 組有顯著低(圖 10)。由此表示睡眠剝奪會增加老鼠頸動脈損傷後新生內膜增生。



第五章 討論

第一節 結果討論

本研究在探討睡眠剝奪是否會增加氣球血管形成術後引發之新生內膜增生。我們的研究結果顯示無論是在氣球血管形成術前或後睡眠剝奪，皆會增加老鼠頸動脈損傷後新生內膜增生。而且氣球血管形成術前及後皆睡眠剝奪更會增加老鼠頸動脈損傷後新生內膜增生。

氣球血管形成術，即冠狀動脈氣球擴張術，雖是治療冠狀動脈疾病之非手術方式，但也會引發血管受傷造成內膜增生並使血管再度狹窄。在血管新生內膜增生的機轉，也已有許多的研究，但並無提及睡眠剝奪與氣球血管形成術後新生內膜增生後引發之現象所做的觀察報告，我們的研究可說是第一個探討睡眠剝奪與氣球血管形成術後新生內膜增生之關係的研究。

血管受傷造成內膜增生之機轉已有很多文獻討論，其中一連串的發炎反應造成內膜增生已被証實，且發炎反應與血管受傷程度成正相關。

睡眠剝奪會引起免疫反應低下，也已被報告過會對傷口造成不良之影響；其可能機轉與受傷引起發炎反應有關。有許多文獻也証實睡眠剝奪會增加血液中細胞激素，如 IL-6、IFN- γ 、TNF- α 等。所以睡眠剝奪可以誘發發炎反應。

由上之討論，氣球血管形成術前或後睡眠剝奪增加老鼠頸動脈損傷後新生內膜增生，及氣球血管形成術前及後皆睡眠剝奪更會增加老鼠頸動脈損傷後新生內膜增生之可能原因應是睡眠剝奪啟動發炎反應造成新生內膜增

生更明顯。於是我們下一個結論：睡眠剝奪會使促使氣球血管形成術後之內膜增生。



第二節 其他相關性討論

臨床上遇到之狀況:病患預施行冠狀動脈氣球擴張術(PTCA)來治療冠狀動脈疾病，但可能因緊張焦慮而失眠，或施行之後睡不好··等。所以可以進一步收集臨床上有睡眠障礙或有睡眠呼吸中止症之病患且曾施行冠狀動脈氣球擴張術者，回溯性分析並探討人類睡眠障礙會對冠狀動脈氣球擴張術之後的影響。探討是否發生再狹窄之機會增加? 或血管平滑肌受損更嚴重? 如何預防?



第三節 研究限制

1. 此研究所用的大白鼠數目較少，每組只有 2 隻，最好每組可以 6 隻以上。
2. 此研究並無測量大白鼠之賀爾蒙或細胞激素，只做組織切片之染色，或許下個論文可以設計其他實驗來探討賀爾蒙或細胞激素之變化。
3. 我們只從形態測定方法計算血管新生內膜及中膜的表面面積比率，分析及評估其比率是否會因睡眠剝奪而增加，並無從病理切片染色或免疫生化染色來觀察新生內膜之細胞變化。
4. 此研究大白鼠皆為公的，不知母的大白鼠是否也有同樣的結果，還是會因懷孕或女性賀爾蒙改變其結果。



第六章 結論與建議

第一節 結論

氣球血管形成術前及後之睡眠剝奪增加老鼠頸動脈損傷後新生內皮增生。



第二節 建議

1. 研究繼續進行，最好累積每組大白鼠數目達 6 隻以上。
2. 將來研究之方向：確定睡眠剝奪會造成氣球擴張後之血管增生及探討促進因子。可從分子細胞、病理切片染色或免疫生化染色、蛋白質或發炎細胞激素層次來設計更進一步之實驗深入探討其原因及機轉，或利用組織螢光染色技術來研究局部血管附近之變化。也可加驗大白鼠之細胞激素，如 IL-6、IFN- γ 、TNF- α 等。
3. 經由本研究，我們建議在臨床上收集有睡眠障礙或有睡眠呼吸中止症之病患且曾施行冠狀動脈氣球擴張術者，回溯性分析其發生冠狀動脈再狹窄之機會是否比一般正常健康者高。
4. 在施行冠狀動脈氣球擴張術之前及後應給予適當之安眠劑或鎮定劑，以免將來增加冠狀動脈再狹窄之機會。

圖

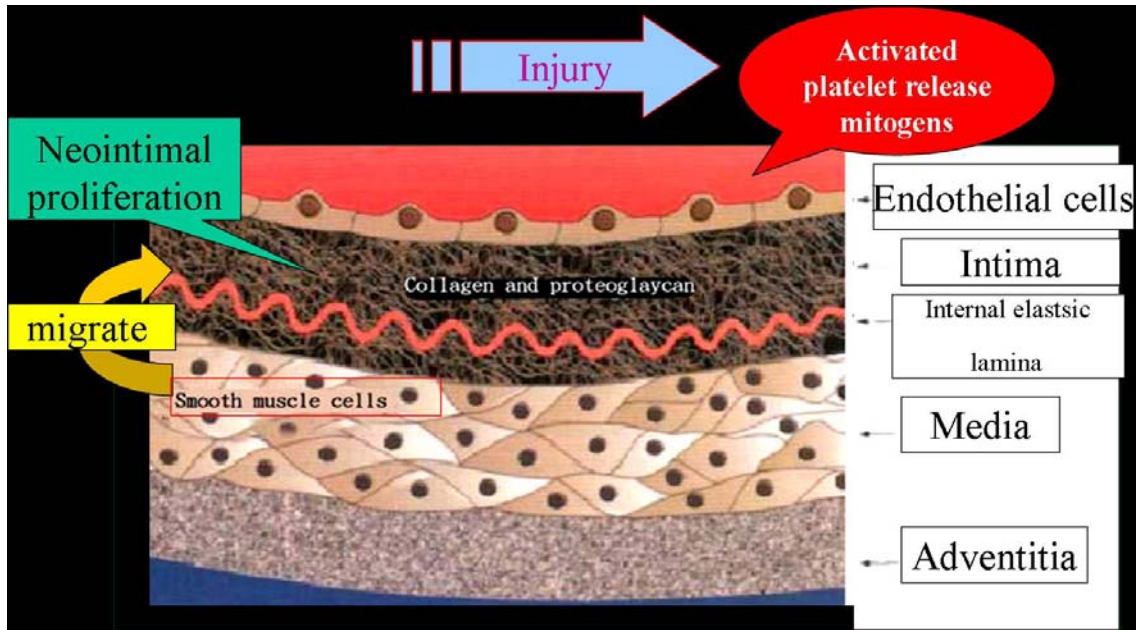


圖 1、血管受損後造成新生內膜增生之機轉模式圖。

血管受損後活化血小板釋放分裂素，使平滑肌肉細胞分裂增生且移行至內膜，造成血管新生內膜增生。

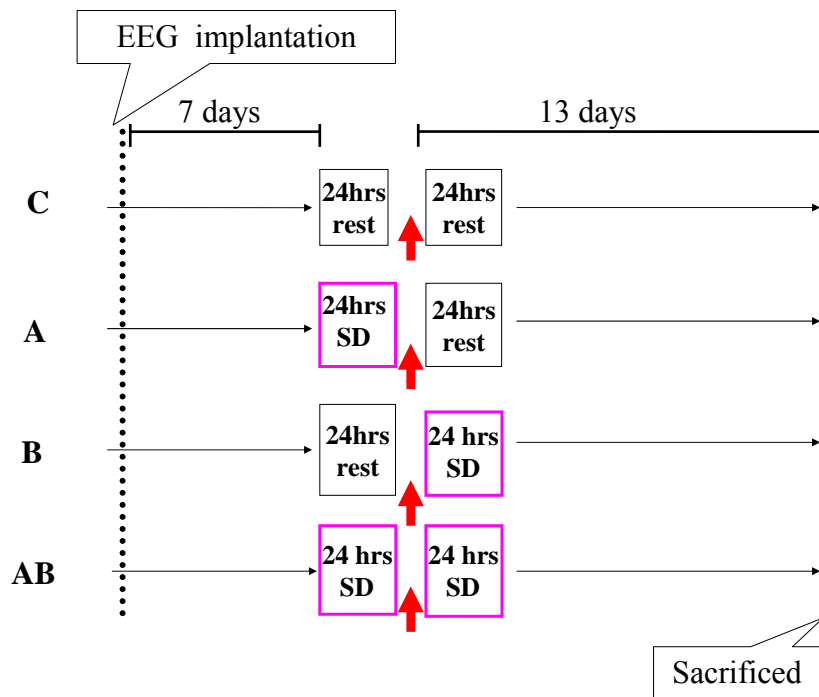


圖 2、實驗設計流程簡圖。

C = C 組，即對照組， A = A 組， B = B 組， AB = AB 組。

SD = 睡眠剝奪

rest = 休息。

EEG implantation = 置入腦波監視器。

Sacrificed = 犧牲大白鼠。

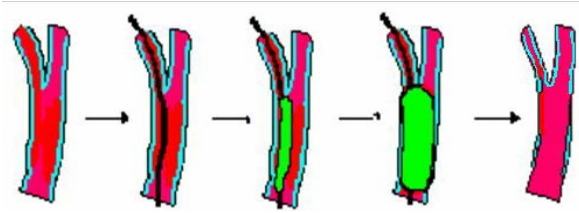


圖 3、頸動脈氣球血管形成術卡通圖示。

使用 2F-Fogarty 氣球導管，打氣至 $1.3\text{kg}/\text{cm}^2$ ，通過大白鼠右側之頸動脈血管 3 次。



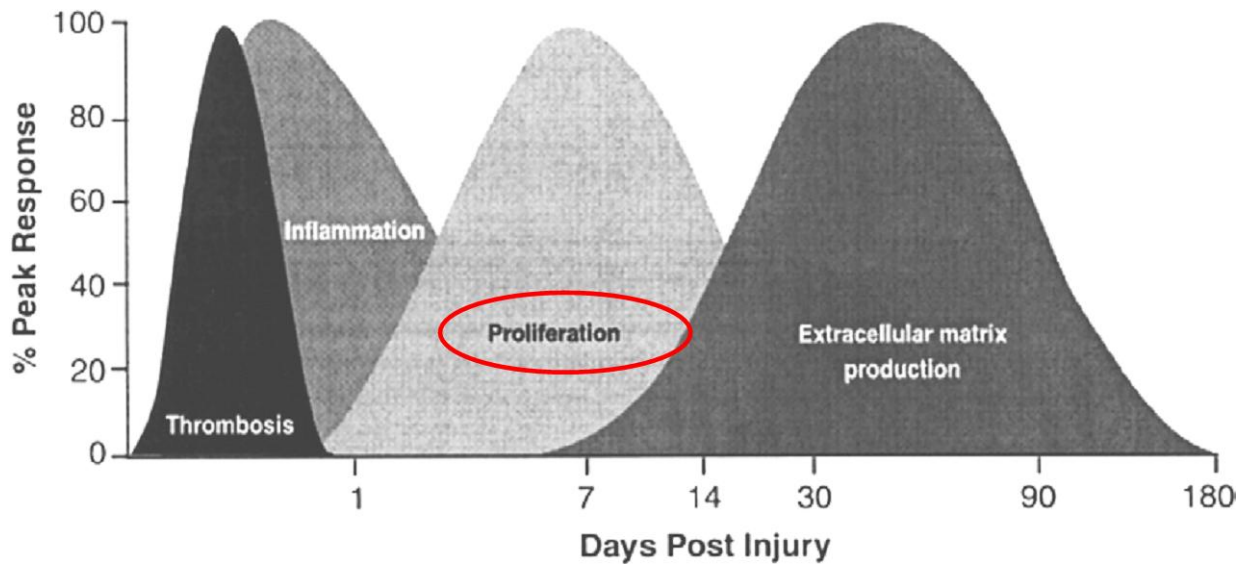


圖 4、血管受傷後造成內膜增生之機轉變化圖示：新生內膜之增生在 7-14 天達最明顯之程度。



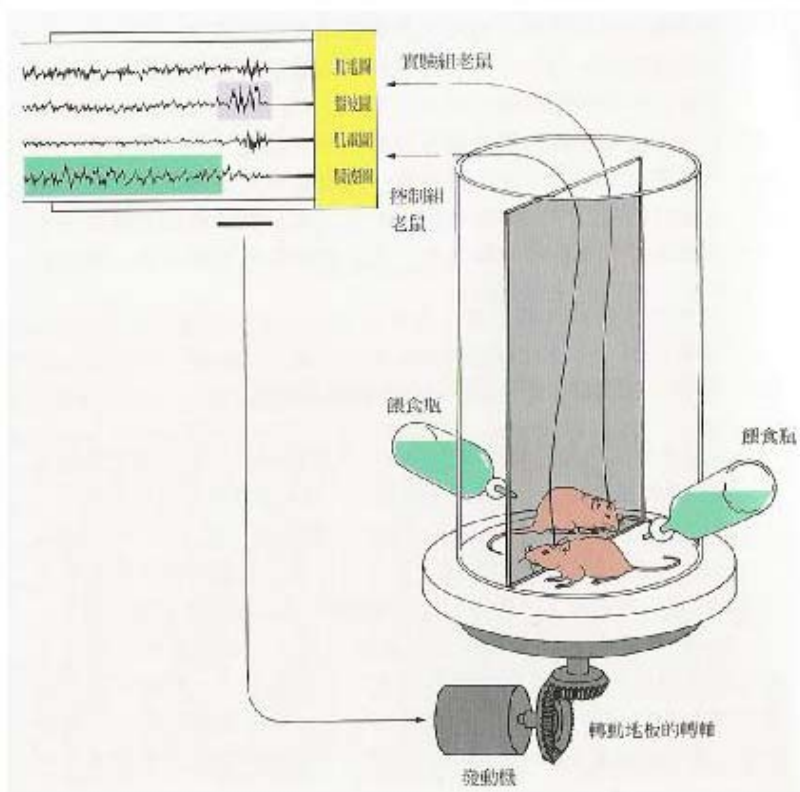


圖 5、雷赫特夏芬(Rechtschaffen)的大白鼠睡眠剝奪實驗設計卡通圖示：
右邊為控制組之大白鼠，左邊為實驗組之大白鼠。





圖 6、本實驗之大白鼠睡眠剝奪實驗圖：
右邊為控制組之大白鼠，左邊為實驗組之大白鼠。
EEG = 連線至腦波監視器。



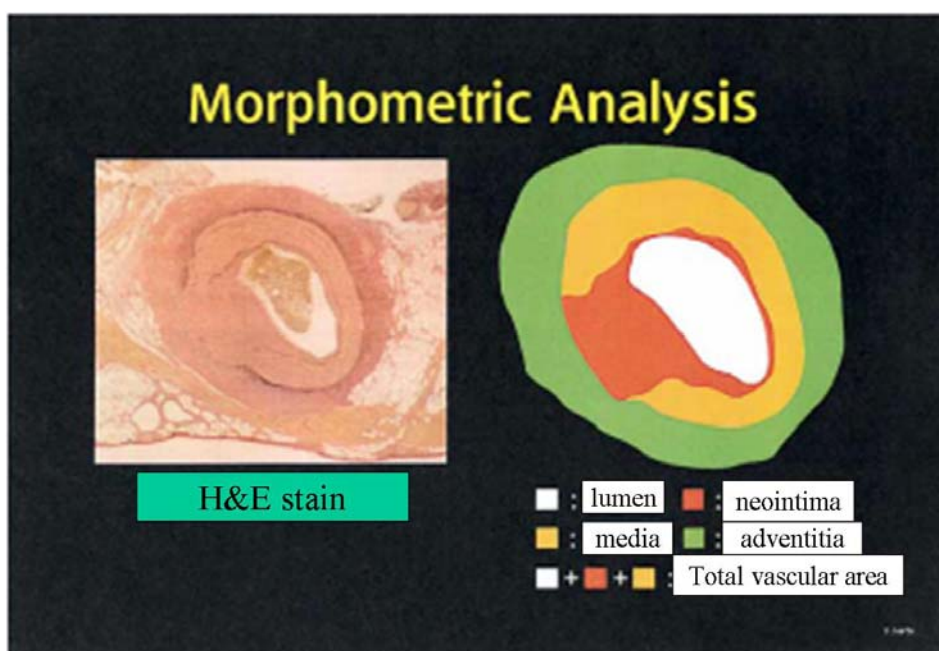


圖 7、形態測定分析方法：

左邊為 H&E stain，右邊為電腦繪圖指示：紅色部份為新生內膜及黃色部份為中膜。



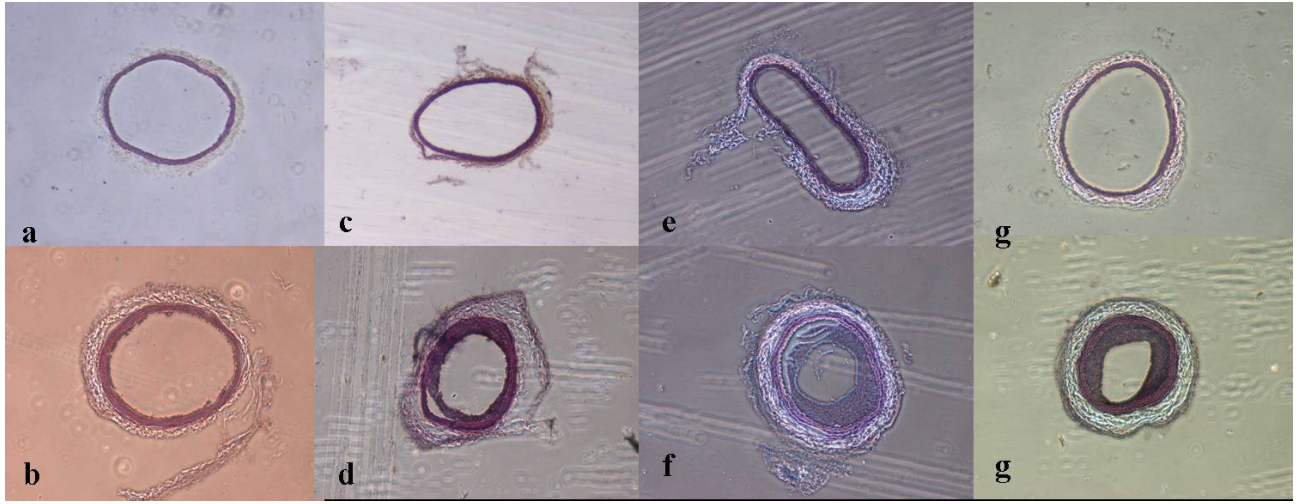


圖 8、各組左側與右側頸動脈血管橫切圖：

上圖為左側頸動脈血管橫切圖片(a 是大白鼠 C1, c 是大白鼠 B1, e 是大白鼠 B1, d 是大白鼠 AB1,)，下圖為右側頸動脈血管橫切圖片(b 是大白鼠 C1, d 是大白鼠 B1, f 是大白鼠 B1, g 是大白鼠 AB1,)。(H&E stain, 放大倍率為 100 倍)



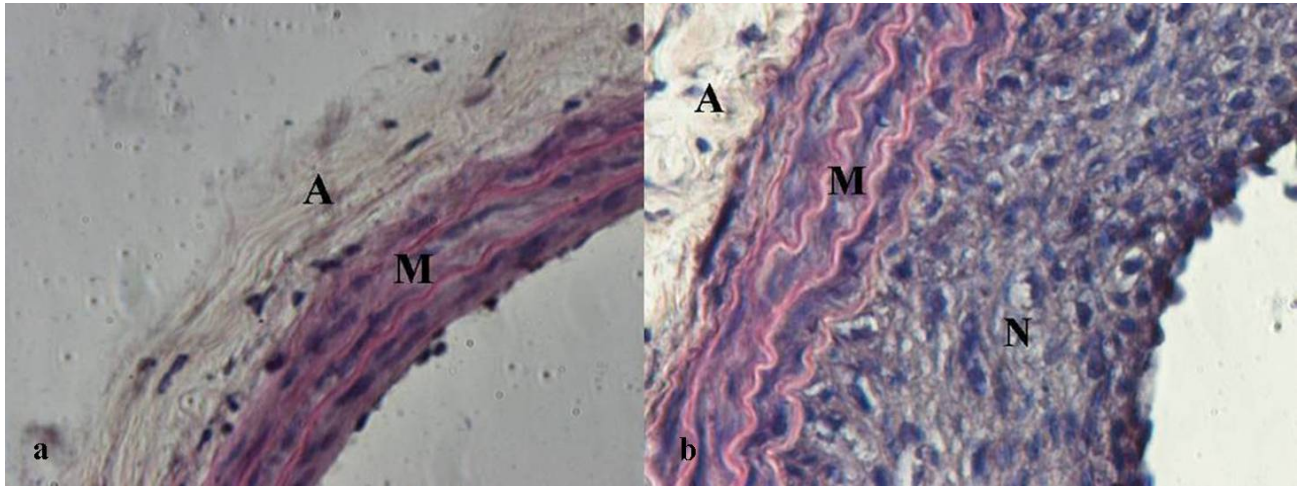


圖 9、頸動脈血管橫切圖片：

a 圖為左側頸動脈血管橫切圖片， b 圖為右側頸動脈血管橫切圖片。(A = adventitia, M= media, N= neointima)

(H&E stain, 放大倍率為 400 倍)



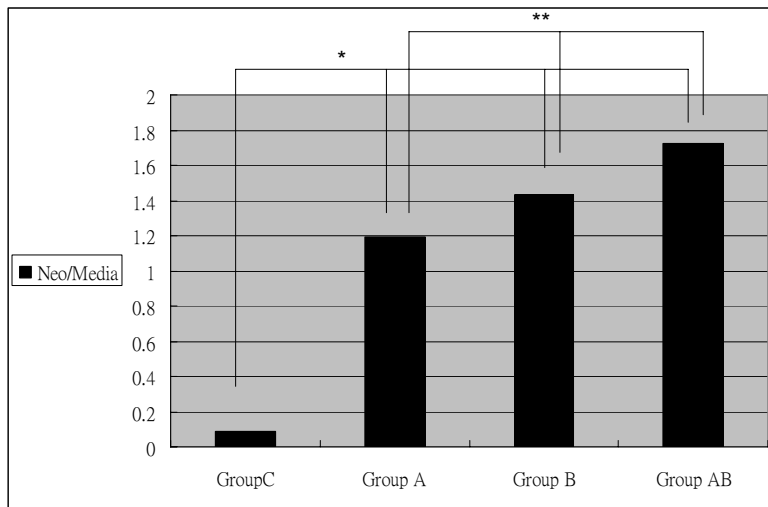


圖 10、C 組、A 組、B 組、AB 組的血管內膜及中膜的表面面積比率柱狀圖：A 組、B 組、AB 組的血管內膜及中膜的表面面積比率皆比 C 組有顯著增加($*=p<0.05$)，且 A 組、B 組的血管新生內膜及中膜的表面面積比率皆比 C 組有顯著低($**=p<0.05$)。

表

	No	Neointima (μm^2)	Media (μm^2)	Neointima-to-media area ratio	Mean ratio
Group C	1	6,029	6,785	0.89	0.900±0.010
	2	5,781	3,371	0.91	
Group A	1	258,524	195,902	1.32	1.195±0.125
	2	31,442	29,477	1.07	
Group B	1	48,040	32,329	1.48	1.435±0.045
	2	58,960	42,417	1.39	
Group AB	1	12,434	7,267	1.71	1.725±0.015
	2	12,554	7,223	1.74	

表 1、各組大白鼠右側頸動脈血管橫切之血管新生內膜及中膜的表面面積，及其比率。



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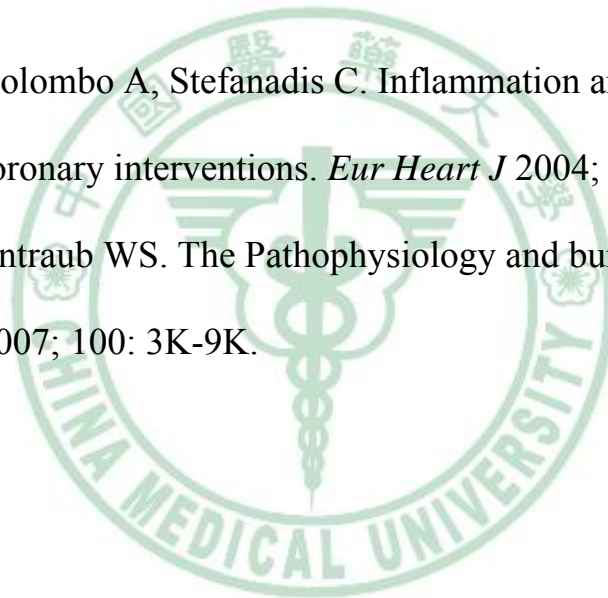
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附錄

附錄一 英文部份

SUMMARY

Coronary artery disease can be treated with percutaneous transluminal coronary angioplasty (PTCA). Although this procedure is efficacious in opening stenotic arteries it may also cause injury and inflammation to the vessel producing neointima formation and subsequently restenosis. Sleep deprivation has major effects on early inflammatory response and may produce numerous untoward effects on cardiovascular disease and wound healing. However, whether sleep deprivation may affect injury-induced neointimal proliferation of the vessel is unknown. This preliminary study is to investigate if sleep deprivation will augment balloon angioplasty induced neointimal proliferation in carotid arteries of rats. Rats were randomly assigned to the following four groups: Group C (control group): balloon angioplasty without sleep deprivation. Group A: balloon angioplasty after 24-hours sleep deprivation. Group B: balloon angioplasty before 24-hours sleep deprivation. Group AB: 24-hours sleep deprivation before and after balloon angioplasty. Twenty four hours sleep deprivation was performed by the disc-on-water method for the rats in Group A, Group B and Group AB. Balloon injury was performed with all rats anesthetized and afterwards subjected to an injury of the right carotid artery with a 2F-Fogarty balloon catheter. The untouched left carotid artery was used as another (self) control. Thirteen days after the balloon injury, all of the rats were

sacrificed and both carotid arteries were removed. The cross sections were later stained with hematoxylin and eosin (H&E) for morphometric analysis. There were 2 rats in each group. The post injury neointima-to-media area ratio in Group C, Group A, Group B and Group AB were 0.90 ± 0.01 , 1.2 ± 0.13 , 1.44 ± 0.05 and 1.73 ± 0.02 , respectively. There were 31.87%, 58.24%, and 87.91% increase in post-injury neointima-to-media area ratio in Group A, Group B and Group AB, respectively, compared with Group C ($p < 0.05$). In addition, the post injury neointima-to-media area ratios in Group A and Group B were lower than that in Group AB ($p < 0.05$). There were no neointimal proliferations in the left carotid artery for all groups. This preliminary study shows that neointimal proliferation induced by balloon angioplasty is significantly increased whether the 24-hours sleep deprivation was before and/or after balloon angioplasty of rats. In addition, sleep deprivation before and after balloon angioplasty had significantly more neointimal proliferation than sleep deprivation before or after angioplasty alone. We concluded that sleep deprivation significantly augments post-injury neointimal proliferation in carotid artery angioplasty of rats.

KEYWORDS: sleep deprivation, balloon angioplasty, neointimal proliferation

INTRODUCTION

Coronary artery disease develops when a combination of fatty material, calcium and plaque builds up in the arteries that supply the heart with blood and is one of the major causes of death and disability. The disease can be treated with percutaneous transluminal coronary artery angioplasty (PTCA) by passing a Fogarty balloon catheter into the lumen of the narrowing artery. However, this procedure can cause the medial smooth muscle cells (SMCs) proliferation within two days and SMCs migration into the intima after four days, where they continue to proliferate for up to two weeks (Olson et al., 2000). These neointimal proliferations and geometric arterial remodeling subsequently result in restenosis, which develops in 30-50% of angioplasty receivers within six months (Stigwart, 1999; Kimura T et al., 1997; Faxon et al., 1984).

Many candidate molecules that regulate neointimal proliferation following vessel injury have been studied including the rennin-angiotensin system, catecholamines, endothelin-1 (ET-1), natriuretic peptides, thrombin, platelet-derived growth factor (PDGF), transforming growth factor- β (TGF- β), fibroblast growth factor, nitric oxide and the estrogen receptor (Berk, 2001; Pawlowski et al., 1997; Gong et al., 1996; Konneh et al., 1995; Rudic et al., 1998; Iafrazi et al., 1997). A numbers of studies carried out in the last decade have consistently indicated that inflammatory mechanisms play a pivotal role in the process of neointimal proliferation and restenosis (Blum et al., 2004; Li et al., 2004; Versaci and Gaspardone, 2004).

Sleep is a major modulator of metabolic and endocrine regulation. Conversely, sleep deprivation has major hormonal and metabolic consequences. Sleep deprivation has been found to affect both behavioral and physiological function, such as memory, cognitive ability, hormone secretion, glucose metabolism, and immune function (Malik and Kaplan, 2005; Van et al., 2007; Zisapel, 2007). It has also been reported that sleep deprivation may produce numerous untoward effects on cardiovascular disease and wound healing (Simpson and Dinges, 2007; Gumustekin et al., 2004). In addition, a study by Vgontzas AN et al. showed that sleep deprivation can provoke a pro-inflammatory response via increased cytokine secretion (Vgontzas et al., 1999). Taken together, these findings provide one possible avenue by which sleep deprivation may affect inflammation and subsequent health.

Since sleep deprivation may provoke inflammation and inflammation has a major role in post-injury neointimal proliferation, we question if sleep deprivation may augment post-injury neointimal proliferation after balloon angioplasty. However, in the literature whether sleep deprivation affects the degree of post-injury neointimal proliferation is unknown. We hypothesize that sleep deprivation may prompt post-injury neointimal proliferation after balloon angioplasty. In the present study, we aimed to investigate whether sleep deprivation augments post-injury neointimal proliferation in carotid arteries of rats by morphometric analysis. As the prevalence of sleep deprivation in the industrialized world appears to be on the rise (Malik and Kaplan, 2005), this

study has important clinical implication in postulating that sleep deprivation may be an important risk factor for post-angioplasty restenosis of the artery.



MATERIALS AND MEDTHODS

To investigate whether sleep deprivation augments neointimal proliferation induced by balloon angioplasty in carotid arteries of rats, the rats were randomly assigned to four groups: Group C (control group): balloon angioplasty without sleep deprivation; Group A: balloon angioplasty after 24-hours sleep deprivation; Group B: balloon angioplasty before 24-hours sleep deprivation; Group AB: 24-hours sleep deprivation before and after balloon angioplasty. **Figure 1** provides a graphic summary of the experimental design.

Experimental Animals

Male Sprague-Dawley rats (5-6 weeks old) weighing 200-300 grams were purchased from the National Laboratory Animal Breeding and Research Center, Taipei, Taiwan. All procedures performed on the animals were approved by the Research Animal Resource Center Committee at China Medical University. The rats were housed in stainless steel cages with 12-h light/dark cycles with free access to food and water. All animal care followed the institutional animal ethical guidelines of the China Medical University Hospital. At the start, all rats had electrodes for cortical electroencephalogram (EEG) monitoring implanted into the brain. The procedure for implantation was as follows: Under Tiletamine-Zolazepam (Zoletil 50®) with xylazine (Rompun®) anesthesia, rats were implanted with four stainless steel screws for EEG

monitoring, one overlaying the right lateral fronto-parietal area, the right and left medial (bregma) parietal area and the left lateral lambda-parietal cortex. One additional pair of nickel–chromium fine wire electrodes were implanted in the dorsal neck muscle for electromyogram (EMG) recording. The electrodes were soldered to a connector, which were fixed to the animal cranium with acrylic dental cement. After the implantation surgery, penicillin and diclofenac were administered in all rats. They were maintained on 12:12 h light-dark cycles at 25 °C for two days for wound healing.

Sleep Deprivation in Rats.

Total sleep deprivation models were elicited using the dish-over-water method with the Rechtschaffen apparatus. The apparatus consists of two rectangular clear plastic chambers, each 60 (length) × 20 (width) × 60 (height) cm³, which are placed side by side to house two rats. Adequate food and water were provided to all rats in the apparatus. Beneath each side of the disc and extending beyond it to the walls of each chamber is a tray with 2-3 cm of water. Before sleep deprivation, all rats were raised ad libitum in the water-disc cages for five days to adapt to the environment. At the start of sleep deprivation, the implanted electrodes were connected to an EEG monitoring system (Biopac System, Inc, MP 150, USA), which functioned with a computer under data acquisition software. The sleep state was controlled by the root mean square of the EEG-theta wave. Upon detecting an EEG sleep state, the computer started

the motor beneath the disc to rotate in a counterclockwise direction at a moderate speed of 3.5 rpm. As the motor rotated the disc, the rats were disturbed and had to walk opposite to the direction of rotation in order not to fall into the water (rats are hydrophobic) thus resulting in total sleep deprivation. Disc rotation stopped when the rats were not sleeping. To avoid bias due to isolation of rats, the functioning apparatus was placed in between 4 other plastic chambers with rats placed for adaptation of their chamber.

Carotid Balloon-Injury Model.

Rats were anesthetized by an intraperitoneal injection of chloral hydrate. Angioplasty of the right carotid artery in each rat was performed using a balloon embolectomy catheter. The balloon catheter (2F Fogarty) (Becton-Dickinson, Franklin Lakes, NJ, USA) was introduced through the right external carotid artery into the aorta, and the balloon was inflated at 1.3 kg/cm^2 using an inflation device. An inflated balloon was pushed and pulled through the lumen three times to damage the vessel. The left carotid artery was left untouched.

Morphometric Analysis.

Thirteen days after balloon angioplasty of the right carotid artery, the

rats were sacrificed with an overdose of desflurane injection. Both the right carotid artery and left carotid artery in each rat were fixed and perfused with 10% formalin. Tissue was harvested for histology at 14th days and fixed with paraformaldehyde at 4 °C overnight. Tissue sections were performed at the desktop microtome with 10 µm thickness and then stained with hematoxylin and eosin (H&E). Tissue slides from each rat were then examined by light microscopy (Nikon, Optophot, Mississauga, ON). Morphometric analysis was carried out using NIH Image analyzer software on a Power PC (G3; Apple Computer, Cupertino, CA) to measure the surface area (µm²). The degrees of neointimal formation of the balloon-injured carotid artery were expressed as the neointima-to-media area ratio, which were subjected to statistical analysis. This is a common mathematical tool that is utilized to normalize the neointimal area for individual differences in arterial sizes (Dalle et al., 2001; Tulis et al., 2001).

Statistics

The neointima-to-media area ratio are expressed as mean ± standard error of the mean (SEM). Statistical analysis was conducted using one-way analysis of variance (ANOVA) with the Duncan test for pairwise comparison. A p-value < 0.05 was considered statistically significant.

RESULTS

Eight rats were enrolled in this study with 2 rats in each group. All eight rats were subjected to balloon angioplasty on the right carotid artery but with their left carotid artery untouched. All rats survived to completion of the three-week study. Cross-sectional segments from both left and right carotid arteries were further analyzed under high of magnification $\times 100$. There were no neointimal formations in the left carotid arteries of the rats in any group (**Figure 2**). In **figure 3**, the histological analysis demonstrated a significant neointimal formation after balloon angioplasty in the right carotid artery but not in the left artery in no.2 group B rat.

Morphometric Analysis.

The morphometric analysis of the balloon angioplasty carotid arteries, including area of neointima and media, and neointima-to-media area ratio are shown in **Table 1**. The post-injury neointima-to-media area ratio of Group A (1.2 ± 0.13), Group B (1.44 ± 0.05) and Group AB (1.73 ± 0.02) were significantly higher ($F= 27.38$, $p=0.0045$) than that of Group C (0.90 ± 0.01) (**Figure 4**). There were 31.87%, 58.24% and 87.91% increase in post-injury neointima-to-media area ratio in Group A, Group B and Group AB, respectively, as compared with Group C ($p<0.05$). In addition, the post-injury neointima-to-media area ratios in Group A and Group B were significant lower ($p<0.05$) than that in Group AB.

However, there was no significant difference in the post-injury neointima-to-media area ratio between Group A and Group B (**Figure 4**).



DISCUSSION

In this study, a rat model of balloon angioplasty on carotid arteries was used to investigate whether sleep deprivation would augment neointimal proliferation triggered by injury to the vessel wall. Neointimal proliferation induced by balloon angioplasty of the vessel was significantly increased whether balloon angioplasty was performed before or after 24-hours sleep deprivation. In addition, neointimal proliferation was also significantly more obvious when sleep deprivation occurred both before and after balloon angioplasty, compared with sleep deprivation occurring only before or only after balloon angioplasty.

Although PTCA and coronary artery stenting have had a tremendous impact on the treatment of coronary vascular disease, these procedures are marked by a high incidence of restenosis (Stigwart, 1999; Kimura T et al., 1997; Faxon et al., 1984). The process of vessel renarrowing occurs because the vascular response to injury triggers a migratory and proliferative response within the SMCs, which exit the quiescent stage G₀ and progress through the G₁ and G₁/S transitions of the cell cycle after arterial injury, resulting in neointimal proliferation (Boehm et al., 2002). Inflammation plays an important role in the development of neointimal proliferation, resulting in restenosis, after balloon angioplasty. More recently, the important role of inflammation in vascular healing has also been increasingly well understood. The focal thrombus formation after artery angioplasty can activate and recruit leukocytes, monocytes, and macrophages from the circulating blood and adventitia at the injury site. The macrophage

number and inflammatory markers could predict the rate of restenosis in patients undergoing PTCA (Moreno et al. 1996). Local inflammation caused by coronary intervention also elicits a systemic inflammatory response initially mediated by inflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor (TNF)- α (Versaci and Gaspardone, 2004).

Although sleep is a fundamental physiologic process, its functions are only starting to be unraveled (Zisapel, 2007; Majde and Krueger, 2005). The main function of sleep is establishing a restorative function for the brain and body, improving the sense of energy and “well-being”, and playing an important role in the cerebral changes that underlie learning and consolidation of memory (Maquet, 1995; Maquet, 2001). Sleep deprivation or disruption, has become a common hallmark of modernity in developed countries, and it can also occur in hospitalized patients after surgery, post-traumatic recovery or other situations. It has multiple effects on immune, endocrine and metabolic function (Malik and Kaplan, 2005; Van et al., 2007; Zisapel, 2007; Simpson and Dinges, 2007; Gumustekin et al. 2004; Vgontzas et al., 1999). Inflammation, one type of non-specific immune response, has the function of directing components of the immune system to a site of injury or infection. Therefore, sleep deprivation can provoke an inflammation increasing response like injury and infection (Vgontzas et al., 1999).

Of cytokines studied thus far, evidence indicates that at least three, IL-1, IL-6 and TNF are involved in the regulation of sleep because plasma levels of these

cytokines increase during sleep deprivation (Zisapel, 2007; Majde and Krueger, 2005). It has been hypothesized that IL-6 is a mediator of sleepiness, and its circadian pattern reflects the homeostatic drive for sleep (Vgontzas, 2005; Wolf and Dittrich, 1992). Based on these data, we postulate that sleep deprivation has deleterious effects on neointimal proliferation after balloon angioplasty, which might be due to elevations in the pro-inflammatory cytokines, such as TNF- α and IL-1.

Neointimal proliferation of the post-injury vessels could be a process of wound healing. A previous similar study of the effects of sleep deprivation on wound healing performed by Gumustekin K et al. showed that sleep deprivation may delay wound healing (Gumustekin et al., 2004). However, the study by Mostaghimi L et al. showed that sleep deprivation did not produce differences in the rate of wound healing, regardless of the timing of the biopsy punch (Mostaghimi et al., 2005). The author states though that it appears to act differently from other types of stressors on wound healing. Another study reported by Landis and Whitney demonstrated that later stages of healing were not affected by sleep deprivation because sleep deprivation might have had greater effects on the early, inflammatory phase of healing (Landis and Whitney, 1997). However, our present study showed that neointimal proliferation was significantly increased whether the 24-hours sleep deprivation was before or after balloon angioplasty, and the neointimal proliferation was also significantly higher when sleep deprivation was both before and after balloon angioplasty.

The number of the experimental rats was only two in each group in this preliminary study; we will enroll more experimental rats in the future. Only male animals were used in our study, and gender-related differences in the degree of neointimal proliferation remain unclear. Furthermore, the mechanism by which sleep deprivation increases neointimal proliferation induced by balloon angioplasty is unclear. In the future, we will design more studies to investigate the role of inflammation in post-injury neointimal proliferation before and after sleep deprivation by detecting macrophage and inflammatory cytokines.

In conclusion, post-injury neointimal proliferation is significantly obvious whether sleep deprivation occurs before or after balloon angioplasty, and sleep deprivation both before and after balloon angioplasty significantly augments post-injury neointimal proliferation. To the best of our knowledge, this is the first study demonstrating that 24-hours sleep deprivation before and after balloon angioplasty augments post-injury neointimal proliferation. This investigation provides the experimental basis toward the clinical implication of sleep deprivation having deleterious effects on patients who have just undergone PTCA. Further studies in clinical settings as well as in animal models are needed to understand the role of sleep in vascular injury in humans.

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Figures

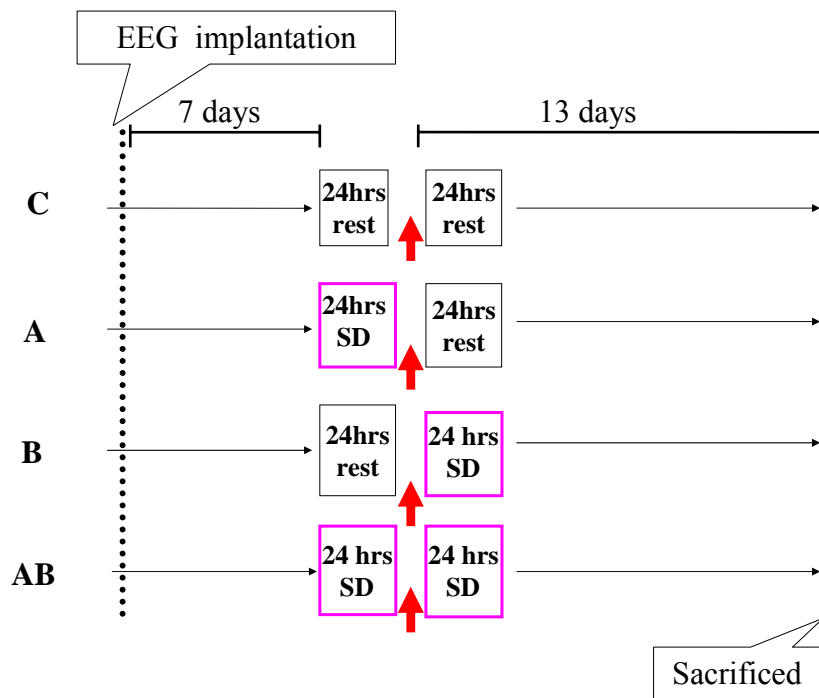


Figure 1. Schematic diagram of the study design. C = Group C, A = Group A, B = Group B, AB = Group AB. EEG = electroencephalogram, SD = sleep deprivation, Red arrows points at time of balloon angioplasty. At the start of the study, electrode implantations for EEG sleep state recording were performed in all rats. A resting postoperative period and environmental adaptation period of 7 days was then provided. Rats were randomly assigned to four groups: Group C (control group): balloon angioplasty in between 24 hours of ad libium activity; Group A: balloon angioplasty after 24-hours sleep deprivation and followed by 24 hours of ad libium activity; Group B: balloon angioplasty after 24 hours of ad libium activity and followed by 24-hours sleep deprivation; Group AB: 24-hours sleep deprivation before and after balloon angioplasty.









Group	Group C No.1 rat	Group A No. 1 rat	Group B No. 1 rat	Group AB No. 1 rat
Left carotid artery				
Right carotid artery				

Figure 2. Samples of Cross-sections of carotid arteries from each group. There were no neointimal proliferations in the left carotid arteries in any group rat. In addition, neointimal areas were more obvious in group A, group B, group AB compared with group C. (H & E staining, original magnification $\times 100$)

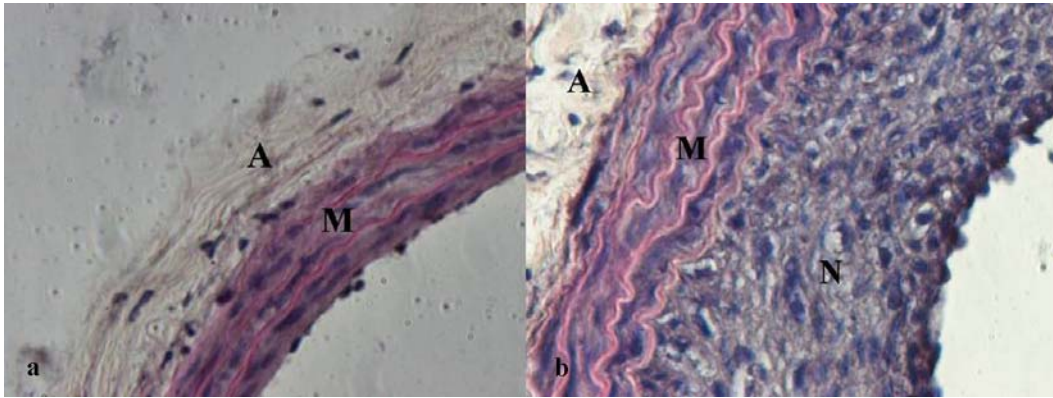
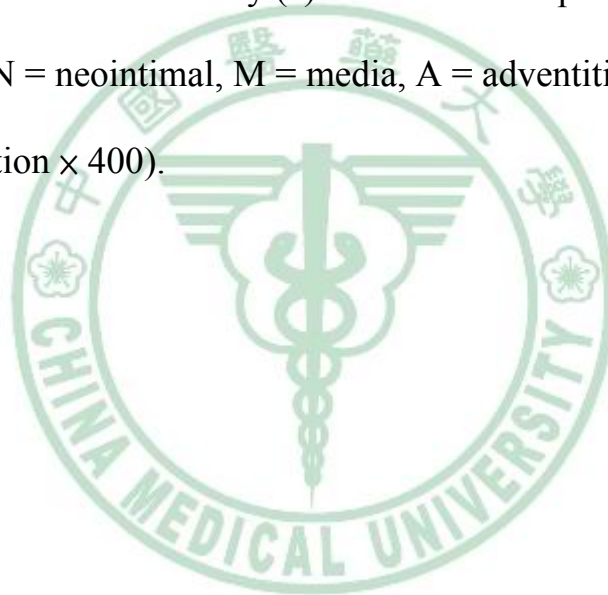


Figure 3. Cross-section of the carotid artery in rat B2, showing no neointimal proliferation in the left carotid artery (a) and neointimal proliferation in the right carotid artery (b). N = neointimal, M = media, A = adventitia. (H & E staining, original magnification $\times 400$).



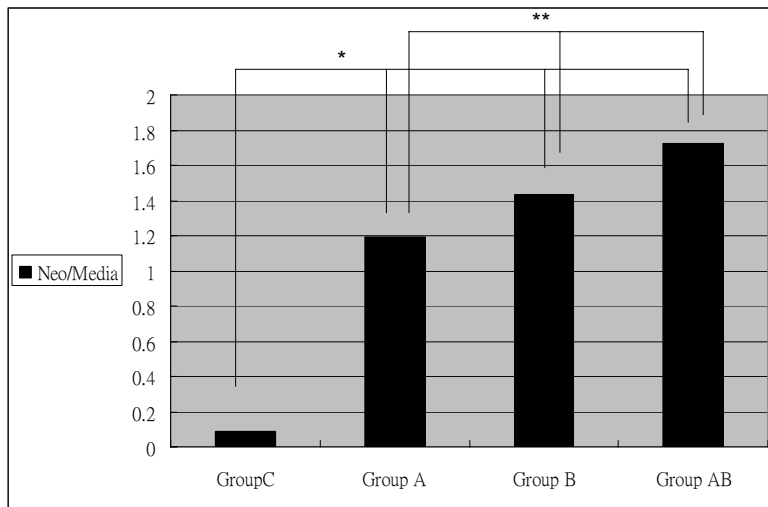


Figure 4. The neointima-to-media area ratio of Group A, Group B and Group AB is significantly higher than that in Group C (* $p < 0.05$). The neointima-to-media area ratio of Group A, Group B is significantly lower than that in Group AB (** $p < 0.05$).

Table

	No.	Neointima (μm^2)	Media (μm^2)	Neointima-to-media area ratio	Mean ratio
Group C	1	6,029	6,785	0.89	0.90±0.01
	2	5,781	3,371	0.91	
Group A	1	258,5 24	195,90 2	1.32	1.20±0.13
	2	31,44 2	29,477	1.07	
Group B	1	48,04 0	32,329	1.48	1.44±0.05
	2	58,96 0	42,417	1.39	
Group AB	1	12,43 4	7,267	1.71	1.73±0.02
	2	12,55 4	7,223	1.74	

Table 1. The area of the neointimal and media of common carotid arteries subjected to balloon angioplasty from rats in each group. The neointima-to-media area ratio was also displayed. Rats were randomly assigned to four groups: Group C (control group): balloon angioplasty in between 24 hours of ad libium activity; Group A: balloon angioplasty after 24-hours sleep deprivation and followed by 24 hours of ad libium activity; Group B: balloon angioplasty after 24 hours of ad libium activity and followed by 24-hours sleep deprivation; Group AB: 24-hours sleep deprivation before and after balloon angioplasty. Values are shown as mean \pm S.E.M.

附錄二 作者簡歷及著作

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著作

(A) Original articles submitted for publication:

1. Chien-Heng Lin, Jeng-Sheng Chang, Ping-Chun Li. The Rescue of Acute Fulminant Myocarditis by Extracorporeal Membrane Oxygenation in Pediatric Patients, Acta Paediatr Tw, 2005; 46 : 201-5 (MI)
2. Chun-Chang Lee, Jeng-Sheng Chang, Chien-Heng Lin, Chih-Shiun Shih. Spontaneous hemopneumothorax in a 17-years-old boy J Pediatr Pulmo 2005; 5: 48-52.
3. Chien-Heng Lin, An-Chyi Chen, Hsiano-Chuan Lin, Wei-Ching Lin, Shu-Fen Wu. Abdominal Actinomyces Complicated with Hydronephrosis: A case Report. J Formos Med Assoc 2005; 104: 669-672 (SCI) (Impact factor:0.474 ; Ranking:48/105)
4. Chien-Heng Lin, Shu-Fen Wu, Wei-Ching Lin , An-Chyi Chen. Wandering Spleen with Torsion and Gastric Vovulus. J Formos Med Assoc 2005; 104: 755-758 (SCI) (Impact factor:0.474 ; Ranking:48/105)
5. Chien-Heng Lin, Ching-Hong Lai, Wei-Ching Lin, Kang-His Wu, An-Chyi Chen, Hung-Chih Lin, Jeng-Sheng Chang. Fulminant Pulmonary Hemorrhage as the Initial Manifestation in Systemic Lupus Erythematosus: A Case Report and Review of the Literature J Pediatr Pulmo 2006; 5: 81-7.
6. Chien-Heng Lin, Shu-Fen Wu, Wei-Ching Lin, An-Chyi Chen. Meckel's Diverticulum Induced Intrauterine Intussusception Associated with Ileal

- Atresia Complicated by Meconium Peritonitis. J Formos Med Assoc 2007; 106: 494-497 (SCI) (Impact factor:0.474 ; Ranking:48/105)
7. Wei-Ching Lin, Jeon-Hor Chen, Chien-Heng Lin, Wu-Chung Shen. Rapidly progressive pancreatic lipomatosis in a young adult patient with transfusion-dependent myelodysplastic syndrome. J Formos Med Assoc 2007; 106: 676-679 (SCI) (Impact factor:0.474 ; Ranking:48/105)
8. Chien-Heng Lin, An-Chyi Chen, Jeng-Dau Tsai, Sung-Hsi Wei, Kai-Chung Hsueh, Wei-Ching Lin. Endoscopic removal of foreign bodies in children. Kaohsiung J Med Sci 2007; 23:447-52. (MI)
9. Lin WC, Chen YF, Lin CH, Tzeng YH, Chiang HJ, Ho YJ, Shen WC, Chen JH*. Emergent Transcatheter Arterial Embolization in Hemodynamically Unstable Patients with Blunt Splenic Injury. Academic Radiology 2007. (SCI) (Impact factor: 1.644; Ranking: 41/84)
10. Preoperative diagnosis of right paraduodenal hernia by multidetector computed tomography Chien-Heng Lin, Yung-Jen Ho, Wei-Ching Lin J Formos Med Assoc 2008; 107: 500-504 (SCI) (Impact factor:0.474 ; Ranking:48/105)
11. Chien-Heng Lin, Wei-Ching Lin Yung-Jen Ho, Jeng-Sheng Chang. Children with Chest Pain Visiting the Emergency Department, Pediatr Neonatal 2008; 49: 26-29. (MI)

(B) Abstract & presentations:

1. 國內研討會

Oral presentation

1. Extracorporeal Membrane Oxygenation in treatment of Fulminant

Myocarditis – report of four cases, Chien-Heng Lin, Jeng-Sheng Chang,

Yung-Chang Lai 兒科醫學會第 44 屆年會第 174 屆學術演講會 台北, 92 年 4 月 26 日

2. Rescue Severe Cardiovascular Failure Children With Extracorporeal

Membrane Oxygenation, Chien-Heng Lin, Jeng-Sheng Chang, Yung-Chang

Lai, Ping-Chun Li*, 兒科醫學會第 176 屆學術演講會, 台北, 92 年 11 月 29 日

3. Experience of percutaneous pigtail catheters for thoracostomy in pediatric

patients Chien-Heng Lin, Jeng-Sheng Chang, Yung-Chang Lai, 兒科醫學會第 45 屆年會第 178 屆學術演講會, 台北, 93 年 4 月 9 日

4. Experience of colonofiberscopy in children in three years, Chien-Heng Lin,

An-Chyi Chen, Shu-Fen Wu, Walter Chen, 兒科醫學會第 45 屆年會第 178 屆學術演講會, 台北 93 年 4 月 10 日

5. Compare chest tubes and percutaneous pigtail catheters for pneumonia with pleural effusion in pediatric patients. Chien-Heng Lin, Jeng-Sheng Chang,

Yung-Chang Lai, 中華民國兒童胸腔醫學會第三屆第一次會員大會暨學術研討會, 桃園, 93 年 4 月 18 日

6. 游離脾併扭轉及胃扭結：一病例報告 李俊美, 林建亨, 吳淑芬, 陳安琪, 陳偉德, 柯世玲, 林哲男 台灣小兒消化醫學會第三屆第一次年會暨學術演講會 台北, 93 年 10 月 3 日
7. Rescue severe enterovirus 71 infection with flexible cardiovascular medication Chien-Heng Lin, Jeng-Sheng Chang, Yung-Chang Lai, 兒科醫學會第 180 屆學術演講會 台北, 93 年 11 月 19 日
8. 以運動不耐為初始表現之兒童真性胸腺增生：一病例報告 吳周潔, 張正成, 林建亨, 馬志豪, 章嘉珍, 林哲男 中華民國兒童胸腔醫學會第三屆第二次會員大會暨學術研討會 高雄, 94 年 4 月 10 日
9. Mortality and Outcome of Pediatric Near-drowning: One Medical Center Report Chien-Heng Lin, Jeng-Sheng Chang, Chih-Hao Ma 台灣兒科醫學會第 46 屆年會暨第 182 屆學術演講會 台北, 94 年 4 月 16 日
10. Chest Pain in Pediatric Patients Presenting to an Emergency Department: One Medical Center Report. Chien-Heng Lin, Jeng-Sheng Chang 台北, 台灣兒科醫學會第 184 屆學術演講會 94 年 10 月 31 日
11. Emergent Gastrointestinal Endoscopy After Foreign Body Ingestion in Children: Retrospective Analysis of 84 Cases. Chien-Heng Lin, An-Chyi Chen, Jeng-Dau Tsai, Sung-Hsi Wei, Kai-Chung Hsueh, Bai-Horng Su 台灣兒科醫學會第 47 屆年會暨第 186 屆學術演講會, 台北, 95 年 4 月 15 日
12. Etiology of Chylothorax in Children: One Medical Center Experience

Chien-Heng Lin, Jeng-Sheng Chang, Hong-Chin Lin, Bai-Horng Su 台灣兒科

醫學會第 47 屆年會暨第 186 屆學術演講會,台北,95 年 4 月 15 日

13. Right lung agenesis with acute airway obstruction: one case report.

Chih-Feng Chang, Chou-Chieh Wu, Tzu-Yao Chuagn, Chien-Heng Lin,

Jeng-Sheng Chang 中華民國兒童胸腔醫學會第三屆第三次會員大會暨學

術研討會, 台中, 95 年 4 月 23 日

14. A Prospective Audit of 100 Consecutive Children with the Presentation of

Right Lower Quadrant Abdominal Pain in Emergency Room. Chien-Heng Lin,

Wei-Ching Lin, Jeng-Dqu Tsai, Sung-His Wei, Kai-Chung Hsueh, Guan-Yi

Lu, Chieh-Mo Lin 台灣兒科醫學會第 188 屆學術演講會, 台北,95 年 11 月

11 日

15. 兒童肺部放線菌病合併腹壁病灶 林建亨, 林維卿, 何永仁, 張嘉麟, 林

玠模 中華民國兒童胸腔醫學會第四屆第一次會員大會暨學術討論會,

台北, 96 年 4 月 16 日

16. Pediatric Application of Abdominal Multidetector Computed Tomography

in Emergency Department. Chien-Heng Lin, Wei-Ching Lin, Yung-Jen Ho,

Jeng-Dau Tsai, Kai-Chung Hsueh, Chieh-Mo Lin 台灣兒科醫學會第 48 屆年

會暨第 190 屆學術演講會, 台北, 96 年 4 月 28 日

Poster

1. 兒童時期之肺膿瘍七年臨床回顧分析 謝承霖, 林建亨, 張正成 中華民

國兒童胸腔醫學會第三屆第一次會員大會暨學術研討會, 桃園, 93 年 4

月 18 日

2. Congenital chylothorax. Chien-Heng Lin, Jeng-Sheng Chang, Hong-Chin Lin, Bai-Horng Su. 中華民國兒童胸腔醫學會第三屆第三次會員大會暨學術研討會, 台中, 95 年 4 月 23 日
3. 全身麻醉下經大腸內視鏡切除兒童大腸直腸瘻肉 林建亨, 吳世銓, 林維卿, 吳淑芬, 陳安琪, 林玠模 中華民國九十六年消化系聯合學術演講年會, 96 年 3 月 17 日
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5. 使用體外膜性氧化器拯救急性猛暴性心肌炎之兒科病患 林建亨, 張正成, 林維卿, 李秉純 2007 年 中華民國重症醫學會第三屆第四次會員大會暨學術演講會
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7. Pancreatic Tumors Metastasis from Other Primary Cancers. Chien-Heng Lin, Wei-Ching Lin, Yung-Jen Ho, Chieh-Mo Lin 2007 年 台灣消化系醫學會秋季學術演講會 96 年 9 月 29-30 日
8. 多探頭螺旋電腦斷層的曲線平面重組影像在診斷阻塞性黃膽的應用 林

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9.以多探頭電腦斷層掃描評估兒童非外傷性腹部急症 林建亨, 林維卿, 何永仁, 林玠模 2008年中華民國放射線醫學會第二十六屆第二次會員大會暨第五十七次學術研討會 97年3月29-30日

10.以多探頭電腦斷層掃之肺血管徵兆來幫助鑑別良性或惡性病灶 林維卿, 蔡伯邦, 陳中和, 沈戊忠, 林建亨 2008年中華民國放射線醫學會第二十六屆第二次會員大會暨第五十七次學術研討會 97年3月29-30日

11.睡眠剝奪對老鼠頸動脈損傷後新生內皮增生之影響-初步研究 林建亨, 吳世銓, 吳介信, 吳憬全, 劉時凱, 黃久珍, 李采娟 2008年台灣睡眠醫學學會 97年度會員大會暨第六屆學術研討會 97年3月29-30日

12.無分葉前腦發育畸形併猴頭畸胎-病例報告 林建亨, 蔡政道, 林維卿, 何永仁 2008年台灣小兒神經醫學會第五屆第一次年會暨第十三次學術演講會 97年5月25日

13.一兒童急診室到院前死亡之流行病學特徵 林建亨、蔡政道、林維卿、林清淵、陳維恭 台灣急診醫學會第八屆第一次會員大會暨學術研討會 97年6月28日

2. 國外研討會

Oral presentation

1. Endoscopic ballon dilatation of esophageal stricture caused by corrosive injury. Shih-Pin Kuo, An-Chyi Chen, Chien-Heng Lin, Shu-Fen Wu, Walter Chen Ninth Congress of the Asian Pan-Pacific Society of Paediatric Gastroneterology, Hepatology and Nutrition 16th-19th June 2005 Malaysian
2. Rescue patients of severe enterovirus 71 infection with flexible cardiovascular medication Jeng-Sheng Chang, Chien-Heng Lin, Shiou-Jien Lin, Bai-Hong Su, The first Congress of Asian Society for Pediatric Research Japan 2005

Poster

1. Colonoscopic polypectomy of colorectal polyps in children – 4 year experience in one medical center. Chien-Heng Lin, An-Chyi Chen, Hsiaou-Chuan, Shu-Fen Wu, Walter Chen. Ninth Congress of the Asian Pan-Pacific Society of Paediatric Gastroneterology, Hepatology and Nutrition 16th-19th June 2005 Malaysian
2. Ileal atresia associated with intrauterine intussusception caused by Meckel's diverticulum. Chien-Heng Lin, Shu-Fen Wu, Tsung-Wen Lin, An-Chyi Chen, Walter Chen, Chia-Lin Chang. Ninth Congress of the Asian Pan-Pacific Society of Paediatric Gastroneterology, Hepatology and Nutrition 16th-19th June 2005 Malaysian

3. Three-dimensional visualization of malrotation in an adolescent patient using multidetector row computed tomography. Shih-Pin Kuo, Shu-Fen Wu, Chien-Heng Lin, Wei-Ching Lin, An-Chyi Chen, Kua-Juei Lin. Ninth Congress of the Asian Pan-Pacific Society of Paediatric Gastroenterology, Hepatology and Nutrition 16th-19th June 2005 Malaysian

