

实验研究

风寒湿刺激对家兔颈部肌肉及血液组织中
氧自由基和前列腺素代谢的影响姜 杰¹ 施 杞²

摘要 为了探讨颈部风寒湿刺激对机体氧自由基及前列腺素的影响,将 24 只新西兰大白兔随机分为正常对照组、风寒湿组、低头位组、复合模型组 4 组,每组 6 只。通过给予风寒湿、低头位刺激,观察受累局部和血液组织中超氧化物歧化酶(SOD)、脂质过氧化物(LPO)、前列腺素 E_2 (PGE_2)、6-酮-前列腺素 $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) 的变化。结果表明,风寒湿与低头位等颈椎病常见的致病因素可使颈部出现无菌性炎症并使受累局部组织和血液中 SOD、LPO、 PGE_2 、6-keto-PGF $_{1\alpha}$ 出现异常。氧自由基及前列腺素代谢异常可能是风寒湿致颈椎病的重要机理之一。

主题词 自由基/代谢 前列腺素/代谢 颈椎病/代谢

流行病学及临床观察显示,风寒湿是颈椎病的重要诱因之一^[1,2]。然而,有关风寒湿致颈椎退变机理尚缺乏实验研究的阐明。氧自由基代谢异常与人体许多疾病的发生发展有密切关系^[3],本课题采用模拟自然致病因素,刺激家兔颈部,对局部及血液组织中超氧化物歧化酶(SOD)、脂质过氧化物(LPO)、前列腺素 E_2 (PGE_2)、6-酮-前列腺素 $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) 含量进行测定,以探讨风寒湿致颈椎病的部分病理机制。

1 材料与方法

1.1 动物分组及造模:3 月龄雄性新西兰大白兔 24 只,体重 2.25 ± 0.27 kg,由上海中医药大学实验动物中心提供(动物合格证 01933,医动字第 02-60 号)。将 24 只大白兔随机分为:(1)正常对照组;(2)风寒湿组;(3)低头位组;(4)复合模型组,每组 6 只。

造模方法:(1)正常对照组:每日缚定时间与低头位组相同,但不做低头处理。(2)风寒湿组:按正常对照组方法处理 8 周后进行风寒湿组造模。按王绪辉等^[4]方法略做修改,剪去家兔颈部毛发,固定动物于恒温恒湿造模箱内,接通喷雾水源、风扇和半导体温度、湿度计电源,调节造模箱内环境至刺激要求强度。条件:温度 $7 \pm 3^\circ\text{C}$,湿度 100%,风力 6 级。刺激强度:每次 2h,休息 1h,间断重复刺激 32h 后按与正常对照组处理至

第 12 周结束。(3)低头位组:按余家阔等^[5]方法改进,自制特殊支架,将家兔固定于低头 45° ,每日 2 次,每次 2h,连续 12 周。(4)复合模型组:按低头位组处理,8 周结束时加同风寒湿组刺激后续按低头位组处理至 12 周结束,该组在造模时意外死亡 1 只家兔。

1.2 标本采集:动物饲养 12 周后,用 40mg/kg 左右戊巴比妥钠麻醉,静脉采血后放血处死,迅速切开家兔颈后部皮肤,在颈 3~7 棘突旁 0.5cm 左右,取皮下肌肉组织约 0.5cm³(操作时避免钳夹),每块组织再切为两半,一半称重后,迅速置冰蒸馏水中漂洗 3 小时,然后取出,加入生理盐水 5ml,在组织匀浆器中持续研磨 10 分钟,制成匀浆,离心取上清液,放入 -20°C 冰箱中备测 SOD、LPO、 PGE_2 、6-keto-PGF $_{1\alpha}$,另一半取部分置于 10% 甲醛溶液中固定,石蜡切片,HE 染色。

1.3 LPO 测定:采用改良的八木国夫法测定^[6]。

1.4 SOD 测定:采用邻苯三酚自氧化抑制法^[7]。

1.5 PGE_2 测定:采用苏州医学院生产的 PGE_2 放免分析药盒,操作按产品说明书。

1.6 6-keto-PGF $_{1\alpha}$ 测定:6-keto-PGF $_{1\alpha}$ 放射免疫分析药盒来源同上,操作按产品说明书。

1.7 颈部肌肉组织形态学观察:常规 HE 染色,在光镜下观察并照像。

1.8 统计学方法:所有数据以 $\bar{x} \pm s$ 表示,SPSS 统计软件包统计处理。

2 结果

2.1 肌肉组织病理学观察:风寒湿组:软组织水肿、充血,伴炎性细胞浸润,血管扩张。低头位组:横纹肌组织肿胀、变性,有少许炎性细胞反应。复合模型组:横纹肌组织广泛肿胀、变性,软组织周围水肿、充血,伴有大量炎性细胞浸润。提示各模型组均有不同程度无菌性炎症表现。

2.2 各组动物血浆、肌肉组织 LPO、SOD、 PGE_2 、6-keto-PGF $_{1\alpha}$ 测定结果比较:由附表可见,风寒湿组、复合模型组家兔血浆 SOD 与正常对照组相比,差异有极显著意义 ($P < 0.01$);低头位组与正常对照组相比有显著意义 ($P < 0.05$);血浆 LPO 组间比较变化趋势

1 暨南大学医学院中医学系,广东省广州市石牌(510632)

2 上海中医药大学骨伤科研究所

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附表 4 组兔血浆、肌肉组织 SOD、LPO、PGE₂、6-keto-PGF_{1α}测定结果比较(±s)

组别	兔数	血浆		肌肉组织			
		SOD(U/g)	LPO(μmol/L)	SOD(U/mg)	LPO(μmol/L)	PGE ₂ (pg/mg)	6-keto-PGF _{1α} (pg/mg)
正常对照组	6	4241.1±675.2	2.405±0.439	24.21±3.170	4.286±0.391	9.02±2.44	144.87±25.68
风寒湿组	6	2867.6±593.3**	3.856±0.988**	16.98±4.225**	5.781±0.602*	16.70±3.71**	223.45±47.44**
低头位组	6	3139.3±760.9*	3.565±0.984*	17.53±2.591**	4.883±0.434	14.18±4.46*	208.11±32.48*
复合模型组	5	2792.2±883.7**	4.112±1.022*	14.90±3.709**	5.924±0.987**	18.56±3.25**	212.56±46.36*

与正常对照组比较, * $P < 0.05$, ** $P < 0.01$

与 SOD 相同。肌肉组织 LPO 测定结果表明, 风寒湿组与正常对照组相比差异有显著性($P < 0.05$); 复合模型组与正常对照组相比差异有极显著性($P < 0.01$); 低头位组与正常对照组之间比较差异无显著性; 各模型组肌肉组织 SOD 含量下降明显(与正常对照组比较, $P < 0.01$)。风寒湿组、复合模型组肌肉中 PGE₂、6-keto-PGF_{1α} 含量与正常对照组比较差异有显著性($P < 0.01$); 低头位组与正常对照组相比差异有显著性($P < 0.05$); 风寒湿组、低头位组和复合模型组之间比较差异无显著性。

3 讨论

颈椎病属中医痹证范畴, 中医学历来都十分重视风寒湿在痹证发病中的重要影响, 认为风寒湿邪是引起痹证的病因^[8,9]。《素问·痹论篇》曰: “风寒湿三气杂至, 合而为痹也”。痹证的病因是虚体与风寒湿邪相结合, 病机是血涩不行, 卫气从之而不通。现代流行病学及临床观察也显示, 风寒湿是颈椎病的重要诱因之一^[1,2]。本实验结合中医痹证理论, 采用模拟自然致病因素, 刺激家兔颈部, 造成颈部痹证模型, 以期研究风寒湿致颈椎病的机理。病理结果显示, 风寒湿侵袭颈部, 可引起软组织水肿、炎性细胞浸润、血管扩张, 肌肉组织发生肌纤维变性、肿胀等无菌性炎症表现; 与以往痹证研究观察结果相符^[10,11]。近年来研究发现, 氧自由基与人体多种退行性变的病理过程有关^[3]。一些研究也发现, 颈椎病患者存在着不同程度的自由基代谢紊乱, 表现为 LPO 较健康人明显增高, 而血清 SOD 活力较健康人明显降低, 这说明颈椎病患者体内自由基代谢非常活跃, 发生机体的过氧化和抗氧化平衡失调^[12~14]。

本实验结果证实, 实验家兔血清及局部肌肉组织中 SOD 活性明显下降, LPO 含量增高, 提示病变局部或全身组织细胞的清除超氧自由基功能受到抑制, 脂质过氧化作用增强, 有害氧化增加。这可能由于风寒湿侵袭, 导致受累组织局部氧化代谢增强, 氧自由基增加, 组织中 SOD 将其清除的同时, 自身也大量耗损, 最终导致 SOD 减少。此外, 风寒湿邪侵袭对颈部组织细胞的损伤, 可能造成细胞内 SOD 合成障碍, 使自由基

引发的过氧化反应发生紊乱; LPO 氧化和还原失控, 细胞膜通透性增加, 线粒体膨胀, 溶酶体释放大量的炎性致病物质, 如前列腺素、5-羟色胺、缓激肽等造成颈部肌肉无菌性炎症和疼痛^[15]。实验中观察到, 受风寒湿影响, 颈部肌肉中 PGE₂、6-keto-PGF_{1α} 含量明显增加。由 LPO 氧化和还原失控引发的肌肉无菌性炎症, 同时组织中酸性代谢产物大量堆积, 产生局部酸胀、僵硬感, 血管壁内外渗透压改变, 造成局部肿胀。长期失治, 产生颈椎病。本实验为风寒湿引发或加重颈椎退行性病变提供了部分实验依据。

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ABSTRACTS FROM ORIGINAL ARTICLES

Clinical Study on Treatment of Senile Vascular Dementia with Shen Ma Yi Zhi Capsule

Luo Zenggang, Zhou Wenquan, Gao Pu, et al

In order to observe clinical therapeutic effect of Shen Ma Yi Zhi Capsule on senile vascular dementia (VD), 68 cases of VD were randomly divided into the treatment group treated with Shen Ma Yi Zhi Capsule and the control group treated with Western medicine Trigonine, and double blind and double simulation methods were adopted for administration of drugs. Results indicated that Shen Ma Yi Zhi Capsule could obviously improve clinical symptoms, increase cumulative score of intelligence rating scale, improve memory and gnosis function, restore nervous function improve daily living capability, indexes of blood rheology and cerebral function. The treatment group in improvement of symptoms was superior to the control group. It is suggested that Shen Ma Yi Zhi Capsule has definite therapeutic effect for senile VD, with no adverse effect.

Authors' address: Department of Postgraduate Students, China Academy of TCM, Beijing 100700;

Key words: Alzheimer's disease/TCM therapy, @ Shen Ma Yi Zhi Capsule

(Original article on page 470)

Clinical Observation on Treatment of 64 Cases of Bronchial Asthma at Acute Attack Stage with Si Chong Qu Feng Gu Ben Decoction

Wu Xinghe

Si Chong Qu Feng Gu Ben Decoction is formulated by the author and the decoction has been used for clinical treatment of bronchial asthma at acute attack stage for about 20 years. In the present paper, 64 cases of bronchial asthma at acute attack stage were used as treatment group treated with Si Chong Qu Feng Gu Ben Decoction and 60 cases who were treated with routine methods for alleviating asthma and strengthening the body resistance and restoring normal function of the body to consolidate the constitution, were served as control group. Results indicated that the therapeutic effect in the treatment group in pulmonary function, relieving rate of asthma, time of producing effect on main symptoms all were superior to the control group ($P < 0.05$ or $P < 0.01$). It is suggested that in treatment of bronchial asthma at acute attack stage, routine treatment plus Si Chong Qu Feng prescription can markedly increase therapeutic effect.

Author's address: Shao Xing Municipal People's Hospital, Zhejiang Province 312000

Key words: Asthma, /TCM therapy, @Si Chong Qu Feng Gu Ben Decoction

(Original article on page 476)

Study on Relation of TCM Syndrome Types of Primary Liver Cancer with Relative Objective Indexes

Lin Lizhu, Lan Shaoqing

Relation of different TCM syndrome types of primary liver cancer with some relative objective indexes, including α -fetoprotein (AFP), alkaline phosphatase (AKP), γ -glutamyl-transpeptidase (GGT), L - lactic dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), globulin (GLO), A/G, total bilirubin (TB), indexes of hepatic reserve (ICG), prothrombin time (PT), natural killer (NK), lymphocyte transformation rate (LBT) and T - lymphocyte subgroups, were analysed for probing into objective indexes of diagnosis in TCM syndrome typing. Results indicated that there were significant differences in AST, ALB, TB, PT, ICG, NK, CD₄ among different syndrome types, and relative indexes for syndrome typing of liver cancer are ALB, TB, NK and CD₄.

Authors' address: The first Affiliated Hospital of Guangdong University of TCM, Guangzhou 510407

Key words: Hepatic Tumor/diagnosis, typing of syndrome differentiation

(Original article on page 486)

Effects of Stimulation of Wind, Cold and Dampness on Metabolism of Oxygen Free Radical and Prostaglandin in Cervical Muscle and Blood in Rabbits

Jiang Jie, Shi Qi

In order to probe into effect of stimulation of wind, cold and dampness to the cervical part on oxygen free radical and prostaglandins, 24 rabbits were randomly assigned to control group and wind - cold - dampness group, head - lowering group and combined model group, 6 rabbits in each group, and changes of superoxide dismutase (SOD), lipid peroxide (LPO), prostaglandin E₂ (PGE₂), 6 - keto - PGF_{1 α} in affected local region and blood were observed. Results indicated that the commonly - seen pathogenetic factors of cervical spondylopathy such as wind, cold, dampness and lowering head might cause aseptic inflammation of the cervical part and abnormality of SOD, LPO, PGE₂ and 6 - keto - PGF_{1 α} in affected local tissue and blood, and abnormal metabolism of oxygen free radical and prostaglandins might be one of important mechanisms of cervical spondylopathy induced by wind, cold and dampness.

Authors' address: Department of TCM, Medical College of Jinan University, Guangdong 510632

Key words: Free radicals/metabolism, prostaglandins/metabolism, cervical spondylopathy/metabolism

(Original article on page 489)