

白首乌抗自由基损伤的研究

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内容提要 本文报告小鼠吸臭氧(O_3)后形成自由基损伤动物模型及中药白首乌抗损伤的功。结果:模型组出现体重减轻、体温降低、体力减弱、御寒能力下降等一系列生理功能减退的变化,并测得脏器过氧化脂含量增加、脑单胺氧化酶-B活性提高等生化改变。这些变化与老龄小鼠的变化相似。服首乌的小鼠以上这些指标都得到改善,血超氧化物歧化酶活性下降,接近正常组水平。证明白首乌有抗自由基损伤功能,是一种预防与治疗 O_3 损伤的有效药物,也可能是一种有前途的延缓自然衰老的药物。

本实验使小鼠吸入臭氧(O_3),在体内诱发较强的自由基反应、导致一系列类似衰老的变化,同时饲以白首乌,检验其抗自由基损伤以及抗衰老的功能。

材料与方法

一、材料:雄性NIH种小鼠,60日龄,体重 $27 \pm 3g$ ($M \pm SD$)。白何首乌(以下简称首乌)系北京西北旺药用植物试验厂栽培,经鉴定为萝藦科耳叶牛皮消。超氧化物歧化酶测定试剂:3-氨基苯二甲酰肼(L),西德E. Merk产品;黄嘌呤氧化酶(HO)与次黄嘌呤(HX),美国Sigma产品。单胺氧化酶测定试剂:盐酸苄胺,上海试剂三厂产品;环己烷,上海试剂一厂产品。过氧化脂测定试剂:硫代巴比妥酸与标准品四甲氧基丙烷,Fluka AG, Bucks产品。

二、方法:将小鼠分为对照、模型及首乌3组,每组9只小鼠,自由取食饮水。首乌组预服药,每天灌饲首乌粉60mg,连续10天。然后与模型组一起进入 O_3 环境(O_3 浓度0.9ppm)10天,首乌组继续喂药。对照组不作特殊处理。实验共20天,第21天全部小鼠作以下观察。

(1)体温测量:北京师范大学产SY-2电子体温计探头插入小鼠肛门,以最后稳定的数字为准。(2)爬绳试验:将小鼠置于水平悬挂的细绳上任其自由爬动,记录其跌落的时间。(3)冻死试验:小鼠先在 $4^\circ C$ 水中游泳2分钟,然后进入 $-8^\circ C$ 冰柜中观察其完全冻僵时间。(4)过氧化脂(LPO)测定:采用TBA法测肝、脑、肺组织的丙二醛(MDA)含量,以此代表LPO量。(5)超氧化物歧化酶(SOD)测定:采用XO-HX-L化学发光系统⁽¹⁾在LKB1250型发光仪上测发光强

度。HO在催化HX生成尿酸的同时产生超氧基,后者能与发光剂L反应发光,SOD能够清除超氧基、抑制发光,从发光强度的被抑制程度测知酶活力的大小,算出抑制率。(6)单胺氧化酶-B(MAO-B)测定:采用苄胺法⁽²⁾,取组织匀浆与苄胺共孵,环己烷提取,在紫外分光光度计上测定,算出MAO-B活性。

结 果

一、体重与体温的变化:模型组体重明显减轻,体温下降。首乌组体重大于模型组而与对照组相近,体温亦高于模型组(表1)。

二、体力的变化:吸入 O_3 前各组体力相似,爬绳时间无区别;吸 O_3 10天后,模型组比对照组爬绳时间明显缩短,饲首乌10天的小鼠爬绳时间较长(表1)。

三、御寒能力的比较:另有3组在实验结束时作冻死实验。首乌组冻死时间与对照组相近,模型组则明显缩短,说明服首乌后小鼠御寒能力得到明显提高(表1)。

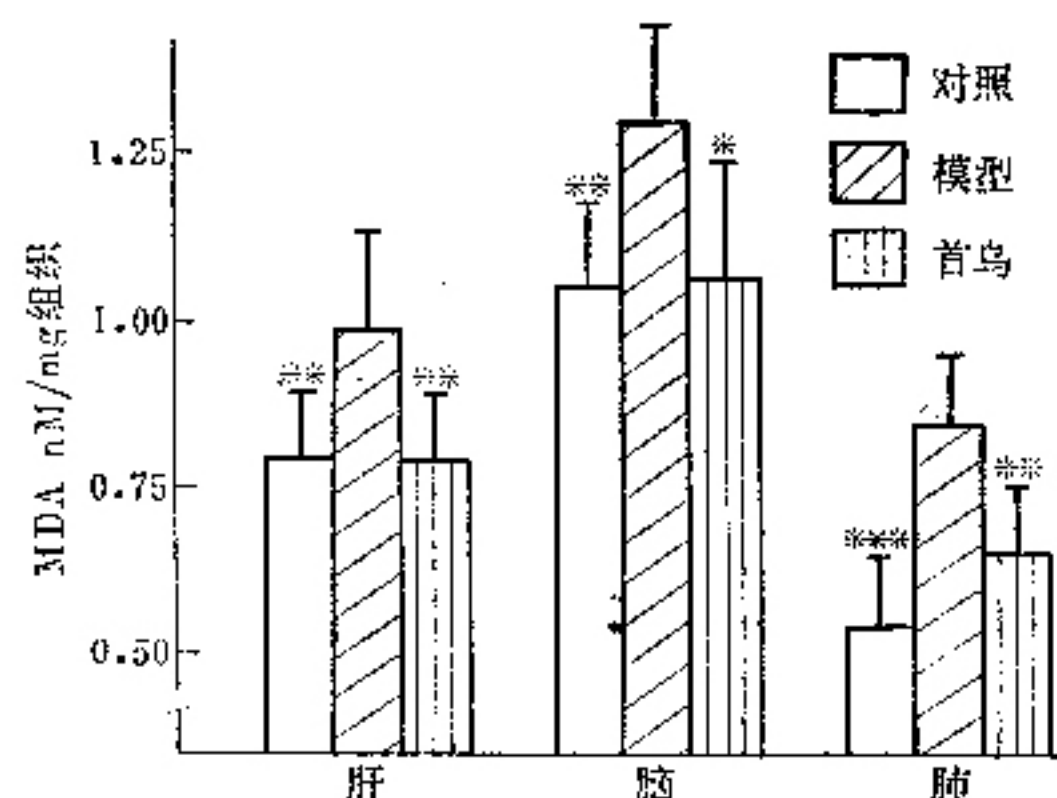
表1 小鼠一些生理功能的变化 ($M \pm SD$)

组别	n	体重(g)	体温($^\circ C$)	爬绳时间(分)	冻死时间(分)
对照	9	29.4 ± 1.4	36.8 ± 0.26	5.4 ± 0.63	20.6 ± 4.9
模型	9	25.1 ± 2.0	36.1 ± 0.58	4.5 ± 0.58	14.2 ± 2.6
首乌	9	30.0 ± 2.8	36.4 ± 0.57	5.6 ± 1.01	19.5 ± 2.5

注:与模型组比(t检验) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ 。下表同

四、过氧化脂含量的比较:与对照组比,模型组肝、脑、肺等组织中的过氧化脂含量均明显升高,首

乌组各相应组织的过氧化脂含量则明显下降, 接近对照组水平(附图)。



附图 各脏器过氧化脂含量的比较

五、红细胞SOD活性的比较: 模型组SOD活性明显升高, 首乌组活性保持在对照组水平(表2)。

表2 红细胞SOD活性的比较 (M±SD)

组别	n	发光抑制率%
对照	8	55.4±1.93***
模型	9	65.4±0.64
首乌	9	54.9±1.64***

6. 单胺氧化酶-B(MAO-B)活性比较: 模型组脑组织MAO-B活性比对照组明显升高, 首乌组此酶活性低于对照组。同时测定的肝MAO-B活性无明显改变(表3)。

表3 脑与肝MAO-B活性(μ/mg/3hr)比较 (M±SD)

组别	n	脑	肝
对照	9	2.81±0.20*	10.66±0.60
模型	9	3.14±0.28	10.68±0.48
首乌	9	2.43±0.22***	10.30±0.90

讨 论

臭氧是全球性空气三大污染物之一, 在常温下几乎能与每一种有机分子迅速反应产生自由基⁽³⁾。衰老的自由基学说认为, 过多的自由基在体内通过加成、交联、均裂反应造成细胞损伤, 影响细胞代谢, 引起病理过程, 促使细胞以至机体的衰老。机体有清除各种自由基的防御性酶系。如机体不断吸入O₃, 大量自由基在体内生成, 超出防御性酶系的清除能力时, 即可引起疾病或机体衰老。动物实验证明, 吸入

O₃能在短期内产生一系列类似衰老的变化⁽⁴⁾。本实验重复同样实验并检验了白何首乌的抗O₃损伤与抗衰老功能。

O₃进入体内所形成的超氧基(O₂⁻)是一种寿命很短的自由基, 较难直接测知。在体内它主要由超氧化物歧化酶(SOD)清除。因此, 一般通过测定SOD活性反映O₂⁻水平⁽²⁾。本实验观察到, 小鼠吸入0.9ppm O₃10天, 红细胞SOD活性明显升高, 比对照组提高16%以上, 与Doglas⁽⁵⁾及Jackson⁽⁶⁾报道的结果一致。这是因为SOD活性在一定时间内呈代偿性增高所致。但SOD活性增高并不足以清除不断增多的O₂⁻, 阻断自由基连锁反应, 因而小鼠体内脂质过氧化物作用增强, 过氧化脂含量增加。过氧化脂是细胞膜上大量不饱和脂肪酸被自由基引发的脂质过氧化作用加强的产物⁽⁶⁾, 其与分解产物丙二醛可引起蛋白质分子内和分子间交联, 影响酶活性, 对细胞膜与细胞器造成损伤, 被认为是细胞衰老的重要原因。本实验O₃组小鼠体内过氧化脂含量升高, 说明O₃损伤有促衰老作用, 与文献报道一致^(7,8)。首乌组小鼠虽然吸入O₃, 由于饲喂首乌粉, 预防了体内O₂升高, 使红细胞SOD活性接近对照组水平。重要脏器组织过氧化脂含量减少可能反映首乌的抗衰老作用。至于首乌是如何使吸O₃小鼠体内SOD水平维持正常、并使过氧化脂含量下降有待进一步探讨。

文献报道老龄动物脑MAO-B活性升高, 单胺能调节作用下降, 出现相应的生理功能和某些行为的改变, 被认为是衰老的重要指征^(3,9)。本实验O₃组小鼠脑MAO-B活性升高, 并表现出与老龄动物相似的生理功能衰退现象, 如体重减轻、体温下降、御寒能力减弱等变化。首乌组小鼠脑MAO-B活性降低, 接近对照组水平, 改善了O₃引起的功能变化。肝组织内MAO起着重要解毒功能, 其活性降低对机体将产生不良影响。检验一种抗衰老药物的作用时, 除证明它能抑制脑MAO-B活性外, 还应对肝MAO-B活性有保护作用。结果说明白何首乌的作用符合作为一种抗O₃损伤与抗衰老药物的要求。

我们曾给15月龄小鼠饲喂白首乌, 发现小鼠脑MAO-B活性与过氧化脂含量降低的同时, 脑和肝的脂褐素含量亦下降, 有助于说明白首乌的抗衰老功效。本实验为传统中药白首乌的抗衰老作用提供了实验依据, 并提示其对抗O₃污染有一定防护作用。

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超氧化物歧化酶及川芎嗪对家兔 缺血心肌再灌注损伤的影响

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心肌缺血最有效的治疗措施是恢复血流供应,但缺血心肌早期再灌注常出现心肌代谢、结构及功能方面的恶性变化。缺血心肌再灌注损伤的机理至今仍不完全清楚,许多学者认为氧自由基(oxygen free radicals)在这个过程中起重要作用。氧自由基作用于心肌组织,产生过氧化脂质(lipid peroxide),过氧化脂质分解为丙二醛(malondialdehyde)使心肌功能进一步受损。本实验观察了氧自由基清除剂超氧化物歧化酶(SOD)及活血化瘀中药川芎嗪对再灌注损伤的影响。

材料及方法 实验用雌性家兔30只,静脉注入20%乌拉坦(5ml/kg),开胸暴露心脏,于冠状动脉左室支中上1/3交界处穿线以备结扎。插颈动脉导管至左心室,通过压力换能器,连接ST-4型多道生理记录仪测左室内压及其变化速率。实验过程中经股静脉恒速(每小时15ml/kg)输入生理盐水以补充液体丢失。实验随机分为3组:组Ⅰ(n=10),结扎左室支1小时,再灌注1小时;组Ⅱ(n=11),结扎左室支1小时,松开结扎线再灌注1小时,在再灌注前5分钟,经股静脉输入SOD(2.5mg/kg,将SOD溶于20ml生理盐水中以每分钟0.5ml/kg输入,SOD系苏州生化制药厂生产);组Ⅲ(n=9),结扎左室支1小时,松开结扎线再灌注1小时,在再灌注前5分钟经股静脉输入川芎嗪(40mg/kg,将盐酸川芎嗪溶于20ml生理盐水中,以每分钟0.5ml/kg输入,盐酸川芎嗪系武汉制药厂生产)。冠脉结扎前后及再灌注后同步连续记录心电图、平均股动脉压、左室内压及其变化速率。观察结束后测定心脏缺血及非缺血部位丙二醛的含量。

结果 冠脉结扎1小时后,心肌收缩性指标+dp/dt max显著下降,与结扎前对照值相比 $P<0.01$ 。再灌注后组Ⅰ、组Ⅱ动物心脏收缩性能下降程度远较组Ⅲ为轻。组Ⅰ动物再灌注后+dp/dt max与对照值百分比每隔10分钟动态变化分别为:10':67.22±6.92%(M±SE,下同),20':65.59±6.45%,30':53.81±7.64%,40':52.35±8.76%,50':55.15±8.34%,60':50.87±7.10%;组Ⅱ动物再灌注后+dp/dt max与对照值百分比的动态变化为:10':79.69±5.52%,20':81.23±5.37%,30':81.75±5.62%,40':80.35±5.63%,50':82.11±5.59%,60':80.79±6.54%;组Ⅲ动物再灌注后+dp/dt max与对照值百分比的动态变化为:10':81.91±5.81%,20':87.47±4.43%,30':79.5±5.17%,40':77.73±5.67%,50':76.79±5.53%,60':73.36±4.53%。组Ⅰ、组Ⅱ与组Ⅲ动物比较,自再灌注20分钟后其差别均有显著性($P<0.01$)。组Ⅰ动物缺血心肌丙二醛含量升高至201.09±22.9%,组Ⅱ动物升高至110.70±7.68%,组Ⅲ动物升至113.34±6.29%,组Ⅰ、组Ⅱ与组Ⅲ比较差异高度显著($P<0.01$)。

实验结果表明超氧化物歧化酶及川芎嗪均有保护缺血心肌免受再灌注损伤的效应,两种药物都可抑制再灌注心肌过氧化脂质的形成。SOD的作用可能与清除超氧自由基有关,川芎嗪作用可能与其活血化瘀,增加冠脉血流,降低心肌氧耗,改善心肌代谢作用有关。这一结果为临床治疗冠心病提供了一定的实验根据。

Comparative Study on Pharmacological Function of Back-Shell and Plastron of Tortoise

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In this dissertation, the results of a comparative study on the pharmacological function and toxicity of back-shell and plastron of tortoise are reported. Since most of the patients with hyperthyroidism were characterized by Yin(阴)-deficiency, rats were prepared as animal models with Yin-deficiency, induced by subcutaneous injection of T_3 50 $\mu\text{g}/100\text{g}$. Over 20 indices on the function of thyroid gland, adrenal gland, immunoactivity and trace elements, which were considered vital in diagnosing Yin-deficiency, were measured. The results indicate that both back-shell and plastron of tortoise possess identical Yin-nourishing property. The mechanism of Yin-nourishing is closely related to decreasing the function of thyroid and adrenal gland, increasing the function of immunoactivity as well as the decreasing of Cu in serum. The maximal tolerant doses (MTD)-250 g/kg prepared from back-shell and plastron are respectively tried on mice, demonstrating very low toxicity. It is thus evident that the above results provide laboratory evidence for the equal utilization of back-shell and plastron.

(Original article on page 279)

Prevention against Free Radical Damage in Mice with *Cynanchum auriculatum*

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Mice (NIH) of 27 ± 3 g were divided into: I. control group, II. model group and III. *Cynanchum auriculatum* Royle ex Wight (CARW) group. Group II was modelled by caging mice in an ozone room of 0.9 ppm concentration for 10 days. A dose of 60 mg CARW/day was given to the mice group III for 20 consecutive days, 10 days prior to and 10 days during the ozone inhalation together with the model mice. The results showed that the ozone inhalation was injurious to mice resulted in significant increase of SOD activity of red blood cell, LPO content in lung, liver and brain-tissue, and MAO content in brain, while in CARW group, the value of corresponding indices mentioned above was lowered and normalized. Physically the CARW mice got restoration of strength and health from debilitated condition caused by ozone damage and were much more tolerant to cold than model mice. These results indicated that CARW displayed an inhibitory effect on ozone induced free radical damage. The action of CARW may be helpful for use as an effective medicine for delaying aging process.

(Original article on page 282)

Study on Dachengqi Decoction(大承气汤) in Treating Rabbits' Respiratory Distress Syndrome

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47 rabbits were randomly divided into 3 groups: (1) 17 animals for the control group; (2) 14 for respiratory distress syndrome (RDS) group; (3) 16 for treated group. The RDS model were made with oleic acid. PaO_2 and morphologic changes of lung tissue were checked after Dachengqi decoction was given per os to the rabbits. Results showed that the PaO_2 raised and the lung lesions improved on the rabbits of RDS which provided the experimental basis for the treatment of RDS.

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Antitumor Effect of Gypenoside

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Investigations were made on the antitumor activity of Gypenoside which was isolated from *Gynostemma pentaphyllum*. (1) Mice were transplanted with S-180 subcutaneously on their backs and drug was given per os or by intraperitoneal injection from third day on for 10 days after the tumor transplantation. The results showed that Gypenoside was able to inhibit the tumor growth. In the group given 30 mg/kg per os inhibitory rate of the tumor growth reached 87% ($P < 0.001$). (2) In vitro, studies showed that Gypenoside was toxic to S-180 cells. When concentrations of the drug were 3.75, 5.5 and 7.5 mg/ml the red staining rate of tumor cells were 54%, 83% and 88% respectively. These results suggest that Gypenoside possesses antitumor activity through a direct cytotoxic action.

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