·基础研究·

养血清脑颗粒对硝酸甘油偏头痛模型大鼠三叉神经脊束核c-Fos蛋白表达的影响

雷小峰　赵佩　牛争平　程焱

【摘要】 **目的**　观察养血清脑颗粒对硝酸甘油偏头痛模型大鼠脑干三叉神经脊束核c-Fos蛋白表达变化的影响。**方法**　共90只雄性Wistar大鼠，分别于皮下注射硝酸甘油建立偏头痛动物模型之前（预防组）、模型制备后（治疗组）接受高剂量（0.32 g/ml）或低剂量（0.16 g/ml）养血清脑颗粒治疗，行为学评价后行免疫组织化学染色检测大鼠三叉神经脊束核神经元c-Fos蛋白表达水平。**结果**　模型组大鼠各观察时间点（30、60、180 min）三叉神经脊束核神经元c-Fos蛋白表达水平与高、低剂量治疗组之间差异无统计学意义（均$P>0.05$）；但高于高剂量预防组（$P=0.031$，0.000，0.000），并于制模后60和180 min时高于低剂量预防组（均$P=0.000$）；制模后30 min时高剂量预防组大鼠三叉神经脊束核神经元c-Fos蛋白表达水平低于低剂量预防组（$P=0.029$）。**结论**　预防性应用养血清脑颗粒可以显著降低三叉神经脊束核c-Fos蛋白表达水平。提示养血清脑颗粒对偏头痛的预防作用大于治疗作用，其机制可能与降低三叉神经脊束核c-Fos蛋白表达水平有关。

【关键词】 偏头痛；　三叉神经核；　原癌基因蛋白质c-fos；　疾病模型，动物

An experimental research on the expression of c-Fos protein in trigeminal nucleus caudalis of migraine rat model induced by nitroglycerin after the intervention of Yangxueqingnao granules

LEI Xiao-feng[1], ZHAO Pei[2], NIU Zheng-ping[3], CHENG Yan[4]

[1]*Department of Neurology, Tianjin Fourth Central Hospital, Tianjin 300140, China*

[2]*Department of Geriatrics, Taiyuan Iron & Steel (Group) Co. Ltd General Hospital, Taiyuan 030008, Shanxi, China*

[3]*Department of Emergency, First Hospital of Shanxi Medical University, Taiyuan 030001, Shanxi, China*

[4]*Department of Neurology, Tianjin Medical University General Hospital, Tianjin 300052, China*

Corresponding author: NIU Zheng-ping (Email: nzp105@sina.com)

【Abstract】 **Objective**　To observe the effect of Yangxueqingnao granules on c-Fos protein expression in trigeminal nucleus caudalis (TNC) of nitroglycerin-induced migraine rats. **Methods**　A total of 108 healthy male Wistar rats were randomly divided into normal control group, model group, high-dose treatment group, low-dose treatment group, high-dose prevention and low-dose prevention group, and then each group was randomly divided into 3 subgroups: 30, 60 and 180 min group. The experimental migraine rat model was established by subcutaneous injection of nitroglycerin. The rats of normal control group were subcutaneously injected with saline solution of the same dosage. The rats of high- and low-dose treatment group were respectively administered with gastric perfusion of high- and low-dose Yangxueqingnao granules once 10 min after the modeling. The rats of high- and low-dose prevention group were respectively administered with gastric perfusion of high- and low-dose Yangxueqingnao granules for 7 d before the modeling. The rats were sacrificed and the brain tissues were collected at corresponding time points. Related behavior indexes of rats in each group were observed and recorded. TNC c-Fos protein expression changes were measured and compared among different groups. **Results**　At all time points, the c-Fos expression level of model group had no statistical difference from high- and low-dose treatment group ($P>0.05$, for all). However, it was higher than that in high-dose prevention group ($P=0.031$, 0.000, 0.000),

doi:10.3969/j.issn.1672-6731.2014.08.012

作者单位：300140 天津市第四中心医院神经内科（雷小峰）；030008 太原钢铁（集团）有限公司总医院老年病科（赵佩）；030001 太原，山西医科大学第一医院急诊科（牛争平）；300052 天津医科大学总医院神经内科（程焱）

通讯作者：牛争平（Email：nzp105@sina.com）

while was only lower than that in low-dose prevention group at 60 and 180 min after modeling (P = 0.000, for all). There was statistical difference between high- and low-dose prevention group only at 30 min after modeling (P = 0.029). **Conclusions** The Yangxueqingnao granules can significantly decrease TNC c-Fos protein expression, which suggests that the preventive effect of Yangxueqingnao granules may be greater than the therapeutical effect on migraine. Its preventive effect may be associated with the decrease of TNC c-Fos protein expression.

【Key words】 Migraine; Trigeminal nuclei; Proto-oncogene proteins c-fos; Disease models, animal

偏头痛(migraine)为临床常见的慢性神经血管性头痛,我国关于偏头痛的最新流行病学调查资料显示,患病率为9.30%[1]。大量研究表明,偏头痛随年龄的增长逐年增加,发作时生活和工作能力下降。偏头痛虽不能治愈,但规范的预防性治疗可显著减少发作,缓解头痛导致的功能障碍,提高生活质量[2]。近年来,关于偏头痛发病机制的研究虽已取得一些进展,但至今尚无定论,各种学说纷争不一。在本研究中,我们观察养血清脑颗粒对硝酸甘油所致偏头痛大鼠三叉神经脊束核c-Fos蛋白表达变化的影响,探讨偏头痛发病机制及养血清脑颗粒预防和治疗偏头痛的作用机制。

材料与方法

一、实验材料

1. 实验动物 健康清洁级雄性Wistar大鼠共108只,体重250~300 g,由山西医科大学医学实验动物中心提供,于安静屏蔽环境喂养,室温22 ℃、相对湿度50%、压强梯度30 Pa,12 h白昼-12 h夜间交替。笼具为白色塑料鼠笼,垫以消毒木质垫料,饮用消毒去离子水、普通颗粒鼠粮饲养。

2. 试剂与药品 硝酸甘油注射液(5 mg/5 ml)购自北京益民药业有限公司。养血清脑颗粒(4 g/袋)购自天津天士力制药集团股份有限公司,并用蒸馏水配制成高剂量(0.32 g/ml)和低剂量(0.16 g/ml)溶液备用。免疫试剂中Ⅰ抗工作液[为兔抗大鼠c-Fos蛋白单克隆抗体(1∶100)]由北京博奥森生物技术有限公司提供,免疫组织化学检测试剂盒[含聚合辣根过氧化物酶(HRP)标记的羊抗兔IgGⅡ抗和体积分数为3%的过氧化氢溶液],以及二氨基联苯胺(DAB)显色试剂盒,均购自武汉博士德生物工程有限公司。

二、实验方法

1. 偏头痛大鼠模型制备 (1)动物分组:依据大鼠体重进行编号后,参照随机排列表法分为正常对照组(对照组)、模型组,以及养血清脑颗粒高剂量(0.32 g/ml)治疗组、低剂量(0.16 g/ml)治疗组、高剂量(0.32 g/ml)预防组、低剂量(0.16 g/ml)预防组,每组18只动物;再根据模型制备后处死大鼠的时间每组再随机分为30、60和180 min共3个亚组,采集脑组织标本。(2)模型制备:根据Tassorelli和Joseph[3]方法,于大鼠皮下注射硝酸甘油诱发偏头痛。当模型制备后3~5 min,大鼠出现前肢频繁挠头、爬笼次数增加、往返运动等烦躁不安表现,并于60 min时达严重状态,180 min后逐渐出现倦怠、活动减少者,提示偏头痛模型制备成功。对照组大鼠仅于皮下注射生理盐水(0.20 ml/100 g),注射后出现短暂性前肢挠头、爬笼动作,但无耳部发红。不同剂量治疗组大鼠在模型制备后10 min分别灌胃养血清脑颗粒溶液(1 ml/100 g)1次,不同剂量预防组大鼠则于模型制备之前分别连续灌胃养血清脑颗粒溶液(1 ml/100 g)7 d。

2. 行为学评价 模型制备后以30 min为时间间隔,共设定6个时间段(0~30、31~60、61~90、91~120、121~150和151~180 min),参照文献[4]方法对不同处理组大鼠各时间点行为学表现进行总体评价(表1)。

3. 免疫组织化学染色检测c-Fos蛋白表达变化 (1)脑组织标本制备:于模型制备后相应时间点,以质量分数为10%水合氯醛溶液(0.30 ml/100 g)腹腔注射麻醉大鼠,经左心室穿刺、升主动脉插管、剪开右心耳快速灌注生理盐水(4 ℃)至肝脏变白,右心耳流出澄清液后灌注质量分数为4%多聚甲醛溶液(pH值7.40,4 ℃)至大鼠肝脏变硬、肢体和鼠尾抽搐变硬。剪开颅骨,剔除硬脑膜,显露脑组织和脑干,于对耳线-4.68 mm、前囟-13.68 mm至对耳线-5.60 mm、前囟-14.60 mm,切取包含三叉神经脊束核的脑干组织,4%多聚甲醛溶液过夜固定、石蜡

包埋。(2)免疫组织化学染色:连续切取层厚为4 μm的脑组织切片5张,脱蜡至水,体积分数为3%过氧化氢溶液室温下反应10 min,灭活内源性酶,蒸馏水冲洗3 min(×3次);置含0.01 mol/L枸橼酸钠缓冲液(pH值6.0)的高压锅内进行抗原高压热修复处理,0.01 mol/L磷酸盐缓冲液冲洗5 min(×3次);滴加封闭液、室温下反应10 min,滴加Ⅰ抗工作液(1∶100)、4 ℃过夜,磷酸盐缓冲液冲洗5 min(×3次);滴加聚合辣根过氧化物酶标记的Ⅱ抗、37 ℃孵育30 min,磷酸盐缓冲液冲洗5 min(×3次)。DAB显色液室温下避光显色,光学显微镜下控制反应时间,蒸馏水洗涤终止显色;苏木素轻度复染,脱水、透明、封片。每张脑组织切片于光学显微镜(×250)下选择5个视野(左上、右上、左下、右下、中央),采用BI-2000医学图像分析系统进行阳性细胞(胞核呈黄褐色)平均灰度值分析。

三、统计分析方法

采用SPSS 17.0统计软件进行数据计算与分析。计量资料以均数±标准差($\bar{x} \pm s$)表示,经正态性检验和方差齐性检验后行单因素方差分析,两两比较采用LSD-t检验;给药顺序、药物剂量、作用时间的交互效应采用析因设计方差分析,统计推断的检验水准均为$\alpha = 0.05$。由于免疫组织化学检测非特异性对照组、阴性对照组阳性细胞数目极少,故未进行统计学检验。

结　果

一、行为学评价

根据行为学评价标准,对不同处理组大鼠各观察时间段挠头、爬笼、咬趾、往返等行为学改变进行综合评价,模型制备后30 min内大鼠活动明显增多,可能系灌药激惹所致。养血清脑颗粒不同剂量治疗组大鼠各观察时间段评分与模型组之间差异无统计学意义(均$P > 0.05$),但不同剂量预防组大鼠各观察时间段评分与模型组之间差异有统计学意义(均$P < 0.05$,表2~5)。

二、三叉神经脊束核c-Fos蛋白表达变化

光学显微镜观察显示,脑干组织切片背景呈浅棕色,其上散在分布胞核呈黄褐色的阳性细胞和胞核呈蓝色的阴性细胞,胞核圆形或类圆形,阳性细胞主要分布在三叉神经脊束核(图1)。在模型制备后的不同观察时间点(30、60和180 min),对照组大鼠脑干三叉神经脊束核c-Fos蛋白表达水平均低于模型组、高剂量治疗组和低剂量治疗组,且差异有统计学意义($P = 0.000$);而模型组与高剂量治疗组和低剂量治疗组相比,差异无统计学意义(均$P > 0.05$,表6)。在模型制备后的不同观察时间点,对照组大鼠三叉神经脊束核c-Fos蛋白表达水平均低于模型组、高剂量预防组和低剂量预防组,且差异有统计学意义($P = 0.000$);而模型组大鼠c-Fos蛋白表达水平高于高剂量预防组($P = 0.031, 0.000, 0.000$),并于60和180 min时高于低剂量预防组(均$P = 0.000$),30 min时高剂量预防组低于低剂量预防组($P = 0.029$,表7)。

析因设计方差分析(2×2×3)显示,预防性给药和治疗性给药对不同观察时间点三叉神经脊束核神经元c-Fos蛋白表达变化均有影响($P = 0.000$),养血清脑颗粒可使不同观察时间点大鼠三叉神经脊束核神经元c-Fos蛋白表达水平下降,且剂量增加对其表达无影响($P = 0.394$);而药物作用时间对其表达亦有影响($P = 0.000$),随着模型制备后时间的延长,c-Fos蛋白表达水平升高。而给药顺序与药物剂量之间存在交互作用($P = 0.023$)、给药顺序与药物作用时间之间同样存在交互作用($P = 0.000$),随着模型制备后时间的延长,预防组大鼠c-Fos蛋白表达升高幅度低于相同剂量治疗组;药物剂量与药物作用时间之间无交互作用($P = 0.264$),给药顺序、药物剂量与药物作用时间之间亦无交互作用($P = 0.777$,表8)。

表1 大鼠行为学评价标准[4]

Table 1. The behavioral score standard of rats[4]

Observation index	Scoring method
Scratching	Record 1 point for 10 times, then each add 0.10 point
Turn round and round	Record 1 point for 2 times, then each add 1 point
Climb the cage	Record 1 point for 3 times, then each add 1 point
Fidgety movement	Record 1 point in the first time, then each add 1 point
Toe/tail biting	Record 1 point for each time
Ear red	Record 1 point once appearing

讨　论

1989年,Iversen等[5]首次发现健康人持续静脉注射硝酸甘油[0.50 μg/(kg·min)]2~2.50分钟即可出现搏动性头痛。1995年,Tassorelli等[3]建立硝酸

表2 治疗组与对照组和模型组大鼠各观察时间段行为学评分的比较($\bar{x} \pm s$,评分)

Table 2. Comparison of behavior scores in different periods of rats from different groups ($\bar{x} \pm s$, score)

Group	N	0–30 min	N	31–60 min	N	61–90 min	91–120 min	121–150 min	151–180 min
Control (1)	18	13.60 ± 3.00	12	5.50 ± 2.30	6	5.40 ± 1.70	5.70 ± 1.10	3.90 ± 1.60	5.80 ± 1.90
Model (2)	18	20.40 ± 3.10	12	23.70 ± 3.80	6	27.50 ± 3.70	21.20 ± 3.20	8.90 ± 4.50	15.60 ± 3.70
HT (3)	18	19.60 ± 3.40	12	23.90 ± 2.40	6	28.60 ± 4.20	23.40 ± 2.50	21.70 ± 3.30	11.20 ± 4.00
LT (4)	18	20.20 ± 3.50	12	24.90 ± 3.50	6	29.10 ± 4.60	19.10 ± 1.70	20.10 ± 3.30	14.70 ± 3.80
F value		18.507		111.378		56.893	74.695	38.193	9.916
P value		0.000		0.000		0.000	0.000	0.000	0.000

HT,high-dose treatment,高剂量治疗组;LT,low-dose treatment,低剂量治疗组

表3 治疗组与对照组和模型组大鼠各观察时间段行为学评分的两两比较

Table 3. Paired comparison of behavior scores in different periods among 4 groups

Paired comparison	0–30 min	31–60 min	61–90 min	91–120 min	121–150 min	151–180 min
(1) : (2)	0.000	0.000	0.000	0.000	0.000	0.000
(1) : (3)	0.035	0.000	0.000	0.000	0.000	0.000
(1) : (4)	0.000	0.000	0.000	0.000	0.000	0.013
(2) : (3)	0.029	0.430	0.365	0.200	0.524	0.645
(2) : (4)	0.559	0.592	0.885	0.187	0.153	0.039
(3) : (4)	0.108	0.798	0.447	0.012	0.418	0.100

表4 预防组与对照组和模型组大鼠各观察时间段行为学评分的比较($\bar{x} \pm s$,评分)

Table 4. Comparison of behavior scores in different periods of rats from different groups ($\bar{x} \pm s$, score)

Group	N	0–30 min	N	31–60 min	N	61–90 min	91–120 min	121–150 min	151–180 min
Control (1)	18	13.60 ± 3.00	12	5.50 ± 2.30	6	5.40 ± 1.70	5.70 ± 1.10	3.90 ± 1.60	5.80 ± 1.90
Model (2)	18	20.40 ± 3.10	12	23.70 ± 3.80	6	27.50 ± 3.70	21.20 ± 3.20	18.90 ± 4.50	15.60 ± 3.70
HP (3)	18	14.30 ± 2.40	12	19.70 ± 4.10	6	19.90 ± 2.20	10.60 ± 2.50	13.40 ± 3.10	9.00 ± 2.90
LP (4)	18	16.40 ± 4.10	12	19.10 ± 3.60	6	17.50 ± 4.00	14.90 ± 4.50	13.10 ± 3.40	11.00 ± 4.30
F value		16.727		60.709		53.371	27.253	21.236	9.078
P value		0.000		0.000		0.000	0.000	0.000	0.001

HP,high-dose prevention,高剂量预防组;LP,low-dose prevention,低剂量预防组

表5 预防组与对照组和模型组大鼠各观察时间段行为学评分的两两比较

Table 5. Paired comparison of behavior scores in different periods among 4 groups

Paired comparison	0–30 min	31–60 min	61–90 min	91–120 min	121–150 min	151–180 min
(1) : (2)	0.000	0.000	0.000	0.000	0.000	0.000
(1) : (3)	0.550	0.000	0.000	0.005	0.000	0.126
(1) : (4)	0.019	0.000	0.000	0.000	0.000	0.017
(2) : (3)	0.000	0.001	0.005	0.000	0.007	0.003
(2) : (4)	0.052	0.000	0.001	0.000	0.005	0.030
(3) : (4)	0.078	0.253	0.646	0.014	0.868	0.347

甘油偏头痛大鼠模型,并在不同地区、不同实验室均复制成功[6-7],具有较好的可重复性,而且实验条件设置合理,复制成功率高达100%,信度良好。该动物模型行为学表现与人类偏头痛有一定相似性,较好地复制了偏头痛神经源性炎症反应机制和痛觉增敏,且对麦角胺反应较为敏感,表面效度和建构效度较佳[8]。由于该模型基本满足了人类疾病所需动物模型的信度和效度要求,且操作简单,便于短期内大量复制,可以作为偏头痛实验模型进行相关病理学和药理学研究。与之相比,电刺激上矢状

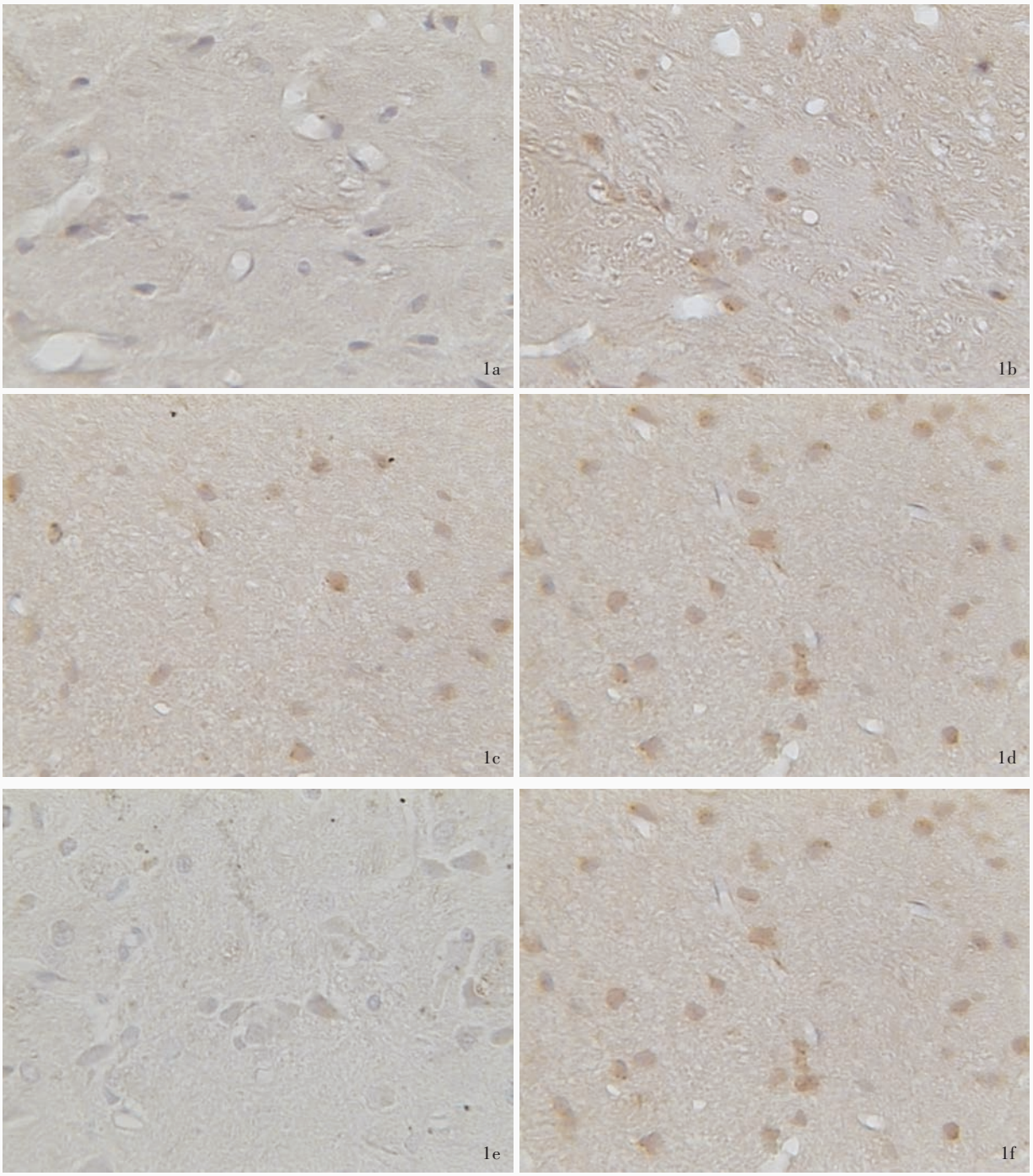

图1　模型制备后180 min时光学显微镜观察所见　免疫组织化学染色(EnVision二步法)　×250　1a　对照组大鼠脑干三叉神经脊束核神经元c-Fos蛋白处于较低水平,并可见散在分布的胞核呈蓝色的阴性细胞　1b　模型组大鼠脑干三叉神经脊束核神经元c-Fos蛋白表达水平高于对照组,并可见较多胞核呈黄褐色的阳性细胞　1c　高剂量治疗组大鼠脑干三叉神经脊束核神经元c-Fos蛋白表达水平降低　1d　低剂量治疗组大鼠脑干三叉神经脊束核神经元c-Fos蛋白表达水平降低　1e　高剂量预防组大鼠脑干三叉神经脊束核神经元c-Fos蛋白表达水平降低,可见大量胞核呈蓝色的阴性细胞,其间散在分布胞核呈黄褐色的阳性细胞　1f　低剂量预防组大鼠脑干三叉神经脊束核神经元c-Fos蛋白表达水平降低

Figure 1　Optical microscopy findings 180 min after modeling.　Immunohistochemical staining (EnVision)　× 250　TNC c-Fos protein expression level of rats in normal control group was low, and scattered negative cells with blue nuclei were seen (Panel 1a). The c-Fos protein expression level of rats in model group was relatively high, and there distributed positive cells with yellowish-brown nuclei (Panel 1b).　The c-Fos protein expression level of rats in high-dose treatment group decreased (Panel 1c).　The c-Fos protein expression level of rats in low-dose treatment group decreased (Panel 1d).　The c-Fos protein expression level of rats in high-dose prevention group decreased.　There were a lot of negative cells with blue nuclei and scattered positive cells with yellowish-brown nuclei (Panel 1e).　The c-Fos protein expression level of rats in low-dose prevention group decreased (Panel 1f).

表6 治疗组与对照组和模型组大鼠各观察时间点三叉神经脊束核神经元c-Fos蛋白表达水平的比较($\bar{x}\pm s$,灰度值)

Table 6. Comparison of TNC c-Fos protein expression levels of rats in different groups at each observation time point ($\bar{x}\pm s$, gray value)

Group	N	30 min	60 min	180 min
Control	6	164.50±2.64	164.93±2.88	164.87±3.66
Model	6	158.65±3.08	152.76±2.97	130.46±3.00
HT	6	160.09±2.44	152.74±2.76	129.01±3.15
LT	6	160.05±2.92	153.08±2.98	130.41±3.00
F value		15.021	77.955	531.166
P value		0.000	0.000	0.000

HT, high-dose treatment, 高剂量治疗组; LT, low-dose treatment, 低剂量治疗组

表7 预防组与对照组和模型组大鼠各观察时间点三叉神经脊束核神经元c-Fos蛋白表达水平的比较($\bar{x}\pm s$,灰度值)

Table 7. Comparison of TNC c-Fos protein expression levels of rats in different groups at each observation time point ($\bar{x}\pm s$, gray value)

Group	N	30 min	60 min	180 min
Control	6	164.50±2.64	164.93±2.88	164.87±3.66
Model	6	158.65±3.08	152.76±2.97	130.46±3.00
HP	6	160.97±3.71	156.60±2.27	149.87±2.53
LP	6	158.62±3.11	155.91±3.00	149.18±2.50
F value		13.847	62.260	407.768
P value		0.000	0.000	0.000

HP, high-dose prevention, 高剂量预防组; LP, low-dose prevention, 低剂量预防组

表8 不同处理组大鼠各观察时间点三叉神经脊束核神经元c-Fos蛋白表达水平的析因设计方差分析表

Table 8. Factorial ANOVA of TNC c-Fos protein expression levels of rats in various groups

Source of variation	*SS*	df	*MS*	*F* value	*P* value
Dosage order (A)	3 141.922	1	3141.922	375.962	0.000
Dosage (B)	6.103	1	6.103	0.730	0.394
Time after modeling (C)	15 966.027	2	7983.013	955.246	0.000
A×B	44.032	1	44.032	5.269	0.023
A×C	4 126.895	2	2063.448	246.912	0.000
B×C	22.413	2	11.207	1.341	0.264
A×B×C	4.226	2	2.113	0.253	0.777

窦模型通常需在麻醉状态下进行,对手术操作和术中生命体征监测相对严格,难度较大,短期内难以大量复制。由于麻醉状态可以减轻疼痛程度,因此不能完全模拟清醒状态下情绪变化的自主神经系统反应。

目前关于偏头痛发病机制有多种学说,包括血管学说、神经源性学说和遗传学说等,但无一能够完全解释偏头痛发病过程中的全部症状[9]。近年来,有学者提出偏头痛急性发作假说[10],认为所有偏头痛急性发作均存在诱发因素,导致"皮质扩散抑制"机制启动,从而诱发偏头痛;诱发因素的第二个靶点位于脑干三叉神经脊束核水平,是偏头痛痛觉传导通路的二级神经元所在部位。另外,该学说还认为,三叉神经脊束核水平存在假设门控,由三叉神经传入的偏头痛痛觉冲动与脑干痛觉调节神经元发出的下行抑制信号在此竞争后才能进一步传导至中枢,而且该门控受5-羟色胺受体调节,从而强调了三叉神经脊束核在偏头痛发作过程中的重要性。

早在20世纪80年代,已有大量证据表明,即刻早期基因(IEGs)*c-fos*表达产物可以作为神经元功能激活的标志[11-12]。随后的研究发现,大鼠初级感觉神经纤维受到刺激可引起突触后脊髓后角神经元c-Fos蛋白表达,主要位于后角外层[13]。Kaube等[14]及Goadsby和Hoskin[15]电刺激猫及平顶猴上矢状窦制备偏头痛动物模型,结果显示,三叉神经脊束核Ⅰ和Ⅱo层、上段颈髓(尤其是C_1节段)后角X层c-Fos蛋白表达水平升高,提示偏头痛的中枢机制可能涉及上述神经核团。此后,三叉神经脊束核和c-Fos蛋白成为偏头痛病理学和药理学机制的研究热点[6,16-24]。

三叉神经系统激活和敏化在偏头痛发作过程中起重要作用,这一过程涉及三叉神经脊束核,然而大量基于三叉神经脊束核激活模式的动物实验研究迄今尚未完全阐明这一过程[22]。Mitsikostas等[23]对关于三叉神经脊束核c-Fos蛋白相关受体,以及抗偏头痛药物如辣椒素受体、5-羟色胺受体、谷氨酸受体、γ-氨基丁酸A(GABAA)受体、神经激肽K-1(NK-1)受体的文献进行分析,发现并非所有下调c-Fos蛋白表达的抗偏头痛药物均具有治疗作用,而且各类受体的临床价值也不均等。最近的一项功能神经影像学研究显示,偏头痛发作时脑桥背外侧和背侧中脑存在激活现象,主要核团包括中脑导水管周围灰质(PAG)、中缝背核、中缝大核、蓝斑核等,这种激活现象可持续到头痛症状完全消失后[24-25],提示脑干激活可能与头痛本身无关。另一项基于上述研究的动物实验提示,电刺激三叉神经节致大鼠三叉神经系统激活,可导致下行网状系统痛觉调节系统激活,但并不能激活

上述偏头痛启动核团[24]。

我们采用皮下注射硝酸甘油的方法复制偏头痛模型，采集大鼠脑干组织标本检测三叉神经脊束核神经元c-Fos蛋白表达变化，探讨偏头痛发作机制和养血清脑颗粒预防和治疗偏头痛的原理。其结果显示，在不同观察时间点，治疗组和预防组大鼠脑干三叉神经脊束核神经元c-Fos蛋白表达水平均高于对照组，尤以60分钟时显著，与Knyihár-Csillik等[21]报告的大鼠三叉神经c-Fos阳性细胞数目增加相符，但与Martin和Martin[26]报告的经静脉注射硝酸甘油后2和8小时未见c-Fos蛋白表达升高的结果不尽一致。推测可能与静脉给药剂量不足有关。另外，c-Fos蛋白表达变化是由缓慢的细胞核改变而后诱导细胞内活动的变化过程，不能反映疼痛发生后的快速细胞内过程，本研究首次制备模型诱发偏头痛发作与采集组织标本间隔1周，有利于c-Fos蛋白的充分表达。

已有大量临床研究结果显示，养血清脑颗粒对偏头痛有预防作用。陈凌等[27]进行的前瞻性随机双盲安慰剂对照临床试验显示，养血清脑颗粒能够减少偏头痛发作次数，推荐作为偏头痛的预防用药。罗盛等[28]进行的多中心随机双盲安慰剂对照临床试验亦显示，养血清脑颗粒可以减少偏头痛发作次数、缩短发作时间，但对偏头痛发作过程中出现的伴随症状无明显治疗效果。进一步研究显示，养血清脑颗粒能够降低患者血清超敏C-反应蛋白(hs-CRP)[29]、IL-1β[30]、溶血磷脂酸(LPA)和肿瘤坏死因子(TNF)表达水平[31]，减轻炎症反应；抑制脑血管收缩，提高脑组织缺氧耐受程度[32-36]，以改善脑组织微循环、降低血管紧张度、改善脑供血、减轻头痛症状[37-40]；同时干预“皮质扩散抑制”机制，缓解典型偏头痛发作症状[40-43]。但是，关于养血清脑颗粒对实验性偏头痛大鼠的研究尚未见诸文献报道。本研究通过观察高、低剂量养血清脑颗粒对硝酸甘油偏头痛模型大鼠的预防和治疗作用，发现模型制备后60分钟时，高、低剂量预防组大鼠脑干三叉神经脊束核c-Fos表达水平明显低于模型组，而高、低剂量治疗组变化不明显，提示养血清脑颗粒对偏头痛的预防作用可能大于治疗作用，与临床试验结果相一致，其预防作用可能与抑制三叉神经脊束核神经元c-Fos蛋白表达有关。虽然，本研究高剂量预防组与低剂量预防组之间未显示出明显的优势，但目前还不能认为预防性应用高剂量养血清脑颗粒能够产生更显著的疗效，尚待扩大样本量、延长实验周期进一步加以证实。另外，我们还发现，部分预防性应用高剂量养血清脑颗粒的大鼠于实验后期出现尿色发黄，建议在未来的相关实验中重点观察养血清脑颗粒的药物不良反应。

参 考 文 献

[1] Yu S, Liu R, Zhao G, Yang X, Qiao X, Feng J, Fang Y, Cao X, He M, Steiner T. The prevalence and burden of primary headaches in China: a population-based door-to-door survey. Headache, 2011, 52:582-591.

[2] D´Amico D, Solari A, Usai S, Santoro P, Bernardoni P, Frediani F, De Marco R, Massetto N, Bussone G; Progetto Cefalee Lombardia Group. Improvement in quality of life and activity limitations in migraine patients after prophylaxis: a prospective longitudinal multicentre study. Cephalalgia, 2006, 26:691-696.

[3] Tassorelli C, Joseph SA. Systemic nitroglycerin induces Fos immunoreactivity in rat brainstem and forebrain structures of the rat. Brain Res, 1995, 682(1/2):167-181.

[4] Zhu XF, Han YC, Xiong WP, Wang HB, Li JF, Liu WW, Fan ZM. Heat coagulation of middle meningeal artery affects plasma CGRP and substance P in migraine rat triggered by nitroglycerin. Lin Chuang Er Bi Yan Hou Tou Jing Wai Ke Za Zhi, 2011, 25:460-462.[朱晓凤, 韩月臣, 熊文萍, 王海波, 李建峰, 刘闻闻, 樊兆民. 热凝脑膜中动脉对硝酸甘油致偏头痛大鼠血CGRP和SP含量的影响. 临床耳鼻咽喉头颈外科杂志, 2011, 25:460-462.]

[5] Iversen HK, Olesen J, Tfelt-Hansen P. Intravenous nitroglycerin as an experimental model of vascular headache. Basic characteristics. Pain, 1989, 38:17-24.

[6] Peng C, Ren YX, Yao G, Chen XL, Liu GP, Wang Y. The expression of c-fos and c-jun in the experimental migrainous animal model. Zhongguo Shi Yan Dong Wu Xue Bao, 2000, 8:112-119.[彭成, 任永欣, 姚干, 陈晓莉, 刘光谱, 王毅. 实验性偏头痛动物模型c-fos、c-jun基因表达. 中国实验动物学报, 2000, 8:112-119.]

[7] Zhou MM, Yang K, Wang YT. Effect on NOS, SP and CGRP receptors in dura mater and trigeminal nuclei of nitroglycerin-induced migraine rats after intervention of Dachuanxiongfang. Zhongguo Yao Li Yu Lin Chuang, 2009, 25:9-10.[周明眉, 杨奎, 王一涛. 大川芎方对硝酸甘油偏头痛模型大鼠硬脑膜血管及三叉神经核NOS、SP和CGRP受体的影响. 中国药理与临床, 2009, 25:9-10.]

[8] Zhang ZX, Cao KG, Gao YH. From bedside to bench: an assessment of reliability and validity of the rat model of migraine induced by glycerol trinitrate. Zhongguo Shi Yan Dong Wu Xue Bao, 2009, 17:312-314.[章正祥, 曹克刚, 高永红. 从临床到基础: 硝酸甘油实验性偏头痛大鼠模型信度和效度评价. 中国实验动物学报, 2009, 17:312-314.]

[9] Tfelt-Hansen PC, Koehler PJ. One hundred years of migraine research: major clinical and scientific observations from 1910 to 2010. Headache, 2011, 51:752-778.

[10] Chakravarty A. How triggers trigger acute migraine attacks: a hypothesis. Med Hypotheses, 2010, 74:750-753.

[11] Goelet P, Castellucci VF, Schacher S, Kandel ER. The long and the short of long-term memory: a molecular framework. Nature, 1986, 322:419-422.

[12] Berridge M. Second messenger dualism in neuromodulation and memory. Nature, 1986, 323:294-295.

[13] Hunt SP, Pini A, Evan G. Induction of c-fos-like protein in

spinal cord neurons following sensory stimulation. Nature, 1987, 328:632-634.

[14] Kaube H, Keay KA, Hoskin KL, Bandler R, Goadsby PJ. Expression of c-Fos-like immunoreactivity in the caudal medulla and upper cervical spinal cord following stimulation of the superior sagittal sinus in the cat. Brain Res, 1993, 629:95-102.

[15] Goadsby PJ, Hoskin KL. The distribution of trigeminovascular afferents in the nonhuman primate brain Macaca nemestrina: a c-fos immunocytochemical study. J Anat, 1997, 190(Pt 3):367-375.

[16] Cutrer FM, Limmroth V, Ayata G, Moskowitz MA. Attenuation by valproate of c-fos immunoreactivity in trigeminal nucleus caudalis induced by intracisternal capsaicin. Br J Pharmacol, 1995, 116:3199-3204.

[17] Mitsikostas DD, Sanchez del Rio M, Waeber C, Moskowitz MA, Cutrer FM. The NMDA receptor antagonist MK-801 reduces capsaicin-induced c-fos expression within rat trigeminal nucleus caudalis. Pain, 1998, 76(1/2):239-248.

[18] Hoskin KL, Kaube H, Goadsby PJ. Central activation of the trigeminovascular pathway in the cat is inhibited by dihydroergotamine: a c-Fos and electrophysiological study. Brain, 1996, 119(Pt 1):249-256.

[19] Classey JD, Knight YE, Goadsby PJ. The NMDA receptor antagonist MK-801 reduces Fos-like immunoreactivity within the trigeminocervical complex following superior sagittal sinus stimulation in the cat. Brain Res, 2001, 907(1/2):117-124.

[20] Schuh-Hofer S, Tayefeh M, Reuter U, Dirnagl U, Arnold G. Effects of parecoxib on plasma protein extravasation and c-fos expression in the rat. Headache, 2006, 46:276-285.

[21] Knyihúr-Csillik E, Toldi J, Mihály A, Krisztin-Péva B, Chadaide Z, Németh H, Fenyo R, Vécsei L. Kynurenine in combination with probenecid mitigates the stimulation-induced increase of c-fos immunoreactivity of the rat caudal trigeminal nucleus in an experimental migraine model. J Neural Transmis, 2007, 114:417-421.

[22] Bergerot A, Holland PR, Akerman S, Bartsch T, Ahn AH, MaassenVanDenBrink A, Reuter U, Tassorelli C, Schoenen J, Mitsikostas DD, van den Maagdenberg AM, Goadsby PJ. Animal models of migraine: looking at the component parts of a complex disorder. Eur J Neurosci, 2006, 4:1517-1534.

[23] Mitsikostasa DD, Sanchez del Rio M. Receptor systems mediating c-fos expression within trigeminal nucleus caudalis in animal models of migraine. Brain Res Brain Res Rev, 2001, 35: 20-35.

[24] Weiller C, May A, Limmroth V, Jüptner M, Kaube H, Schayck RV, Coenen HH, Diener HC. Brain stem activation in spontaneous human migraine attacks. Nature Med, 1995, 1:658-660.

[25] May A, Kaube H, Büchel C, Eichten C, Rijntjes M, Jüptner M, Weiller C, Diener HC. Experimental cranial pain elicited by capsaicin: a PET study. Pain, 1998, 74:61-66.

[26] Martin RS, Martin GR. Investigations into migraine pathogenesis: time course for effects of m-CPP, BW723C86 or glyceryl trinitrate on appearance of Fos-like immunoreactivity in rat trigeminal nucleus caudalis (TNC). Cephalalgia, 2001, 21:46-52.

[27] Chen L, Lü L, Hu WH, Zhi YH, Zhao N. A clinical observation of Yangxueqingnao granules in preventive treatment of migraine. Zhejiang Zhong Yi Za Zhi, 2009, 44:184-185.[陈凌, 吕磊, 胡万华, 支英豪, 赵娜. 养血清脑颗粒预防偏头痛发作的临床观察. 浙江中医杂志, 2009, 44:184-185.]

[28] Luo S, Wang DX, Kuang PG, Jia JP, Yang ZJ, Zhou BY, Yu HF, Chang SY, Ma WY. A clinical study of Yangxueqingnaokeli in preventive treatment of migraine. Zhonghua Shen Jing Ke Za Zhi, 2001, 34:291-294.[罗盛, 王德新, 匡培根, 贾建平, 杨志杰, 周宝玉, 余华峰, 常蜀英, 马维雅. 养血清脑颗粒预防和治疗偏头痛的临床研究. 中华神经科杂志, 2001, 34:291-294.]

[29] Huang SE, Li JM, Deng SQ. Effect of Yangxue Qingnao Grain on C reactive proteins in patients with migraine. Zhongguo Yi Yuan Yong Yao Ping Jia Yu Fen Xi, 2008, 8:58-59.[黄赛娥, 李建明, 邓士钦. 养血清脑颗粒对偏头痛患者超敏C反应蛋白的影响及疗效的观察. 中国医院用药评价与分析, 2008, 8:58-59.]

[30] Cai XJ, Luo S, Wang XD. Effect of Yangxueqingnao granules on interleukin-1 beta in patients with migraine. Zhongguo Lin Chuang Kang Fu, 2002, 6:1464.[蔡晓杰, 罗盛, 王新德. 养血清脑颗粒对偏头痛患者血中白介素-1β的影响研究. 中国临床康复, 2002, 6:1464.]

[31] Qi JX, Zhao L, Zhu HW, Sun JK, Zhao YL, Fan ZP. The effect of Yangxueqingnao Granula on LPA and TNF level in patients with migraine. Zhongguo Shi Yong Shen Jing Ji Bing Za Zhi, 2009, 12:32-35.[齐进兴, 赵亮, 朱华伟, 孙建奎, 赵彦玲, 范仲鹏. 养血清脑颗粒对偏头痛患者LPA、TNF水平影响的研究. 中国实用神经疾病杂志, 2009, 12:32-35.]

[32] Xu JH, Hu XZ. The effect of Yangxueqingnao granules on blood flow velocity in 80 patients with migraine. Jiangxi Zhong Yi Yao, 2002, 33:14.[徐建华, 胡喜招. 养血清脑颗粒对偏头痛80例脑动脉血流速度的影响. 江西中医药, 2002, 33:14.]

[33] Huang YR, Xu H, Zhao L. A clinical observation of Yangxueqingnao granules on TCD in patients with migraine. Zhong Cao Yao, 2003, 34:747-748.[黄友荣, 徐海, 赵玲. 养血清脑颗粒治疗偏头痛TCD临床观察. 中草药, 2003, 34:747-748.]

[34] Qin GC, Chen LX, Zhou JY. Research progress in chronic migraine. Zhongguo Teng Tong Yi Xue Za Zhi, 2011, 17:176-178.[秦光成, 陈力学, 周冀英. 慢性偏头痛研究进展. 中国疼痛医学杂志, 2011, 17:176-178.]

[35] Zhang ZX, Huang XM, Cao KG, Fan JP. The regulation strategy and traditional Chinese medicine in the treatment of migraine. Jiangxi Zhong Yi Yao, 2010, 41:18-19.[章正祥, 黄晓明, 曹克刚, 范吉平. 偏头痛的治疗策略及中成药的调节作用. 江西中医药, 2010, 41:18-19.]

[36] An XX, Xu HL. Observation of the effect of Yangxueqingnao granules combined with flunarizine in the treatment of migraine. Zhongguo Shi Yong Shen Jing Ji Bing Za Zhi, 2011, 14:84-85.[安新献, 徐海丽. 养血清脑颗粒联合盐酸氟桂利嗪治疗偏头痛疗效观察. 中国实用神经疾病杂志, 2011, 14:84-85.]

[37] Feng JL, Zeng AY, Du YP. The effect of Yangxueqingnao granules on microcirculation and cerebral blood flow in patients with migraine. Zhongguo Zhong Xi Yi Jie He Za Zhi, 2004, 24:357-358.[俸军林, 曾爱源, 杜云鹏. 养血清脑颗粒对偏头痛患者微循环和脑血流的影响. 中国中西医结合杂志, 2004, 24:357-358.]

[38] Liu CM, Zhou JS. The advance in international classification, diagnosis standard, pathogenesis, prevention and treatment of migraine. Yi Nan Bing Za Zhi, 2010, 9:953-955.[刘春梅, 周俊山. 偏头痛的国际分类诊断标准发病机制与防治研究进展. 疑难病杂志, 2010, 9:953-955.]

[39] Sun XT, Cai GH, Chen JK, Yu J, Zhang HF, Zhao YF, Tie R. Experimental study on the effect of aromatherapy in migraine rats. Shanxi Yi Xue Za Zhi, 2012, 41:390-392.[孙湘桐, 蔡国洪, 陈健康, 于军, 张海锋, 赵玉峰, 铁茹. 芳香疗法对大鼠偏头痛疗效的实验研究. 陕西医学杂志, 2012, 41:390-392.]

[40] He YQ. Curative effect observation fluoxetine hydrochloride combined with flunarizine in treatment of 50 cases with migraine. Zhongguo Zhong Yi Yao Zi Xun, 2011, 3:120.[何永桥. 盐酸氟西汀联合盐酸氟桂利嗪治疗偏头痛50例疗效观察. 中国中医药资讯, 2011, 3:120.]

[41] Yang J, Dong WW. The effect and clinical significance of Yangxue Qingnao Granules on MEP and SPECT of typical

migraine patients. Zhongguo Yi Yuan Yong Yao Ping Jia Yu Fen Xi, 2002, 2:348-349.[杨军, 董为伟. 养血清脑颗粒对典型偏头痛患者MEP和SPECT的影响及临床意义. 中国医院用药评价与分析, 2002, 2:348-349.]

[42] Du S, Zhang YJ, Tong YL, Liu LH, Liu JJ. The effect of Shunaoxin Pill on meningeal tissue morphology and brainstem c-fos gene mRNA expression of migraine model rat. Shanxi Zhong Yi, 2010, 31:1245-1247.[杜帅, 张艳军, 佟永领, 刘丽华, 刘俊静. 舒脑欣滴丸对偏头痛模型大鼠脑膜组织形态及脑干c-fos基因mRNA表达的影响. 陕西中医, 2010, 31:1245-1247.]

[43] Zhou LH. Curative effect observation of self-made slow tight decoction in the treatment of chronic tension headache. Zhong Yi Yao Lin Chuang Za Zhi, 2010, 22:45-46.[周丽华. 自拟慢紧汤治疗慢性紧张性头痛疗效观察. 中医药临床杂志, 2010, 22:45-46.]

（收稿日期：2014-05-26）

·临床医学图像·

颅内皮样囊肿破裂

doi：10.3969/j.issn.1672-6731.2014.08.019

Ruptured intracranial dermoid cyst

HAN Tong, LI Hui

Department of Neuroradiology, Tianjin Huanhu Hospital, Tianjin 300060, China

Corresponding author: HAN Tong (Email: mrbold@163.com)

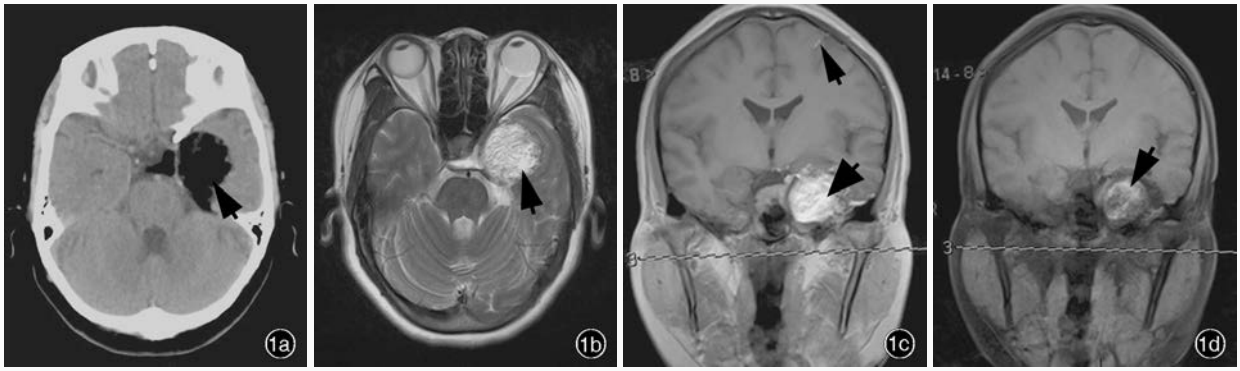

图1 女性患者，37岁。主因头痛1周就诊。影像学检查提示，左侧鞍旁占位性病变，考虑皮样囊肿破裂，不排除畸胎瘤可能。术后组织病理学证实为皮样囊肿 1a 横断面CT显示，左侧鞍旁边界清晰的低密度影（箭头所示）。病变内侧（鞍上池后部）可见同样强度的小片状低密度影 1b 横断面T_2WI显示，左侧鞍旁类圆形占位性病变，呈不均匀高信号影（箭头所示），边界锐利，左侧颞叶内侧面受压 1c 冠状位T_1WI显示，左侧鞍旁病变呈高信号（粗箭头所示），左侧额叶脑沟内、鞍上池内散在点状和线样高信号，提示病变破裂，脂质内容物进入蛛网膜下隙（细箭头所示） 1d 冠状位抑脂T_1WI显示，左侧鞍旁病灶内高信号消失（箭头所示），提示为脂肪成分

Figure 1 A 37-year-old female had suffered from headache for a week and came to clinic. MRI showed a space-occupying lesion in the left parasella, which was considered as ruptured dermoid cyst, or possible teratoma. Postoperative pathological diagnosis revealed dermoid cyst. Axial CT showed a low density lesion with clear boundry in left parasella (arrow indicates). There existed an irregular low density close to the medial wall of the lesion in the back of suprasellar cistern (Panel 1a). Axial T_2WI showed an oval lesion with heterogeneously high intensity (arrow indicates) and sharp edge compressing the adjacent medial left temporal lobe (Panel 1b). Coronal T_1WI showed the lesion with slightly high intensity (thick arrow indicates). There existed multiple hyperintense foci in the subarachnoid space suggestive of fat deposition from dermoid rupture (thin arrow indicates, Panel 1c). Coronal T_1WI with fat suppression showed the hyperintense signal within the lesion could be suppressed by fatty content (arrow indicates, Panel 1d).

颅内皮样囊肿为临床少见的、起源于外胚层的先天性良性异位肿瘤，占颅内肿瘤的0.20%～1%，可发生于任何年龄段，无明显性别差异。内含皮肤附属器官如毛发、毛囊、汗腺和皮脂腺；囊壁由两层结构组成，即外层的纤维组织和内层的皮肤组织。肿瘤多位于中线，常见于颅后窝、颅前窝或鞍旁，亦可见于颅骨骨缝和脑室内。临床主要表现为头痛、呕吐、癫痫，囊肿破裂后可引起化学性脑膜炎、血管痉挛、脑卒中，甚至死亡。CT呈边缘清晰的低密度影（图1a），脂质成分浮于上方；囊壁较厚，可发生钙化。T_2WI呈混杂高信号（图1b）、T_1WI呈脂肪样高信号（图1c），脂肪抑制序列高信号消失（图1d）；增强后病灶无明显强化。囊肿破裂时可见蛛网膜下隙和脑室内脂肪滴（图1c），CT宽窗显示脂肪性低密度影，T_1WI呈高信号、与低信号的脑脊液和中等信号的脑实质形成鲜明对比。根据颅内皮样囊肿典型的CT和MRI表现，特别是皮样囊肿破裂时的影像学和临床特征性表现，可明确诊断。但须注意与颅内积气、脂肪瘤、表皮样囊肿、畸胎瘤、蛛网膜囊肿等肿瘤性病变相鉴别。

（天津市环湖医院神经放射科韩彤 李慧供稿）