·实验研究·

# 施今墨抗衰老方制备液对小鼠脑和肝B型单胺氧化酶活性及果蝇寿命的影响

北京大学生物系　戴尧仁　高崇明　田清涞　殷　莹

**内容提要**　本文测定了用施今墨抗衰老方制备液饲喂90天后的小鼠脑和肝中B型单胺氧化酶(MAO-B)活性，发现该液可抑制小鼠脑中MAO-B活性达50%，而肝中MAO-B活性只有较轻微的抑制。果蝇寿命试验表明，该液可延长果蝇平均寿命约21%。表明施今墨抗衰老方确实具有一定的抗衰老作用。

七十年代以来，许多研究者发现人脑中单胺氧化酶(MAO，E·C·1·4·3·4)的活性，在45岁以后随年龄增长而急剧增加[1~3]。根据Finch[4]和Samorajski[5]的学说，脑儿茶酚胺的变化是定位于下丘脑的中央寿命钟的主发条，而使用单胺氧化酶抑制剂(MAOI)，能影响脑儿茶酚胺的水平，从而调节衰老过程。到目前为止，已发现及合成的MAOI约有140种之多，但多数有严重的副作用，如"奶酪效应"。另一方面，中国传统医学文献中有许多著名的"抗衰老"方，对于延缓衰老确有一定的作用。从单胺氧化酶的抑制作用的角度来研究这些药方的药理作用，可能具有一定的意义。因此，我们研究了已故著名中医施今墨的抗衰老方制备液(下称施今墨方)对小鼠脑和肝中MAO-B活性及果蝇寿命的影响。

## 材料和方法

一、施今墨方含黄芪、枸杞、桑椹、茯苓、炙实各20g，党参、黄精、首乌、黑豆、白术、玉竹、五味子、紫河车、葡萄干、大生地、菟丝子各10g，乌梅2g，麦冬、莲子、丹参、大熟地、柏子仁、山萸肉、炙甘草、怀山药、龙眼肉各5g。实验所用生药均从市场购入。

二、施今墨方制备液：将各种生药粉碎后，按上述剂量配合[6]，加水1 120ml煮沸30分钟，浓缩至160ml，然后在3 000rpm下离心20分钟，取上清液备用，其最终浓度为每毫升含总生药量1.60g。

三、实验动物及处理：本实验除离体脑MAO-B活性测定使用6月龄雄性ICR/JCL种小鼠外，其余均使用6月龄NIH系雄性小鼠。小鼠分为两组，每组10只，单只饲养。一组以饮水方式饲喂施今墨方制备液(每毫升浓缩液稀释至67ml)，每只小鼠平均每日折合饲喂总生药量130mg。另一组喂自来水作为对照，其他固体饲料相同。小鼠共饲喂90天，断头处死，迅速取出脑和肝备用。

四、MAO-B的制备：参照Kan和Benedetti的方法[7]，并加修改。取出脑和肝组织，分别加入10倍体积0.2M磷酸缓冲液(pH7.4)，先后在预冷的研钵和玻璃匀浆器中研磨，匀浆液在24KC下超声两次，每次30秒，间隔30秒，再在1 000g下离心10分钟(4°C)，弃去沉淀，上清液再在17 000g下离心30分钟(4°C)，弃去上清液，沉淀用0.2M磷酸缓冲液重新悬浮，此即为粗酶液。

五、MAO-B活性的测定：参照Mc Ewen的方法[8]进行测定。反应体系为8mM苄胺盐酸0.3ml，适量酶液，用0.2M磷酸缓冲液(pH7.4)补足到3ml，在37°C下振荡保温2.5小时。取出加60%过氯酸以终止反应，再加入3ml环己烷，振荡混合以提取单胺氧化产物。然后在3 000rpm下离心10分钟，取上层液在242nm下测定其光密度。空白样品在保温前不加苄胺盐酸，保温终止后再加，其他操作相同，酶活性以苄醛生成nmol/hr·mg蛋白质表示。离体试验在反应体系中加入施今墨方制备液1.0ml，其他操作相同。蛋白质采用Spector法[9]，应用小牛血清蛋白为标准蛋白。

六、果蝇寿命试验：本试验采用美国野生型果蝇(*Drosoplila melanogaster*, American wild type)，卵、蛹、幼虫培养在25ml玻璃管中，收集24小时内孵出的成虫，用乙醚麻醉后进行性别鉴定，雌雄成虫分别饲养。培养基含玉米10%、红糖3%、蔗糖3.7%、干酵母粉1%、苯甲酸0.15%，pH为6.4～6.8。药物处理在特别设计的玻璃瓶中进行。瓶中无培养基，基

部放置加入蔗糖的施今墨方制备液（蔗糖浓度为15%），用硬纸板与上部空间相隔开。硬纸板中央插入用滤纸卷成的轴，上部滤纸剪成细条状，药液通过滤纸扩散上行至细条部，将果蝇移入容器中，放置3天。对照组处理相同，只是在瓶子的下部放置的是的15%蔗糖。处理结束后，将果蝇移回具培养基的玻璃管中，每24小时统计存活率，并记录每只果蝇死亡时间，求出平均寿命值。

## 结　果

一、饲喂施今墨方制备液90天的小鼠，其脑中MAO-B特异活性为128.35nmol/hr·mg蛋白质，而对照组为250.29nmol/hr·mg蛋白质，其抑制率达50%左右，肝MAO-B活性，与对照组相比，表明有较弱的抑制作用（表1）。体外试验中也观察到施今墨方制备液对小鼠脑MAO-B活性的抑制作用，其抑制率亦达50%左右（表2）。

**表1**　施今墨方制备液对小鼠脑、肝MAO-B活性的影响

| | 动物数 | MAO-B活性*（nmol/hr·mg蛋白质） | |
|---|---|---|---|
| | | 脑 | 肝 |
| 对　照 | 9 | 250.29±1.13 | 156.83±0.67 |
| 施今墨方制备液 | 9 | 128.35±2.75 | 121.05±1.65 |
| P　值 | | <0.01 | <0.01 |

* 表中数据系三次试验的平均值(M±SE)

**表2**　施今墨方制备液对离体小鼠脑MAO-B活性的影响

| | 动物数 | MAO-B活性* | |
|---|---|---|---|
| | | 苄　醛（nmol/3hr） | 相对活性(%) |
| 对　照 | 10 | 33.82±1.02 | 100.06±3.04 |
| 施今墨方制备液 | 10 | 14.37±2.83 | 42.45±8.37 |
| P　值 | | <0.01 | <0.01 |

* 表中数据为三次试验的平均值(M±SE)

二、饲喂施今墨方制备液对小鼠体重无明显影响。在观测58天内，对照组9只小鼠，平均体重增加0.80±0.78g(M±SE)，施今墨方制备液组9只小鼠，平均体重增加0.5±0.10g，两组相比，无明显差异($P>0.05$)。

三、果蝇寿命试验中，饲喂施今墨方制备液三天后，对照组为42.7±3.30天(M±SE)，喂药组平均寿命为51.9±0.76天(M±SE)，延长21.63%($P<0.05$)。表明施今墨方对寿命有显著的延长作用。

## 讨　论

本研究中观察到，施今墨方制备液在相对较长时间（90天）饲喂的条件下，表现出对脑MAO-B活性的一定的抑制作用。这表明施今墨方药物能通过调节脑神经单胺水平，影响中央衰老钟的运行，从而起到延缓衰老的作用。体外试验中也观察到对脑MAO-B活性的类似程度的抑制作用，有力地证明施今墨方药物对脑MAO-B活性的直接的抑制作用。因此，从脑单胺类神经介质水平的调节角度部分地解释了施今墨方的抗衰老功效。此方除了影响脑MAO-B活性外，还可能通过其它途径影响衰老进程，尚需进一步研究。试验表明，施今墨方制备液对肝的MAO-B活性抑制较弱，不影响肝对体内某些有害的单胺的氧化分解，从而表明，施今墨方在临床上是可以应用的。此外，体重变化的观察也证明，施今墨方对动物的正常生长无不良影响，这一点也同样表明它是可以用于临床的。值得注意的是，果蝇寿命的试验结果表明，施今墨方制备液能使果蝇平均寿命延长20%以上，相当于人类15年的寿命。果蝇寿命测定是衰老生物学中常用的方法，具有一定的参考价值。

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### Effects of Qing Court Shoutao Pill (清宫寿桃丸) on Concentrations of Zn, Cu, Na and Br in Senile Hair

Luo Shihua (罗世华), Chen Keji* (陈可冀), et al
*Institute of High-Energy Physics, Academia Sinica, Beijing*
**Research Dept. of Geriatrics, Xiyuan Hospital, China Academy of TCM, Beijing*

This paper reports the clinical effect of Qing Court Shoutao pill (QCSP) on the concentrations of Zn, Cu, Na and Br in senile hair. 25 over 60 years old patients suffering from senile symptom-complexes were divided randomly into two groups and treated with QCSP (12 cases) and vitamin E (13 cases) respectively. The concentrations of Zn, Cu, Na and Br in senile hair collected before and after eight weeks of treatment were separately measured with neutron activation analysis. It was shown that after eitht weeks of treatment with QCSP or vitamin E, Cu and Na concentrations decreased, Zn concentration remained almost constant, and thus Zn/Cu ratio increased. This indicated that QCSP as well as vitamin E possessed the effect of slowing down the process of aging (Original article on page 216)

### Observation on Effect of Anti-Fluorine No.1 in Treating Endemic Fluorosis

Ai Hongfei (艾鸿飞), Geng Man* (耿　满), et al
*Liaoning Academy of TCM; *Shenyang Research Institute of TCM, Shenyang*

Two clinical treatment groups divided randomly were observed. The experimental group (33 cases) was treated with Chinese remedy Anti-fluorine No.1, while the control group (32 cases) administered with routine Western medicines. Result: The general symptoms of 97% patients of the experimental group were improved, and 94% of that of the neckache, backache and arthralgia were reduced, while the effective rates of the control group were 58～77% and 64% respectively. In the experimental group, before and after treatment, the mean gripping power of left and right hand was 16.27 kg, 17 kg and 20.1 kg, 21.23 kg respectively, which showed a significant difference ($P<0.005$). In the control group, the mean values were 19.61 kg, 18.69 kg and 22.38 kg, 23.07 kg respectively, the difference was not significant statistically ($P>0.05$). Before treatment the mean urinary fluorine in the experimental and control group were 3.39 mg/L and 3.23 mg/L respectively. After treatment, the former excretion increased to 5.15 mg/L ($P<0.05$) and the latter was 4.47 mg/L showing no statistic significance. (Original article on page 219)

### Analysis of Pulse Condition in 643 Cases of Healthy Adults

Zhao Guanying (赵冠英), et al
*Department of TCM, General Hospital of PLA, Beijing*

This study deals with observation on the pulse condition in 643 healthy adults, 342 males, 301 females, whose age ranged from 16 to 80. It was found that pulse was present in nine types: Normal pulse in 86 cases, normal smooth in 73, smooth in 26, small smooth in 79, wiry smooth in 88 cases, small wiry in 130 cases, wiry pulse Ⅰ in 109, wiry pulse Ⅱ in 18 and wiry pulse Ⅲ in 34 cases. Features of these types of pulse and the relationship between their features of various types of pulse and age were analysed statistically. The results showed that pulse condition in healthy adults changed from normal or normal smooth or small smooth to smooth or small wiry, to wiry smooth, and finally to wiry Ⅰ～Ⅲ with the advancing age.
(Original article on page 221)

### Effect of Preparation of Shi Jinmo's (施今墨) Anti-aging Recipe on B Type Monoamine oxidase Activity in Mice and Life Span of *Drosophila melanogaster*

Dai Yaoren (戴尧仁), Gao Chongming (高崇明), Tian Qinglai (田清涞), et al
*Dept. of Biology, Peking University, Beijing*

The preparation of the anti-aging recipe of Shi Jinmo, a famous experienced doctor of TCM, showed about 50% inhibition on brain MAO-B activity when the preparation was fed to 6-month male NIH mice for 90 days. The inhibitory effect of MAO-B activity partly explained the anti-aging efficacy of Shi Jinmo's remedy in terms of the regulation on brain level of monoamine transmitters, the shift of which was