上海医科大学校庆七十周年论文汇编

主　编　　姚　泰

上海医科大学出版社

前　言

为迎接学校建校七十周年，反映我校近年来的科研成就和学术水平，决定编辑出版《上海医科大学校庆七十周年论文汇编》。在征稿过程中，共收到校内作者论文 3 421 篇，校友论文 394 篇，为此专门成立了编委会。经编委会讨论，最后选出论文 491 篇（校内作者的 333 篇，校友的 158 篇），以摘要形式刊登（每人限登一篇）；其余的校友论文以题录形式刊登，校内作者的将以专门题录集出版。此外，为反映我校毕业的院士在医学领域的成就，征集了院士近期发表或完成的论文 13 篇，以全文刊登。

编委会

1997.7

目　录

论 文

小肝癌研究的过去、现在与未来
——临床与分子生物学方面

汤钊猷

（上海医科大学中山医院、肝癌研究所）

根据1995年我国卫生部的统计，原发性肝癌（简称肝癌）在我国癌症死亡中的位次已由过去的第3位上升为第2位，在农村仅次于胃癌，在城市则次于肺癌；90年代初，肝癌的死亡率为20.40/10万（胃癌为25.21/10万）[1]。预期我国胃癌将与日本一样逐渐下降，而肝癌下降的速度可能将不如胃癌。Parkin (1993) 报道1985年全世界新的肝癌病人数为315 000人，其中137 500人发生在中国[2]。

肝癌自建立起其科学基础以来已有百余年历史，但其险恶的预后改变甚慢。美国肝癌的相对5年生存率在1974～1976、1980～1982、1986～1992的三个年代，白人分别为4%、4%、和7%；而黑人则分别为1%、2%、和5%[3]。70年代，由于甲胎蛋白 (AFP) 用于无症状人群的普查，导致小肝癌的早期发现和切除，而且获得值得鼓舞的5年生存率[4～8]。80年代由于医学影像学、局部治疗、对亚临床复发的再切除，使小肝癌的生存率得到进一步提高[9～14]。由于综合治疗的进步，不能切除肝癌的缩小后切除成为小肝癌研究的一个延伸[15]。在西方对小肝癌作肝移植其疗效优于切除[16,17]。基础研究已深入到分子水平，从而对切除后进一步提高生存率有潜在意义，尤其在控制复发和转移方面。在我国，小肝癌的研究已概括在题为《亚临床肝癌》（英文版）一书中[18]。

小肝癌研究的意义

小肝癌研究的意义可概括为以下方面：

(a) 是获得肝癌长期生存者的重要途径。我所曾报道生存长的肝癌病人[19,20]。随访至1995年，我所共有239例生存5年以上的肝癌病人，而1905～1970年间的世界文献中仅45位肝癌病人生存5年以上 (Curutchet 等，1971)。分析239例所用的治疗方法提示小肝癌切除占第1位，其次为大肝癌切除，然后是不能切除肝癌的缩小后切除和切除以外的姑息性外科治疗。

(b) 为提高肝癌5年生存率的重要途径[21]。我所住院病人的5年生存率自1958～1970年 (178例) 的4.8%，1971～1983年 (646例) 的12.2%，提高到1984～1995年 (1 815例) 的46.7%，与小肝癌切除在肝癌病人中比例的提高有关，这三个时期分别为1.7%、9.8% 和31.9%。

(c) 为治疗上事半功倍之道。小肝癌切除 (645 例) 的 5 年生存率与大肝癌切除 (950 例) 比为 61.3% 对 33.6%。

(d) 小肝癌研究促进了肿瘤标记、定位诊断、复发转移和分子生物学的研究。

(e) 促进了肝癌临床一系列概念的更新，包括肝癌的自然病程等。小肝癌病人用药物治疗其 1～、2～、3～、4～、5～年生存率分别为 72.7%、36.4%、13.6%、13.6% 和 0%；这一结果与 Ebara 所报道者 (<3cm 小肝癌) 相仿，其 1～、2～、3～年生存率分别为 90.7%、55.0% 和 12.8%[22~24]。此外，小肝癌切除后有长期生存者也提示肝癌也有单中心发生者[25]。

70 年代和 80 年代临床格局的改变

70 年代小肝癌研究的主要特征包括：①证实通过 AFP 对普通和高危人群的普查监测，可有效查出亚临床肝癌，其中大多数为小肝癌。②证明对 AFP 和 SGPT 的联合分析对小肝癌的早期诊断有用。③对合并肝硬化的小肝癌倡用局部切除以代替肝叶切除，导致切除率的提高、手术死亡率的降低和生存率的提高。最近的结果表明，局部切除(包括左外叶切除)的 5 年生存率甚至比右半肝切除者更高，为 64.2% 对 55.8%。④小肝癌作手术切除其 5 年生存率远高于非手术切除者，分别为 62.9% 和 25.2%。

80 年代以来小肝癌研究的进展包括：①强调在肝癌高危人群(有肝炎史，有家族肝癌史和乙型肝炎病毒携带者)作普查。对高危人群普查，肝癌检出率提高，比正常人群高 34 倍[26]。②在普查中强调 AFP 与超声联合应用。③随着医学影像学的迅速发展，非侵入性的超声 (US)、电子计算机 X 线断层显像 (CT)、磁共振显像 (MRI) 等已成为小肝癌定位的常规，目前直径 1cm 的小肝癌已不难检出。④小肝癌的治疗强调多种方法的应用，如反复瘤内无水酒精注射或术中冷冻治疗是不宜手术切除小肝癌治疗的可选用的方法，而经导管动脉化疗栓塞的疗效较差。我所 56 例小肝癌用冷冻治疗的 5 年生存率为 53.1%[27]。⑤证明根治性切除后定期用 AFP 和超声监测可发现亚临床期复发，对亚临床期复发的再切除可进一步提高根治性切除后的生存期[14]。⑥采用 HBV-DNA 整合和 p53 基因型的分析，证明小肝癌切除后复发既有单中心发生，也有多中心发生者[28,29]。⑦另一进展为使大肝癌转变为小肝癌[15]。

总之，70 年代的努力主要是改进早期发现、早期诊断和早期治疗的效果。而 80 年代则主要是在高危人群普查、用新的医学影像学技术作定位、探索切除以外的局部治疗、早期发现和治疗肝癌切除后的亚临床期复发，以及通过缩小疗法使不能切除大肝癌变为可切除的小肝癌。预期如供肝来源和费用问题得到解决，肝移植可望成为部分小肝癌病人的有效疗法，而生物学特性的研究则可能是小肝癌切除后控制复发转移的一个重要研究目标。

由大肝癌转变来的小肝癌

小肝癌可来自对高危人群的普查、对慢性肝炎与肝硬化的监测和肝癌根治性切除后的随访。近年由于癌症局部治疗和综合治疗的进展，一些仍局限的不能切除的大肝癌已可能转变为可切除的小肝癌[15,30~34]。文献中这类转变见于肝母细胞瘤[35]。我所在 663 例手术证实不能切除肝癌中有 72 例转变为可切除者。成功的缩小疗法主要是采用肝动脉结扎

(HAL)、肝动脉插管灌注(HAI)、放射免疫治疗,或超分割局部外放射等的三联或二联治疗。72 例序贯切除的手术死亡率为 1.4%,5 年生存率为 62.1%,可与小肝癌切除者相比(61.3%,645 例);这是由于肿瘤的中位直径已由 10cm 转为 5cm。分析表明,单个结节、包膜完整、位于右叶或肝门区、伴小结节肝硬化、用三联或二联治疗者,其二期切除率较其相对应组高;单个肿瘤局限一半肝、无癌栓、手术标本无残癌者生存期较长。对缩小疗法而言,三联或二联治疗较单一治疗更有效[33]。自 1985 年起,我们在 HAL 和 HAI 的基础上又加上放射免疫治疗[36~39]。局部超分割外放射是放射免疫治疗的一种代替[40]。序贯切除率为:用 HAL+HAI+放射免疫治疗或放射治疗的三联治疗为 14.3%~34.0%,HAL+HAI 的二联治疗为 10.1%,而单一治疗仅为 1.1%。近年由于经导管动脉内化疗栓塞(TACE)的进步,为不能切除的肝癌提供了一种非手术的缩小疗法。我所也曾报道 TACE 后的序贯切除,最近总结的 59 例,其 5 年生存率为 56%[41]。

复发的治疗

小肝癌根治性切除后的 5 年复发率可高达 43.5%[42]。为此我们强调在小肝癌根治性切除后每 2~3 月用 AFP 与超声监测 5~10 年,以发现亚临床期的复发。对肝内的亚临床期复发或单个肺转移,再切除是最好的疗法[43]。我所 147 例再切除的 5 年生存率自第 1 次手术起算为 48.9%[21]。吴孟超等报道 72 例再切除,5 年生存率自第 1 次手术起算为 49.5%[44]。我所小肝癌切除的 5 年生存率由 1971~1983 年的 52.4% 提高到 1984~1995 年的 63.0%,主要是由于复发再切除数的增多。但复发的多中心发生仍然是一个障碍。

复发的细胞起源

从临床的角度,阻碍肝癌生存率进一步提高的主要因素是复发和转移。再切除无疑可延长生存期,但这一办法由于肝癌的侵袭性以及多中心发生而受到限制。对复发的细胞来源有多种方法可进行研究:①通过研究 HBV-DNA 整合的位点,证实复发和多发结节中既有单中心又有多中心发生[45,46]。②也有报道染色体 16 杂合性的丢失(LOS)也有助多中心发生的诊断[47]。③p53 杂合性的丢失也有被用于鉴别复发细胞来源的[48]。我所用 HBV-DNA 整合和 p53 基因型的方法证实复发肝癌同时存在单中心和多中心发生[28,29]。通常切除后 1 年内复发者多来自切除灶的播散,而若干年后出现者则可能为另一个中心。

小肝癌与大肝癌的比较

小肝癌与大肝癌相比,前者局部切除率高(70.1% 对 40.8%),从而导致切除率较高(93.2% 对 49.5%)、手术死亡率较低(1.3% 对 4.0%)以及 5 年生存率较高(60.0% 对 22.2%),10 年生存率也较高(42.7% 对 16.1%)。前者在病理方面以单个肿瘤较多(79.1% 对 54.1%)、包膜完整者较多(74.2% 对 36.4%)、门静脉癌栓较少(31.3% 对 45.2%)、Edmondson 分级为 3~4 级肝癌者也略少(9.7% 对 16.7%)。丛文铭等比较小肝癌(<3cm)和大肝癌后发现:66.7% 的小肝癌为二倍体,包膜受侵者较少(16%),癌栓较少(20%),切除后

5 年生存率较高 (75%)；而大肝癌中 92.3% 为异倍体，84% 有包膜受侵，80% 有癌栓，5 年生存率仅为 46.2%[49]。为此，3cm 可能是一个分界线。总之，病理研究提示小肝癌常分化较好，二倍体较多，多为单个有包膜和肿瘤；然后随着肿瘤的增大而变为分化差，异倍体多，多个结节者多，包膜也欠完整[22,50]。

小肝癌复发转移机制与阻断的实验研究

已如上述，小肝癌复发可因肝癌的多中心发生以及与肝癌侵袭性有关的肝内播散所引起。前者属病因预防，后者是另一个需要认真研究的问题。我所发现不少癌基因和生长因子与肝癌的侵袭性有关，如：p16(CDKN2) 突变、p53 突变，以及 p21、TGFa、EGFR、c-erbB-2 等。例如有肝内播散的肝癌其 p16 突变率达 64.3%，而无肝内播散者仅为 10%；侵袭性肝癌 p21 的阳性率为 43.2%，而非侵袭性肝癌者仅为 16.7%；有复发转移的肝癌 p21 的阳性率达 38.6%，而无复发转移者其为 0%；有肝内播散的肝癌 p53 突变的阳性率达 73.7%，而无肝内播散者仅为 33.5%。此外还发现 nm23/TIMP-2 的表达与预后呈正相关，即肝癌表达 nm23/TIMP-2 者，其预后也好。但所有上述者在小肝癌和大肝癌中表达的差别尚无统计学意义 (p16 突变为 20.0% 对 47.4%，p21 为 26.5% 对 32.5%，p53 突变为 42.9% 对 60.0%，TGFa 为 43.2% 对 48.5%，EGFR 为 40.5% 对 54.5%，c-erbB-2 为 87.9% 对 96.9%，nm23-H1 为 60.5% 对 46.9%，TIMP-2 为 57.9% 对 42.9%)。说明即使小肝癌的预后仍主要取决于肝癌的生物学特性[51-54]。

为了进一步研究小肝癌的生物学特性并发展新的生物治疗和转移复发阻断途径，建立高转移模型是必由之路。近年根据"种子与土壤"学说，我所将 30 例肝癌手术切除标本移植于裸鼠肝脏，获得 1 株裸鼠人肝癌转移模型，为国际上首例。此模型的肝内、肺和淋巴结的转移率可达 100%，其染色体 90% 为异倍体，已传 30 余代，仍保持转移的特性，是研究肝癌转移的可取模型[55,56]。

转移的实验性干预：肝癌转移是一个多环节、多因素参与的过程，现对这一复杂过程的分子机制已有不少认识。针对不同的环节进行阻断，都有可能预防播散。我所的初步实验表明，反义 H-ras、抗 CD3/ 抗 HBx 的双特异抗体合并 LAK 治疗、基质金属蛋白酶抑制剂 BB-94、血管生成抑制剂 TNP-470 等，在转移模型的实验性治疗中有抑制转移的作用；对用 TNF 基因治疗也作了尝试[57-60]。关于实验性干预治疗的文献报道如：用 C11 单抗阻断肿瘤与肝细胞的相互作用；用反义 TGFa；用分化诱导剂维甲酸后可使 c-myc 上升，c-fms 下降，IGF-II 下降；用肝细胞生长因子 (Shiota 等，1996)；最新的报道认为 IL-10 有抗转移作用 (Kundu 等，1996)。有关肝癌的实验性基因治疗如：腺病毒介导 p53、纤维母细胞介导 IFNa 基因 (Cao 等，1995)、自杀基因和细胞因子基因的联合，TIMP-2 基因 (Imren 等，1996) 等。最近越来越多的科学家注意到控制肿瘤血管是一条十分重要的途径。

未来的挑战

以下问题有待研究：①普查的"耗费与效益"仍然是早期发现小肝癌的一个实际问题。②小肝癌的生物学特性，尤其是即使很小的小肝癌也存在的肝内门静脉的侵犯，是导致

小肝癌切除后复发转移、影响小肝癌预后的首要因素。③肝癌的多中心发生是处理小肝癌切除后复发的另一障碍。④肝移植将起重要作用，但供肝来源和经费问题尤其在发展中国家是一个困难的问题。⑤合并肝硬化，尤其是 Child C 者，也是巨大的挑战[61~63]。

参考文献

[1] 中华人民共和国卫生部.1995 中国卫生统计提要.北京：中华人民共和国卫生部，1995:73

[2] Parkin DM, Pisani P, Ferlay J. Estimates of worldwide incidence of eighteen major cancer in 1985. Int J Cancer 1993; 54:594～606

[3] Parker SL, Tong T, Bolden S, et al. Cancer statistics, 1997. Ca-Cancer J Clin, 1997; 47:5～27

[4] Okuda K, Kotoda K, Obata H, et al. Clinical observation during a relatively early stage of hepatocellular carcinoma, with special reference to serum alpha fetoprotein levels. Gastroenterology, 1975; 69:226～234

[5] 余业勤，宋礼安整理.原发性肝癌的早期诊断及手术体会.肿瘤防治研究，1975; 3:1～5

[6] Shanghai Coordinating Group for Research in Liver Cancer, China (Tang ZY, Yu EX, Wu CE, Gu XY). Diagnosis and treatment of primary hepatocellular carcinoma in early stage-report of 134 cases. Adv Med Oncol Res Edu, Proc 12th Int Cancer Cong, 1979; 9:291～295

[7] Tang ZY,Yu YQ, Lin ZY, et al. Small hepatocellular carcinoma-Clinical analysis of 30 cases. Chin Med J, 1979; 92:455～462

[8] 朱源荣.启东地区甲胎蛋白普查和肝癌的早期诊断.中华肿瘤杂志，1981; 3:35～37

[9] Tang ZY. Surgical treatment of subclinical cases of hepatocellular carcioma. In: Okuda K, Ishak KG,eds. Neoplasms of the liver. Tokyo:Springer, 1987.367～373

[10] Tang ZY, Yu YQ, Zhou XD, et al. Surgery of small hepatocellular carcinoma-analysis of 144 cases. Cancer, 1989; 64:536～541

[11] Tang ZY, Yu YQ, Zhou XD, et al. Small hepatocellular carcinoma-three decades' experience. In: Jiang SJ, Xiao SD(eds). Proc 1992 Shanghai Int Symp Gastroenterology. Shanghai: Shanghai Sci & Technical Lit Publ, 1992.52～59

[12] Tang ZY, Yu YQ, Zhou XD, et al. Subclinical hepatocelluar carcinoma:an analysis of 391 patients. J Surg Oncol, 1993; 3:55～58

[13] Makuuchi M, Kosuge T, Takayama T, et al. Surgery of small liver cancers. Semin Surg Oncol, 1993; 9:298～304

[14] Tang ZY, Yu YQ, Zhou XD. An important approach to prolonging survival further after radical resection of AFP positive hepatocellular carcinoma. J Exp Clin Cancer Res, 1984; 3: 359～366

[15] Tang ZY, Yu YQ, Zhou XD, et al. Cytoreduction and sequential resection: A hope for unresectable primary liver cancer. J Surg Oncol, 1991; 47:27～31

[16] Bismuth H, Chiche L. Comparison of hepatic resection and transplantation in the treatment of liver cancer. Semin Surg Oncol, 1993; 9:341～345

[17] Iwatsuki S, Starzl TE. Role of liver transplantation in the treatment of hepatocellular carcinoma.Semin Surg Oncol, 1993; 9:337～340

[18] Tang ZY(ed). Subclinical hepatocellular carcinoma. Beijing:China Acad Publ; Berlin: Springer. 1985.1～366

[19] Zhou XD, Tang ZY, Yu YQ, et al. Prognostic factors of primary liver cancer:report of 83 patients surviving 5 years or more compared with 811 patients surviving less than 5 years. J Exp Clin Cancer Res, 1991:10:81～86

[20] Tang ZY, Yu YQ, Zhou XD, et al. Analysis of one hundred and twenty five patients with primary liver cancer surviving more than five years. Jpn J Cancer Chemother, 1992; 19:1202～1206

[21] Tang ZY, Yu YQ, Zhou XD. Evolution of surgery in the treatment of hepatocellular carcinoma from the 1950s to the 1990s. Semin Surg Oncol, 1993; 9:293～297

[22] Tang ZY, Ying YY, Gu TJ. Hepatocellular carcinoma: changing concepts in recent years. In:Popper H, Schaffner F(eds)Progress in liver diseases. Vol VII. New York:Grune & Stratton, 1982:637～647

[23] Tang ZY. A new concept on the natural course of hepatocellular carcinoma. Chin Med J. 1981; 94:585～588

[24] Ebara M, Ohto M, Shinagawa T, et al. Natural history of minute hepatocellular carcinoma smaller than three centimeters complicating cirrhosis: a study in 22 patients. Gastroenterology, 1986; 90:289～298

[25] Tang ZY. The role of unicentric origin in subclinical hepatocellular carcinoma. In:Tang ZY, (ed). Subclinical hepatocellular carcinoma. Berlin: Springer, 1985.162～170

[26] Yang BH, Tang ZY. Alpha fetoprotein serosurvey in high risk population for early detection of hepatocellular carcinoma. Hepatol Rapid Literat Rev, 1986; 16:703(Abstr).

[27] Zhou XD, Tang ZY, Yu YQ, et al. The role of cryosurgery in the treatment of hepatic cancer: a report of 113 cases. J Cancer Res Clin Oncol, 1993; 120:100～102

[28] Liang XH, Loncarevic IF, Tang ZY, et al. Resection of hepatocellular carcinoma: Oligocentric origin of recurrent and multinodular tumors. J Gastroenterol Hepatol, 1991; 6: 77～86

[29] He B, Tang ZY, Liu KD, Zhou G. Analysis of the cellular origin of hepatocellular carcinoma by p53 genotype. J Cancer Res Clin Oncol, 1996; 122:763～766

[30] Tang ZY, Yu YQ, Ma ZC, et al. Conversion of surgically verified unresectable to resectable hepatoellular carcinoma-a report of 26 patients with subsequent resection. Chin J Cancer Res, 1989; 1:41～47

[31] Yu YQ, Tang ZY, Zhou XD, et al. Resection of huge hepatocellular carcinoma by two stage operation:report of 48 cases. Asian J Surg, 1994; 17:17～19

[32] Tang ZY, Yu YQ, Zhou XD, et al. Treatment of unresectable primary liver cancer: with reference to cytoreduction and sequential resection. World J Surg, 1995; 19:47～52

[33] Tang ZY, Yu YQ, Zhou XD, et al. Cytoreduction and sequential resection for surgically verified unresectable hepatocellular carcinoma: evaluation with analysis of 72 patients. World J Surg, 1995:19:784～789

[34] Sitzmann JV, Abrams R. Improved survival for hepatocellular cancer with combination surgery and multimodality treatment. Ann Surg, 1993; 217:149～154

[35] Reynolds M, Douglass EC, Finegold M, et al. Chemotherapy can convert unresectable hepatoblastoma. J Pediatr Surg, 1992; 27:1080～1084

[36] Tang ZY, Liu KD, Bao YM, et al. Radioimmunotherapy in the multimodality treatment of hepatocellular carcinoma with reference to second-look resection Cancer, 1990; 65:211～215

[37] Tang ZY, Zeng ZC, Liu KD, et al. Intrahepatic arterial ^{131}I anti hepatocellular carcinoma (HCC) monoclonal antibody combined with hepatic artery ligation for treatment of unresectable HCC. Antibody Immunoconj Radiophar, 1993; 6:167～175

[38] Tang ZY, Yu YQ, Zhou XD, et al: The role of targeting therapy in cytoreduction and sequential resection of unresectable hepatocellular carcinoma. Chin J Cancer Res, 1994; 6: 24～30

[39] Liu KD, Tang ZY, Fan Z, Lu JZ, Yu YQ, Zhou XD. Radioimmunotherapy in treatment of unresectable hepatoma: a report of 43 cases. Chin J Cancer Res, 1994; 6:74～78

[40] Lu JZ, Li BX, Liu KD, et al. Alternating chemotherapy and fractionated radiotherapy as a modality for the treatment of primary liver cancer. Chin J Cancer Res, 1994; 6:69～73

[41] Yu YQ, Xu DB, Zhou XD, et al. Experience with liver resection after hepatic arterial chemoembolization for hepatocellular carcinoma. Cancer, 1993; 71:62～65

[42] Tang ZY. Small hepatocellular carcinoma. In:Tang ZY, Wu MC, Xia SS, (eds). Primary liver cancer. Berlin: Springer, 1989:191～203

[43] Zhou XD, Tang ZY, Yu YQ, et al. Recurrence after resection of alpha fetoprotein positive hepatocellular carcinoma. J Cancer Res Clin Oncol, 1994; 120:369～373

[44] Wu MC, Chen H, Yan YQ. Rehepatectomy of primary liver cancer. Semin Surg Oncol, 1993; 9:323～326

[45] Esumi M, Aritaka T, Arii M, et al. Clonal origin of human hepatoma determined by integration of hepatitis B virus DNA. Cancer Res, 1986; 46:5767～5771

[46] Chen PJ, Chen DS, Lai MY, et al. Clonal origin of recurrent hepatocellular carcinomas. Gastroenterology, 1989; 96:527～529

[47] Tsuda H, Oda T, Sakamoto M, Hirohashi S. Different pattern of chromosomal allele loss in multiple hepatocellular carcinoma as evidence of their different origin. Cancer Res, 1992; 52 :1504～1509

[48] Hsu HC, Peng SY, Lai PL, et al. Allelotype and loss of heterozygosity of p53 in primary and recurrent hepatocellular carcinoma: a study of 105 patients. Cancer, 1994; 73:42～47

[49] Cong WM, Wu MC. Small hepatocellular carcinoma-DNA content and biological characteristics. Chin Med J 1989; 102:783～785

[50] Kondo F, Kondo Y. Hepatocellular carcinoma of the early stage: morphology and developmental process. J Surg Oncol, 1993:3:1665～1675

[51] Tang ZY: Studies on small hepatocellular carcinoma:clinicalaspect and molecular biology. Hepatol Rapid Lit Rev, 1996;(1):VII-XVI

[52] Wang XM, Tang ZY, Xue Q, et al. Transforming growth factor a induces proliferation and expression of epidermal growth factor receptor in hepatocellular carcinoma cells. J Exp Clin Cancer Res, 1995; 14:179～184

[53] Qin LX, Tang ZY, Liu KD, et al. p53 mutations may be related to tumor invasiveness of human hepatocellular carcinoma in China. Oncol Rep, 1995; 2:1175～1179

[54] Qin LX, Tang ZY, Liu KD, et al. Alterations of CDKN2(P16/MTS1)exon 2 in human hepatocellular carcinoma. Oncol Rep, 1996; 3:405～408

[55] Sun FX, Tang ZY, Liu KD, et al. Establishment of a novel "patient-like" metastatic model of human hepatocellular carcinoma in nude mice using orthotopic implantation of histologically intact tissue. Int J Cancer, 1996; 66:239～243

[56] Sun FX, Tang ZY, Liu KD, et al. Metastatic models of human liver cancer in nude mice orthotopically constructed by using histologically intact patient specimens. J Cancer Res Clin Oncol, 1996; 122:397～402

[57] Liao Y, Tang ZY, Liu KD, et al. Preparation and application of anti-HBx/anti-CD3 bispecific monoclonal antibody (BsAb)retargeting effector cells for lysis of human hepatoma xenografts in nudemice. Oncol Rep, 1996; 3:637～644

[58] Liao Y, Tang ZY, Liu KD, et al. Apoptosis of human BEL-7402 hepatocellular carcinoma cells released by antisense H-ras DNA-in vitro and in vivo studies. J Cancer Res Clin Oncol. 1997; 123:25～33

[59] 廖　勇，汤钊猷，孙坊宪，等.反义寡脱氧核苷酸对人肝癌裸鼠模型生长及转移的影响.中华医学杂志，1996; 76:650～653

[60] 冯学胜，汤钊猷，薛　琼，等.重组人肿瘤坏死因子治疗原发性肝癌的实验研究.肿瘤，1995; 15:373～375

[61] Tang ZY, Yang BH. Secondary prevention of hepatocellular carcinoma. J Gastroenterol Hepatol, 1995; 10:683～690

[62] Tang ZY. Small hepatocellular carcinoma:past, present and future. Chin Med J, 1996; 109: 21～24

[63] Tang ZY, Yu YQ, Zhou XD, et al: Recurrence and metastasis: the next target on the study of small hepatocellular carcinoma. In: Xiao SD, (ed). Proc 1996 Shanghai Int Conf Gastroenterol. Shanghai, 1996. 88～92

Eur J Pharmacol, 1995; 280: R13 ~ 15

Physical Withdrawal in Rats Tolerant to Δ^9-Tetrahydrocannabinol Precipitated by a Cannabinoid Receptor Antagonist

Kang Zou (邹冈), SL Patrick, JM Walker

(Departments of Psychology and Neuroscience, Brown University, Providence, RI 02912, USA)

Abstract Tolerance to Δ^9-tetrahydrocannabinol (Δ^9-THC) was produced in rats by twice daily injections (15 mg/kg,i.p.)for 6.5 days. Administration of the cannabinoid antagonist SR 141716A(i.p. or i.c.v.)induced a profound precipitated withdrawal syndrome in Δ^9-THC-tolerant animals. The syndrome was characterized by a disorganized pattern of constantly changing brief sequences of motor behavior. Autonomic signs were not evident. THC-tolerant animals that were treated with vehicle remained quiet throughout the observation period.

Abrupt discontinuation of heavy use of marijuana results in only mild withdrawal symptoms, if they occur at all(reviewed by Hollister, 1986). Although anecdotal reports of an abstinence syndrome in rats and monkeys have appeared (e.g. Kaymakalan, 1978), quantitative behavioral and physiological studies have revealed at most only mild withdrawal signs (McMillan et al, 1971). These failures to observe profound abstinence signs following discontinuation of chronic use of Δ^9-tetrahydrocannabinol (Δ^9-THC) may result from its long half-life in plasma (Wall et al, 1983), because the slowly waning levels of drug could permit adaptation to occur.

Following chronic heavy intake of opiates, with-drawal symptoms can be precipitated by administration of the competitive opiate antagonist naloxone. This approach to cannabinoid withdrawal was impossible until the development of the competitive cannabinoid receptor antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride (SR141716 A) which was accomplished recently by Rinaldi-Carmona et al (1994). Here we report that SR141716A precipitates a profound withdrawal syndrome in rats rendered tolerant to Δ^9-THC by repeated injections.

Tolerance was produced in rats ($n=10$) by injections of 15 mg/kg Δ^9-THC i.p. (National Institute on Drug Abuse, Rockville, MD, USA; suspended in an ethanol: alkamuls-emulphor:saline solution, 1:1:18)every day between 8:00 and 10:00 and again between 16:00-18:00 for 6.5 days. Control animals ($n=10$) received the vehicle at the same times.

Examination of the hypothermic effects of Δ^9-THC revealed that tolerance had occurred. Administration of Δ^9-THC produced a marked drop in core temperature in 5 animals tested on the first day of this regimen (-0.8 ± 0.05℃), but this effect

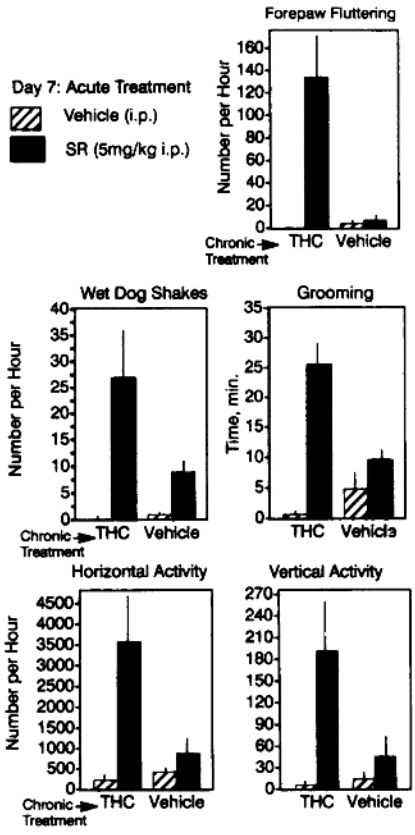

Fig. 1. Rats were either rendered tolerant to Δ^9-THC by twice daily injections for 6.5 days as described in the text, or they served as controls and received the vehicle at the same times. On the test day, half the animals from each of these groups received the selective cannabinoid receptor antagonist SR141716A (5 mg/kg, i.p.); the other half received the vehicle. Approximately 10 min following administration of the antagonist, animals that were tolerant to Δ^9-THC displayed a novel behavioral syndrome characterized by hyperactivity and disorganization of behavior, as described in the text. The following types of behavior were observed and tested statistically using analysis of variance (ANOVA) with the Newman-Keuls (NK) post-hoc test for mean differences: bouts of forepaw fluttering, ANOVA: $F(3,15)=10.1$, $P<0.001$, NK: $P<0.05$; wet dog shaking $F(3,15)=5.8$, $P<0.01$, NK: $P<0.05$; time spent grooming, ANOVA: $F(3,15)=20.7$, $P<0.0001$, NK: $P<0.05$); horizontal activity, ANOVA: $F(3,15)=8.44$, $P<0.002$, NK: $P<0.05$; and vertical activity, $F(3,15)=4.79$, $P<0.02$, NK: $P<0.05$. In no case did the antagonist produce a significant effect in non-tolerant rats compared to acute treatment with vehicle.

failed to occur on the third day of treatment with the agonist (-0.06 ± 0.06℃). Furthermore, vocalization and 'popcorn' behavior occurred in 4/5 animals on the first day of the regimen, but these behaviors never appeared in any animal on the third day. These findings are consistent with previous reports of cannabinoid tolerance (McMillan et al, 1971).

To test for precipitated withdrawal, animals were placed in an activity chamber (Digiscan, Columbus Instruments, Columbus, OH, USA) 30 min following the last injection. After 1 h, SR141716A (5 mg/kg, $n=5$ dissolved in 100% dimethyl sulfoxide) or the vehicle ($n=5$) was administered i.p. to both THC-tolerant and nontolerant rats. Approximately 10 min following administration of the cannabinoid receptorantagonist to Δ^9-THC-tolerant animals, a dramatic abstinence syndrome appeared and lasted throughout the 1 h observation period (Fig. 1).

In nearly constant motion, abstinent rats rapidly alternated between different sequences of behavior, each sequence rarely lasting more than 2 s. Analysis of videotapes revealed sequences such as the following: full turn left, walk backwards two steps, full turn right, raise hindpaw, abort movement-lower hindpaw to floor, wet dog shake, sniff three times, rear, return to horizontal position, half turn to left. Abstinent rats exhibited numerous instances of for-

epaw fluttering, a tremor-like movement characterized by rapid repetitive medial-lateral movements of the forepaws. This behavior was rarely observed in untreated animals and does not result from any other drug treatment that we are aware of. The significant increases ($P<0.01$) in horizontal and vertical activity, grooming, wet dog shaking and forepaw fluttering in abstinent rats compared to controls were the result of these unique, rapid and profoundly disorganized patterns of motor activity (Fig.1). Because the antagonist failed to produce similar effects in non-tolerant animals, it would appear that the syndrome we observed was in fact precipitated withdrawal rather than any effect of the antagonist itself.

A second experiment was carried out to determine whether the withdrawal syndrome was mediated by an effect of the antagonist on periventricular structures. In these animals ($n=48$) cannulae were implanted in the left lateral ventricle and, following recovery, they underwent 6 days of injections as above. Twenty-four hours following the last injection, animals received either SR141716A (100 μg,i.c.v) or the vehicle. Withdrawal signs were evident, but the magnitude and complexity of the syndrome was less than that observed following i.p. injection of the antagonist. The most dramatic signs of precipitated abstinenc. were frequent wet-dog shaking and marked increase in the time spent grooming.

The most striking aspect of the withdrawal syndrome was the rapidly alternating sequences of what appeared to be aborted fragments of organized behavior. This aspect of the syndrome appears to be unique to cannabinoid withdrawal and is not characteristic of the acute effects of any known drug. The site(s) in the brain mediating these effects cannot be stated with certainty. However, it is notable that the highest densities of cannabinoid receptors, are found in the basal ganglia (Herkenham et al, 1991), a group of neural circuits whose function may be to organize sequences of behavior (Aldridge et al, 1993; Benecke et al, 1987). Conceivably, the profound disturbance in the sequencing of behavior may have resulted from alterations in the physiology of these circuits.

References

[1] Aldridge JW, Berridge KC, Herman M, et al. Neuronal coding of serial order. Psychol Sci. 1993; 4:391

[2] Benecke R, Rothwell J C, Dick J B. Disturbance of sequential movements in patients with Parkinson's disease. Brain 1987; 110:361

[3] Herkenham M, Lynn AB, Johnson MR, et al. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci. 1991; 11:563

[4] Hollister L E. Health aspects of cannabis. Pharmacol Rev, 1986, 38:1

[5] Kaymakalan S. Pharmacological similarities and interactions between cannabis and opi-

oids. In: Nachas DE, Paton WD, eds. Marihuana: biological effects. Oxford: Pergamon. 1978.591

[6] McMillan D E, Dewey W L, Harris L S. Characteristics of tetrahydrocannabinol tolerance. Ann NY Acad Sci, 1971; 91:83

[7] Rinaldi-Carmona, M, Barth F, Heaulme M, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor, FEBS Lett, 1994; 350:240

[8] Wall M E, Sadler B M, Brine D, et al. Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women. Clin Pharmacol Ther, 1983; 34:352

Microsurgery, 1995; 16:547~551

Effects of Nerve Growth Factor on Crushed Sciatic Nerve Regeneration in rats

Zhongwei Chen (陈中伟), Minsheng Wang

(Microsurgery Research Laboratory, Department of Orthopedics, Zhongshan Hospital, Shanghai Medical University)

Abstract The effects of nerve growth factor (NGF) on crushed sciatic nerve regeneration were studied in 30 rats, with 60 bilateral nerves. The nerves were crushed at a site 6 mm distal to the sciatic notch by the standard technique and 3 mm wide crush injuries were created. Then 2.1 μl of normal saline in the control groups and an equal volume of NGF solution (containing 1 μg of NGF) in the NGF-treated groups was injected into the crush sites and followed for 12,28, and 56 days, respectively. At the end of the observation, electrophysiological evaluation was carried out; then samples 10mm distal to the crush site were removed and prepared for histological and morphometric studies. Evoked muscle action potential (MAP) was recorded in 50% of the NGF-treated group at 12 days but not in the control group; the difference was statistically significant ($P<0.05$). The motor nerve conduction velocity (MNCV) was increased in NGF-treated groups compared with control groups at 28 and 56 days ($P<0.05$). Morphometrically, significantly more regenerated myelinated fibers (RMFs) were seen at 12 days, and larger diameter RMFs were found at 12,28, and 56 days in NGF-treated groups than in control groups. These results indicate that topically applied NGF stimulates nerve regeneration and promotes function recovery in crushed rat sciatic nerves.

The discovery of nerve growth factor (NGF) by Levi-Montalcini and Hamburger in 1951[1]revolutionized the study of the biology of nerve regeneration. More recently, demonstration that the peripheral nerve microenvironment allows the regeneration of central nervous tissue as well as peripheral axons has stimulated significant interest in and brought much talent to the study of peripheral nerve regeneration.[2]Over the past decade, numerous research studies on NGF, both in vivo and in vitro, have been reported.[3,4]It has been demonstrated that NGF is present in peripheral nerves and that the levels of NGF, NGF mRNA, NGF receptors, and NGF receptor mRNA increase in nerves after injury,[5~7]indicating that the increase in nerve growth factor at injury sites may play an important role in the regeneration of nerves. The present study was performed to determine the effects of topically applied NGF on the regeneration of crushed rat sciatic nerves.