动物营养学报 » 2011, Vol. 23 » Issue (08) :1378-1385 DOI: 10.3969/j.issn.1006-267x.2011.08.017

沙棘总黄酮、槲皮素对鸡成骨细胞碱性磷酸酶活性作用机制的体外研究

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Mechanisms of Action for Total Flavones of Hippophae Rhamnoides and Quercetin on Alkaline Phosphatase Activity in Chicken Osteoblasts

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摘要 本试验旨在研究在体外培养条件下沙棘总黄酮（total flavones of Hippophae rhamnoides，TFH）及槲皮素（quercetin）对爱拔益加（AA）肉鸡鸡胚成骨细胞碱性磷酸酶（alkaline phosphatase，ALP）活性的影响及其作用机制。成骨细胞分别采用基础培养液、基础培养液添加β-雌二醇(β-estradiol，E2)、TFH或槲皮素培养细胞24 h；细胞经雌激素受体（estrogen receptor，ER）拮抗剂处理2 h，再分别于基础培养液、基础培养液添加TFH或槲皮素中培养24 h；细胞经胞外信号调节激酶(extracellular signal-regulated kinase，ERK)抑制剂处理2 h，再分别于基础培养液、基础培养液添加TFH或槲皮素培养24 h；收集细胞，测定细胞ALP活性。基础培养液添加TFH、槲皮素，于孵育0、2、5、15、30、60 min 6个时间点收集细胞，ELASA法测定磷酸化胞外信号调节激酶（phosphorylated extracellular-regulated kinase，p-ERK）浓度。细胞采用基础培养液培养细胞24 h后经基础培养液、基础培养液分别添加E2、TFH、槲皮素培养细胞30 min；经ER拮抗剂处理15 min，再分别于基础培养液、基础培养液添加E2、TFH或槲皮素培养30 min；收集细胞，ELASA法测定p-ERK浓度。结果表明：1）培养液添加TFH、槲皮素可以显著提高ALP活性(P<0.05)，ER拮抗剂、MEK抑制剂可完全阻断这2种黄酮的作用(P<0.05)；2）培养液添加TFH在培养后5、15 min，槲皮素在培养后2、5、15、30、60 min ERK磷酸化水平显著提高(P<0.05)；3）培养液添加TFH、槲皮素可显著提高细胞ERK磷酸化水平(P<0.05)，ER拮抗剂可完全阻断这一作用(P<0.05)。结果提示：TFH、槲皮素可以显著提高ALP活性和ERK磷酸化水平，具有雌激素样作用，二者可能是通过ER途径和ERK途径调节ALP活性。

关键词： 沙棘总黄酮 槲皮素 成骨细胞 碱性磷酸酶 胞外信号调节激酶

Abstract： The experiment was conducted to study the effects of total flavones of Hippophae rhamnoides (TFH) and quercetin on alkaline phosphatase (ALP) activity in osteoblasts of arbor acres (AA) broilers' embryo in vitro and its mechanisms of action. Cells were cultured in basal culture medium, basal culture medium supplemented with β-estradiol (E2), TFH and quercetin for 24 h, respectively; after treated by estrogen receptor (ER) antagonist for 2 h, the cells were cultured for another 24 h in basal culture medium, basal culture medium supplemented with TFH and quercetin, respectively; after treated with extracellular signal-regulated kinase (ERK) inhibitor for 2 h, the cells were cultured for another 24 h in basal culture medium, basal culture medium supplemented with TFH and quercetin, respectively; the cells were collected for the analysis of ALP activity. Cells were cultured in basal culture medium supplemented with TFH or quercetin, and collected at six time points (0, 2, 5, 15, 30, 60 min) for the determination of phosphorylated extracellular-regulated kinase (p-ERK) concentration by ELASE. Cells were cultured in basal culture medium, basal culture medium supplemented with E2, TFH or quercetin for 30 min, respectively; after treated by ER antagonist for 15 min, the cells were cultured for 30 min in basal culture medium, basal culture medium supplemented with E2, TFH and quercetin, respectively; cells were collected for the determination of p-ERK concentration by ELASE. The results showed as follows: 1) culture medium supplemented with TFH or quercetin significantly increased the ALP activity (P<0.05), and the effects of which were totally prevented by ER antagonist and MEK inhibitor (P<0.05); 2) the phosphorylation level of ERK were significantly increased in culture medium supplemented with TFH after 5, 15 min incubation and with quercetin after 2, 5, 15, 30, 60 min incubation (P<0.05); 3) culture medium supplemented with TFH or quercetin significantly increased the phosphorylation level of ERK, the effects of which were totally prevented by ER antagonist (P<0.05). The results indicate that TFH and quercetin can significantly increase the ALP activity and ERK phosphorylation level, exert the estrogen-like effect, and may adjust the ALP activity via ERK and ER pathways. ［Chinese Journal of Animal Nutrition, 2011, 23（8）: 1378 -1385］

Keywords： total flavones of Hippophae rhamnoides， quercetin， osteoblast， alkaline phosphatase， extracellular signal-regulated kinase

基金资助:

国家科技支撑项目（2006BAD14802-8-1）；东北农业大学创新团队发展计划项目(CXT006-2-1)

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引用本文:

. 沙棘总黄酮、槲皮素对鸡成骨细胞碱性磷酸酶活性作用机制的体外研究[J]. 动物营养学报, 2011,V23(08): 1378-1385

. Mechanisms of Action for Total Flavones of Hippophae Rhamnoides and Quercetin on Alkaline Phosphatase Activity in Chicken Osteoblasts[J]. Chinese Journal of Animal Nutrition, 2011,V23(08): 1378-1385.

链接本文:

http://211.154.163.124/Jweb_dwyy/CN/10.3969/j.issn.1006-267x.2011.08.017 **或**
http://211.154.163.124/Jweb_dwyy/CN/Y2011/V23/I08/1378

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