Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 802 (2004) 217–223
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Phytoalexin resveratrol attenuates the mutagenicity of the heterocyclic amines 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline

Antony Boyce [a], Johannes Doehmer [b], Nigel J. Gooderham [a,*]

[a] *Molecular Toxicology, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, London SW7 2AZ, UK*
[b] *GenPharmTox BioTech AG Fraunhoferstraße 9, D-82152 Martinsried/Planegg, Germany*

Abstract

Resveratrol is a phytoalexin, that belongs to a family of naturally occurring stilbenes. It has been reported that resveratrol can inhibit chemical carcinogenesis in experimental animals and although the mechanisms involved are unknown, an anti-mutagen mechanism has been proposed. We have explored this hypothesis using mutagenicity assays based on bacterial (*Salmonella typhimurium*) and eukaryotic cells (Chinese hamster V79 cells). We found resveratrol to be potent in both systems, blocking the mutagenicity of the food-derived heterocyclic amines (HA) 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) at micromolar concentrations. Furthermore, in cells capable of activating 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine to cytotoxic derivatives, resveratrol was able to attenuate cytotoxicity. Paradoxically, in cells lacking the ability to activate PhIP, resveratrol itself was toxic and co-incubation with PhIP reduced this toxicity. Our data confirm the potent anti-mutagenic activity of resveratrol and support its potential as a chemopreventative.

Keywords: Heterocyclic aromatic amines mutagenicity; Resveratrol; 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline

1. Introduction

A large proportion of human cancers are associated with diet [1]. Tumours of the breast in females and colon/rectum in males are those most commonly linked to dietary habits and in the Western world such cancers have an incidence second only to that of lung cancer [2]. Predisposition to colorectal tumours seems to be strongly linked to meat consumption, particularly well-cooked meat products [3]. Such foods have been found to contain a number of heterocyclic amine (HA) compounds that are genotoxic in prokaryotic and mammalian cells [4,5]. 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) are two food-derived HA pro-mutagens, each reported to account for approximately 20% of the total mutagenicity (Ames test) of cooked beef [6]. Of the two, MeIQx is the more potent bacterial mutagen but PhIP is present at significantly higher levels in cooked meats [6–8]. PhIP and MeIQx both require metabolic activation involving an initial N-oxidation to their N-hydroxy derivative by the liver specific cytochrome P4501A2 (CYP1A2) and subsequently esterification to generate their genotoxic species [9,10]. Other CYPs, including CYP1A1, CYP3A4, CYP2A3 and CYP1B1 have also been found to carry out the oxidative reaction, but generally to a much lesser extent [11,12]. The N-hydroxy derivatives undergo further bioactivation catalysed by phase II esterification enzymes (e.g. sulphotransferases and acetyl transferases) to their genotoxic products [13].

Resveratrol is a phytoalexin, that belongs to a family of naturally occurring stilbenes, and has been observed in a number of Spermatophyta, of which grapes, peanuts and pines belong. It is also a member of a group of chemicals known as the viniferins, whose role in plant physiology is to combat fungal infections [14]. Red wine contains resveratrol and is thought to constitute one of the highest concentration sources of the polyphenol in the human diet [15]. It has recently been reported that resveratrol inhibits

* Corresponding author. Tel.: +44-20-7594-3188; fax: +44-20-7594-3050.
E-mail address: n.gooderham@ic.ac.uk (N.J. Gooderham).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.10.057

the carcinogenesis process at the initiation, promotion and progression stages and decreases tumour growth in a rat model [14]. Furthermore, red wine based supplements have been shown to delay the onset of tumours in transgenic mice [16]. This may be in part due to its anti-oxidant activity [14] and in part due to its inhibitory actions on the cell cycle [17,18].

In this investigation we set out to explore the hypothesis that resveratrol was anti-mutagenic and examined the effects of the compound on the cytotoxicity and mutagenicity of the cooked-meat derived HA's, MeIQx and PhIP. Since MeIQx is a powerful bacterial mutagen, we examined the effects of resveratrol using the *Salmonella typhimurium* mutagenicity assay and since PhIP is a potent mammalian cell mutagen, we used a Chinese hamster V79 cell based mutagenicity assay with *hprt* gene as target.

2. Experimental

2.1. Chemicals

PhIP, MeIQx and 3,8-dimethyl-2-nitroimidazo[4,5-*f*]quinoxaline (nitro-MeIQx) were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum were from Gibco BRL (Paisley, UK). All other reagents were from Sigma Chemical Co. (Poole, UK).

2.2. Ames test

The Ames *S. typhimurium* bacterial mutagenicity test was used as described previously [10]. Nitro-MeIQx was used as a direct acting positive control. In some experiments metabolic activation was employed using mouse liver microsomes or rat liver S9 and an NADPH regenerating system. To investigate the effect of resveratrol on the growth of bacteria, resveratrol (1–1000 μM) was incubated with a suspension of *S. typhimurium* TA98 (10^9 cells/ml) at 37 °C. Cell growth was estimated by measuring the absorbance at 660 nm.

2.3. Cell lines

The Chinese hamster fibroblast V79 cell line (V79MZ) and the genetically engineered variants with stable expression of human CYP1A2 and 1B1 (V79MZh1A2 and V79MZh1B1, respectively) were as previously described [19]. The V79MZ and V79h1A2 cell lines were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin. The V791B1 cell line was cultured in high glucose DMEM supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. All cell lines were maintained at 37 °C in a humid atmosphere with 5% carbon dioxide 95% air.

2.4. Treatment of cells

Cells were maintained in exponential growth, in Dulbecco-Vogt's modified Eagle medium supplemented with HAT (5×10^{-5} M hypoxanthine, 4×10^{-7} M aminopterine and 5×10^{-6} M thymidine) to reduce the background *hprt*$^-$ mutation rate. When using engineered cell lines, G148 solution (5 μg/ml) was added to the medium to select for cells containing the transfected plasmid. Twenty-four hours before treatment, 1.5×10^6 cells in exponential growth were seeded into 75 cm^2 tissue culture flasks in fresh culture medium. Following this the cells were exposed to PhIP, resveratrol or a combination of the two (prepared in anhydrous dimethyl sulphoxide [DMSO] to give a final DMSO concentration of <0.5%); cells treated with vehicle only (DMSO) were used as control. At the end of the treatment (after 24 h), the cells were washed with Hank's balanced salt solution twice and with phosphate buffered saline (PBS) once, trypsinised, counted, and split into two groups for assessment of colony forming ability and mutation.

2.5. V79 Cell survival (colony forming ability)

Cells were seeded at a density of 100 cells per well in 5 ml of fresh medium (DMEM) and incubated as before at 37 °C for 7–10 days after which the medium was discarded and the wells flooded with methylene blue in 50% methanol to stain cells. Colonies that contained more than 50 cells were counted and scored as survivors. The cloning efficiency for the cells from the DMSO group ranged from 50 to 70%.

2.6. V79 Cell mutagenicity studies

Cells were seeded in to 25 cm^2 tissue culture plates containing fresh medium (DMEM) and maintained in exponential growth for 7 days to facilitate phenotypic expression of *hprt*$^-$ mutants. After 7 days, cells were trypsinised, counted and seeded at 250,000 cells/10 cm petri dish (a minimum of 10^6 cells per treatment), in fresh medium containing 6-thioguanine (6-TG, 5 μg/ml). Resistance to the lethal effect of 6-TG was used to select for *hprt*$^-$ mutant clones. After 2 weeks growth, the medium was discarded and the petri dishes were flooded with methylene blue (in 50% methanol) and stained colonies that contained more than 50 cells were scored as mutants.

3. Results

3.1. Effect of resveratrol on MeIQx mediated bacterial mutagenicity using S. typhimurium TA98

Resveratrol showed a clear dose-dependent inhibition of liver mediated MeIQx (10 ng per plate) induced mutation (Fig. 1). The absolute number of histidine independent *S. typhimurium* mutant colonies was much higher using

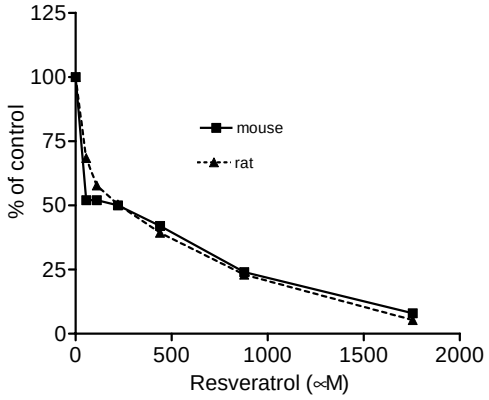

Fig. 1. Effect of resveratrol on the mutagenicity of MeIQx (10 ng/plate) in *S. typhimurium* TA98. The values are means of two separate experiments and are expressed as percent of control (no resveratrol). Using rat liver S9, the MeIQx (10 ng/plate) control generated an average of 262 mutant colonies. Using mouse liver microsomal protein, the MeIQx (10 ng/plate) control generated an average of 1036 mutant colonies. At all concentrations tested, resveratrol on its own failed to induce mutation above the background spontaneous rate (<20 revertant colonies per plate). In the absence of metabolic activation, MeIQx failed to induce mutation (<20 revertant colonies per plate) whereas the direct acting mutagen nitro-MeIQx (10 ng/plate) induced 1808 ± 56 revertant colonies per plate.

mouse liver microsomal activation compared to the rat liver S9. This is consistent with previous studies with MeIQx in our laboratory. Irrespective of the source of activation, at 100 μM resveratrol, inhibition of MeIQx induced mutation was approximately 40% of the control value (Fig. 1). In the absence of a metabolic activation system, MeIQx failed to induce mutation (<20 revertant colonies per plate), whereas the direct acting nitro-MeIQx (10 ng/plate) generated 1808 ± 38 revertant colonies per plate. On its own, resveratrol, in the presence or absence of a metabolic activation system, failed to induce the mutation frequency above background (∼20 revertant colonies per plate). At high concentrations of resveratrol (>1 mM), inhibition of activated MeIQx mutation was >75%. This effect at high concentration prompted us to examine the effect of resveratrol on bacterial cell growth. Growth curves with *S. typhimurium* confirmed that at doses of resveratrol >1 mM, growth was almost completely inhibited, whilst at concentrations of up to 100 μM, the effect on growth was minimal (Fig. 2).

3.2. Effect of resveratrol on growth of V79 cells

Fig. 3 shows the effect of resveratrol on the growth of V79h1A2 cells. The cells were maintained in the presence of resveratrol for 3 days, then growth was assessed by counting cell density. At the highest concentration of resveratrol used (1000 μM resveratrol), the cell count was massively reduced, compared to the DMSO control. Inhibition of growth was also noted at the 10 and 100 μM resveratrol concentrations, which after 3 days dropped to 50 and 12% of control, respectively. We therefore used 10 μM resveratrol concentrations in subsequent experiments.

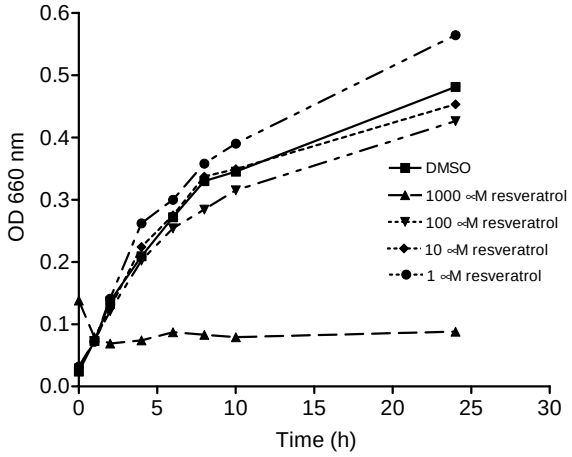

Fig. 2. Effect of resveratrol on the growth of *S. typhimurium* TA98.

3.3. Effect of resveratrol on PhIP-induced cytotoxicity in mammalian cells

The effect of resveratrol on PhIP toxicity is shown in Fig. 4. The food-derived HAs require metabolic activation before they form genotoxic and cytotoxic derivatives, thus, the cytotoxicity of PhIP to the V79h1A2 cells was not unexpected (Fig. 4a). The effect of PhIP was dose-dependent with colony survival inhibited by over 50% (of control value) by 50 μM PhIP, increasing to 70% with 100 μM PhIP. Interestingly, the potent cytotoxicity of the positive control, EMS (100 μM) was apparently unaffected by resveratrol. In contrast, when PhIP was incubated with the metabolically non-competent V79MZ cells, there was no significant reduction in colony survival at PhIP concentrations up to 100 μM, confirming that in the absence of metabolic activation, PhIP was not cytotoxic (Fig. 4c). Exposing cells to resveratrol (10 μM) for 24 h had little effect on V79h1A2 cell viability, as measured in the clonogenicity assay (Fig. 4a). Yet co-incubation with cytotoxic concentrations

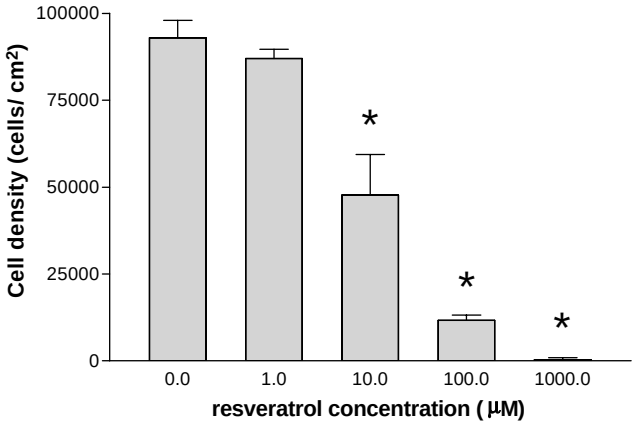

Fig. 3. The growth of V79h1A2 cells cultured in the presence resveratrol for 3 days. Values are the mean ± S.E.M., $n = 3$ separate experiments. *$P < 0.05$ when compared to DMSO control.

of PhIP significantly prevented PhIP-mediated toxicity (Fig. 4a). Paradoxically, incubations with resveratrol for 24 h was apparently toxic to V79MZ cells (Fig. 4c), and yet when co-incubated with PhIP, the toxic effect was reduced (Fig. 4c). Indeed at 100 μM PhIP/10 μM resveratrol, colony survival was significantly enhanced in the V79MZ cell line (Fig. 4c). Thus, in V79MZ and V79h1A2 cells, resveratrol and PhIP appear to mutually antagonise toxicity.

In some experiments we used V79h1B1 cells. These have been engineered to express human CYP1B1. Like CYP1A2, it is claimed that CYP1B1 can both activate and detoxify PhIP by competing metabolic pathways [21,22]. In our experiments there was no cytotoxicity noted in the V79h1B1 cells incubated with PhIP for 24 h (Fig. 4b). Even at concentrations as high as 100 μM, where a 70% reduction in colony survival was observed in V79h1A2 cells, there was no evidence of toxicity in V79h1B1 cells. On its own, 10 μM resveratrol had no significant effect on V79h1B1 colony survival, however when PhIP (10/100 μM) and

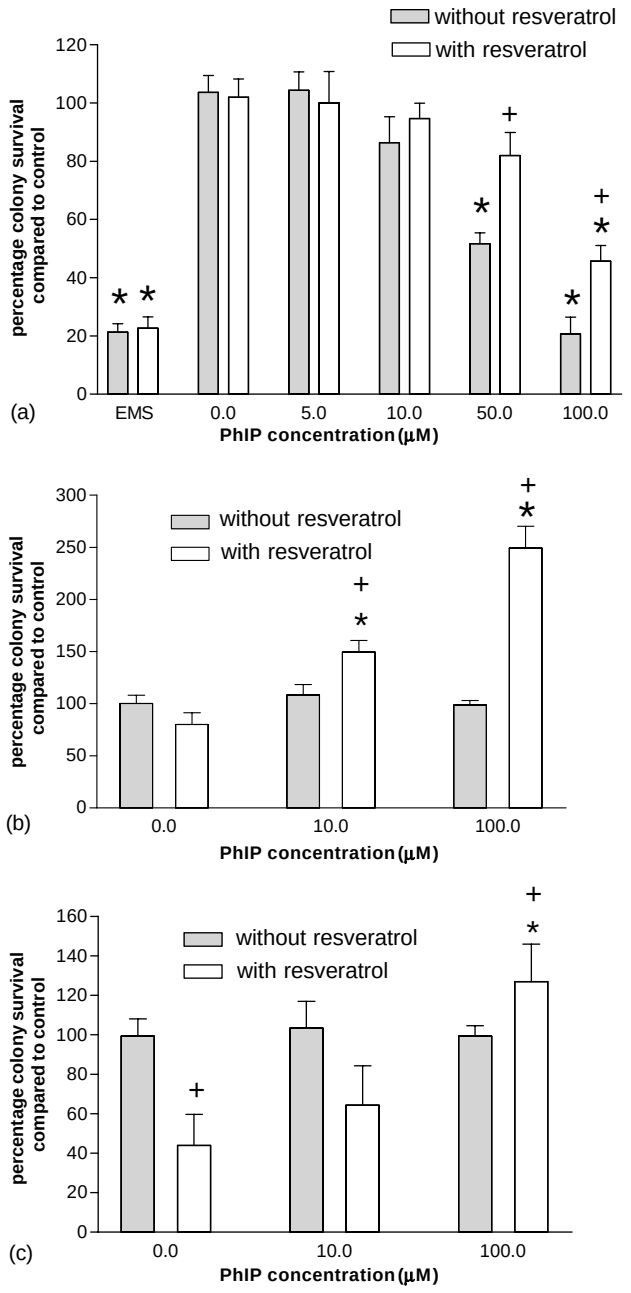

Fig. 4. Colony survival of V79 cells after treatment with PhIP or PhIP and resveratrol (10 μM) for 24 h at 37 °C. (a) V79h1A2 cells, (b) V79h1B1 cells and (c) V79MZ cells. The cells were plated immediately after treatment and colonies scored after 7 days as described in chapter 3 section. Bars are standard deviation for three separate experiments each performed in triplicate. $*P < 0.05$ when compared to no PhIP control. $+P < 0.05$ when compared to PhIP alone.

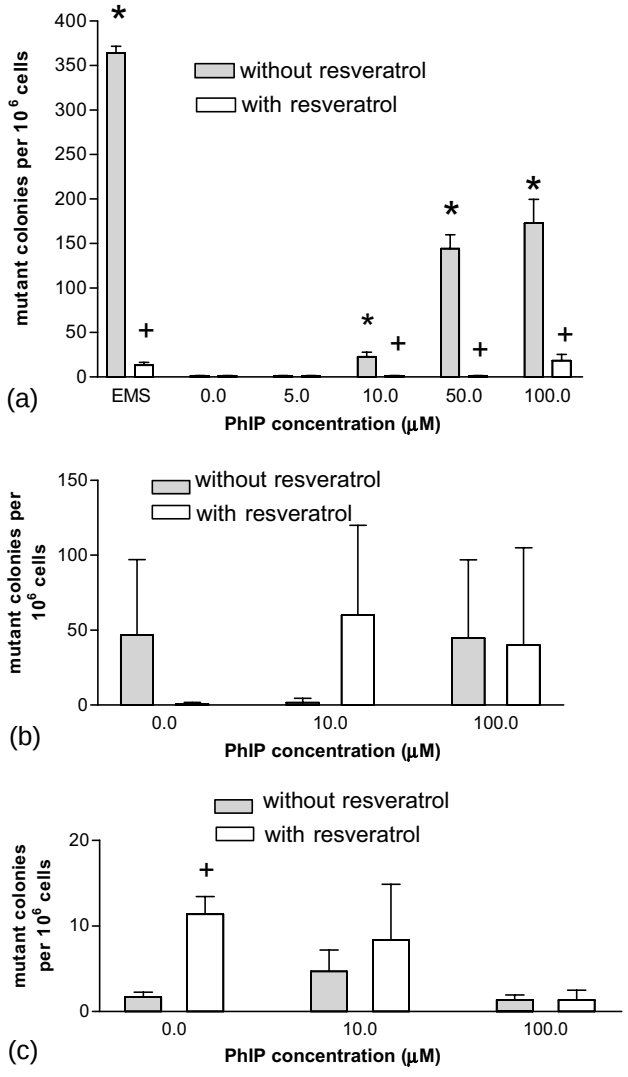

Fig. 5. Induction of mutation to 6-thioguanine resistance in V79 cells treated with PhIP or PhIP and resveratrol (10 μM) for 24 h at 37 °C. (a) V79h1A2 cells, (b) V79h1B1 cells and (c) V79MZ cells. Frequencies of 6-TG mutants are corrected for the background mutant frequency. Bars are standard deviation for three separate experiments each performed in triplicate. $*P < 0.05$ when compared to no PhIP control. $+P < 0.05$ when compared to PhIP alone.

resveratrol (10 μM) were incubated together colony survival increased significantly (2.5-fold) over the control value (Fig. 4b).

3.4. Effect of resveratrol on PhIP-induced mutagenicity in mammalian cells

The positive control, EMS, is a direct acting mutagen that showed a powerful mutagenic response (Fig. 5a). Like PhIP-induced cytotoxicity, PhIP-induced mutation was dependent on a functional activation system. Dose-dependent genotoxicity was evident when PhIP (5–10 μM) was incubated with V79h1A2 cells (Fig. 5a), presumably due to oxidation of the HA to its genotoxic N^2-OH-derivative by the human CYP1A2. In the absence of functional CYP1A2 (V79MZ cells, Fig. 5c), PhIP itself was not mutagenic.

As previously indicated, CYP1B1 has been reported to be able to metabolise PhIP to both its N^2-OH-PhIP genotoxic derivative and also to the 4-OH detoxication product. Surprisingly however, when PhIP was incubated with V79h1B1 cells expressing the human form of CYP1B1, a mutagenic response was not observed (Fig. 5b). These results suggest that either this cell line had a poor ability to bioactivate PhIP to toxic species or alternatively that the cell line may have had a more efficient detoxication activity (4-OH PhIP derivative), or a combination of the two.

On its own, resveratrol did not induce mutation in V79h1A2 nor in V79h1B1 cells, although a marginal effect was apparently found with the V79MZ cells (Fig. 5c). More importantly, treatment of V79h1A2 cells with 10 μM resveratrol appeared to promote extensive inhibition of mutation throughout the PhIP concentration range (5–100 μM), suggesting that the compound possesses potent anti-mutagenic activity. Remarkably, in contrast to the lack of effect of resveratrol on EMS cytotoxicity, the compound was extremely effective at inhibiting the potent mutagenicity of EMS (Fig. 5a).

4. Discussion

It has previously been shown that resveratrol can suppress the induction of mutation by 3-amino-1, 4-dimethyl-5H-pyridol[4,3-b]indole (Trp-P-1) in the Ames assay using *S. typhimurium* TA98 [22] and we now show that the phytoalexin can also inhibit MeIQx mediated mutation of *S. typhimurium* TA98. The present mammalian cell experiments extend the established anti-mutagenic effect of resveratrol to eukaryotic systems, however the mechanism of inhibition is still unclear. Conventional mutation tests like those outlined above cannot distinguish between chemicals that inhibit DNA-adduct formation (blocking agents) and those that suppress mutant colony promotion (suppressing agents). However in toxicity studies with *S. typhimurium* TA98, we determined that only high resveratrol concentrations (1000 μM), inhibited bacterial growth. This suggests that the inhibition of mutation observed in the bacterial cells at concentrations <100 μM was not an inhibitory action on cell growth but an attenuation of the mutation process.

Previous reports have shown that the heterocyclic amines are well absorbed and bioavailable in humans [23–25], and oxidation of the compounds by human CYP1A2 can efficiently produce N-hydroxy derivatives. Subsequent metabolic esterification generates potent DNA damaging species [13]. In this study we used three Chinese hamster V79 cell lines, one of which was metabolically incompetent (V79MZ), one that stably expressed transfected human CYP1A2 (V79h1A2) and the other expressed human CYP1B1 (V79h1B1). Both of these enzymes have been shown to bioactivate the heterocyclic amines including PhIP [20]. Of the three lines used, V79h1A2 were the most susceptible to the cytotoxic and genotoxic effects of PhIP. This confirmed the requirement to metabolically activate PhIP to toxic species while the mutagenic potency of PhIP in these cells was consistent with the notion that human CYP1A2 was particularly efficient at catalysing these reactions.

In the V79h1A2 cell line there was a dose-response relationship for both decreased colony survival and increasing mutagenicity due to PhIP, consistent with the report by Yadollahi-Farsani et al., [5]. The mechanism of PhIP induced cytotoxicity is not clear from these experiments but may be explained by the ability of PhIP to accumulate genetic damage during S-phase arrest of the cell and induce apoptosis [26,27]. Similar biochemical effects have also been observed in colonic epithelium in vivo [28]. It has been suggested that there may be a correlation between the potential for the induction of apoptosis and induction of carcinogenesis. Because there is abundant evidence showing that PhIP is a powerful carcinogen in animal models, the apoptotic pathway may be central to the cytotoxic effects of the heterocyclic amines.

In contrast to the V79h1A2 cells, colony forming ability was not compromised when high concentrations of PhIP (100 μM) were incubated with V79h1B1 cells. Despite reports suggesting that CYP1B1 can activate PhIP to toxic derivatives [21] our data show that the efficiency of the V79h1B1 cells to activate PhIP was low, or the detoxication pathway to 4-hydroxy PhIP (also catalysed by CYP1B1) predominated.

The apparent selective inhibition of colony survival induced by resveratrol in V79MZ and V79h1B1 cells contrasted to the lack of effect with V79h1A2 cells. It may be possible that CYP1A2 was metabolising resveratrol to non-toxic species, however several lines of evidence suggest that this is not the case. Resveratrol has a poor affinity for the human form of CYP1A2 (IC50 = 1.2 mM) [29]. Furthermore, the cytotoxicity induced by resveratrol in gingival epithelial S–G cells was not altered by the addition of aroclor-induced rat hepatic S9 microsomal fractions to incubates [30], suggesting that CYP enzymes have no

effect on the biological properties of the compound. Interestingly prolonged incubation of V79h1A2 cells with 10 μM resveratrol for three days produced a decrease in growth suggesting that resveratrol cytotoxicity may require time to emerge. Additionally, it should be noted that resveratrol itself can be oxidatively metabolised to hydroxylated derivatives, catalysed by CYP1B1 [31]. Furthermore, hydroxy stilbenes (including resveratrol) have also been found to be inhibitors of CYP1B1, in the case of resveratrol, the Ki is 23 μM [32]. Although the dose of resveratrol used in the present study was less (10 μM), it is possible that CYP1B1 mediated metabolism was altered and this influenced the survival of the CYP1B1 expressing cells.

High concentrations of resveratrol can inhibit cellular proliferation. This anti-proliferative property appears to maintain a degree of selectivity for malignant cell lines and it has been suggested that this may be due, in part, to its ability to inhibit DNA polymerase and ornithine decarboxylase, an enzyme enhanced in many tumour growths which is involved in polyamine biosynthesis [33].

Resveratrol has been also shown to inhibit cycloxygenase-2 (Cox-2) [14,18]. Cox-2 is not expressed constitutively in normal tissue, but like other early response gene products it can be rapidly induced. Induction of Cox-2 has been observed in many different types of tumours and transformed cells. Inappropriate up-regulation of Cox-2 is thought to prolong the survival of transformed cells, promote tumour growth and help lead to physical changes in cells that have oncogenic potential. The exact biochemical regulation of Cox-2 is not well understood, however it is thought that NF-κβ may play an important role. The nuclear transcription factor, NF-κβ, is one of the most ubiquitous eukaryotic transcription factors, and has the ability to regulate gene expression involved in the proliferation and growth of cells, inflammation responses and many more biological processes, such as positive regulation of Cox-2 [34]. There is evidence to suggest that resveratrol can inhibit the activity of NF-κβ by attenuating IKK activity, a key regulator of NF-κβ activity [35].

The inability of V79MZ and V79h1B1 cells to metabolise PhIP to genotoxic derivatives was apparent from the mutation studies. In contrast, V79h1A2 cells were very responsive to PhIP treatment, showing increased mutation in a dose-dependent manner. Despite the potency of the genotoxic effect of PhIP in this cell line, resveratrol almost completely attenuated mutation throughout the range of PhIP concentrations employed (0–100 μM). This anti-mutagenic effect was also extended to the positive control EMS. Collectively, our bacterial and eukaryote cell experiments emphasise that resveratrol has the capability to inhibit mutation induced by powerful DNA-reactive chemicals such as MeIQx, PhIP and EMS.

It is likely that multiple mechanisms, including blocking the formation of DNA-adducts and by inhibiting the cell cycle are involved in the anti-mutagenic effects of resveratrol. As such mechanisms are central to the tumorigenesis process, studies that investigate the potential of resveratrol to act as a chemoprotective are warranted.

Acknowledgements

Supported by the UK Food Standards Agency, contract T01009.

References

[1] R. Doll, R. Peto, J. Natl. Cancer Inst. 66 (1981) 1191.
[2] WCRF, Food, Nutrition and the Prevention of Cancer: A Global Perspective, AICR, Washington, USA, 1997.
[3] M. Gerhardsson de Verdier, U. Hagman, R.K. Peters, G. Steineck, E. Overvick, Int. J. Cancer 49 (1991) 520.
[4] T. Sugimura, S. Sato, Cancer Res. 43 (1983) 2415.
[5] M. Yadollahi-Farsani, N.J. Gooderham, D.S. Davies, A.R. Boobis, Carcinogenesis 17 (1996) 617.
[6] J.S. Felton, M.G. Knize, N.H. Shen, B.D. Andresen, L.F. Bjeldanes, F. Hatch, Environ. Health Perspect. 67 (1986) 17.
[7] J.S. Felton, M.G. Knize, Mutat. Res. 259 (1991) 205.
[8] S. Murray, A.M. Lynch, M.G. Knize, N.J. Gooderham, J. Chromatogr. 616 (1993) 211.
[9] K. Rich, B.P. Murray, I. Lewis, N. Rendell, D.S. Davies, N.J. Gooderham, A.R. Boobis, Carcinogenesis 13 (1992) 2221.
[10] K. Zhao, S. Murray, D.S. Davies, A.R. Boobis, N.J. Gooderham, Carcinogenesis 15 (1994) 1285.
[11] M.A. Butler, M. Iwasaki, F.P. Guengerich, F.F. Kadlubar, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 7696.
[12] H. Yamazaki, Y. Inui, S.A. Wrighton, F.P. Guengerich, T. Shimada, Carcinogenesis 16 (1995) 2167.
[13] M.H. Buonarati, K.W. Turteltaub, N.H. Shen, J.S. Felton, Mutat. Res. 245 (1990) 185.
[14] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, H. Beecher, H. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon, J.M. Pezzuto, Science 275 (1997) 218.
[15] R. Lu, R.G. Serrero, J. Cell. Phys. 304 (1999) 297.
[16] A.J. Clifford, S.E. Ebeler, J.D. Ebeler, N.D. Bills, S.H. Hinrichs, P.L. Teissedre, A.L. Waterhouse, Am. J. Clin. Nutr. 64 (1996) 748.
[17] D.S. Jang, B.S. Kang, S.Y. Ryu, I.M. Chang, K.R. Min, Y. Kim, Biochem. Pharmacol. 57 (1999) 705.
[18] K. Subbaramaiah, W.J. Chung, P. Michaluart, N. Telang, T. Tanabe, H. Inue, M. Jang, J.M. Pezzuto, A.J. Dannenberg, J. Biol. Chem. 273 (1998) 21875.
[19] A. Luch, W. Schober, V.J. Soballa, G. Raab, H. Greim, J. Jacob, J. Doehmer, A. Seidel, Chem. Res. Toxicol. 12 (1999) 353.
[20] F.G. Crofts, P.T. Strickland, T.R. Sutter, Carcinogenesis 18 (1997) 1793.
[21] F.G. Crofts, T.R. Sutter, P.T. Stickland, Carcinogenesis 19 (1998) 1969.
[22] F. Uenobe, S. Nakamura, M. Miyazawa, Mutat. Res. 373 (1997) 197.
[23] N.J. Gooderham, S. Murray, J.C. Rice, A.R. Boobis, D.S. Davies, Drug Metab. Rev. 20 (1989) 285.
[24] A.M. Lynch, M.G. Knize, A.R. Boobis, N.J. Gooderham, D.S. Davies, S. Murray, Cancer Res. 52 (1992) 6216.
[25] H. Ushiyama, K. Wakabayashi, M. Hirose, H. Itoh, T. Sugimura, M. Nagao, Carcinogenesis 12 (1991) 1417.
[26] H. Zhu, A.R. Boobis, N.J. Gooderham, Cancer Res. 60 (2000) 1283.
[27] R. Duc, P.M. Leong-Morgenthaler, Mutat. Res. 486 (2001) 155.
[28] Y. Hirose, S.N. Sugie, N. Yoshimi, K. Matsunaga, A. Hara, H. Mori, Cancer Lett. 123 (1998) 167.

[29] Y.J. Chun, M.Y. Kim, F.P. Guengerich, Biochem. Biophys. Res. Commun. 262 (1999) 20.
[30] H. Babich, A.G. Reisbaum, H.L. Zuckerbraun, Toxicol. Lett. 114 (2000) 143.
[31] G.A. Potter, L.H. Patterson, E. Wanogho, P.J. Perry, P.C. Butler, T. Ijaz, K.C. Ruparelia, J.H. Lamb, P. Farmer, L.A. Stanley, M.D. Burke, Br. J. Cancer 86 (2002) 774.
[32] F.P. Guengerich, Y.-J. Chun, D. Kim, E.M.J. Gillam, T. Shimada, Mutat. Res. 523–534 (2003) 173.
[33] N.J. Sun, S.H. Woo, J.M. Cassady, R.M. Snapka, J. Nat. Prod. 61 (1998) 362.
[34] M. Jang, J.M. Pezzuto, Drugs Exp. Clin. Res. 25 (1999) 65.
[35] M. Holmes-McNary, A.S. Baldwin Jr., Cancer Res. 60 (2000) 3477.