

# 氧化苦参碱在鸭原代肝细胞中抗鸭乙肝病毒(DHBV)作用的研究

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**摘要** 通过建立鸭原代肝细胞-DHBV 感染模型研究氧化苦参碱抗 DHBV 的作用。分别在 DHBV 感染前、感染同时以及感染后给药, 利用打点杂交、Southern 印迹核酸杂交和荧光定量 PCR 方法分别检测培养细胞上清及细胞内病毒核酸, 观察氧化苦参碱在病毒感染的各个环节所起的抗病毒作用。实验结果显示 1mg/ml 氧化苦参碱处理细胞后, 鸭原代肝细胞培养上清及细胞内的 DHBV 核酸明显低于病毒感染对照组, 病毒抑制率达 91.6%; 在病毒感染同时加药对病毒的抑制率可达 98.5%; 感染后持续用药能使不同培养天数的鸭肝细胞内的 DHBV 核酸降低 60.5%~96.6%。氧化苦参碱与 DHBV 共孵育后, 可以使病毒感染力下降 69.6%。结果说明氧化苦参碱可以在 DHBV 感染鸭原代肝细胞的多个环节, 包括病毒吸附、进入细胞及细胞内复制等方面发挥抗病毒作用。

**关键词** 鸭乙肝病毒; 氧化苦参碱; 鸭原代肝细胞; 抗病毒作用

**中图分类号** R373.2 **文献标识码** A **文章编号** 1000-872X(2006)05-0369-06

氧化苦参碱(Oxymatrine, OM)是从中药苦豆子中提取的一种生物碱, 与苦参碱同属四环的喹诺里西啉(Quinolizidine)生物碱。在临床乙肝治疗中发现此类药物具有一定的抗病毒疗效<sup>[1,2]</sup>, 其安全浓度范围较大, 无明显的临床不良反应<sup>[3]</sup>; 并且与其它抗病毒药物联用可提高疗效<sup>[4]</sup>, 是我国自主研发的一种抗乙肝病毒药物。由于乙肝病毒具有严格的宿主与组织嗜性, 体外很难建立支持其自然感染的细胞模型, 故以往多采用稳定转染 HBV 基因组 DNA 的 HepG2 2.2.15 细胞研究氧化苦参碱的抗病毒复制作用<sup>[5]</sup>。嗜肝 DNA 病毒的复制周期起始于病毒对易感细胞的吸附, 进而通过内吞作用进入细胞, 之后是脱壳、基因组复制、装配及释放子病毒。抗病毒药物可能通过作用于病毒复制的一个或多个环节发挥作用。为了评价氧化苦参碱在病毒感染的不同环节的抗病毒效果, 此研究利用鸭原代肝细胞-DHBV 感染模型, 模拟病毒的自然感染过程, 在病毒感染前、感染同时及感染后等不同时间给药, 以研究在 DHBV 感染的进入、吸附以及细胞内复制等环节氧化苦参碱的抗病毒作用。

## 材料与方法

1. 动物和毒株 樱桃谷鸭(*Anas platyrhynchos*)由上海市畜牧兽医研究所种鸭基地提供。颈静脉采血分离血清, 利用 PCR 方法检测血清样本中的 DHBV 病毒 DNA。选择 DHBV 病毒阴性雏鸭作为肝细胞供体。DHBV QP-1 由 DHBV 先天感染的鸭分离血清而得, 含 DHBV 病毒  $3 \times 10^6$ /mL, 由本实验室保存。

2. 药物 氧化苦参碱(Oxymatrine, OM), 分子式  $C_{15}H_{24}N_2O_2 \cdot H_2O$ , 分子量 282.38, 白色结晶, 纯度大于 97%, 由江苏正大天晴药业股份有限公司惠赠。用不含血清的细胞培养液配制为 50mg/mL, 0.22 $\mu$ m 滤膜过滤除菌。

3. 试剂 细胞培养基 Leibowitz's L-15 Medium, collagenase, Liver Perfusion Medium, Hepatocyte Wash Medium 购于 GIBCO 公司; Insulin, Hydrocortisone 21-hemisuccinate sodium salt 购于 Sigma 公司; EX Taq HS, 5 $\times$  real-time PCR buffer, dNTPs Mixture 等购于 TaKaRa 公司; 阳离子尼龙膜为 Roche 公司产品; 医用 X 光胶片购于柯达公司; 蛋白酶 K 购于 E. MERCK 公司; 其它试剂为国产分析纯试剂。

4. 鸭原代肝细胞的分离、培养与感染 3 日龄雏鸭经颈静脉采血, PCR 检测 DHBV 先天感染(引物 S: CTCACCTTCTGTGATCTCATTTG, AS: ATGGGATAGTCCGGGTTGG), DHBV 感染阴性的雏鸭用作肝细胞分离培养。雏鸭经戊巴比妥钠麻醉后, 打开腹腔, 分离门静脉, 自门静脉灌注 Liver Perfusion Medium 100mL 后, 用含 1mg/mL 胶原酶 IV 的无酚红 Hank's 液继续灌注(50mL), 流速 5mL/min。随后取出肝脏, 揭去肝包膜后研磨, 用 Hepatocyte Wash Medium 冲洗肝细胞, 离心, 弃上清, 用培养液重悬细胞, 以  $6 \times 10^6$ /mL 密度将细胞铺于 60mm 细胞培养皿, 加入培养液 5mL L-15 培养基, 含 5% 胎牛血清, 1% 双抗, 1% L-谷氨酰胺, 1 $\mu$ g/mL in-

收稿日期: 2006-03-20; 修回日期: 2006-08-16

基金项目: 国家自然科学基金(30070693)

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