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Invited review

Predicting the physiological relevance of *in vitro* cancer preventive activities of phytochemicals[1]

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Abstract

There is growing interest in the ability of phytochemicals to prevent chronic diseases, such as cancer and heart disease. However, some of these agents have poor bioavailability and many of the in-depth studies into their mechanisms of action have been carried out *in vitro* using doses which are unachievable in humans. In order to optimize the design of chemopreventive treatment, it is important to determine which of the many reported mechanisms of action are clinically relevant. In this review we consider the physiologically achievable doses for a few of the best studied agents (indole-3-carbinol, diindolylmethane, curcumin, epigallocatechin-3-gallate and resveratrol) and summarize the data derived from studies using these low concentrations in cell culture. We then cite examples of *in vitro* effects which have been observed *in vivo*. Finally, the ability of agent combinations to act synergistically or antagonistically is considered. We conclude that each of the compounds shows an encouraging range of activities *in vitro* at concentrations which are likely to be physiologically relevant. There are also many examples of *in vivo* studies which validate *in vitro* observations. An important consideration is that combinations of agents can result in significant activity at concentrations where any single agent is inactive. Thus, for each of the compounds reviewed here, *in vitro* studies have provided useful insights into their mechanisms of action in humans. However, data are lacking on the full range of activities at low doses *in vitro* and the benefits or otherwise of combinations *in vivo*.

Introduction

In recent years there has been increasing interest in the use of biologically active phytochemicals in cancer prevention. In particular, many *in vitro* studies using a wide range of natural products have demonstrated a preferential induction of cell cycle arrest or apoptosis in tumor cell lines compared to lines derived from non-tumor tissue. On further investigation, phytochemicals have been found to modulate the expression or activity of a large number of cellular proteins which are key for cell survival and the transformed phenotype. However, there is also much concern that many of these effects[1–3] are irrelevant *in vivo*, since the concentrations used are often orders of magnitude greater than appear to be achievable in the human body.

In this review, we have attempted to address this concern for a few of the most studied diet-derived compounds. For each agent, we have assimilated reported *in vivo* concentrations, based where possible on human data. We have then surveyed the *in vitro* biological effects at these or lower doses. For the indoles only, we have included data on phase I drug metabolizing activity relating to altered estrogen metabolism. Encouragingly, a significant amount of published data validates some of these changes *in vivo*.

For a number of reasons, some guesswork was involved in deciding on the most relevant *in vitro* doses for consideration. First, adding a compound directly to a cell culture may deliver a much higher local dose than occurs following

ingestion in the body. Second, some if not all of the compounds may undergo metabolism *in vivo* to other more or less active derivatives and such metabolism may not be possible in culture. Third, higher *in vivo* doses than those so far reported may be achievable by administration of a pure compound rather than a dietary source, or an optimized formulation of a pure compound. Fourth, some target tissues may receive a higher (or more prolonged) dose than the reported peak levels in plasma. Fifth, some target tissues, such as skin, oral cavity, gastrointestinal tract, and bladder, may receive higher doses because they are not dependent on circulating levels. The colon, for example, can be exposed to significant amounts of (unabsorbed) excreted material and the lining of the bladder may be exposed for substantial periods of time to any compounds concentrated in urine. Finally, where diet is concerned, any one compound may be poorly bioavailable, but with dozens, even hundreds, of active molecules being ingested together, the cumulative dose of similar acting compounds may be significantly higher.

It should not be forgotten that some natural products have exhibited toxicity *in vivo* when given in high doses, so there is also an argument for the use of higher concentrations *in vitro* to indicate the full range of activity of these molecules.

Indole-3-carbinol and diindolylmethane

Indole-3-carbinol (I3C) is derived from glucobrassicin, found in cruciferous vegetables. Diindolylmethane (DIM) is an acid condensation product formed from 2 molecules of I3C. *In vivo*, this is thought to occur in the acid conditions of the stomach. Data for mammals only are considered here.

Bioavailability of I3C and DIM in humans Information on the bioavailability and tissue distribution of I3C or DIM in humans is very limited[4–6]. However, there are a number of studies in which oral administration of I3C resulted in a biochemically or clinically measurable outcome, indicating that the absorption of I3C and/or its acid-condensation products does occur. Administered doses of I3C have ranged from 200 to 500 mg/d (~ 6–7 mg/kg), typically for periods of 1–6 months, although treatments up to 82 months have also been reported[7–14]. A dose-dependent effect (placebo, 200 and 400 mg/d for 3 months) was observed in the treatment of cervical intraepithelial neoplasia[7], and 200 or 400 mg/d were similarly effective against vulval intraepithelial neoplasia[14]. In dose-escalation studies for breast cancer prevention, 300 mg/d (minimum) increased the urinary estrogen metabolite ratio of 2-hydroxyestrone to 16 alpha-hydroxyestrone; 800 mg/d did not provide additional benefits over 400 mg/d in adult women[15,16]. Elevated cytochrome P450 activity was responsible for an increase in 2-hydroxylation of estrogen, increasing the ratio of 2-OH:16-OH estrone[16], which is regarded as favorable for the prevention of breast cancer and human papilloma virus (HPV)-related neoplasias[7–14].

Two studies detected DIM in plasma (2.5 μmol/L maximum at 2 h, gradually decreasing by 12 h) or in urine following oral administration of I3C (Table 1). I3C was not detected in plasma or serum following oral doses of 400–1200 mg[4,5]. DIM was also detected in the urine of a patient receiving DIM[4]. In a pilot study using a formulation of enhanced absorption DIM (BioResponse DIM, 108 mg/d for 30 d), increased 2-hydroxylation of estrogen was also reported[6].

Bioavailability of I3C and DIM in animals Radio-labeled I3C was used to follow distribution and tissue content in several studies, although this method did not differentiate between I3C and related products. In rats receiving 50 mg ^{14}C-I3C by gavage, I3C equivalents peaked at 28 μmol/L in the blood and 121 μmol/L in the liver after 30 min. The labeled product was detectable in the 100 μmol/L range from 10 min to 2 h following dosing[17]. High maximal concentrations of the I3C equivalent, but not I3C itself, were detected in the liver (1154 μmol/L), kidney, lung (436 μmol/L), blood (320 μmol/L), and tongue of the rats given ^{3}H-I3C in the diet for 1 week (0.88+/–0.074 mmol/kg/d)[18]. Once a steady state had been reached, excretion in feces and urine accounted for 75% dose/d, the majority of this being present in the feces by 110 h, indicating that either the dose was not absorbed or that a major excretory route was via bile. When ^{14}C-I3C was

Table 1. Bioavailability of DIM in humans following oral administration of I3C.

I3C dose mg/day	Peak time point (h)	DIM plasma μg/mL (μmol/L)	DIM urine μg/mg creatinine	Reference
200			12.1	4
400			15.6	
400–1200	2 (1 g)–3 (400 mg)	0.3 (400 mg)–2.5 (1g)		5
400 chronic	1.3	0.3		

given to pregnant mice, it was detected in the fetal liver, stomach, kidney, intestine, and lung (100–300 μmol/L) after 8 h of maternal exposure[19].

Anderton *et al* detected I3C and DIM in tissues following the dosing of mice with 250 mg/kg I3C (Table 2) using an HPLC method allowing the simultaneous identification and quantification of I3C and its derivatives[20]. The maximum level of 28 μmol/L I3C was observed at 15 min, falling below the level of detection by 1 h after dosing. I3C was detected in the liver (170 μmol/L)>kidney (116 μmol/L)>lung and heart>plasma>brain. The levels of DIM peaked at around 2 h in the liver (16 μmol/L)>lung and kidney>heart>brain>plasma (4 μmol/L), and by 24 h, were still detectable at approximately 0.5 μg/g in the brain and liver. The presence of linear trimer, 1-(3-hydroxymethyl)-indolyl-3-indolylmethane and indolo(3, 2b)carbazole, together with oxidative metabolites of I3C, was also documented. In a further study, Anderton *et al* compared concentrations of DIM in tissues of mice dosed with either pure DIM (250 mg/kg; Table 2) or an equivalent dose of the enhanced absorption BioResponse DIM[21]. The tissue distribution of DIM was similar to that reported previously following the administration of I3C with maximal concentrations around 160 μmol/L in the liver[20]. The BioResponse DIM resulted in levels approximately 50% higher than those obtained with unformulated DIM.

Thus, following the oral administration of I3C, both I3C and DIM were detectable at μmol/L concentrations in the blood and multiple organs. I3C was rapidly absorbed and cleared from the blood and tissues within 1 h, while DIM peaked slightly later and was more persistent. The observation of I3C in the blood and tissues at these very early time points belies previous assumptions that I3C is not absorbed, but undergoes complete acid condensation in the stomach. Several studies have revealed distinct responses to I3C and DIM in animal models[22–26]. Therefore, the *in vivo* activity of dietary I3C cannot be attributed completely to the production of DIM, although response due partially to DIM conversion is probable.

Physiologically relevant concentrations of I3C and DIM As no data are available for achievable levels of I3C in humans, we extrapolated from animal studies[20,21]. The maximum plasma and tissue concentrations attained in mice for I3C were 28 and 170 μmol/L (15 min) and for DIM, 4 and 16 μmol/L (2 h), respectively (Table 2). By allometric scaling, the I3C dose given to mice would equate to a 20 mg/kg dose in humans (1200 mg/d), which yielded the maximal serum concentration 2.5 μmol/L DIM (2 h), with no I3C detectable after 1 h[5]. Therefore, maximal detectable DIM concentrations following I3C administration are similar in mice and humans, and the discrepancy in I3C detection is likely to be caused by the sensitivity of methods and selection of time points.

The maximum levels of DIM achieved in animals following a dose of a pure compound range from 24–200 μmol/L (Table 2), with BioResponse DIM resulting in 50% higher bioavailability. The dose of BioResponse DIM used in humans was 108 mg (~1.3–1.9 mg/kg)[6], which might be expected to give levels in the range 3–30 μmol/L.

For the purposes of this review, the effects of the physiological concentrations up to 150 μmol/L I3C and 50 μmol/L DIM *in vitro* have been considered.

***In vitro* mechanistic studies using low doses of I3C or DIM** Several mechanisms are responsible for the chemopreventive activities of I3C and DIM, as summarized in Tables 3 and 4. Both agents induce activity of phase I and II enzymes involved in the biotransformation and elimination of carcinogens and steroid hormones. While detailed molecular interactions involved have not been completely elucidated, DIM interacts with the aryl hydrocarbon receptor (AhR), resulting in its nuclear translocation and induction of the genes encoding phase1 and II enzymes[27]. Several lines of evidence suggest that DIM exerts agonist and/or modulator activity on the AhR[28,29]. I3C can activate the NF-E2-related factor-2 (Nrf2) transcription factor which interacts with the antioxidant response element in the promoter of many cytoprotective enzymes, as described later. The induction of cytochrome P450 (CYP450) by physiological concentrations of I3C and DIM was observed in cancer cells[30] and confirmed by an analysis of mRNA expression profiles[31]. Increased CYP450 activity led to increased

Table 2. Bioavailability of I3C and DIM in animals following oral administration.

Dose	Time point	C_{max} plasma μg/mL (μmol/L)	Tissue concentration μg/g (μmol/L)	Reference
I3C (250 mg/kg)	15 min	I3C 4 (28)	27–25 (20–170)	20
	2 h	DIM 1 (4)	1–4 (4–16)	
DIM (250 mg/kg)	0.5–1 h	DIM 6 (24)	8–50 (32–200)	21

Table 3. Bioactivity of I3C *in vitro*.

Model	Dose (μmol/L)	Biomarkers affected	Outcome	Reference
Breast cancer	100	↓ p-Akt	Growth IC_{50} 30 μmol/L,	40, 41, 180, 181
MDA-MB468	25–100	↓ MUC1 protein and mRNA	apoptosis	
	125	↓ mitochondrial potential, ↑ caspase-9, ↑ Src ⇒ EGFR signaling, ↓ EGFR		
MDA-MB231	50	↑ 2-hydroxylation of E	Apoptosis	182
MDA-MB435	30–60	↑ Bax and induced translocation to mitochondria	Apoptosis	183
MCF7	Sub μmol/L -50	↑ 2-hydroxylation of E	Growth IC_{50} 50 μmol/L	32, 33, 37, 43,
			Apoptosis, G_0/G_1 arrest	181, 184–190
	10–50	↓ E2-induced ER–DNA binding and ERE		
	50–60	reporter activity; ↓ E responsive genes;		
		↓ E2-stimulated ER phosphorylation;		
		↑ BRCA1 and BRCA2 mRNA and protein		
		↑ CYP1A		
	25–50	↓ MUC1 protein, mRNA, and promoter activity		
	100	↓ cdk6; ↓ p-Rb; ↑ p27; ↑ p21; ↓ cdk2 activity; altered cdk2/cyclin E complex and localization; ↓ ERα mRNA and protein		
MCF10CA1a	60–100	↓ p-Akt; ↓ Akt activity; ↓ $BclX_L$; ↓ NF-κB–DNA-binding	Apoptosis	49, 191
T47D	50	↑ 2-hydroxylation of E; ↓ E2-induced ER-DNA binding; ↓ cell migration	Growth inhibition	32, 186, 192 43
	60	↑ BRCA1 and BRCA2 mRNA and protein		
Prostate cancer	30–60	↓ p-Akt; ↓ Bad; ↓ $BclX_L$; ↓ EGFR	Apoptosis	31, 193
PC-3	60	↓ gene expression of EGFR, PI3K, TGF-β2, FGF, cyclin E2, ATF, Bcl2		
LNCaP	30–90	↑ DR4 and DR5, ↑ BRCA1 and BRCA2 mRNA and protein	Growth inhibition, ↑ TRAIL-induced apoptosis	43, 194
DU145	60	↑ BRCA1 and BRCA2 mRNA and protein		43
Myeloid and leukemia cells (Jurkat, KBM-5, U266, MM.1), epithelial cancers H1299, SCC-4,	25–50	↓ TNFα-induced activation of NF-κB, ↓ activity of IKK and phosphorylation of IκBα, ↓ NF-κB DNA binding, ↓ NF-κB-regulated expression of a reporter gene and endogenous genes		52
A293	1–25		↑ cytotoxicity, induced by TNFα, cisplatin, and doxorubicin	
Colon cancer HCT-116	50–100	↑ NAG-1 expression	Growth inhibition	179
HCT-116 clones	10	PARP cleavage, caspase-9 activation, ↓ Bcl-X_L	$IC_{50} \cong$ 5–10 μmol/L	195

estrogen metabolism and the degradation of estradiol (E2)[27], which is required for the growth of estrogen receptor-alpha (ERα)-positive cancer cells. I3C (50 μmol/L) inhibited the estradiol-stimulated growth of estrogen-responsive MCF7, T47D and ZR75.1 breast and cervical cells. It inhibited receptor phosphorylation and DNA binding as well as estrogen-dependent reporter gene activity in breast tumor cells and cervical cancer cell lines[32,33]. DIM (10 μmol/L) also

Table 4. Bioactivity of DIM *in vitro*.

Model	Dose (μmol/L)	Biomarkers affected	Outcome	Reference
Breast cancer MCF7	10–50	↑ CYP1A1; ↑ formation of Ah-receptor nuclear complex	Apoptosis, G_1 cell cycle arrest, growth inhibition	27, 34, 37, 45, 48, 69, 196–198
	1–10	↑ ER-DNA binding, ↑ ER-regulated pS2 mRNA and reporter gene expression, ↓ ERα mRNA		
	50	↑ GADD proteins; ↑ IFNγ expression; ↑ p-JNK; ↑ p-p38; ↑ p-Jun; ↑ p-ATF-2, ↑ p21Cip1 mRNA and protein, ↓ cdk2 activity, ↑ Sp1 DNA binding, ↓ Bcl-2; inhibits mitochondrial H^+-ATP synthase, ↑ ROS		
MCF10CA1a	15–50		Growth inhibition	50
	30–50	↓ NF-κB–DNA-binding		
	50	↓ p-Akt		
MDA-MB231	40–50	↑ mRNA levels for p21Cip1, p57Kip2, ↓ mRNA levels for genes involved in cell proliferation and survival as well as angiogenesis and metastasis, ↓ Bcl-2	Apoptosis, G_1 cell cycle arrest, growth inhibition, IC_{50} 60 μmol/L	196, 198, 199
Endometrial cancer Ishikawa	1–30	↑ p-ERK1/2; ↑ p-CREB; ↑ ERE-reporter gene activity; ↑ ER-responsive genes (TGF-α, alkaline phosphatase, PR)	Growth inhibition	35, 36
HEC-1B (ER transfected)	30	↑ ERE-reporter gene activity		36
Prostate cancer LNCaP	10	↓ PSA protein and mRNA levels; binds toAR and blocks nuclear translocation, ↓ expression of AR-regulated reporter gene expression, ↓ AR function. ↓ DHT-induced NF-κB DNA binding	Growth IC_{50} 40 μmol/L	38, 53, 200
	10–50 B-DIM			
	50	↓ expression of androgen receptor	Decreased proliferation	39
PC-3	40	↓ gene expression of EGFR, PI3K, TGF-β2, FGF, cyclin E2, ATF, Bcl2	Growth IC_{50} 40 μmol/L	31, 51, 200
	15–60	↓ p-Akt, ↓ PI3K activity; ↓ NF-κB-DNA-binding, ↓ EFGR	Apoptosis	
DU 145	25–50	↓ p-Akt, ↓ cdk4, ↓ cdk6, ↑ Ca^{2+} mobilization	Apoptosis, growth inhibition, G_1 cell cycle arrest, IC_{50} 20 μmol/L	47, 200, 201
Colon cancer HCT-116	12–25	↑NAG-1 expression, ↑NAG-1-promoter regulated reporter gene expression		179
	25–50	↑ ATF3		
HCT-116 clones	20	PARP cleavage, caspase-9 activation, ↓ Bcl-X_L,	$IC_{50} \cong 11$ μmol/L	195
HT29		Blocked exit from G_1	Growth IC_{50} 10 μmol/L	202
HepG2 (hepatoma)	30–50	Inhibits topoisomerase IIα	Growth inhibition, G_1 cell cycle arrest	44
Adrenocortical carcinoma H295R	<10	↑ EROD activity		203
Pancreatic cancer Panc-1 and Panc-28	20	↑ endoplasmic reticulum stress, ↑ GRP78, CHOP, ↑ DR5, ↑ caspase-8, ↑ PARP cleavage, ↑ caspase3	Decreased survival, apoptosis	46

inhibited the estradiol-stimulated growth of MCF7, but in the absence of 17β-estradiol it appeared to stimulate growth[34].

DIM also exerted other estrogenic effects in these and endometrial cells[34–36]. Conversely, earlier data indicated an inhibitory effect of DIM on E2-regulated reporter activity and ER DNA binding[27]. Both I3C (75–100 μmol/L) and DIM (1–25 μmol/L) reduced the expression of ERα mRNA in MCF7 cells[33,37]. DIM also interfered with androgen receptor expression, DNA binding, and signaling[38,39].

Both compounds are growth inhibitory to a wide range of tumor cell lines, including ER-negative cancer cells. The increased loss of viability of several breast cancer cell lines when grown in a 3-D environment in the presence of I3C implied greater susceptibility *in vivo* than in a monolayer cell culture[40]. Recent studies have shown that I3C decreases proliferation and induces apoptosis by reducing the expression and signaling of the genes essential for tumor cell viability, such as ER and epidermal growth factor receptor (EGFR) in breast cells of luminal A and basal-like subtypes[33,41]. I3C (50 μmol/L) can also inhibit phosphoinositide-3-kinase (PI3K)[42], resulting in the inhibition of protein kinase B (Akt) phosphorylation and decreased survival in cancer cells dependent on this pathway. A mechanism dependent on breast cancer-related protein (BRCA)1/2 upregulation has also been proposed[43].

DIM, which is considerably more potent than I3C, also inhibits the growth of a range of cells (Table 4). Topoisomerase II-alpha and mitochondrial H^+-ATP synthase were identified as direct targets of DIM. The inhibition of the latter enzyme results in increased mitochondrial reactive oxygen species (ROS) production and signaling via the p38 stress activation pathway[44,45]. Apoptosis via the activation of the endoplasmic reticulum stress pathway has also been reported[46,47].

Both agents have a significant effect on several other signaling pathways, such as those involving p38[45,48] and NF-κB signaling[49–53]. They modulate a variety of growth-, cell cycle-, and apoptotic-regulatory proteins at the mRNA or protein level, including EGFR, PI3K, transforming growth factor (TGF)-β2, fibroblast growth factor (FGF), cyclin E2, activating transcription factor (ATF), B cell lymphoma Bcl2, $BclX_L$, Bad, and Bax.

In vitro* effects of I3C or DIM observed *in vivo Both I3C and DIM clearly inhibit tumor cell growth *in vivo* in a range of animal models[54]. The reduced incidence and multiplicity of mammary tumors was concurrent with increased phase I and II drug metabolizing enzymes in I3C-treated animals[55–57]. An analysis of transgenic $Nrf2^{-/-}$ mice indicated that the I3C-induced upregulation of phase II enzymes required the Nrf2 transcription factor[58,59]. The upregulation of CYP450 activity by DIM *in vivo* has been proposed to occur via a mechanism involving the AhR[30], as reported in MCF7 cells *in vitro*[27]. The induction of phase I and II enzymes has been reported in liver, small intestine, and lungs of rodents receiving I3C (in the diet or by gavage)[30,54,57,60–63]. DIM also induced P450 activity and flavin-containing monooxygenase 1 in the rat liver[30,60].

Apoptosis in response to I3C was observed *in vivo* in initiated mammary glands with activation of caspases-8, -9, and -3[25] and in cervical epithelium of transgenic mice (HPV16), developing cervical cancer in response to estrogen[56]. Few studies have investigated the effect of either agent on signal transduction intermediates *in vivo*, but in one study, dietary I3C (0.5%) caused a significant decrease in total tyrosine phosphorylation and ornithine decarboxylase activity in the rat liver[62]. Many of the signaling events modulated by I3C *in vitro* involve tyrosine phosphorylation[41], but interestingly, changes in ornithine decarboxylase activity in breast and colon cells *in vitro* were only observed at relatively high concentrations (>100 μmol/L)[62,64]. The downregulation of NF-κB-regulated genes by I3C occurring in a variety of cancer cells *in vitro*, was also observed in mouse xenografts of MDA-MB231 cells[52,65].

Evidence for I3C or DIM acting synergistically/ antagonistically In rats, I3C (5 mg/kg) reversed vinblastine- or vincristine-induced P-glycoprotein levels[66]. This group also showed that a very high dose of I3C (10 mmol/L) decreased P-glycoprotein levels *in vitro* in the multidrug resistant cell line K562/R 10, sensitizing it to vinblastine, but had no growth-inhibitory effect on the parent K562 cell line[67]. I3C (333 or 500 mg/kg per day) also reversed the MDR phenotype of the B16/hMDR1 (drug-resistant MDR1-expressing murine melanoma) tumor *in vivo*, and in combination with vinblastine, actually reduced the tumor mass[68]. In the same study, an I3C acid-condensation mixture (12.5 μmol/L) sensitized the B16/hMDR1 cell line *in vitro* to vinblastine, while DIM (45 μmol/L) increased the drug content of cells by 50%[68]. Combined treatments using I3C (50–125 μmol/L) in combination with Src or/and EGFR inhibitors reduced the viability of breast cancer cells MDA-MB468 and MCF7[41].

DIM (25 μmol/L) plus genistein (5 μmol/L) synergistically induced growth arrest and DNA damage-inducible (GADD)34 protein levels and apoptosis, and at higher concentrations induced estrogen receptor response element (ERE)-driven reporter gene activity[69]. I3C (50 or 100 μmol/L) has been shown to cooperate with tamoxifen (1 μmol/L) *in vitro* to increase cell growth inhibition and G_0/G_1 cell cycle arrest of MCF7 cells[70]; *in vivo*, it reduces tumor mass and increases latency of mammary cancers[71]. An

I3C acid condensation mixture also enhanced the efficacy of vinblastine in mouse melanoma cells, while I3C itself had no effect[68].

In vivo, dietary I3C, together with crambene (1-cyano 2-hydroxy 3-butene), another glucosinolate from vegetables, showed a greater than additive induction of glutathione-S-transferase (GST) and quinone reductase activity[72].

Curcumin

Curcumin (diferuloylmethane) is a major constituent of the spice turmeric, derived from the roots of *Curcuma longa*. The major dietary source is curry, but it is also used as a food coloring and in some medicines.

Bioavailability of curcumin in humans Curcumin exhibits poor gastrointestinal absorption, with much of an oral dose passing unchanged through the gastrointestinal tract, and a further proportion undergoing conjugation, without absorption, prior to fecal loss. Absorbed curcumin undergoes sequential reduction and conjugation (glucuronidation and/or sulfation) within the gastrointestinal tract and liver, with the resultant formation of metabolites and low systemic levels of the parent compound[73,74].

Studies in humans have demonstrated that the oral administration of curcumin furnishes very low systemic levels, mostly in the low nanomolar range (Table 5). An exception is the study by Cheng *et al*, which reported serum levels in the low micromolar range using the maximum tenable dose (8 g/d)[75]. Other groups have failed to replicate this finding, with Sharma *et al,* for example, administering up to 3.6 g/d of curcumin to patients for up to 4 months, yet only achieving levels in the 10 nmol/L range[76]. The discrepancy between these studies remains to be explained, but may have resulted from the use of different formulations of curcumin.

Due to its poor bioavailability, curcumin levels in tissues beyond the gastrointestinal tract are also in the low nanomolar

Table 5. Bioavailability of curcumin in humans following oral administration.

Source	Maximum dose (mg/d)	C_{max} plasma/ serum (T_{max})	Urinary/ fecal excretion		Tissue levels (nmol/g)	Metabolites		Reference
Curcumin	8000 (3 months)	1.77 μmol/L[1] 60–120 min	Urine	ND				75
Curcuma extract (8.2% curcumin)	2200 (up to 4 months) (=180 mg/d curcumin)	ND	Urine	ND		Plasma/urine	ND	76
			Faeces (dried)	64–1054 nmol/g		Feces	CS detected in 1case	
Curcumin	3600 (7 d)	low nmol/L (systemic/portal) (Sampled 60 min post-dose)			Liver ND Bile ND	Blood Liver	CS/CG(low nmol/L) Hexahydrocurcumin and hexahydrocurcuminol(trace)	204
Curcuminoid capsules (90% curcumin)	3600 curcumin (up to 4 months)	11.1 nmol/L (60 min)	Urine Faeces (dried)	1.3 μmol/L 25–116 nmol/g		Plasma Urine Faeces	CS (8.9 nmol/L); CG (15.8 nmol/L); CS (45 nmol/L); CG (510 nmol/L) CS (trace)	76
Curcuminoid capsules (90% curcumin)	3600 curcumin (7 d)	<3 nmol/L (Sampled 60 min post-dose)			Colorectal 7.7 mucosa Colon CR cancer 12.7	Plasma	ND CS/CG detected	77
Curcumin extract (75% curcumin)	12000 (1 dose)	150 nmol/L[2] (60 min)						205

[1] Serum level declined within 12 h; figures given are maximum levels achieved unless otherwise indicated. [2] Curcumin detectable in only 1 of 3 patients receiving this dose; no curcumin was detectable at doses ≤8000 mg/d. ND, not detected; CS, curcumin sulfate; CG, curcumin glucuronide.

range or below. Garcea *et al* were unable to detect curcumin in normal liver or colorectal liver metastases in patients who had received 3.6 g/d for 1 week. In the only human study to examine colorectal tissue to date, this oral dose resulted in levels in the 10 μmol/L range[77].

Bioavailability of curcumin in animals A number of groups have examined the bioavailability of curcumin in animals, following oral, intragastric (ig) or intraperitoneal (ip) dosing (Table 6). Studies suggest that oral dosing may give rise to significant levels of curcumin within the gastrointestinal tract. In a rat model, approximately 1.8 μmol curcumin/g of tissue was demonstrated in colonic mucosa following the dietary administration of 1200 mg/kg daily[78]. Perkins *et al* reported 750 mg/kg of curcumin/d to result in ~100 nmol/g in mouse small intestine mucosa and 500 nmol/g within colonic mucosa[79]. Following oral dosing of 400 mg per rat, liver and kidney levels were less than 20 μg per tissue[80]. Significant levels of curcumin may also be achieved locally when administered topically to the skin or within the oral cavity, but the exact dose achieved in these scenarios remains to be confirmed.

It is neither practicable nor desirable to increase the oral dose of curcumin above that already investigated. Recent animal studies, however, have demonstrated that the reformulation of curcumin may enable further improvements in bioavailability. It has previously been shown that the formulation of drugs with phosphatidylcholine increases their plasma bioavailability. Such a formulation led to significantly higher levels of curcumin within plasma and the liver compared with the parent compound, although lower levels within the intestinal mucosa[81]. Several other animal studies have also found curcumin bioavailability to be significantly increased by its administration as a phospholipid complex[82,83]. These increases in bioavailability now require confirmation in human studies. Although the bioavailability data are lacking, *in vitro* and animal studies have also shown promising anticancer potential for a liposomal preparation of curcumin[84,85]. In addition, nanoparticle-encapsulated curcumin may provide an alternative means to increase the bioavailability of this agent[86].

Physiologically relevant concentrations of curcumin The bioavailability data suggest that *in vitro* studies with curcumin in the 10 μmol/L range or below might have human physiological relevance, but that its role as a chemopreventive agent may lie primarily within the gastrointestinal tract.

***In vitro* mechanistic studies using low doses of curcumin** The anticancer effects of curcumin have been demonstrated in multiple cell types, at concentrations between 5 and 50 μmol/L[87]. Selected studies demonstrating the anticancer activity of curcumin at or below the 10 μmol/L level achievable in the human colon *in vivo* are summarized in Table 7. Where available, data are presented from studies using colorectal cell lines; results from other cell types using a maximal dose of 10 μmol/L are also included. In addition to these studies, curcumin also inhibited the proliferation of squamous carcinoma SCC-25 cells[88] and the proliferation and invasion of HBL100 breast cells[89].

In vitro* effects of curcumin observed *in vivo In a rat model, dietary curcumin significantly increased the apoptotic index in azoxymethane-induced colonic tumors[90]. Rao *et al* demonstrated the effect of a curcumin-containing diet on azoxymethane-induced rat carcinogenesis[91]. Curcumin significantly reduced tumor volume, as well as colonic mucosa and tumor prostaglandin $(PGE)_2$ expression by over 38%. Similarly, it enhanced 2-amino-1-methyl-6-phenylimidazol(4,5-b)pyridine-induced apoptosis in Min/+ mice and inhibited tumorigenesis in the proximal small intestine. Also in mice, Mahmoud *et al* found dietary curcumin to normalize enterocyte proliferation and restore the level of enterocyte apoptosis to that of wild-type animals[92]. In rats, a gavage administration of curcumin (200 or 600 mg/kg) inhibited diethylnitrosamine (DEN)-induced hepatic hyperplasia and inflammation. Specifically, the increased expression of p21ras and p53 in the liver was prevented. The decreased expression of proliferating cell nuclear antigen, cyclin E, and cdc2 was also observed, along with the inhibition of DEN-induced NF-κB activation[93].

While there are no *in vitro* studies for comparison, there is evidence from both animal and human studies showing that curcumin suppresses malondialdehyde-deoxyguanosine adduct (M_1dG) adduct formation in DNA[77,78]. However, Garcea *et al*, while noting decreased M_1dG adduct formation in the colorectum following curcumin treatment, found no alteration in cyclooxygenase 2 (COX2) protein levels[77].

Despite the low bioavailability of curcumin, there are examples in animal studies of its biological activity at sites distant from the locus of absorption, where levels are expected to be inefficacious based upon the results of *in vitro* studies. Sharma *et al*, for example, demonstrated increased hepatic GST expression and the attenuation of hepatotoxin-induced adduct formation following curcumin treatment[78]. Oral curcumin also led to the complete suppression of tumor NF-κB activation in an orthotopic mouse model of pancreatic cancer[94]. Anticancer activity has also been reported at a number of other sites distant from the gastrointestinal tract, including the breast[95], prostate[96], lung[97], and liver[98].

Table 6. Bioavailability of curcumin in animals.

Source	Model	Maximum dose[1]	C_{max} plasma/serum (T_{max})	Urinary/fecal excretion (% of dose)	Tissue levels	Metabolites	Reference
Curcumin	Rat	1000 mg/kg (po)	Trace	Urine trace Feces 75%	Bile trace		206
Curcumin	Rat	600 mg (*po*) 600 mg (ip)		Urine 6% Feces 89% Feces 73%	Bile 11% excreted	Major biliary metabolites-glucuronides	207
Curcumin	Rat	400 mg (*po*)	Systemic ND Portal <5 μg/mL (13.5 μmol/L)*	Urine ND Feces 38% remaining in large intestine (24 h)	Liver <20 μg Kidney <20 μg	Urine-glucuronides and sulfates	80
Curcumin	Rat	400 mg (*po*)	Detectable in blood	Urine Minimal Feces Major route	Detectable in liver and kidney		208
Curcumin	Mouse	1000 mg/kg (ig)	0.22 μg/mL (60 min)[2] (0.6 μmol/L)		Following IP: Kidney 7.5 μg/g (20 μmol/L) Spleen 26.1 μg/g (71 μmol/L) Liver 26.9 μg/g (73 μmol/L) Brain 0.4 μg/g (1 μmol/L) Intestine 117.0 μg/g (316 μmol/L)	99% plasma curcumin conjugated with glucuronide	209
		100 mg/kg (ip)	2.25 μg/mL (15 min)[3] (6.1 μmol/L)				
Curcumin (91% curcumin)	Rat	1200 mg·kg^{-1}·d^{-1} (14 d in diet)	Systemic/portal ND	Faeces (dried) 8.6 μmol/g	Colon mucosa 1.8 μmol/g (mmol/L) Liver 0.8 nmol/g (μmol/L)	CS/CG ND in plasma/ tissues/ feces	78
		200–400 mg/kg (diet)	<10 nmol/L (30 min)		Colon mucosa 279 nmol/g (μmol/L) Liver 0.1 nmol/g (μmol/L)	Plasma conjugates ND	
		500 mg/kg (ig)	30 nmol/L (30 min)		Colon mucosa 1.7 nmol/g (μmol/L) Liver 0.1 nmol/g (μmol/L)	Plasma conjugates detected	
Curcumin	Rat	500 mg/kg (ig)	<20 nmol/L			Plasma CG (1.7 μmol/L), CS (0.35 μmol/L), CG and others detectable	73
Curcumin (97% curcumin)	Mouse	300–750 mg·kg^{-1}·d^{-1} (7 d in diet)	~5 pmol/L	Urine ND Feces 3.2–3.8 μmol/g	Small intestine[4] 39–240 nmol/g (μmol/L) Colon[4] 15–715 nmol/g (μmol/L) Liver[4] 119 pmol/g (nmol/L)	Colon and feces-CS (trace)	79

(Continue)

Source	Model	Maximum dose[1]	C_{max} plasma/serum (T_{max})	Urinary/fecal excretion (% of dose)	Tissue levels	Metabolites	Reference
Curcumin Meriva (curcumin+ phosphatidylcholine)	Rat	340 mg/kg (ig)	6.5 nmol/L 33.4 nmol/L	30 min 15 min	Liver <10 ng/mL Intestinal mucosa 2.8 mg/g Liver 80 ng/mL Intestinal mucosa 2 mg/g	Plasma metabolites detected. Levels of metabolites in liver and gastrointestinal mucosa higher following administration of Meriva	81
Curcumin (>99% purity)	Rat	100 mg (*po*)	266.7 ng/mL (0.72 μmol/L)	97 min			82
Curcumin-phospholipid complex		300 mg (*po*)	600.98 ng/mL (1.63 μmol/L)	140 min			
Curcumin	Rat	500 mg/kg (*po*)	0.06 μg/mL (0.16 μmol/L)	42 min			210
Curcumin	Rat	g/kg (*po*)	0.5 μg/mL (1.36 μmol/L)	45 min			83
Curcumin-phospholipid complex		1.0 g/kg of curcumin (*po*)	1.2 μg/mL (3.26 μmol/L)	90 min			

[1] Single dose unless otherwise stated; [2] Levels ND at 6 h; [3] Levels decline rapidly within 1 h; [4] Tissue levels decline to ND by 3–6 h; *Figures in brackets are derived from the published data. *po*, per orum/os; ND, not detected; CS, curcumin sulfate; CG, curcumin glucuronide; CGS, curcumin glucuronide sulfate.

Evidence for curcumin acting synergistically/antagonistically The treatment of MCF7 breast cells with curcumin (10 μmol/L) and genistein (25 μmol/L) demonstrated a synergistic effect, leading to the total inhibition of proliferation induced by an endosulfane/chlordane/DDT mixture[99]. Curcumin also synergistically potentiated the inhibitory effect of celecoxib on pancreatic carcinoma cells[100] and additively inhibited the growth of colorectal cancer with celecoxib in the 1,2-dimethylhydrazine rat model[101].

In an *in vitro* model of oral cancer, EGCG blocked cells in the G_0/G_1 phase, while curcumin blocked in the G_2/M phase of the cell cycle. The combination showed synergistic interactions in growth inhibition[102]. While tea or curcumin individually decreased the number and volume of dimethylbenzanthracene (DMBA)-induced oral tumors in hamsters, only the combination decreased the proliferation index of squamous cell carcinoma[103].

LNCaP prostate cancer cells are relatively insensitive to tumor necrosis factor related, apoptosis-inducing ligand (TRAIL). At low concentrations, neither TRAIL (20 ng/mL) nor curcumin (10 μmol/L) produced significant cytotoxicity, whereas cell death was markedly enhanced by the combination. Both agents together induced the cleavage of procaspases-8, -9, and -3, the truncation of Bid, the release of cytochrome c, and apoptosis[104].

Recent studies have also demonstrated promising interactions between curcumin and established chemotherapeutic agents. In colorectal carcinoma lines, the antiproliferative and pro-apoptotic effects of curcumin and oxaliplatin increased markedly when cells were treated with both agents[84,105]. Similarly, curcumin potentiated the pro-apoptotic effects of gemcitabine and paclitaxel in bladder cancer cell lines[106] and the antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer. Antagonistic interactions have also been demonstrated, however, with curcumin shown to inhibit chemotherapy-induced apoptosis in breast tumor lines. Camptothecin-, mechlorethamine-, and doxorubicin-induced apoptosis in MCF7, MDA-MB231 and BT474 cells was inhibited by as much as 70%, following 3 h exposure to as little as 1 μmol/L curcumin. The inhibition of both c-jun N-terminal kinase (JNK) activation and cytochrome c release occurred[107]. The same authors, using an *in vivo*

Table 7. Bioactivity of curcumin *in vitro*.

Model	Minimum effective dose (μmol/L)	Biomarkers affected	Outcome	Reference
Colo320	12.5		Growth inhibition; apoptosis	211
HCT-116	5		Apoptosis	212, 213
	10	↑ GADD153 mRNA and protein expression; ↑ DNA damage		
Moser	15	↑ PPARγ; ↓ cyclin D1; ↓ EGF signaling	Growth inhibition	214, 215
	10	↓ EGFR gene expression	Growth inhibition	
HCA-7	5	↓ CD/PMA-induced COX2 and PGE_2 production		216
HCEC	10	↓ TNFα-induced COX2 mRNA		217
HT29	5	↓ COX2 mRNA and protein	Growth inhibition	218, 219
	10	↓ Arachidonic acid metabolism		
SK-Hep-1	10	↓ MMP-9 secretion;	↓ invasion (matrigel)	220
MDA MB468	1–5		Growth inhibition, ↓invasion (matrigel)	89
	10	↓ JNK activation by anisomycin		
MCF7	10		Apoptosis (p53-dependent); growth inhibition; G_2/M arrest	99, 221, 222
KB-V1	1	↓ MDR1 mRNA		
	5	↓ P-glycoprotein expression		223
B cell lymphoma	10	↓ egr-1; ↓ c-myc; ↓ Bcl-X_L; ↓ p53; ↓ NF-κB	Growth inhibition; apoptosis	224
Jurkat	5–10	↓ JNK activation by PMA and ionomycin	Growth inhibition	225
HL-60	3.5	↓ Bcl-2 expression	Apoptosis	226
U266; RPMI 8226; MM.1S	10	↓ constitutive STAT3 phosphorylation	↓ IL-6 induced proliferation	227

xenograft model, found dietary curcumin (25 g/kg) decreased the level of cyclophosphamide-induced tumor regression, again with decreased JNK activation and less apoptosis.

Epigallocatechin-3-gallate (EGCG)

Green tea and its constituent molecules, including EGCG, have been found to prevent tumor formation in a wide range of tissues in animal models. However, the possible influence of green tea on cancer in humans has been difficult to interpret due to confounding factors, such as diversity in types of tea used, preparation methods, including temperature of infusion, and frequency of tea drinking.

The relevance of *in vitro* studies with EGCG has been reviewed by Lambert and Yang[108], who concluded that the effectiveness of tea consumption in cancer prevention remained unclear and required a better understanding of bioavailability and fundamental mechanisms.

Bioavailability of EGCG in humans A number of studies have reported the bioavailability of EGCG in various human body fluids (Table 8) following the administration of green tea or EGCG. Levels in plasma up to a maximum of 7.3 μmol/L (±3.6) have been reported, but more often are in the submicromolar range. Bioavailability in two early studies found plasma levels at 0.2%–2% of the ingested amount of EGCG (up to 4 μmol/L), but higher plasma concentrations have since been reported in fasting patients compared to those who consumed catechins with food[109–111]. The oral administration in human patients resulted in high plasma clearance levels and volume distribution, suggesting that the bioavailability

Table 8. Bioavailability of EGCG in humans following oral administration.

Source	Dose	C_{max} plasma	T_{max} plasma	Elimination half-life	Reference
Decaffeinated tea solids	1.5 g (in 500 mL)	119 ng/mL (0.26 μmol/L)*	1.4–2.4 h	5.0–5.5 h	228
>98%purity	3.0	326 ng/mL (0.71 μmol/L)			
	4.5	321 ng/mL (0.70 μmol/L)			
EGCG	2.78 mg/kg	0.15 μmol/L	2 h	2.7 h	229
Green tea	20 mg/kg	77.9 ng/mL (0.17 μmol/L)	1.6 h	3.4 h	230
Decaffeinated green tea	20	24.4 ng/mL (0.05 μmol/L)	1.2 h	2.3 h	
EGCG	2	34.7 ng/mL (0.08 μmol/L)	1.6 h	3.7 h	
Green tea	2–3 cups/d	4.8–22 μg/mL (10–40 μmol/L) in saliva		10–20 min	115
EGCG	200 mg	74 ng/mL (0.16 μmol/L)	127.1±76.6 min	118.0±77.0 min	109
	400	119 ng/mL (0.26 μmol/L)	108.7±26.4	162.3±84.3	
	600	169 ng/mL (0.37 μmol/L)	180.0±84.8	183.7±67.6	
	800	439 ng/mL (0.96 μmol/L)	240.6±84.6	114.0±33.3	
EGCG	50–1600 mg	130–3392 ng/mL (0.28-7 μmol/L)	1.3–2.2 h	1.9–4.6 h	231
EGCG	688 mg	1.3 μmol/L	2.9 h	3.9 h	232
EGCG	225 mg	0.66 μmol/L	1.5 h	6.9 h	110
	375	4.3			
	525	4.4			
Black tea	16.74 mg EGCG 4×/6 h	0.02 μmol/L	5 h		233
Polyphenon	(mg of EGCG)	(free EGCG, ng/mL)	(free EGCG, min)	(free EGCG, min)	112,
E containing EGCG	400 mg fed	141.8±89.1 (0.30 μmol/L)	122.9±83.7	145.2±129.1	234
	400 mg fasting	798.7±573.1 (1.74 μmol/L)	93.9±59.0	170.5±104.6	
	800 mg fed	294.0±113.5 (0.64 μmol/L)	154.9±78.3	220.9±209.3	
	800 mg fasting	1522.4±1357.8 (3.32 μmol/L)	83.3±31.0	156.5±77.5	
	1200 mg fed	923.6±755.3 (2.01 μmol/L)	175.1±74.6	254.9±59.9	
	1200 mg fasting	3371.6±1651.2 (7.34 μmol/L)	90.6±28.4	228.4±75.3	
Polyphenon E containing EGCG	618 mg	0.7±0.4 μmol/L	2.5 h±1.1		235
Purified EGCG	580 mg	0.5±0.2 μmol/L	2.6 h±1.2		

*Figures in brackets are derived from published data.

of EGCG in the blood may be low, similar to the situation found in rodents[109,112]. Dvorakova *et al* suggested that topical application to skin of an ointment containing 10% EGCG was likely to result in substantial intradermal uptake, but very poor systemic absorption[113].

It has been found that holding a green tea solution (1.2 g/200 mL water) in the mouth for 1 min resulted in salivary EGCG concentrations (mean) of 27 μmol/L, with values up to 48 μmol/L recorded, several fold higher than that achieved through normal drinking, and many more times greater than plasma concentrations[114,115]. However, holding the tea in the mouth for 5 min resulted in salivary concentrations 4–5 times higher, whilst taking tea solids in capsules resulted in no detectable salivary catechin levels. Thus, drinking tea slowly may be an effective way of delivering relatively high concentrations to the oral cavity and esophagus.

EGCG can undergo metabolism through glucuronidation, sulfation, methylation, or ring fission[108], processes which are subject to interindividual variation.

Bioavailability of EGCG in animals Surprisingly, few studies have documented the bioavailability of EGCG in animals. Most have shown a maximum plasma concentration in the nmol/L to low μmol/L range, similar to the human situation, although 1 study using a large dose of EGCG in rats reported plasma concentrations up to 20 μmol/L (Table 9). No animal studies have examined the effect of fasting on bioavailability. Fang *et al*, using liposomes for the local (injection) delivery of EGCG, found that a liposomal cocktail containing deoxycholic acid and ethanol greatly increased the tumor uptake of EGCG in both melanoma and colon murine tumor models[116,117]. However, liposomal delivery was not superior following topical application.

Lambert and colleagues[118] reported that piperine from black pepper enhanced the bioavailability of EGCG in mice. Small intestinal levels following EGCG administration alone resulted in a C_{max} of 37.5±2.5 nmol/g at 60 min, decreasing to 5.1±1.7 nmol/g at 90 min. Following cotreatment with piperine, the C_{max} was 31.6±15.1 nmol/g at 90 min, with levels still above 20 nmol/g at 180 min. The appearance of EGCG in the colon and feces was slower in the cotreated mice.

In rats and mice 24 h after the intragastric administration of radiolabeled EGCG, 10% of the dose was present in the blood, with around 1% in tissues, such as liver, kidney, heart, lung, and prostate[119]. Major elimination occurred in the feces. In line with these findings, another study suggested that the transporter-mediated intestinal efflux of catechins may play a role in the systemic elimination of these compounds[120]. Following an intravenous (iv) dose in rats, >70% was eliminated in bile and 2% in urine[119,121]. A study in rats, in which different green tea catechins were administered by iv or ip, suggested that first-pass hepatic elimination did not play a major role in the metabolism of orally-

Table 9. Bioavailability of EGCG in animals.

Source	Model	Dose	C_{max} plasma	T_{max} plasma	Tissue			Excretion		Reference
EGCG	Mouse	0.3% (*po*)	90.6 nmol/L		Liver	42.9 ng/g	(94.6 nmol/L)*	Urine	26.9–98.6 μmol/L	229
		0.1% (*po*)	22.4			13.4	(29.3 nmol/L)		13.4–40.3	
		in drinking			Kidney	14.3	(31.5 nmol/L)	Feces	18–89 μg/g	
		water				4.8	(10.6 nmol/L)		24–53	
					Intestine	14.4	(31.7 nmol/L)			
						14.8	(32.6 nmol/L)			
EGCG	Mouse	21.8 μmol/kg (iv)	2.70 μmol/L		Lung, Prostate	0.31nmol/g (μmol/L)				236
		163.8 (ig)	0.28		Colon	7.9				
					Intestine	45.2				
EGCG	Rat	10 mg/kg (iv)		85.5 min	Intestine	High		Bile		237
		75 mg/kg (ig)			Kidney, Lung, Liver	Low				
EGCG	Rat	6000 μg (iv)	≥10 μg/mL	Approx 30 min						122
		6041 μg (ip)	(21.8 μmol/L)							

*Figures in brackets are derived from the published data.

administered epigallocatechin, epicatechin, and EGCG[122].

Physiologically relevant concentrations of EGCG The plasma bioavailability of EGCG, whether administered as tea or a pure compound, is in the range of 0.1–7 μmol/L in humans, with concentrations over 100 μmol/L observed in saliva. No significant excretion occurred in urine (generally <0.1% of dose). Rodent data indicate levels up to 20 μmol/L may be achievable. Based on these data, we chose 20 μmol/L as the maximum concentration at which to consider *in vitro* findings.

***In vitro* mechanistic studies using low doses of EGCG** Many *in vitro* studies show that EGCG, at concentrations ≤20 μmol/L, inhibits growth and induces cell cycle arrest or apoptosis in a variety of cell types (Table 10). A wide range of signaling molecules is affected, including growth factor receptors [EGFR, platelet derived (PDGFR), fibroblast (FGFR), vascular endothelial (VEGFR)], survival signaling pathway components [extracellular signal regulated kinase (ERK), p38, activating protein-1 (AP-1), signal transducer and activator of transcription (STAT), PI3K, Akt, and NF-κB], cell cycle regulators [cyclin D1, p21, p27, phosphorylated retinoblastoma (pRb), cyclin-dependent kinases (cdk)2/4/6], and regulators of apoptosis [Bcl2, Bax, Bad, caspases-3/7/8/9, and poly (ADP ribose) polymerase (PARP)]. One interesting effect of EGCG at the lowest doses (0.01–0.1 μmol/L) is the inhibition of VEGF-dependent phosphorylation of the VEGFR (Table 10), an anti-angiogenesis effect which also occurs *in vivo,* as discussed later.

In vitro* effects of EGCG observed *in vivo There is substantial evidence that the effects of EGCG or green tea recorded *in vitro* have also been observed in animal models or humans. Green tea polyphenols inhibited the growth of 4T1 breast cancer cells and their metastasis to lungs in BALB/c mice. A reduction in tumor weight, increased survival time, and later tumor appearance were observed. The ratio of Bax/Bcl2 was altered in favor of apoptosis, along with a decrease in proliferating cell nuclear antigen and the activation of caspase-3[123]. The topical application of EGCG to SKH-1 hairless mice that had been pretreated twice weekly with UVB light decreased the multiplicity of skin tumors by 44%–72% and increased the apoptotic index by 56%–92%, again measured by increased caspase-3 activity[124]. Fang and colleagues, who demonstrated that the liposomal delivery of EGCG resulted in increased tumor uptake, also found that this delivery system led to increased antiproliferative activity in basal carcinoma cells *in vitro*, where the EGCG concentration in the liposomes was 21.3 μmol/L[117].

A study investigating the effect of EGCG in murine colon 26-L5 cells found that, using *in vitro* assays, 1,1-diphenyl-2-picryl-hydrazyl free radicals were reduced with an ED_{50} of 2.9 μmol/L, and cell growth was inhibited with an IC_{50} of 41.8 μmol/L. Following the injection of these colon cells into female BALB/c mice to analyze the effect on lung metastases, they found that EGCG, administered ip, reduced the number of tumor nodules in a dose-dependent manner[125].

When green tea extract (400–500 mg/cup, 5 cups/d) was administered for 4 weeks to 3 heavy smokers[126], smoking-induced DNA damage was decreased, cell growth (keratinocytes) was inhibited, and the percentage of cells in S phase was reduced, with accumulation in the G_0/G_1 phase. DNA content became less aneuploid and p53 and caspase-3 were increased. Li and colleagues found that in hamsters, 0.6% green tea inhibited DMBA-induced oral tumor number and volume, with increased apoptosis and a decreased proliferation index and microvessel density[103]. *In vitro* EGCG inhibits AP-1 transcriptional activation, and this was also observed *in vivo* in UVB-treated transgenic mice carrying a luciferase reporter gene with an AP-1 binding sequence[127].

EGCG (10 or 50 μg/mL) significantly decreased the proliferation of bovine capillary endothelial cells, and at 1–100 μg per disc, it also inhibited neovascularization in the chick chorioallantoic membrane assay[128]. These authors also showed that green tea in drinking water (1.25% containing 708 μg/mL EGCG, giving plasma levels of 0.1–0.3 μmol/L) could significantly suppress VEGF-induced corneal neovascularization. Such results suggest that EGCG may be a useful *in vivo* inhibitor of angiogenesis.

Green tea consumption in 2 study groups, 1 in China and 1 in the USA, decreased oxidative DNA damage (8-hydroxydeoxyguanosine in white blood cells and urine), lipid peroxidation (malondialdehyde in urine), and free radical generation (2,3-dihydroxy benzoic acid in urine) in smokers. Non-smokers (USA group) also exhibited a decrease in overall oxidative stress, which was correlated to decreased levels of free radicals[129].

A recent clinical trial involving 60 volunteers with (premalignant) prostate intraepithelial neoplasia, conducted by Bettuzzi *et al*, showed that after 1 year, only 1 man (3%) in the group receiving 600 mg/d green tea compounds in (oral) capsule form, presented with cancer compared to 9 (30%) from the placebo group[130]. No significant side-effects were reported. Therefore, despite the apparent poor bioavailability of green tea catechins in many studies, they appear to have great promise as chemopreventive agents.

Evidence for EGCG acting synergistically/antagonistically There are a number of reports documenting an enhanced chemopreventive effect when EGCG or green tea is used in combination with another chemopreventive agent or a therapeutic drug.

Table 10. Bioactivity of EGCG *in vitro*.

Model	Dose	Biomarkers affected	Outcome	Reference
HT29	10 μmol/L 10–20	↓ p-ERK1/2; ↓ VEGF (serum starved) ↓ VEGF reporter gene activity ; ↑ AP-1 reporter activity; ↑ cyclin D1 ↑ NF-κB reporter gene activity		238, 239
HT29 spheroids	10–20		Inhibition of spheroid formation	240
LNCaP	1–10 μmol/L 10 μmol/L 20 μmol/L– 10 μg/mL (22 μmol/L)	↑ p27; ↑ IκB-α ↓ Arachidonic acid-stimulated PGE_2 levels ↓ COX2 mRNA; ↓ PI3K (p85); ↓ p-Akt; ↑ p-ERK1/2 ↓ Chymotrypsin-like activity of 20S proteasome	↓ arachidonic acid-stimulated cell growth; G_0/G_1 arrest; Apoptosis, Growth inhibition	241-243
DU145	From 10 μg/mL (22 μmol/L)	↓ PI3K (p85); ↓ p-Akt; ↑ p-ERK1/2	Growth inhibition	243, 244
Jurkat T	From 1 μmol/L 10 5–20	↑ p27 ↓ chymotrypsin-like activity of 20S proteasome (IC_{50} 18 μmol/L)	G_0/G_1 arrest	241
CLL B	5–10 μg/mL (10-22 μmol/L) 3 μg/mL (6.5 μmol/L)	 ↑ caspase 3 and PARP cleavage; ↓ Bcl-2; ↓ XIAP; ↓ Mcl-1 ↓ VEGF-R1; ↓ VEGF-R2 phosphorylation	Apoptosis	245
Foreskin keratinocytes	10 μg/mL (22 μmol/L)	↑ involucrin promoter activity; ↑ cornification	Growth inhibition	246
HCL14	From 5.45 nmol/L	↓ UVB-induced AP-1 activity	No effect	247
HaCaT	7.5–10 μmol/L	↓ UVB-induced c-fos; ↓ UVB-induced p38 activation		248
A-375	1–5 μg/mL (0.5–10 μmol/L) 2.5–10 μg/mL (1.5–22 μmol/L) 10 μg/mL (22 μmol/L)	↓ colony formation ↑ Bax; ↓ Bcl2; ↑ Bax:Bcl2 ratio; ↑ caspase 3, 7, 9; ↑ PARP cleavage; ↑ p21; ↑ p16; ↓ cdk2 ↓ cyclin D1; ↑ p27	Growth inhibition; G_0/G_1 arrest; apoptosis	249
Hs-294T	1–5 μg/mL (0.5-10 μmol/L) 2.5–10 μg/mL (1.5–22 μmol/L)	↓ colony formation ↑ Bax; ↑ Bax:Bcl2 ratio; ↑ caspase 7; ↑ PARP cleavage ↓ cyclin D1; ↓ cdk2; ↑ p16; ↑ p27; ↑ p21	Growth inhibition; ↑S phase; apoptosis	249
JB6 (Cl 41)	1 μmol/L 5–20	↓ UVB-induced phospho-p70S6K (possible direct effect) ↓ p-Akt and PI3K activity and expression ↓ TPA-induced cell transformation; ↓ TPA-induced NF-κB activity; ↓ NF-κB DNA-binding; ↓ TPA-induced p-IκBα; ↓ AP-1–DNA binding and reporter activity		250-252

(Continue)

Model	Dose	Biomarkers affected	Outcome	Reference
A431	0.5–1 μg/mL (0.5–2 μmol/L) 10 μmol/L–10 μg/mL (22 μmol/L)	↓ tyrosine kinase activity of EGFR, PDGFR, FGFR ↓ NF-κB–DNA binding; ↓ nuclear NF-κB (p65); ↑ caspases-3,-8,-9 activation	Apoptosis	253-255
Immortalized ECE16-1	10 μmol/L 20	↓ p-ERK1/2; ↓ERK1/2 and Akt activity (direct effect); ↓ p-Bad ↑ p21; ↑ p27; ↑ p53	Growth inhibition	256
HeLa cells	1–25 μmol/L	↓ oxidative stress (↓ 8-OHdG and HMdU)		257
NBT-II	10 μmol/L 20 10–40	↓ p-Rb ↓ cyclin D1 ↓ cdk 4/6	Growth inhibition G_0/G_1 arrest Apoptosis	258
YCU-N861 YCU-H891	10 μg/mL (22 μmol/L) 0.1–1.0 μg/mL (0.2–2 μmol/L)	↑ p21; ↑ p27; ↓ cyclin D1; ↓ pRb; ↓ p-EGFR; ↓ p-ERK1/2; ↓ p-STAT3; ↓ Bcl2; ↓ $BclX_L$; ↓ Bax; ↓ p-HER2	Growth IC_{70} 10 μg/mL; G_0/G_1 arrest; apoptosis ↑ growth inhibition by 5-FU and Taxol	133, 134
NOE (normal) MSK-Leuk1 MSK-Leuk1S 1483	5.5 μmol/L 8 μmol/L 11.5 μmol/L 18 μmol/L	↓ pRb; ↑ hypo-pRb	ED_{50} 5.5 μmol/L; G_0/G_1 arrest ED_{50} 8 μmol/L; G_0/G_1 arrest ED_{50} 11.5 μmol/L; G_0/G_1 arrest ED_{50} 18 μmol/L; G_0/G_1 arrest	259
KYSE	<10 μmol/L 20 μmol/L	↓ DNA methyltransferase activity (*in vitro* assay) ↓Hypermethylation; ↑mRNA expression $p16^{INK}$, *RARβ*, *MGMT*, *hMLH1*	Growth inhibition	260
Bovine capillary cells	From 10 μg/mL (22 μmol/L)		Growth inhibition ↓ *in vitro* angiogenesis	128
BAEC	0.01–1 μmol/L	↓ VEGF-dependent phosphorylation of VEGFR-2;	↓ *in vitro* angiogenesis	261
HMVEC	2–20 μmol/L 20	↓ VEGF-stimulated VE-cadherin; ↓ *in vitro* VEGF-induced angiogenesis ↓ VEGF-stimulated p-VE-cadherin and p-Akt		262
Vascular smooth muscle cells	3 and 10 μmol/L 1,3, and 10 μmol/L	↓ Thrombin-induced activation of MMP-2 at 10 μmol/L ↓ Thrombin-induced activation of membrane-type MMP-1 at 1, 3, and 10 μmol/L	Prevent smooth muscle cell invasion (at 30 μmol/L, lower concentration not tested)	263
HEK293 cells	5 and 10 μmol/L	↓ nuclear and membrane-associated β-catenin and membrane-associated E-cadherin		264
A549 cells	10 μmol/L	↑ PGE_2		265
PC-3 cells	10 μmol/L		↓ viability	266
SH-SY5Y cells	1 μmol/L		↓ Bad	267

Suganuma *et al* found that epicatechin significantly enhanced the uptake of labeled EGCG into human lung PC-9 cells and suggested that whole green tea was a better preventive agent than EGCG alone[131]. In this study, the pro-apoptotic effects of EGCG were also increased by tamoxifen or sulindac. Another study using the prostate cancer cell lines PC-3, LNCaP, and CWR22Rv1 showed that while 10 μmol/L EGCG only resulted in a 12%–21% inhibition in cell viability, the addition of 10 μmol/L NS-398 (a COX2 inhibitor), resulted in a 44%–49% inhibition, greater than the additive effect of either agent alone. These results corresponded to decreases in Bcl-2, procaspases-6 and -9, phospho-p65 and peroxisome proliferator-activated receptor (PPAR)γ, and increases in Bax and PARP[132].

EGCG at 0.1 μg/mL (equivalent to serum concentrations) markedly enhanced the growth inhibitory effects of 5-fluorouracil in head and neck squamous carcinoma cells[133]. The IC_{50} values for 2 different cell lines were reduced by ~4-fold (sensitive line) and 45-fold (resistant line). EGCG on its own at this concentration had no effect. The same group also found that EGCG enhanced the sensitivity of HNSCC (0.1 μg/mL) and breast (1.0 μg/mL) cells to Taxol[134].

Min/+ mice treated with a combination of white tea (1.5%) and sulindac (80 ppm) had significantly fewer intestinal tumors than mice treated with either agent alone. While β-catenin and β-catenin/T cell factor-4-regulated genes, cyclin D1, and c-jun were detected in polyps, the expression of these proteins was markedly reduced in the normal intestine[135]. A combination of EGCG and sulindac was also found to be efficacious in preventing azoxymethane-induced colon cancer in rat, where the combination synergistically enhanced apoptosis[136].

Resveratrol

The phytoalexin resveratrol is found largely in grape products and peanuts, with red wine a major source of human consumption. Its potential role in disease prevention is well documented, as it exhibits vasorelaxing, anti-inflammatory, antilipidemic, anti-estrogenic, antioxidant, antifungal, and antibacterial properties[137–139].

Whilst resveratrol appears to have great potential *in vitro*, the relevance to *in vivo* effects in both humans and animals is less clear, as its chemopreventive effect *in vivo* depends greatly on its absorption, metabolism, and tissue distribution.

Bioavailability of resveratrol in humans Several studies have looked at the rate of uptake in healthy human volunteers via the oral administration of either resveratrol in its pure form or when present in foodstuffs (Table 11). Estimates of the amount of resveratrol in red wine (mainly trans), vary from 0.3 to 10.6 mg/L (1.3–46 μmol/L)[140–142]. Recent studies by Boocock *et al*[143,144] found peak resveratrol plasma concentrations of 0.3–2.4 μmol/L following single oral doses of 0.5–5 g in healthy human volunteers. Observed peak plasma concentrations for resveratrol metabolites ranged between 0.92 and 4.3 μmol/L (mono-glucuronides), and 3.7–14 μmol/L (resveratrol 3-sulfate). Plasma half-lives for the parent compound and major conjugates were of a similar order (2.9–10.6 h).

Subjects receiving a lower dose of 10–25 mg pure resveratrol/70 kg body mass were similarly found to have serum resveratrol levels between 1.83 and 2.06 μmol/L at 30 min post-dose, returning to baseline by 4 h[145]. Wang *et al*[146] found both resveratrol and resveratrol glucuronide present

Table 11. Bioavailability of resveratrol in humans following oral administration.

Source	Dose	C_{max} plasma		Clearance	Urinary excretion (% of intake)	Reference
Resveratrol	25 mg/70 kg body weight	491 ng/mL	(2 μmol/L)*	~9 h		268
White wine	25 mg/70 kg body weight resveratrol	416 μg/L	(1.82 μmol/L)		16.8	
Grape juice		416 μg/L	(1.82 μmol/L)	~4 h	16.0	145
Vegetable juice		471 μg/L	(2.06 μmol/L)		17.0	
Resveratrol	0.5–5 g	73–539 ng/mL	(0.3–2.4 μmol/L)			143, 144
Red wine	7.5 μg/kg body weight		0–26 nmol/L			269

*Figures in brackets are derived from the published data.

at up to 6 h following a dose of 1 g. It is likely that conjugated resveratrol is the main component in the circulation[147], and plasma concentrations around 2 μmol/L appear typical (Table 11).

Several major metabolites of resveratrol are found in human subjects, including the sulfate-glucuronide, monoglucuronides, and mono- and di-sulfates[143,144], with sulfation thought to occur primarily via the sulfotransferase 1A1[148] and glucuronidation via the UDP-glucuronosyltransferases[149]. The rate of glucuronidation in the human liver ranges between 0.23 and 1.2 nmol/min/mg and the rate of sulfation is 80 pmol/min per mg[141,150]. The presence of sulfated products *in vivo* may vary depending upon whether pure resveratrol is administered, as quercetin (also present in red wine) is known to inhibit its sulfation. Resveratrol glucuronide has been assumed to be pharmacologically inactive, although it has been suggested that glycosylation of polyphenols is an important step in protecting them from enzymatic oxidation, so extending their half-life and biological properties[151]. However, it is possible that the glucuronide may be converted back to resveratrol *in vivo* by the action of beta-glucuronidases[152]. The aqueous solubility of resveratrol is low, and it is thought that albumin is the main carrier in plasma, with little free resveratrol[153]. The presence of fatty acids increases binding to serum albumin, which may have important consequences for the delivery of resveratrol to cell membranes and thus signaling events.

Bioavailability of resveratrol in animals Marier *et al*[154] observed that resveratrol was absorbed within minutes following an oral dose of 50 mg/kg to rats. Resveratrol aglycone (parent compound) plasma concentrations dropped from 10 μmol/L to levels at the limit of detection by 12 h. The glucuronide, however, was present at ~100 μmol/L, falling to 3 μmol/L after 12 h, again suggesting that resveratrol is absorbed from the intestine mainly in this form. A lower dose of 20 mg/kg gave a maximal resveratrol plasma content of 3 μmol/L, falling to <0.1 μmol/L after 1 h[140], with a 5 mg/kg dose showing maximal plasma levels of 1.5 μmol/L [155].

The disposition of resveratrol revealed far higher levels in tissues involved in absorption and excretion, such as the intestine, liver, and kidney, than in plasma. In mice, the highest accumulation was in intestinal mucosa[156], brain[157], kidneys, and liver, reaching 25–30 μmol/L following a dose of 5 mg/kg[155]. The major tissue metabolites were resveratrol-3,4'-disulfate, -3,4',5-trisulfate and β-*D*-glucuronide[158]. Following a 20 mg/kg dose, the rat lung contained 0.8 nmol/g resveratrol, the mouse liver 1.03 nmol/g, and the mouse kidney 0.17 nmol/g[140].

Physiologically relevant concentrations of resveratrol Animal and human studies consistently indicate resveratrol levels of 1–2 μmol/L in plasma (Tables 11, 12). Concentrations 10–20 times higher than this have been achieved through ig or iv dosing in animal studies. An examination of tissue distribution revealed that concentrations in some tissues can be significantly higher than those in plasma. Therefore, in considering the relevance of *in vitro* studies, we have focused on those which have reported effects at concentrations of 10 μmol/L or less.

***In vitro* mechanistic studies using low doses of resveratrol** Cell culture studies with resveratrol indicate anticancer potential over a range of doses and in a wide variety of tissues, including breast, colon, pancreas, stomach, prostate, head and neck, ovary, liver, lung, and cervix[137] (Table 13). At physiological concentrations of 10 μmol/L or less, resveratrol exhibits a range of activities which modulate signal transduction. These include the downregulation of growth factors (EGF and VEGF), alterations to survival signaling (ERK, JNK, AP-1, and NF-κB), cell cycle regulators (cyclinD1, cdk4/6, p21, and p53) and apoptosis regulators (PARP, ceramide, and caspases). In U937 monocytes, concentrations as low as 0.1 μmol/L effectively inhibited the production of ROS, with the inhibition of Akt phosphorylation at 10 μmol/L [159].

In vitro* effects of resveratrol observed *in vivo Whether or not the IC_{50} values *in vitro* are achievable *in vivo* depends to some extent on the target tissue. It is likely that the highest concentration of resveratrol and its metabolites following ingestion would be achieved in colorectal mucosal tissue and in the liver. Several studies administering resveratrol via ingestion of red wine have used the mean serum antioxidant capacity as a marker of efficacy. In healthy volunteers consuming 300 mL red wine over a 30 min period, blood taken up to 2 h post-dose showed significantly raised serum antioxidant capacity[160].

MDA-MB231 xenografts in nude mice exhibited an increase in the apoptotic index and decreased angiogenesis when treated daily with 25 mg/kg resveratrol for 3 weeks, while the same cell line in the culture did not undergo apoptosis at concentrations less than 100 μmol/L[161]. Conversely, when B16M tumor cells were inoculated into mice, 20 mg/kg resveratrol did not affect tumor growth (tumor concentration of 0.04 nmol/g), even though in the culture the cells underwent 60% apoptosis following a 5 μmol/L treatment for 24 h[140]. The rats inoculated with the Yoshida ascites hepatoma receiving daily ip injections (1 mg/kg) of resveratrol exhibited decreased tumor growth due to the induction of apoptosis and a G_2/M cell cycle arrest. This effect was not seen *in vitro* using resveratrol in the range of

Table 12. Bioavailability of resveratrol in animals.

Source	Model	Dose	C_{max} plasma (μmol/L)		T_{max} plasma	Clearance	Reference
Grape juice	Rat	2 mg/kg resveratrol (ig)	1.2				270
Resveratrol	Rat	20 mg/kg (*po*)	1.2		5 min	2 h	140
	Mouse		2.6				
	Rabbit		1.1				
	Mouse	2.6 mg·kg^{-1}·d^{-1} (*po*)	0.05–0.1				
	Rabbit	20 mg/kg (iv)	38.4–47.2				
Resveratrol	Mouse	1 mmol/kg (ig)	32		10 min	1 h	156
Resveratrol	Rat	15 mg/kg (iv)	~20		0.08 h	8 h	154
		50 mg/kg (*po*)	~7			12 h	
Resveratrol (^{14}C)	Mouse	5 mg/kg (*po*)	1.5		1.5 h (earliest measured)	High urinary content by 6 h	155
Resveratrol	Rat	2 mg/kg (*po*)	0.175 mg/L (0.7 μmol/L)*				271
Resveratrol	Mouse	20 mg/kg (ip)	Resveratrol	<0.5	15 min	Metabolites cleared by 2 h	147
			Resveratrol sulfate	13 (ip); 5 (ig)			
		20 mg/kg (ig)	Resveratrol glucuronide	3 (ip); 1 (ig)			
		60 mg/kg (ig)	Resveratrol	<0.5	15 min		
			Resveratrol sulfate	300			
			Resveratrol glucuronide	175			

*Figures in brackets are derived from the published data.

15–30 μmol/L over 24 h[162]. Daily ip injections of 40 mg/kg resveratrol (estimated serum level of 25 μmol/L) reduced neuroblastoma growth in rats and increased survival by 70%. In culture, resveratrol was also cytotoxic to neuroblastoma cells in a range from 10 to 100 μmol/L [163]. Resveratrol at 100 mg/kg per day prolonged the survival time for rats with intracerebral tumors generated from RT-2 glioma cell xenografts. The IC_{50} for RT-2 cells in the culture equated to 12.8 μmol/L following a 48 h treatment, with 39% cells undergoing apoptosis at the higher concentration of 25 μmol/L[164].

A number of reports have shown that resveratrol can inhibit NF-κB activation *in vitro*. Banerjee *et al* found that in rats, 10 ppm produced striking reductions in DMBA-induced breast tumor incidence and multiplicity, while extending tumor latency[165]. They reported that resveratrol suppressed DMBA-induced COX2 and matrix metalloproteinase (MMP)-9 expressions through the downregulation of NF-κB activation.

Resveratrol treatment may also inhibit preneoplastic conditions. In both an experimentally induced model of colitis[166] and the Min/+ mouse[167], resveratrol was able to reduce damage/adenoma load and COX2 protein expression. The spontaneous development of mammary tumors in HER2/neu mice was delayed with the reduction in both size and number of tumors following resveratrol treatment[168]. In rats, azoxymethane treatment caused the formation of aberrant crypt foci, the number of which was significantly reduced in the presence of resveratrol (200 μg/kg per day for 100 d), with decreased bax and increased p21 expression in the crypts[169]. The treatment of dimethylhydrazine-induced aberrant crypt foci with resveratrol (8 mg/kg per day) resulted in a marked reduction in tumor incidence and degree of histological lesions[170]. Similarly, rats fed a diet containing 15% grape extract showed a decrease in the number and area of GST-P^{+ve} foci[171].

Table 13. Bioactivity of resveratrol *in vitro*.

Model	Dose (μmol/L)	Biomarkers affected	Outcome	Reference
LNCaP	2.5	↓ pAkt; ↓ Bcl2:Bax ratio	Growth inhibition; apoptosis; G_0/G_1 arrest.	272
	1–5		Growth inhibition; apoptosis	273
A2780/CP70	12.5	↓ HIF-1α; ↓ VEGF		274
Ishikawa	10	↓ EGF	Growth inhibition	275
MCF7	0.1–10	↓ CYP1A1 mRNA and protein		276–278
	1–10	↑ adenylate cyclase	Growth inhibition	
	1–15	↓ progesterone receptor and pS2		
	10		Increased growth	279
T47D	0.1–10	↓ CYP1A1 mRNA and protein		276, 278
	1–15	↓ Progesterone receptor; ↓ pS2	Growth inhibition	
MDA-MB231	16	PARP cleavage; ↑ ceramide	Apoptosis	280
184B5/HER	2.5–20	↓ COX2 mRNA and activity		281
BHP2–7, 18-21	1, 10	↑ activation of ERK1/2, ↑ p53	Apoptosis	282
JB6	5-20	↑ p53 activation	Growth inhibition; Apoptosis; G_0/G_1 arrest	283, 284
	10–20	↑ JNK activation; ↑ p53 phosphorylation		
HCT-116	10–20	Bax-independent	Apoptosis	163
CaSki	10	↓ MMP-9 transcription; ↓ AP-1 activation		285
BAEC	6–20		↓ migration, tube formation, ↓angiogenesis	286
A431	5–10	↑ p21; ↓ cyclin D1; ↓ cdk4, 6	Growth inhibition, Apoptosis; G_0/G_1 arrest	287
HL60	4-8	↓ caspase activation, ↑ DNA fragmentation	Apoptosis	288
MEF (p53+,p53-)	10	↑ p53 activation	Apoptosis	289
U937	0.1–20	↓ ROS production		159, 290
	5–10	↓ AP-1 activation; ↓ NF-κB activation		
	10	↓ p-Akt		
HeLa; glioma (H4); Jurkat	5–10	↓ AP-1 activation; ↓ NF-κB activation		290
BEPD2; BEAS-2B	10	↓ B[a]P-induced adducts; ↓ CYP1A1/CYP1B1 mRNA		291
Melanoma	5	↑ p53; ↑ quinone reductase 2	Growth inhibition; S- and G_2/M arrest	292
SCC-9; HEPG2	5–10	↓ hypoxia-induced HIF-1α; ↓ VEGF; ↓ pERK; ↓ pAkt		293

Evidence for resveratrol acting synergistically/antagonistically Resveratrol sensitized colon cancer cells to CD95 and the TRAIL-mediated induction of apoptosis, and at 10 μmol/L, sensitized HT29 cells to cisplatin-induced apoptosis[172]. Fulda and Debatin found that pretreatment with resveratrol cooperatively enhanced doxorubicin, cytarabine, actinomycin D, Taxol, and methotrexate-induced apoptosis and cell cycle arrest in neuroblastoma cells[173], and enhanced TRAIL-mediated apoptosis in neuroblastoma and Jurkat T cells[174]. Resveratrol (10 μmol/L) also enhanced the apoptotic effects of paclitaxel in A549, EBC-1, and Lu65 lung cancer cell lines[175], and of cisplatin and doxorubicin in OVCAR-3 and Ishikawa cells, respectively[176].

Resveratrol has been used in combination with other phytochemicals, such as beta-siterol, resulting in enhanced growth inhibition due to an arrest at both the G_1 and G_2/M phases of the cell cycle in PC-3 cells[177]. The combination of quercetin/ellagic acid with resveratrol resulted in a synergistic effect on caspase-3 activation leading to apoptosis[178]. Lee *et al*[179] found individual concentrations of resveratrol (0.5 μmol/L) or I3C (50 μmol/L) to induce the non-steroidal, anti-inflammatory, drug-activated gene (NAG)-1, a TGF-β superfamily gene associated with pro-apoptotic and anti-tumorigenic activity. However, when used in combination, the doses could be reduced to 0.025 and 1 μmol/L, respectively.

Conclusion

Plasma concentrations in humans, following normal dietary intake or administration of supplements or formulations, have been measured or can be estimated from animal studies for each of the agents reviewed. In general, achievable plasma concentrations were in the low micromolar range, although animal studies revealed the possibility of considerably higher concentrations in some tissues. In summarizing data from many cell culture studies, which have been carried out using the low concentrations achievable *in vivo*, it is apparent that all the compounds still exhibit biological activity. However, the range of activities is more limited, compared to that using a much wider dose range. While this may reflect genuine lack of activity at low doses, it is partly due to the fact that many studies have chosen to use only higher doses and shorter time points. There may therefore be many more useful preventive possibilities to be identified using lower doses.

Two very encouraging themes emerge from the data reviewed here. First, while not all *in vitro* findings are matched *in vivo*, many observations have been validated in animals or humans, giving credibility to the value of cell cultures for screening and more detailed mechanistic studies. However, some caution is required in extrapolation, as in many cases it is not known whether exactly the same signaling mechanisms are operational *in vivo*. There can also be significant discrepancies in the effective doses, even to the extent that where low levels are active *in vivo*, much higher concentrations are required to achieve the same effect *in vitro*.

Second, there is a growing body of evidence to suggest that even if single agents are inactive at low concentrations, combinations of 2 or more compounds might be much more efficacious. Combinations with chemotherapeutic drugs also offer the possibility of lowering the dose of the latter, with a consequent reduction of unwanted side-effects.

Thus, studies in cell culture have provided valuable insights into chemopreventive mechanisms of action, and there is now a need to pursue these at physiological doses and in novel combinations. One further aspect, which has not been tackled in any detail here, is the need to address more rigorously the question of tissue specificity and cancer subtype.

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