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原花青素激活 Nrf2 信号通路抗氧化损伤的系统评价

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摘　要:系统评价原花青素的抗氧化损伤效果,为揭示原花青素通过激活 Nrf2 信号通路拮抗氧化损伤的作用机制提供参考。对 CNKI、VIP、Cochrane、PubMed、Springer 等数据库进行文献检索,查找原花青素干预实验动物的文献资料,对文献资料进行 Meta 分析,以标准化均数差(standard mean difference,SMD)描述组间差别。与氧化损伤模型组比较,原花青素干预组 SOD[SMD 为 2.11,95% *CI*(1.45,2.78)]、GSH-Px[SMD 为 2.88,95% *CI*(1.94,3.82)]、GSH[SMD 为 4.45,95% *CI*(3.09,5.81)]、Nrf2[SMD 为 3.75,95% *CI*(2.35,5.15)]、NQO1[SMD 为 4.92,95% *CI*(0.65,9.19)]、HO-1[SMD 为 5.13,95% *CI*(2.01,8.25)]、GST[SMD 为 4.77,95% *CI*(2.31,7.22)]水平均高于氧化损伤模型组,差异有统计学意义(P 均 <0.05);MDA[SMD 为-3.00,95% *CI*(-3.59,-2.41)]水平低于氧化损伤模型组,差异有统计学意义($P<0.05$)。原花青素可以通过激活 Nrf2 信号通路拮抗氧化损伤;亚组分析发现,高剂量、长时间的原花青素干预抗氧化作用更强。

关键词:原花青素类;NF-E2 相关因子 2;氧化性应激;综述;Meta 分析

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Effects of Proanthocyanidins on Oxidative Damage via Nrf2 Signaling Pathway: A Systematic Review and Meta-Analysis

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Abstract: The systematic review was performed to investigate the protective effects of proanthocyanidins on oxidative damage, which provides reference for revealing the mechanism of anti-oxidative damage by activating the Nrf2 signaling pathway. Literature was searched by independently searching databases, including the China National Knowledge Infrastucture, VIP, Wanfang data, CBM, Cochrane, PubMed, Springer, Web of Science to search for the data of intervention on experimental animals of proanthocyanidins. And a meta-analysis was to retrieve experimental data on PC and oxidative damage published both in China and worldwide in the past few years. The difference between groups was described by standard mean difference (SMD). Levels of SOD [SMD = 2.11, 95% *CI* (1.45, 2.78)], GSH-Px [SMD = 2.88, 95% *CI* (1.94, 3.82)], GSH [SMD = 4.45, 95% *CI* (3.09, 5.81)], Nrf2 [SMD = 3.75, 95% *CI* (2.35, 5.15)], NQO1 [SMD = 4.92, 95% *CI* (0.65, 9.19)], HO-1 [SMD = 5.13, 95% *CI* (2.01, 8.25)], GST [SMD = 4.77, 95% *CI* (2.31, 7.22)] in the PC intervention group was higher than that of the oxidative injured model group ($P<0.05$). Levels of MDA [SMD = -3.00, 95% *CI* (-3.59, -2.41)] in the PC intervention group was lower than that of the oxidative injured model group ($P<0.05$). While Nrf2 [SMD = 2.79, 95% *CI* (0.64, 4.94)], HO-1 [SMD = 2.03, 95% *CI* (0.17, 3.89)], GST [SMD = 2.23, 95% *CI* (0.42, 4.03)] in the PC group was higher than control group ($P<0.05$). Subgroup analysis demonstrated that intervention time (≥35d or 24h) of PC was found to promote the production of Nrf2 ($P=0.02$); high intervention dose (>200mg/kg or 1mg/L) of PC increase the expression of Nrf2 ($P=0.04$). PC can protect against oxidative damage by activating the Nrf2 signaling pathway. High-dose and long-time intervention of PC can play a better role in antioxidative damage.

Key words: proanthocyanidins; NF-E2-related factor 2; oxidative stress; review; meta-analysis

原花青素(proanthocyanidins,PC)是一类广泛存在于葡萄籽、山楂、松树皮、银杏等植物中的天然多酚化合物,具有极强的抗氧化能力和清除自由基能力[1]。原花青素是由不同数量的儿茶素或表儿茶

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素缩合而成的多聚体,其二聚体具有很强的抗氧化活性[2]。

大量研究表明[3-5],氧化应激能够直接或间接损伤细胞内蛋白质、脂质、核酸等大分子物质的生理功能,是众多疾病发生的病理生理基础。而近年来研究发现,核因子 E2 相关因子 2(Nuclear factor-erythroid 2-related factor 2,Nrf2)是细胞氧化应激反应中的关键因子,可以通过与抗氧化反应元件(antioxidant-response element,ARE)相互作用来调节编码保护性蛋白,这是迄今为止发现的最为重要的内源性抗氧化应激通路之一[6,7]。这些保护性蛋白包括抗氧化蛋白和Ⅱ相解毒酶,如血红素氧合酶-1(HO-1)、醌氧化还原酶 1(NQO1)、谷胱甘肽-S-转移酶(GST)、超氧化物歧化酶(SOD)等[8]。

国内外有文献报道[9-11],原花青素干预氧化损伤动物后可使其体内的 Nrf2 及其下游的 HO-1、NQO1 等靶基因的表达量升高;但另有文献的试验结果与之相反,赵娇[12]、周礼华[13]、王维芬等[14]报道的原花青素干预组动物的以上指标表达量较低,而氧化应激组动物的以上指标表达量高于原花青素干预组。本研究从系统综述的视角,全面搜集原花青素干预实验的国内外文献资料,以期客观评价原花青素抗氧化作用的有效性,为其作用机制提供理论线索。

1　对象与方法

1.1　纳入标准

1.1.1　研究类型

实验性研究,限中、英文。

1.1.2　研究对象(Patient)

细胞、动物。

1.1.3　干预措施(Intervention/Comparison)

实验组 1:原花青素干预组,对照组 1:氧化损伤模型组;实验组 2:原花青素组,对照组 2:正常对照组。

1.1.4　评价指标(Outcome)

核因子 E2 相关因子 2(Nuclear factor-erythroid 2-related factor 2,Nrf2),醌氧化还原酶 1(NAD(P)H:quinone oxidoreductase 1,NQO1),血红素氧合酶-1(Heme Oxygenase-1,HO-1),谷胱甘肽-S-转移酶(Glutathione S-transferase,GST),谷胱甘肽(Glutathione,GSH),谷胱甘肽过氧化物酶(Glutathione peroxidase,GSH-Px),超氧化物歧化酶(Superoxide dismutase,SOD),丙二醛(Malondialdehyde,MDA)。

1.2　排除标准

(1)文献语言非中、英文;(2)对同一篇文献在多个数据库中被检索到的情况,仅保留一篇文献;(3)综述文献;(4)仅能检索到摘要的文献;(5)无本研究所需对照或无所需提取数据的文献。

1.3　文献检索策略(Study design)

按 PICOS 原则采用中文检索式"原花青素 AND(Nrf2 OR NQO1 OR HO-1 OR GST)"、"原花青素 AND 氧化应激"在 CNKI、VIP、Wanfang data、CBM 等中文数据库进行检索。采用英文检索式"Proanthocyanidins AND (Nrf2 OR NQO1 OR HO-1 OR GST)"在 Cochrane、PubMed、Springer、Web of Science 等数据库进行检索。检索日期均到 2017 年 5 月 31 日。

1.4　文献筛选、资料提取

利用检索策略共检测出 513 篇相关文献,经过筛选,本次研究共纳入 53 篇文献[9-14,17,23,29-73],检索流程见图 1。

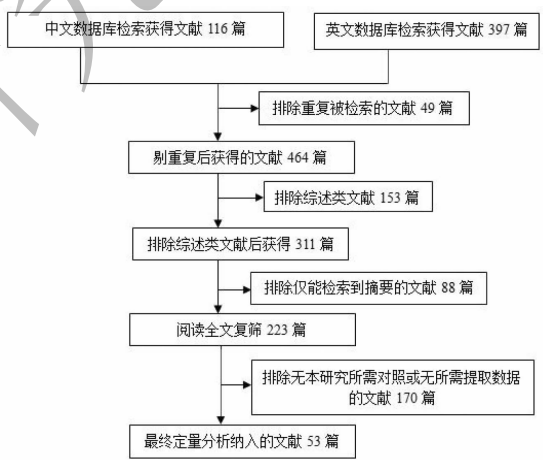

图 1　文献筛选流程及结果

Fig. 1　Flow chart of the study selection

由两位研究者按照纳入与排除标准独立筛选文献,用事先制定好的 Excel. 2010 表格提取资料,提取内容主要包括:(1)纳入文献基本信息,包括文献题目、第一作者、发表时间及发表杂志;(2)实验组和对照组的基本数据资料(n,$\bar{x} \pm s$);(3)实验动物基本信息(品系,性别,周龄,体重等);(4)原花青素的作用方式和干预时间;(5)测量指标及其来源。两名研究者交叉核对纳入文献的提取结果,当遇到结果不一致时,请相关专家进一步核实。

1.5　方法学质量评价

采用 Cochrane 协助网推荐的偏倚风险评估方

法,应用 RevMan 5.2 软件对本次研究对所纳入的 53 篇文献进行质量评价,处于“Low risk”的比例占到 75% 以上。

1.6 统计分析

应用 RevMan 5.2 软件进行 Meta 分析。对各个指标所纳入的文献进行异质性检验:若文献间异质性较小(I2 <50% 且 $P>0.1$)则采用固定效应模型;若文献间异质性较大(I2 ≥50% 且 $P\leq0.1$),则分析异质性来源,对可能导致异质性的因素进行亚组分析,并采用随机效应模型。计算各个指标的合并效应标准化均数差(Standard Mean Difference,SMD)值和 95% 可信区间(95% confidence interval,95% CI),做出森林图分析各个指标的合并效应,并对发表偏倚进行漏斗图分析。采用 Stata 12.0 软件进行敏感性分析,检验结果的稳定性。

2 实验结果

2.1 合并效应的 Meta 分析

与氧化模型组比较,原花青素干预组 SOD、GSH-Px、GSH 水平均高于氧化模型组,差异有统计学意义(P 均 <0.05);MDA 水平低于氧化模型组,差异有统计学意义($P<0.05$)。见表 1。

表 1 原花青素干预组与氧化损伤模型组氧化水平指标蛋白表达 Meta 分析的合并效应

Table 1 Combined effect of meta-analysis of protein expression of oxidative level indicators of PC intervention group and oxidative injured model group

Index	*N*	*n*	SMD	95% *CI*	*P*
SOD	15	116	2.11	1.45,2.78	<0.00001
GSH-PX	16	171	2.88	1.94,3.82	<0.00001
GSH	15	147	4.45	3.09,5.81	<0.0001
MDA	40	421	-3.00	-3.59,-2.41	<0.00001

注:N:纳入文献数量;n:实验组/对照组样本量
Note:N:included literatures;n:experimental/control

与氧化模型组比较,原花青素干预组 Nrf2[SMD 为 3.75,95% CI(2.35,5.15)]、NQO1[SMD 为 4.92,95% CI(0.65,9.19)]、HO-1[SMD 为 5.13,95% CI(2.01,8.25)]、GST[SMD 为 4.77,95% CI(2.31,7.22)]水平均高于氧化模型组,差异有统计学意义(P 均 <0.05)。见图 2-3。

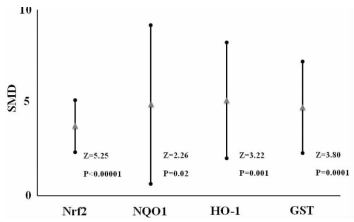

图 2 原花青素干预组与氧化损伤模型组 Nrf2 通路各指标蛋白表达 Meta 分析的合并效应

Fig. 2 Combined effect of meta-analysis of different indicators protein expression of Nrf2 signaling pathway of PC intervention group and oxidative injured model group

与正常对照组比较,原花青素干预组 Nrf2[SMD 为 2.79,95% *CI*(0.64,4.94)]、HO-1[SMD 为 2.03,95% *CI*(0.17,3.89)]、GST[SMD 为 2.23,95% *CI*(0.42,4.03)]水平均高于正常对照组,差异有统计学意义(P 均 <0.05)。见图 4。

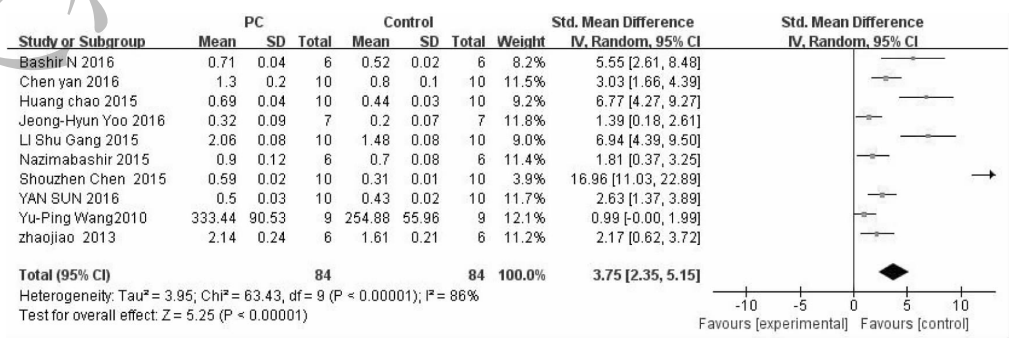

图 3 原花青素对氧化损伤模型 Nrf2 表达的影响

Fig. 3 Effects of proanthocyanidins on Nrf2 expression of oxidative injured models

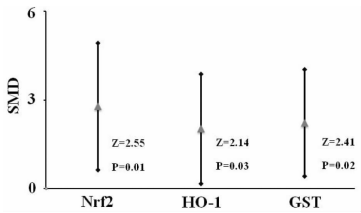

图 4　原花青素组与正常对照组各指标蛋白表达 Meta 分析的合并效应

Fig. 4　Combined effect of meta-analysis of different indicators protein expression of PC group and control group

2.2　亚组分析

按照原花青素的干预时间(<35 d & 24 h,≥35 d & 24 h)、干预剂量(≤200 mg/kg & 1 mg/L, >200 mg/kg & 1 mg/L)分为不同亚组(N:纳入文献数量;n:实验组/对照组样本量)。

2.2.1　原花青素干预时间亚组分析

干预时间 <35 d&24 h 时,原花青素干预组 Nrf2、HO-1、GST 水平均高于对照组,差异有统计学意义(P 均 <0.05),NQO1 水平与对照组相比,差异无统计学意义(P = 0.16);干预时间≥35 d & 24 h 时,原花青素干预组 Nrf2、HO-1、NQO1、GST 水平均高于对照组,差异有统计学意义(P 均 <0.05)。长时间亚组的 Nrf2 水平高于短时间亚组,组间差异有统计学意义(P = 0.02),见表 2。

表 2　原花青素干预时间亚组分析

Table 2　Subgroup analysis of different intervention time of PC

Index	<35d&24h				≥35d&24h				组间差异(P)
	N	n	SMD	95% CI	N	n	SMD	95% CI	
Nrf2	2	12	1.98 *	0.92,3.03	8	72	4.45 *	2.63,6.27	0.02
HO-1	6	48	6.30 *	2.34,10.25	4	83	6.78 *	3.21,10.35	>0.05
NQO1	4	35	4.60	-1.83,11.04	2	16	5.81 *	3.37,8.26	>0.05
GST	3	26	6.99 *	1.33,12.65	4	33	4.29 *	0.76,7.83	>0.05

注:N:纳入文献数量;n:实验组/对照组样本量; * P <0.05,与氧化损伤组比较;P:组间差异。

Note:N:included literatures;n:experimental/control; * P <0.05,compared with oxidant injury group;P:Difference between groups.

2.2.2　原花青素干预剂量亚组分析

干预剂量≤200 mg/kg & 1 mg/L 时,原花青素干预组 Nrf2、HO-1、GST 水平均高于对照组,差异有统计学意义(P 均 <0.05),NQO1 水平与对照组相比,差异无统计学意义(P = 0.15);干预剂量 >200 mg/kg&1 mg/L 时,原花青素干预组 Nrf2、HO-1、NQO1、GST 水平均高于对照组,差异有统计学意义(P 均 <0.05)。高剂量亚组的 Nrf2 的水平高于低剂量亚组,组间差异有统计学意义(P = 0.04)。见表 3。

表 3　原花青素干预剂量亚组分析

Table 3　Subgroup analysis of different doses of PC

Index	≤200 mg/kg&1 mg/L				>200 mg/kg&1 mg/L				组间差异(P)
	N	n	SMD	95% CI	N	n	SMD	95% CI	
Nrf2	5	35	2.40 *	1.39,3.41	8	49	5.82 *	2.71,8.92	0.04
HO-1	8	63	3.48 *	0.31,6.65	2	20	15.23 *	1.02,29.45	>0.05
NQO1	4	32	5.71	-2.04,13.46	2	19	5.04 *	0.07,10.00	>0.05
GST	5	43	3.13 *	1.07,5.18	2	16	17.95 *	0.39,35.50	>0.05

注:N:纳入文献数量;n:实验组/对照组样本量; * P <0.05,与氧化损伤组比较;P:组间差异。

Note:N:included literatures;n:experimental/control; * P <0.05,compared with oxidant injury group;P:Difference between groups.

2.3　发表偏倚

在本次纳入的 53 篇文献中,对文献进行发表偏倚分析,以 Nrf2 为例,结果可见文献呈现如下分布,提示存在一定发表偏倚。见图 5。

2.4　敏感性分析

以 Nrf2 为例进行敏感性分析,可见所有研究结果围绕合并效应分布,并未发现对本次研究结果产生较大影响的文章,提示结果稳健。

3　讨论与结论

近年来,原花青素(PC)作为安全无毒的天然抗氧化剂之一,在欧美等地区的医药、保健食品等领域

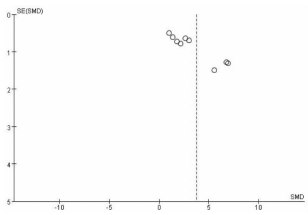

图 5 Nrf2 的入选文献发表偏倚检验漏斗图

Fig. 5 Funnel plot of included literatures of Nrf2

得到了广泛的应用[15,16]。原花青素的提取、性质、生物活性等方面的研究已取得较大进展;但对其发挥抗氧化作用分子机制的文献报道还较少,多数作用机制仍无足够的证据。

反映机体氧化水平的可靠指标包括 SOD、GSH-Px、GSH、MDA 等,SOD 可维持氧化应激过程中机体内的氧化/抗氧化平衡;GSH 和 GSH-Px 可有效清除自由基,提高机体抗氧化能力;MDA 是氧化损伤作用过程中形成的重要产物,可反映机体氧化损伤程度[17]。本分析结果显示,与氧化损伤组比较,原花青素干预组小鼠或细胞中的 SOD、GSH-Px、GSH 水平显著升高,MDA 水平明显降低。这些指标的变化说明原花青素可能通过增加抗氧化物质的活性与含量,提高机体抗氧化能力,缓解氧化应激。并且原花青素含有的多个酚性羟基在体内被氧化后释放出 H^+,竞争性地与自由基及氧化物结合,阻断自由基链式反应[18],使脂质过氧化作用最终分解产物 MDA 的含量明显减少。

核因子 E2 相关因子 2(Nrf2)是重要的抗氧化转录因子,Keap1-Nrf2-ARE 信号通路是迄今为止发现的最为重要的内源性抗氧化应激通路之一[6]。本分析结果显示,与氧化损伤组比较,原花青素干预组小鼠组织中的 Nrf2 及下游产物 NQO1、HO-1、GST 的蛋白表达量明显提高,这表明原花青素可能激活了 Keap1-Nrf2-ARE 信号通路。现在研究发现 Nrf2 通路可通过磷酸化的方式激活[19-23],故此过程可能是原花青素的代谢产物竞争性与 Kelch 样环氧氯丙烷相关蛋白 1(Keap1)结合,使 Nrf2 泛素化降低,同时使之磷酸化。磷酸化的 Nrf2 与 Keap1 发生解离并转移进入细胞核内,与小分子蛋白 Maf 结合并形成异二聚体,识别抗氧化反应元件(ARE)上的结合位点并与之结合,启动重要的下游靶基因的转录,激活 NQO1、HO-1、GST 以及 SOD 等抗氧化蛋白和Ⅱ相解毒酶的表达,从而参与组织细胞抗氧化反应和氧化还原的调节[24-26]。抗氧化蛋白和Ⅱ相解毒酶催化自由基转变为无毒物质,增加其水溶性利于排出[27],达到拮抗氧化损伤的效果,维持了氧化还原平衡。这为将原花青素开发为抗氧化应激的药物提供了更充分的理论依据。原花青素干预无氧化损伤小鼠或细胞的结果分析发现,Nrf2、HO-1、GST 蛋白表达含量显著高于无氧化损伤组,这表明原花青素对于氧化还原平衡的机体可能有预防氧化损伤的作用,为原花青素在保健品和化妆品领域的应用提供了理论依据。

通过亚组分析发现,长时间、高剂量干预组 Nrf2 表达量显著高于短时间、低剂量组。这说明较长时间的原花青素干预可更好地发挥抗氧化作用;且原花青素的抗氧化活性在一定范围内可能存在剂量-效应关系,这与 Bagchi D 等的报告[28]中的结论相一致。这为原花青素作为抗氧化药物在临床上的应用提供了理论依据。

综上所述。原花青素可以通过激活 Nrf2 信号通路拮抗氧化损伤,且高剂量、长时间的原花青素干预可以发挥更强的抗氧化作用。在本研究纳入的部分文献中,原花青素的聚合度、纯度等未作详细描述;灌胃小鼠的原花青素生物有效剂量可能存在偏差,这均可能使合并效应存在一定误差。今后应继续研究原花青素发挥作用的分子机制方面,为更加深入地揭示原花青素激活 Nrf2 信号通路拮抗氧化损伤的作用机制提供参考。

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