Antioxidative Properties of Phenolic Acids against Hydroperoxide-Induced Oxidative Stress in Cultured Rat and Human Hepatocytes

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Natural phenolic acids are widely distributed in the plant kingdom and known for their interesting biological activities, namely as antioxidants. This study compares the antiradical and antilipopeoxidative activities of six phenolic acids – caffeic (CA), rosmarinic (RA), chlorogenic (CHA), ferulic (FA), 3-(3,4-dihydroxyphenyl)lactic (PLA) and protocatechuic (PA).

The free radical scavenging potentialities were tested by using methanolic solution of 1,1-diphenyl-2-picrylhydrazyl radical model (DPPH). The 50% decolirisation of DPPH by phenolic acids was expressed as IC_{50} (1). Action against lipid peroxidation was evaluated on model primary rat and human hepatocyte cultures intoxicated by *tert*-butylhydroperoxide (TBH). Rat hepatocytes were isolated according to a two-step collagenase perfusion technique (2). The hepatocytes were suspended in supplemented Wiliam's E medium. Human hepatocytes were obtained by method of *Pichard* (3). Primary rat and human hepatocyte cultures were incubated with TBH (0.5 mM, 90 min) following pretreatment with phenolic acids (1.0 mM, 20 hours). The damage of hepatocytes was determined by the level of lactate dehydrogenase (LDH) in the medium. Lipoperoxidation was measured by the production of thiobarbituric acid reactive substances (MDA). The cellular content of gluthathione (GSH) was determined by the reaction with 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB).

Free radical scavenging activity of phenolics corresponds with reults obtained on rat and human hepatocytes (Table 1.) The values of MDA and GSH, which are in good correlation with LDH, are not shown.

Our results on rat and human hepatocytes supplement data published to date on other *in vitro* models. From the studied phenolic acids are the most potent RA and CA. The antioxidant activity of acids is generally ascribed to the number of hydroxyl groups. *Ortho*- or *para*-position of these groups increases the activity due to the resonance stabilisation and quinone formation. The weakest antioxidant activities of ferulic acid confirm this opinion.

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Table 1. Scavenging (IC_{50}) and Cytoprotective Effect of Phenolic Acids (1.0 mM) on Primary Rat and Human Hepatocyte Cultures Intoxicated by TBH (0.5 mM)

Compound	IC_{50} (μM)[a]	LDH (%)[a]	
		Rat hepatocytes	Human hepatocytes
CA	4.75 ± 0.15	41.88 ± 5.84	34.52 ± 2.24
RA	5.15 ± 0.25	15.16 ± 1.645	24.83 ± 1.54
CHA	6.94 ± 0.09	54.47 ± 6.87	50.0 ± 5.78
PA	8.87 ± 0.23	66.14 ± 11.47	51.10 ± 4.78
PLA	11.11 ± 0.84	54.14 ± 6.94	59.27 ± 4.21
FA	37.1 ± 1.05	85.16 ± 7.43	68.84 ± 5.67

[a]All values were expressed as average ± s.d. of triplicates of 3 experiments. [b]Control values were 14.89 ± 1.64% (rat hepatocytes) and 23.56 ± 1.87% (human hepatocytes); toxicity of TBH was 100 ± 9.08%.