行政院原子能委員會

委託研究計畫研究報告

醣質藥物於肝衰竭之臨床前應用研究

Preclinical Application Research of Glyco-Drug on Hepatic Failure

計畫編號：**1002001INER088**

受委託機關(構)：國立臺灣大學醫學院附設醫院 核子醫學部

計畫主持人：顏若芳

聯絡電話：**(02)23123456 #65581**

E-mail address：rfyen@ntu.edu.tw

核研所聯絡人員：王美惠

報告日期：**2011** 年 **11** 月 **14** 日

目　錄

中文摘要

本計畫目標在建立 ASGPR image biomarker 可為殘餘肝功能檢測指標之臨床前資料庫。Asialoglycoprotein receptors（ASGP-R）是一種肝臟細胞膜上的受體，在各種肝病變的過程中，ASGP-R 的數量會有所變化，本實驗室在 98 年的計畫中，以核研所開發之可與 ASGP-R 有專一性結合的醣質藥物，應用於慢性肝炎與急性肝炎的動物模式上，評估醣質藥物作為肝功能造影劑的可行性，初步已獲得令人滿意的成果，實驗結果顯示在兩種動物模式中，醣質藥物在肝臟的攝取量都有明顯的降低。100 年我們增加病毒性肝炎土撥鼠的動物模式，建立臨床定量造影的 protocol，並評估 ASGPR image marker 應用於病毒性肝炎作為殘餘肝功能檢測指標的可行性。土撥鼠屬於自然病毒感染的動物模式，比較能模擬人體實驗的情形；本年度並將根據臨床前動物實驗的結果撰寫臨床試驗程序書，做為下一步申請人體臨床試驗的重要參考資料。

關鍵字：肝病變、醣質藥物、肝造影劑、單光子斷層影像

Abstract

The purpose of this project is to setup preclinical database for ASGPR image biomarker for an indicator of residual liver function. Asialoglycoprotein receptors（ASGP-R）, located on the hepatocyte membrane, can recognize galactose or N-acetylgalactosamine terminal of desialylated glycoprotein or glycopeptide. Sawamura et al reported that decrease in the number of ASGP-R led to an accumulation of asialoglycoprotein in the sera of galactosamine-treated rats, and the number of these receptors decreases in patients with chronic liver diseases. In the first year, we studied the ASGP-R targeting efficacy by fibrotic and acetaminophen induced hepatitis mice. In this study, we focus on the animal model suffering hepatitis B. ASGP-R biomarker prepared by INER will be performed to demonstrate their in-vivo liver-targeting efficacy and biodistribution by SPECT. Meanwhile, we will gather all the animal studies data to complete the draft of clinical trial, which will be a valuable and important document for our future application of clinical trial.

Key words：liver disease、ASGP-R biomarker、Liver imaging agent、SPECT(single photonemmision tomography)

壹、計畫緣起與目的

目前臨床殘餘肝功能方法有 ICG、Child-Pugh、與 Tc-99m phytate。ICG 適合用在肝癌切除開刀病患，但對肝硬化病患很容易有 false negative 的結果，很難判定其肝臟殘存量；Child-Pugh 係用在肝硬化病患，但即使是 C grade 也很難再細分其差異，無法及早找出極需換肝的病患；Tc-99m phytate 是由肝臟巨噬細胞所吞噬，非真正肝臟細胞之功能。如何判斷一個病人必定要肝移植，否則無法存活；或是這個病人可以不必肝移植也可恢復，因此免去終生使用抗排斥藥物？這個問題一直是臨床醫師最想了解的，以便給與病人最適切的醫療。準確性高的預測指標在臨床上相當重要，但是目前仍缺少大家公認準確的指標。

Asialoglycoprotein receptors（ASGP-R）是一種肝臟細胞膜上的受體，在各種肝病變的過程中，ASGP-R 的數量會有所變化，開發可與 ASGP-R 相結合的藥劑，可以作為肝功能之預測。本計畫的 ASGP-R biomarker 由核研所所製備。

98,99 年我們將重點放在藥毒性肝炎動物模式的建立，以核研所之 ASGP-R biomarker，應用於藥毒性肝纖維化與急性肝炎的動物模式上，評估 ASGPR image biomarker 可為殘餘肝功能檢測指標可行性。初步實驗結果顯示注射 500mg/kg acetaminophen 會導致一半 20g 左右的 mice 發生肝衰竭現象，其發生急性肝炎的時間約在 acetaminophen 靜脈注射後 4 小時，由體內藥物生物分佈實驗及 microSPECT/CT 電腦斷層造影證實，醣質核醫藥物在肝臟的攝取量於藥毒性肝纖維化與急性肝炎動物模式有明顯的降低，而且在數據分析後，發現醣質核醫藥物在肝臟的攝取量低於某一閥值時，小鼠一週內一定會死亡。在膽管結紮肝纖維大鼠，除產生大量腹水外，醣質核醫藥物在肝臟的攝取量也有明顯的降低。這些計畫研究成果皆顯示核

研醣質藥物有潛力應用於藥毒性肝纖維化與肝炎之肝功能評估，未來有進入 IND 的可能。

此外土撥鼠可自然感染土撥鼠肝炎病毒(WHV)，其病程和所產生的病理變化及後遺症都和人類感染 B 型肝炎病毒(HBV)非常類似，包括慢性肝炎和肝癌。在此動物模式中，將人類長達 20~30 年的致癌過程縮短在 2~3 年內發生，是目前用來研究 HBV 引起肝病變 pathogenesis 的最佳動物模式。

本計畫主要目的在

1. 以假體 data 來建立單光子造影的定量模式
2. 探討 ASGP-R 在病毒性肝炎土撥鼠中肝臟組織的分布
3. ASGP-R 於 B 肝土撥鼠分子造影數據的建立, 與 ASGPR biomarker 的相關性, 並用為殘餘肝功能檢測指標
4. 臨床試驗計畫書草案之撰寫。。

貳、研究方法與過程

一、 於2011年4月26日取得行政院原子能委員會核能研究所委託研究計畫合約書 (計畫編號：1002001INER088)，開始進行本委託研究。

二、 於2011年6月完成Tc-99m 之phantom模擬影像SPECT造影檢查。

三、 於2011年5月至6月完成土撥鼠編號 4679、5088、5505之病理切片以anti-ASGPR染色。

四、 於2011年9月完成病毒肝炎土撥鼠動物模式分子影像數據建立。初步結果並在中華民國核醫學學會、台灣分子生物影像學會2011年聯合年會暨2011年尖端生物醫學暨分子影像國際學術研討會以壁報發表。

五、 開始進行臨床試驗計畫書撰寫準備及文獻回顧。

參、主要發現與結論

一、Tc-99m Phantom SPECT Study

(一) 以目前臨床所用 SPECT/CT 掃描儀，以 CT 影像做衰減校正 (attenuation correction)及 partial volume correction，並利用疊代重組演算法做影像重組 (iterative reconstruction)，應該可以做定量分析. 所以首先我們以假體來做掃描儀的內在反應 (intrinsic response) 分析.

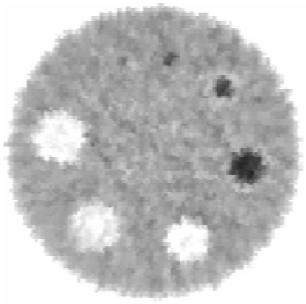
(二) Phantom:

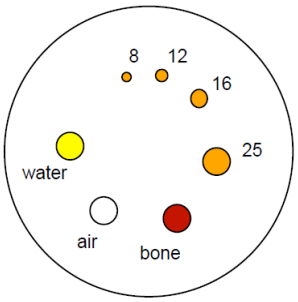
使用 Flangeless Esser PET Phantom™

Specifications of PET Lid:

Refillable thin-walled cylinders: 8, 12, 16, 25 (x3) mm

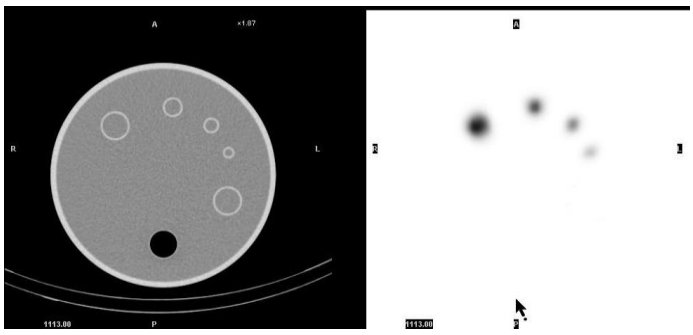
Cylinder height: 1.5 in





(三) 放射性同位素: Tc-99m 5mCi 加入 1000cc Normal saline(NS), 混合均勻後. 以空針抽取 dilute 後濃度為 5uCi/cc 之 NS 注射入直徑分別 25mm, 16mm, 12mm, 8mm 之圓柱體中

(四) 分別於 0, 3, 6, 9, 12 小時進行 SPECT 照影 (每 6 度一個 step, 每個 step 30 秒), 所得到的 raw data 以 OSEM algorithm 進行影像重組, 影像如下:

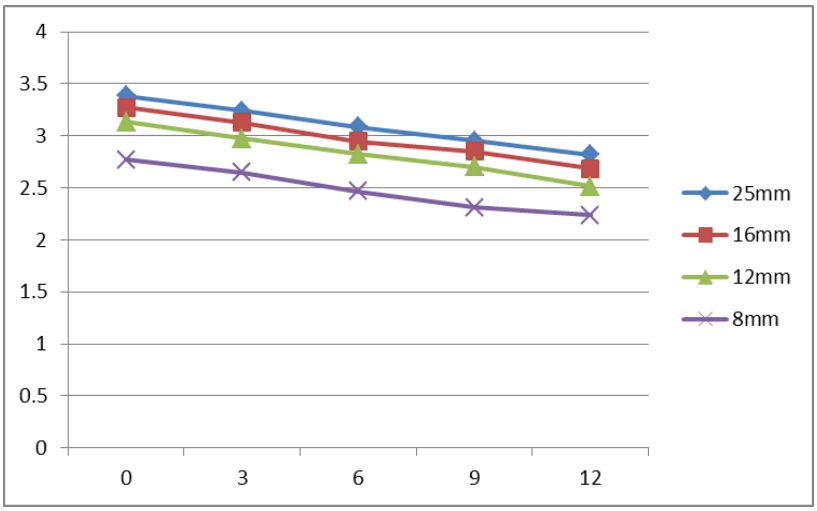


(五) 每一個時間點所得到的 SPECT 影像以一半圓柱體直徑的 ROI 測出每個 ROI 內的 average count 及 maximal count 如下:

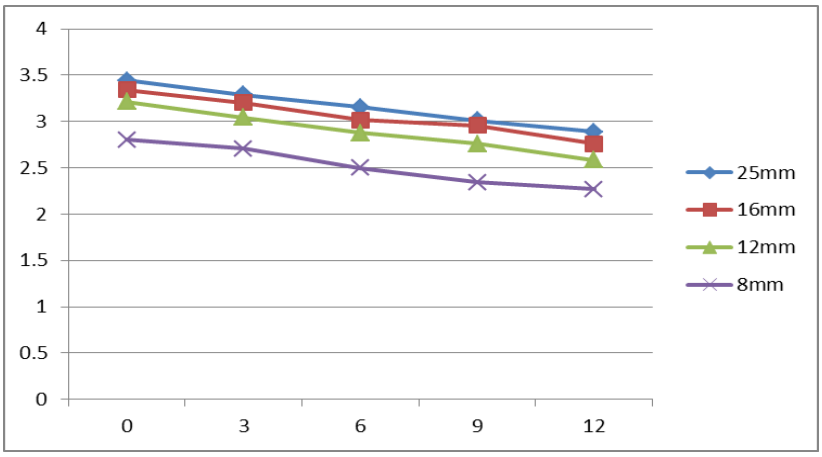
	Average					Maximal			
	25mm	16mm	12mm	8mm		25mm	16mm	12mm	8mm
0	2425	1870	1362	589	0	2767	2203	1644	638
3	1740	1341	937	445	3	1952	1606	1098	509
6	1224	887	666	293	6	1425	1036	758	314
9	891	707	502	206	9	1029	907	574	222
12	659	487	326	172	12	774	574	382	187

(六)　做圖如下: 橫軸: 時間(小時), 縱軸: Log(count)

ROI(average)



ROI(max)



(七) Interpretation and results

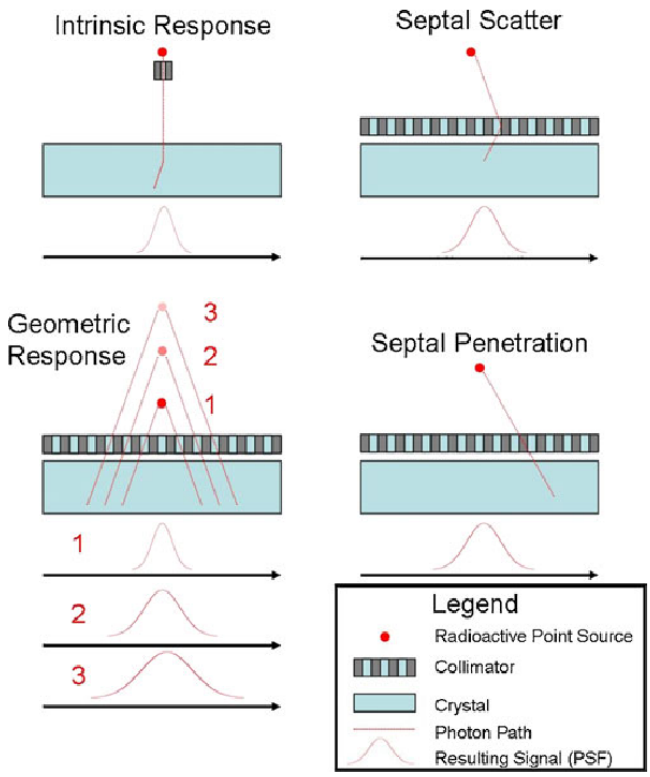
i. 無論以 average count 或 maximal count 來定量, 都有很好的線性關係

ii. SPECT resolution 在同位素濃度在 1.25uCi/cc 時(兩個半衰期)仍可以達 8mm 以上

iii. 仍需定量出 resolution 為 8mm 之最小濃度

(八) Quantification

由 phantom 所注入同位素濃度和 SPECT/CT 測得的 count 可以計算出該 scanner 的 intrinsic response, 進一步計算組織的 kilobecquerels per cubic centermeter.



二、土撥鼠組織病理

之前的小鼠實驗以 ASGPR stain 染色，可見小鼠正常肝臟 ASGPR 數量較纖維化肝臟為多，且具統計上顯著差異.

所以我們由吳慧琳老師提供三隻土撥鼠的肝臟組織，以 anti-ASGPR stain，觀察肝臟組織內 stain 的分佈:

(一) 正常肝藏細胞細胞膜上均有 ASGPR 染色

(二) liver cirrhosis 時 fibrosis 組織的 anti-ASGPR stain 染色減少

三、土撥鼠活體影像模型

(一) Background and objectives

In-111 DTPA-hexa lactoside (HexLac) is an asialoglycoprotein receptor (ASGP-R) targeting agent developed by Institute of Nuclear Energy Research (INER). In the meanwhile, the eastern woodchuck (*Marmota monax*) has been demonstrated to be a good model for studying chronic hepatitis B and HBV-associated hepatocellular carcinoma (HCC). Woodchuck harbors a DNA virus (Woodchuck hepatitis virus [WHV]) that is similar in structure and replicative life cycle to the human hepatitis B virus (HBV). The woodchuck HCC, which usually develops within 1–4 y after WHV infection, is a naturally occurring tumor model that bears similarities to human HCC caused by HBV. It is therefore a better animal model for studying hepatitis B and HBV-associated HCC than mouse model. In addition, the sizes of woodchucks and their tumors are more suitable for imaging diagnosis than those of smaller rodents. Thus, it enablea an easier translation of interventional procedures to future human application.

The aim of this study was to imaging the hepatic uptakes of this agent in healthy, chronic woodchuck hepatitis B virus (WHV)-infected and HCC–bearing woodchucks.

(二) Material and Method

A commercial SPECT/CT (Symbia T2, Siemens Healthcare) equipped with medium energy collimator was used in this study. In-111 and HexLac cold kits were provided by INER. The radiotracers were freshly labeled just before each injection procedure. Dynamic and SPECT imaging were performed for 3 woodchucks, one healthy control, one with chronic hepatitis B, and one with HBV-associated hepatoma. Dynamic study for 30 minutes was performed for each woodchuck immediately after tracer injection (total volume of 10 cc with infusion speed of 300 ml/hr) followed by SPECT/CT acquisition. SPECT/CT imaging was taken by 60 projections over a circular orbit of 360° [30 second per angle] and its data were stored in 128 x 128 matrices. CT transmission scan was performed after the completion of emission data acquisition. Emission data were reconstructed by FLASH-3D algorithm. For data analysis in dynamic study, the regions of interest were drawn on the heart and liver and total count/sec for each ROI was recorded. For SPECT images, the average radioactivity count for liver, heart, kidneys, urinary bladder was obtained by placing the region of interest (ROI) inside the corresponding organs. Specific ASGP-R binding potential was calculated for each woodchuck by the equation: (Mean count ROI (liver)- Mean count ROI (heart))/Mean count ROI (heart).

(三) Results

The analysis of the time-activity curves showed that the liver uptake reached equilibrium in 5 minutes after the completion of tracer injection. There was no significant abdominal tracer activity up to 60 minutes after tracer injection. There were only mild activity in kidneys and urinary bladder. The total tracer uptake ratio of liver/heart and specific ASPG-R binding potential was significantly higher for the healthy woodchuck than the other two WHV infected ones. In addition, very low uptake was noted in hepatoma lesion of woodchuck No. 3.

(四) Study limitations

1. 因為土撥鼠進入冬眠，目前只完成三隻土撥鼠的活體實驗，等明年春天土撥鼠冬眠結束 計劃再做 1-2 批實驗.
2. 所用同位素劑量及掃描時間都比照臨床核醫檢查，所以影像品質沒有一般動物實驗清晰. 未來應該可以增加劑量及掃描時間.

(五) 尚待克服之困難/尋求協助之議題

1. In-111 long physical half life (2.8 d)，考量受試動物 radiation dosimetry 之故，只能用較低的 dose, images 的 quality 可能較低，定量準確性也可能較不精確，如果能發展出 T-99m 標誌藥物應該更為理想.
2. 為進入人體臨床試驗，同位素藥物的安全性測試需要取得認證.

(六) 未來研究方向

1. 建立好土撥鼠模式
2. 準備 phase 1 and phase 2 臨床試驗

(七) Conclusion

In-111 DTPA-HexLac is a highly specific ASGP-R targeting agent which reflect the viability of hepatocytes. The tracer uptake is significantly correlated with the hepatic function reserve. Hepatoma was found to be In-111 DTPA-HexLac non-avid. This may indicate lack of ASGP-R in this malignancy.

肆、參考文獻

1. YC Lee, RR Townsend, MR Hardy, J Lonngren, J Arnarp, M Haralsddon and H Lonn Binding of Synthetic Oligosaccharides to the Hepatic Gal/GalNAc Lectin JBC 258, pp199-202, 1983.
2. SK Ha-Kawa and Y Tanaka　A Quantitative Model of Technetium-99m-DTPA-Galactosyl-HAS for the Assessment of Hepatic Blood Flow and Hepatic Binding Receptor J Nucl Med 1991;32:2233-2240.
3. C Plank, K Zatloukal, M Cotton, K Mechtler and E Wagner Gene Transfer into Hepatocytes Using Asialoglycoprotein Receptor Mediated Endocytosis of DNA Complexed with ab Artificial Tetra-Antennary Galactose Ligand Bioconjugate Chem 3:533-539, 1992.
4. JR Merwin, GS Noell, WL Thomas, HC Chiou, ME DeRome, TD McKee, GL Spitalny and MA Findeis Targeted Delivery of DNA Using YEE
5. (GalNAcAH)3, a Synthetic Glycipeptide Ligand for the Asialoglycoprotein Receptor Bioconjugate Chem 5:612-620, 1994.
6. JJ Hangeland, JT Levis, YC Lee and POP　Ts’o Cell-Type Specific and Ligand Specific Enhancement of Cellular Uptake of Oligodeoxynucleoside Methylphophonates Covalently Linked with a Neoglycopeptide, YEE(ah-GalNAc)3 Bioconjugate Chem 6:695-701,1995.
7. JJ Hangeland, JE Flesher, SF Deamond, YC Lee, POP Ts’o and JJ Frost Tissue Distribution and Metabolism of the P-32 Labeled Oligodeoxynucleoside Methylphosphonate-Neoglycopeptide Conjugate, [YEE(ah-GalNAc)3]-SMCC-AET-pUmpT7 in the mouse

Antisense and Nucleic Acid Drug Development 7:141-149, 1997.

8. H Toyama, K Suzuki, A Naito, M Kuroda, K Kikukawa, Y Komori, A Hasumi, K Matsumura, T Fujiwara, K ito, K Ejiri, K Senda, A Takeuchi and S Koga Evaluation of asialoglycoprotein receptor imaging agent as a marker of hepatic ischemia-reperfusion injury and recovery. Annals of Nuclear medicine 13(3):155-160, 1999.
9. AH Kwon, T Inoue, and SK Ha-Kawa Characterization of the Asialoglycoprotein Receptor Under Hypoxic Conditions in Primary Cultured Rat Hepatocytes J Nucl Med 46:321-325, 2005.
10. T. Sawamura, H. Nakada, H. Hazama, Y. Shiozaki, Y. Sameshima, Y. Tashiro Hyperasailoglycoproteinemia in ptients with chronic liver diseases and/or liver cell carcinoma: asialoglycoprotein receptor in cirrhosis and liver cell carcinoma. Gastroenterology. 1984; 87:1217-1221.
11. GY Wu, JM Wilson, F. Shalaby, M. Grossman, DA Shafritz and CH Wu Receptor-mediated gene delivery in vivo J Biol Chem. 266(22):14338-14342, 1991.
12. Zhu JY, Leng XS, Dong N, Qi GY, Du RY. Measurement of liver volume and its clinical significance in cirrhotic portal hypertensive patients. WJG. 1999;5:525-526.
13. Kaibori M, Ha-Kawa SK, maehara M, Ishizaki M, Matsui K, Sawada S, Kwon AH. Ann Nucl Med. 2011 epub.
14. Yang W, Mou T Shao G Wang F, Zhang X, Liu B. J Nucl Med. 2011; 52:978-985.
15. Kew MC. Synergistic interaction between aflatozin B1 and hepatitis B virus in hepatocarcinogenesis. Liver Int. 2003;23:405-409.
16. Huang KW, Wu HL, Lin HL, Liang PC, Chen PJ, Chen SH, et al. Combining antiangiogenic therapy with immunotherapy exerts better therapeutical effects on large tumors in a woodchuck

hepatoma model. PNAS. 2010;107:14769-14774.