

实验研究

分散和植块法培养获得大量雪旺氏细胞的研究

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摘要 SD 乳鼠坐骨神经匀浆应用分散培养, 在差速粘附贴壁的基础上, 联合应用低浓度血清和抗分裂剂, 可抑制和消除快速分裂的成纤维细胞, 得到纯净的雪旺氏细胞。同时, 取乳鼠坐骨神经和钳伤的青年大鼠坐骨神经, 分别采用“反复种植法”培养, 经免疫组化 (抗 S-100 蛋白) 染色证实, 长出的细胞为雪旺氏细胞。

关键词 雪旺氏细胞 坐骨神经 免疫组织化学法

鉴于雪旺氏细胞 (Schwann's cell, SC) 在周围神经再生中的重要作用^[1], 有关 SC 的培养、分离、纯化、生物特性和临床应用等研究, 日益受到人们的重视^[2-5]。本实验采用分散培养法和植块培养法, 均获得了大量 SC, 现报告如下。

材料和方法

1. 分散培养实验用出生 1~3 天的 SD 乳鼠 10 只。清洁消毒后, 在超净工作台上, 用眼科剪和眼科镊小心地剥取双侧坐骨神经, 放入 D-Hanks 液中, 在解剖显微镜下剥除神经外膜, 将神经剪成组织匀浆, 加入 0.25% 胰蛋白酶和 0.03% 胶原酶 (Sigma), 置于 37℃ 水浴 30~40 分钟。匀浆中的组织块可用吸管吹打, 待组织块消化后, 再加培养液至 10ml, 离心 (1000 转/分) 8 分钟, 吸去上清液至 4ml, 再加培养液至 10ml, 再离心, 反复 3 次。最后吸去上清液剩 2ml, 计数后接种至涂有鼠尾胶原的培养瓶内, 入 37℃, 5%CO₂ 培养箱 30~40 分钟, 每 10 分钟轻摇一次, 收集未贴壁的细胞重新接种。24 小时后更换含阿糖胞苷 (Ara-C) 10⁻⁹ M 的培养液作用 48 小时。间隔 10 小时后, Ara-C 再次作用 24 小时。培养液成分: 小牛血清 (10%)、D-MEM、葡萄糖 (6g/L)、青霉素 (100u/ml)、链霉素 (100u/ml)。从培养第 5 天起, 更换含 20% 小牛血清的培养液。以 PAP 方法做抗 S-100 蛋白免疫细胞化学染色。细胞接种后, 每天倒置显微镜下观察、计数, 并以台盼兰染色, 计算活细胞百分比。

2. 植块培养 取出 1~3 天 SD 乳鼠之坐骨神经, 剥除神经外膜, 以 D-Hanks 液洗净, 将神经剪切成 0.5mm³ 大小植块, 加培养液浸湿。将植块贴附于覆有鼠尾胶原的培养瓶瓶底, 底朝上加入适量培养液静置 37℃、5%CO₂, 饱和湿度的二氧化碳培养箱。24 小时后翻转培养瓶, 使植块没入培养液中, 每周换液两次。

5 天左右, 从植块长出的细胞基本汇合, 此时用 0.25% 胰蛋白酶加 0.01% EDTA (1:1) 消化传代, 植块按上述法重新接种, 如此反复数次。

另取 10 周龄 SD 大鼠, 先钳伤其右侧坐骨神经, 48 小时后, 剪取钳伤段坐骨神经, 剥除神经外膜, 其余方法同乳鼠坐骨神经植块培养。在培养的不同时间, 以免疫细胞化学法染色、每天倒置显微镜下观察、计数。

结 果

1. 分散培养: 反复离心后, 从 10 只乳鼠中, 可获得 SC 数量为 10⁶ 细胞。台盼兰排除法表明, 所获的 SC, 95% 以上为活细胞。按细胞的形态和免疫组化方法进行 SC 的纯度鉴定, 反复贴壁三次, 加 Ara-C 作用两次后, 可获得纯净度 99% 的 SC。

2. 乳鼠坐骨神经植块法培养: 植块初次接种后 24 小时, 植块周围长出细胞晕, 5 天时, 长出一细胞层, 逐渐连成片, 呈放射状排列, 细胞多呈多角形, 基本是纤维细胞。第二次长出的细胞排列较为整齐, 呈旋涡状、栅栏状排列, 具有明显的“端端相连”的生长排列特点, 基本是 SC 细胞纯净度在 95% 以上。

3. 青年鼠坐骨神经植块培养: 接种 3~4 天时, 植块周围长出细胞晕, 细胞成份围绕植块周围呈放射状排列, 7~8 天时, 植块间细胞互相连结成片, 早期 (1 周左右) 多为成纤维细胞, 后期 (8~10 天), 有部分双极的 SC 长出。第二次接种后长出的细胞为轮廓清晰、双极梭形的 SC, 纯净度为 92% 以上。

讨 论

研究用的 SC, 其来源都必须借助体外培养, 并要求有较高的纯净度。由于周围神经内膜, 束膜和外膜组织中含有较多的成纤维细胞, 解剖镜下只能剥除其外膜, 而且在体外培养时, 成纤维细胞分裂快, 时间稍久,

其比例增加,最后将 SC 取代。因此欲获得纯净的 SC,消除成纤维细胞成为重要的研究课题。周围神经中的细胞成分主要是两种,SC 和成纤维细胞。利用后者的贴壁速度较 SC 快且粘附性强这一特性,将细胞悬液接种于涂有鼠尾胶原的培养瓶中,贴壁一段时间,收集未贴壁的 SC 重新接种,可清除一部分成纤维细胞。培养时,成纤维细胞分裂较快,一般 2~3 天一次,而 SC 7~8 天分裂一次。低浓度血清能减慢成纤维细胞的分裂,Ara-C 是一种抗分裂剂,在成纤维细胞的指数生长期使用,能有效地抑制和杀死快速分裂的成纤维细胞,当撤除 Ara-C 后,剩余的成纤维细胞重新开始分裂,再次用 Ara-C 处理,所剩成纤维细胞极少,此时给予高浓度血清培养,有利于 SC 生长。本实验运用物理(差速贴壁)和化学(Ara-C)方法,可获得高纯度的 SC。

体外培养 SC,一般取乳鼠坐骨神经为材料,这种取材方法较为困难,工作强度大。本实验采用 Bolin^[6]方法,以钳伤青年大鼠坐骨神经为材料,反复植块培

养,同样培养出较纯净的 SC。此方法具有方法简便、经济、成活率高的特点,缺点是费时较长。

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郁金白芨汤治疗胸肋闪挫伤 59 例

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笔者用自拟郁金白芨汤治疗胸肋闪挫伤 59 例,介绍如下。

临床资料 59 例中男 47 例,女 12 例;年龄 12~80 岁;病程最短在半天至三天以内 24 例,病程在 4 至 7 天以内 18 例,病程七天以上 17 例,其中有 3 例病程在半年以上。

治疗方法 处方:郁金 15g 白芨 20g 当归 12g 川芎 10g 降香 12g 柴胡 8g 枳壳 10g 甘草 6g 米酒 50~100g。胸肋瘀肿明显加红花、桃仁;胀痛明显加香附、青皮;气质虚弱加准山药、党参;陈旧性胸肋部挫伤加丹参、鸡血藤、首乌。

煎服法:米酒与中药加清水同煎,三碗清水煎至一碗,分二次服,每次半碗,每日一剂。

治疗结果 59 例经内服中药后均达胸部肿胀疼痛及压痛消失,呼吸舒畅。治疗时间短为一天,长为 14 天。

体会 胸肋闪挫伤又称“努伤”,属于胸肋内伤范

围,是胸部遭受暴力,致使软组织损伤,经络受阻,气血流行不畅,气滞作痛,血瘀作肿,肝肺气机受损而出现胸闷,肋助刺痛,呼吸不畅,深呼吸及用力活动时伤处牵引掣痛等症状。

笔者对胸部挫伤在临床辨证施治上,根据气滞血瘀的病理变化特点,自拟郁金白芨汤,功效着重宽胸理气,活血止痛。从 59 例的疗效观察表明郁金白芨汤对胸肋闪挫伤疗效卓著。方中郁金、白芨行气止痛活血,柴胡、枳壳、降香宽胸理气,理顺胸部气机,达到气行则血行的目的,再以当归,川芎活血祛瘀,推动气血运行,解除胸部气滞血瘀,甘草调和药性。上药与米酒同煎,因酒能通经脉行气血,带药直达病所。在运用本方时,根据病情不同而灵活动用。如瘀积痛明显者加红花、桃仁,增强活血祛瘀。全胸胀痛明显者加青皮、香附,增强宽胸理气,如气质虚弱有加党参,准山药,陈旧性挫伤者加丹参、鸡血藤、首乌等扶助气血。

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Abstract of Original Articles

clinical study on break down of lumbar chondral plate Jiang Wei — zhuan et, al Institute of Orthopaedics and Traumatology, China Academy of TCM(100700)

Eighty one cases of break down of lumbar chondral plate were summerized. There were special manifestations on clinic, X—ray, CT etc. imaging changes and pathology as well. Bread down, dislocation and ossification of chondral plate were concluded andcorresponding therapeutic measures were suggested. Through analysis of clinical data, a comparative study of break down of chondral plate and annulus fibrosa were carried on.

Key words Lumbar Break down of chondral plate Imaging diagnosis

(Original article on page 3)

Clinical analysis of operative treatment of senile prolapse of lumbar intervertebral disc Zhou Zhang — wu et, al. Affiliated Hospital of Anhui College of TCM(230031)

Twenty eight cases of senile prolapse of lumbar intervertebral disc operated with total laminectomy, removal of the nucleus, cutting off the thickening ligamentum flavum etc. with satisfactory results. Operative technique, clinical characteristics and rehabilitation were discussed. Key words Prolapse of lumbar intervertebral disc

Key Words Operative treatment Senile person

(Original article on page 6)

Study on large amount of Schwann's cell obtained in the culture of scattered andlock implantation method Li Yi — kai, Shi Yin — yu, Shi Guan — tong Shu Guang Afiliated Hospital, Shanghai University of TCM(200021)

SD suckling rat sciatic nerve even paste was cultured separately, on the basis of differential adhesion stick wall, combined application of low concentration serum and anticlaveage agent to inhibit and diminish quick cleavage of the fibroblast in order to obtain pure Schwann's cell. Meanwhile suckling and clamp injured juvenile rat were used in repeatedly implantation cul-

ture. Through immunohistochemical stain (anti—S—100 protein) proved that the growing cell is Schwann's cell.

Key words Schwann's cell Sciatic nerve Immunohistochemical method

(Original article on page 8)

Prolapse of lumbar intervertebra disc treated by tridimensional multifunctional traction Bi Da — wei, Zheng Han — guang, Qian Ying Hangzhou Red Cross Hosqital(310004)

One hundred and fourteen cases of prolapse of lumbar inner vertebral disc were treated by tridimensional multifunctional traction and epidural space instillation, the total near efficiency being 91%. It is realized that lumbar intervertebral disc bears tridirectional axis of motion, thus according to biomechanical principle tridimensional joint traction could exert fully stress on the intervertebral disc and the prolapsed disc could be reduced through quick traction so long as protruded disc had not been adhered by surrounding tissues or didn't bear degenerative changes or break down. Based on chemical neuritis theory, epidural space continuous instillation of the nerve root. It is an effective supplementary therapy.

Key words Traction therapy Epidural instillation Prolapse of lumbar intervertebral disc

(Original article on page 12)

Fracture of mid—lower segment of the humerus treated with over—joint splint fixation and hanging plaster of Paris Pu Xing — hai First Afiliated Hospital, Lanzhou College of Medical Science(730000)

Twenty seven cases of fracture of mid—lower segment of the humerus were treated with over—joint splint fixation plus hanging plaster of Paris. Follow—up study with X—ray and they were all healed within 6—8 weeks. No delayed union or non—union was found. It is realized that combination of the abovetwo methods bears the advantage of fixation and traction, maintaining the stability of the fracture, alleviation of local shear force and promoting healing.

Key words Fracture of mid—lower segment of the