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Modulation by (iso)flavonoids of the ATPase activity of the multidrug resistance protein

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Abstract The multidrug resistance protein (MRP) is an ATP-dependent transport protein for organic anions, as well as neutral or positively charged anticancer agents. In this study we report that dinitrophenyl-*S*-glutathione increases ATPase activity in plasma membrane vesicles prepared from the MRP-overexpressing cell line GLC4/ADR. This ATPase stimulation parallels the uptake of DNP-SG in these vesicles. We also show that the (iso)flavonoids genistein, kaempferol and flavopiridol stimulate the ATPase activity of GLC4/ADR membranes, whereas genistin has no effect. The present data are consistent with the hypothesis that certain (iso)flavonoids affect MRP-mediated transport of anticancer drugs by a direct interaction with MRP.

Key words: Multidrug resistance; Multidrug resistance protein; (Iso)flavonoids; Flavopiridol; Dinitrophenyl-*S*-glutathione; ATPase

1. Introduction

A major problem in the treatment of cancer is the occurrence of cellular resistance to cytotoxic drugs. Cancer cells can be simultaneously resistant to a wide spectrum of natural agents, such as anthracyclines and vinca alkaloids. One form of resistance is caused by overexpression of the drug transporter P-glycoprotein (Pgp), which belongs to the superfamily of ATP binding cassette (ABC) transporter proteins [1]. Characteristic of these proteins are their nucleotide bindings domains (NBD), which are essential for ATP binding and hydrolysis. Another ABC transporter causing multidrug resistance (MDR) is the multidrug resistance protein (MRP), which has been cloned from a drug-resistant small-cell lung cancer line [2]. MRP appears to mediate the transport of a broad range of drugs across cellular membranes. On the one hand, it was concluded from the increased cellular drug efflux from MRP-overexpressing or transfected tumour cell lines that MRP functions as a transporter of the typical 'MDR' anticancer agents [3,4]. On the other hand, the observation that the efflux of anticancer drugs from MRP-overexpressing cells was glutathione-dependent [5,6] suggested that MRP is a transporter of anionic compounds and/or glutathione-conjugated drugs [7,8]. Important evidence for the identification of MRP as a transporter of multiple organic anions (MOAT) came from experiments showing ATP-dependent uptake of glutathione conjugates, such as leukotriene C4, into inside-out plasma membrane vesicles prepared from MRP-overexpressing cells [7,9].

In recent years much effort has been directed at the identification of transport-modulating agents in order to circumvent Pgp- and MRP-mediated drug resistance. Among others, several members of one group of natural compounds, the so-called (iso)flavonoids, were found to be able to modulate Pgp- and MRP-mediated drug transport in tumour cell lines [10,11]. These compounds are important constituents of our daily food, since they are metabolic products of many plant species, such as vegetables, red wine and tea [12]. Several biologically important activities of (iso)flavonoids are phosphotyrosine kinase inhibition [13–15], topoisomerase II inhibition [16], and radical scavenging [17,18]. The (iso)flavonoid genistein was shown to inhibit the efflux of daunorubicin from small-cell lung cancer GLC_4/ADR cells [11] in a competitive way [19], although it was not investigated whether genistein itself was transported by MRP. Recently a competitive inhibition of daunorubicin transport by genistein was also shown, using plasma membrane vesicles from the MRP-transfected NIH 3T3 cells [20]. Because of broad interest in (iso)flavonoids, more insight into the interaction of these compounds with MRP is important.

In the present work we have used plasma membrane vesicles prepared from GLC_4/ADR cells to demonstrate that the transport of the MRP substrate dinitrophenyl-*S*-glutathione (DNP-SG) into these vesicles is associated with an increase in ATPase activity. Subsequently, we show that the MRP-related ATPase activity is stimulated by several (iso)flavonoids.

2. Materials and methods

2.1. Chemicals

[^{3}H]GSH was purchased from New England Nuclear (Dreieich, Germany). Dithiothreitol (Sigma, St. Louis, MO) was separated from [^{3}H]GSH according to Butler et al. [21]. [^{3}H]DNP-SG was synthesised as described earlier [23]. 1-Chloro-2,4-dinitrobenzene (CDNB) was from Sigma (St. Louis, MO). The flavonoid kaempferol, the isoflavonoid genistin, malachite green base, ammonium molybdate, Triton N101, benzbromarone and ATP were from Sigma (St. Louis, MO). The isoflavonoid genistein was from ICN Biomedicals (Zoetermeer, The Netherlands). The synthetic flavone derivative flavopiridol (L86-8275) (−)*cis*-5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl)-piperidinyl]-4H-benzopyran-4-one was kindly provided by Dr. Sedlacek and Dr. Czech (Behringwerke A.G., Marburg, Germany). The (iso)flavonoids were dissolved as a stock solution of 40 mM in DMSO (Across Chimica, Belgium) and stored at -20°C. Before experiments 1:1 dilutions were made in ethanol, followed by a dilution in ethanol/water (1:3, v/v) to the appropriate concentrations. The maximal concentration of DMSO and ethanol was 0.5% (v/v), which was also added in the controls. Membrane filters OE67 were from Schleicher and Schuell (Dassel, Germany).

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Abbreviations: DMSO, dimethyl sulfoxide; DNP-SG, dinitrophenyl-*S*-glutathione; GSH, glutathione; GSSG, oxidised glutathione; MDR, multidrug resistance; MOAT, multiple organic anions transporter; MRP, multidrug resistance protein; Pgp, P-glycoprotein

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Vinblastine was from Eli Lilly Nederland B.V. (Nieuwegein, The Netherlands). Doxorubicine hydrochloride was from Laboratoire Roger Bellon (France).

2.2. Cells

The human small-cell lung cancer cell line GLC_4 and its adriamycin-selected MRP-overexpressing subline GLC_4/ADR [22] were cultured in RPMI medium (Flow Labs., Irvine, Scotland, UK), supplemented with 7.5% fetal calf serum (Gibco, Paisley, UK). GLC_4/ADR were cultured in the presence of 1.2 μM doxorubicin until 7–14 days before experiments.

2.3. Plasma membrane vesicles

Plasma membrane vesicles were prepared from parental GLC_4 and drug-resistant GLC_4/ADR cells as described [23]. Cells were harvested by centrifugation (275×*g*, 5 min) and washed twice in phosphate-buffered saline. Subsequently, the cells were incubated in 100 mM KCl, 5 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride and 50 mM HEPES/KOH (pH 7.4) for 1 h at 0°C, in order to allow the cells to swell. Then the cells were ultrasonicated at 20% of the maximum power of an MSE sonicator, Soniprep 150, for 3 bursts of 15 s. The suspension was centrifuged (1500×*g*, 10 min). The post-nuclear supernatant was layered on top of a 46% sucrose cushion. After centrifugation (100000×*g*, 60 min) the interface was removed and washed in 100 mM KCl/50 mM HEPES/5 mM $MgCl_2$ buffer (pH 7.4). The final membrane preparations were stored at −80°C at a protein concentration of ~4 mg/ml. The enrichment of Na^+K^+-ATPase was about 5-fold [23].

2.4. Uptake of substrates into vesicles

Uptake of labelled substrates into vesicles was measured by rapid filtration as previously described [24]. Vesicles were incubated in KCl/HEPES buffer (pH 7.4) at 37°C (protein concentration ~0.25 mg/ml), in the presence of 10 mM $MgCl_2$ with or without 1 mM ATP and tritium labelled DNP-SG (1.6 KBq/pmol, 32 Bq/μl). The reaction was stopped by adding ice-cold KCl/HEPES buffer. After rapid filtration, the filters were washed twice with KCl/HEPES buffer. Radioactivity on the filters was measured using liquid scintillation counting.

2.5. ATPase activity determinations

To measure the rate of ATPase activity in plasma membrane fractions we used a colorimetric method to estimate amounts of inorganic phosphate [25] with some modifications. Plasma membranes were incubated in KCl/HEPES buffer (pH 7.4) at 37°C, in the presence of 1 mM EGTA, 1 mM sodium azide and 0.1 mM ouabain. When indicated, 1 mM ATP was included. Reactions were stopped by addition of the colour reagent (0.034% w/v malachite green base, 1.05% w/v ammonium molybdate and 0.025% v/v Triton N101). After 1 min, sodium citrate was added to a final concentration of 3.6% (w/v), in order to prevent ATP hydrolysis after having stopped the enzymatic reaction. Light absorption was measured in an ELISA reader at a wavelength of 595 nm. No ATP hydrolysis was seen without the addition of membrane vesicles.

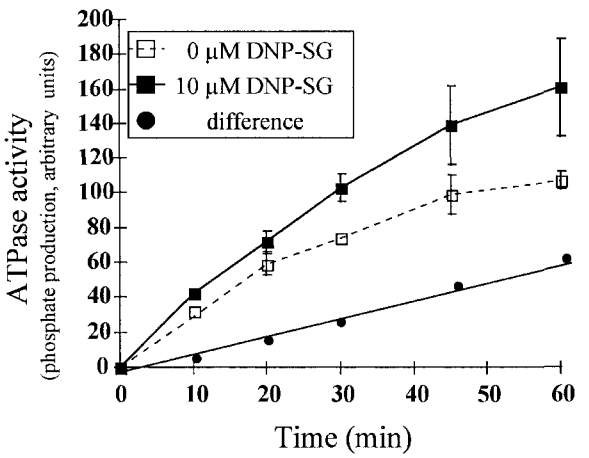

Fig. 1. ATPase activity of GLC_4/ADR membranes in time. Phosphate production in the presence or absence of DNP-SG is depicted. Difference between 10 and 0 μM DNP-SG was also calculated (*n*).

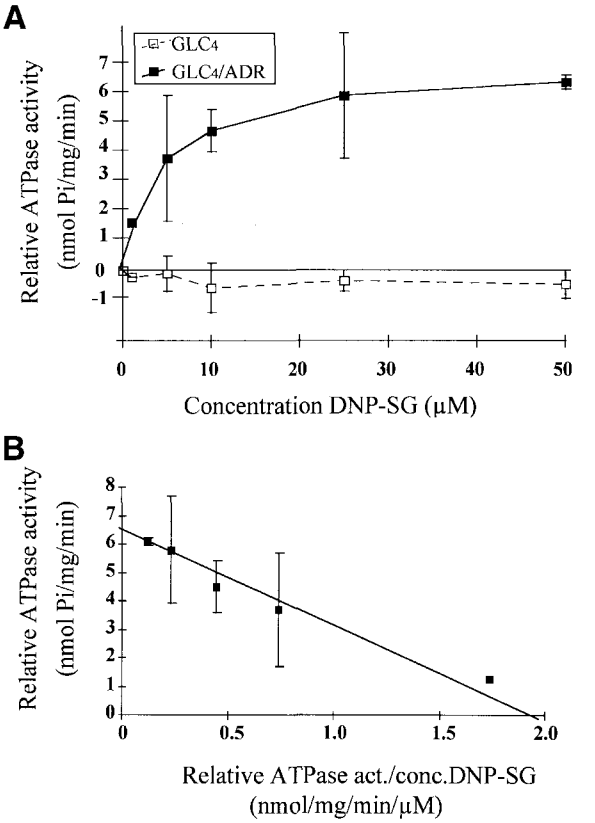

Fig. 2. A: Dose response of DNP-SG-stimulated ATPase activity of GLC_4 and GLC_4/ADR membranes. Incubation $t=60$ min. ATPase activity at 0 μM DNP-SG was set at 0 nmol/mg/min. Data are means ± SD of 5 independent experiments with different batches of vesicles. B: Eadie-Hofstee plot calculated from the data of (A).

3. Results

3.1. Transport of DNP-SG into plasma membrane vesicles from GLC_4 and GLC_4/ADR cells

The time dependence of the uptake of [^{3}H]DNP-SG into vesicles prepared from GLC_4 and GLC_4/ADR cells was measured in the presence or absence of 1 mM ATP. Under these conditions the DNP-SG uptake could reach a maximum rate of 0.56 ± 0.08 nmol/mg/min in the GLC_4/ADR vesicles [23]. In contrast, the uptake rate in GLC_4 vesicles was very low, consistent with the low MRP expression in these latter cells [26,27] and the relatively low uptake of the MRP substrate leukotriene C4 in GLC_4/ADR vesicles [9].

3.2. ATPase activity of MRP

In order to obtain information on the interaction of (iso)-flavonoids with MRP we wanted to study their effect on MRP-associated ATPase activity. Since MRP-associated ATPase activity, induced by MRP substrates, has not been reported before, we first measured the effect of the established MRP substrate DNP-SG on the ATPase activity in GLC4 and GLC4/ADR plasma membranes.

Using 1 μM of DNP-SG we observed no significant stimulation of the ATPase activity of both GLC4 and GLC4/ADR membranes (data not shown). In contrast, 10 μM DNP-SG clearly stimulated the ATPase activity of the GLC4/ADR membranes. Fig. 1 shows the time course of the phosphate production by GLC4/ADR membranes in the presence or in the absence of 10 μM DNP-SG.

Subsequently, the substrate concentration dependence of the stimulation of the ATPase activity of GLC4 plasma membranes and GLC4/ADR plasma membranes was studied. The basal ATPase activity, which was apparently not completely blocked by the added inhibitors EGTA, azide and ouabain, as shown for GLC4/ADR in Fig. 1, was similar in both cell lines (data not shown). Stimulation by DNP-SG was only seen in GLC4/ADR membranes (Fig. 2A,B). This stimulation was dose-dependent, as shown in Fig. 2A.

The kinetic parameters of this DNP-SG stimulation of ATPase activity were calculated from an Eadie-Hofstee plot (Fig. 2B). The apparent K_M was 3.9 ± 0.8 μM, with a maximum stimulated phosphate production rate of 6.6 ± 1.2 nmol phosphate/mg protein/min.

We also measured the stimulation of the ATPase activity in GLC4/ADR membranes by two other putative MRP substrates. In addition to DNP-SG, oxidised glutathione (GSSG) showed a small but significant stimulation of ATPase activity in GLC4/ADR membranes. At a concentration of 100 μM GSSG this stimulation was $10\% \pm 4$ ($n = 4$). In contrast, vinblastine did not significantly stimulate the ATPase activity in GLC4/ADR membranes.

3.3. Modulation of the ATPase activity of MRP by (iso)flavonoids

After having shown a DNP-SG stimulatable ATPase activity in GLC4/ADR membranes, which is consistent with a high overexpression of MRP in this cell line and with the ATP-dependent uptake of DNP-SG in vesicles, we tested the modulating effects of several (iso)flavonoids on the ATPase activity of GLC_4 and GLC_4/ADR membranes. The membranes were incubated with one of the (iso)flavonoids kaempferol, genistein, genistin or flavopiridol (see Fig. 4), in the absence or in the presence of DNP-SG. None of the tested (iso)flavonoids affected the ATPase activity of GLC4, shown as an example for flavopiridol in Table 2. However, the ATPase activity of GLC4/ADR membranes was stimulated significantly by the (iso)flavonoids kaempferol, genistein and flavopiridol, but not by genistin. This stimulation was observed whether DNP-SG was present or not (Tables 1 and 2 and Fig. 3).

Table 1
Effect of genistein on ATPase activity of GLC4/ADR membranes

	Genistein (μM)	Stimulation (%)	P value (t test)
−DNP-SG	0	0	0
	10	5	0.019
	50	6	0.029
	100	9	0.038
	150	9	0.026
	200	11	0.025
+DNP-SG	0	23	0.006*
	10	30	0.033
	50	35	0.009
	100	35	0.004
	150	40	0.003
	200	36	0.004

*P value compared to −DNP-SG. Other P values for genistein compared to corresponding value minus genistein.

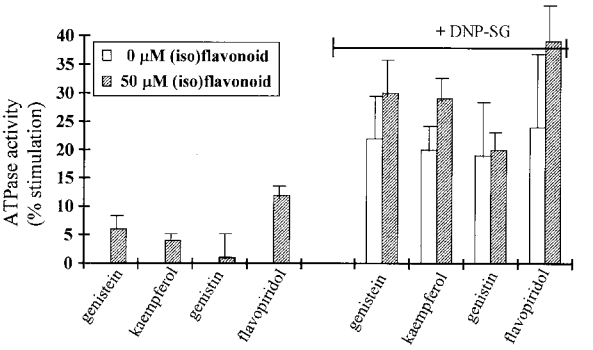

Fig. 3. Effect of (iso)flavonoids on the ATPase activity of GLC_4/ADR membranes in the presence or absence of 10 μM DNP-SG. Data are means of 5 independent experiments with 5 different batches of vesicles. Effects of the (iso)flavonoids were significantly different from controls, except for genistin, as calculated with a Student's t test ($P < 0.05$).

The specific MRP modulator benzbromarone [28] inhibited the DNP-SG-stimulated ATPase activity of GLC4/ADR membranes (Table 2). The inhibition of the DNP-SG effect (at 10 μM) by 10 μM benzbromarone was about 80%. The ATPase stimulation by the (iso)flavonoids flavopiridol, genistein and kaempferol, was also inhibited by this compound, as is presented for flavopiridol in Table 2.

4. Discussion

(Iso)flavonoids are plant polyphenols which are thought to be important constituents of our daily food. Estimations of the amounts of daily intake vary from milligrams per day [29] to 1 g per day [12]. In addition to their antioxidant properties [17,18], a number of interesting activities have been described for some of these compounds, which may relate to their effects on tumour cell proliferation. Among these activities are inhibition of phosphotyrosine kinases, as was shown for e.g. genistein [13–15]. Inhibition of topoisomerase II [16] and protein kinase C [30] has also been described. Finally, cyclin-dependent kinases can also be inhibited by certain (iso)flavonoids [31–34]. The synthetic flavonoid flavopiridol, for instance, showed to be a potent inhibitor of cdc2 kinase activity [32].

Of relevance to their use as anticancer agents alone or in combination with other agents [34] may be their interaction with the drug transporters Pgp and MRP, which are frequently expressed in human tumours [10,11].

In this study we have performed an initial analysis of the effects of some of the typical members of the large group of (iso)flavonoids on MRP activity. We have used isolated plasma membrane vesicles from parental GLC_4 cells and from drug resistant, MRP-overexpressing GLC_4/ADR cells to study the transport of MRP substrates, such as DNP-SG and GSSG [23]. Since certain (iso)flavonoids are thought to interact with ATP-binding sites of their target proteins (e.g. topoisomerase II, phospho-tyrosine kinases) we studied putative effects of these compounds on the MRP-related ATPase activity.

Our results show that the uptake of DNP-SG is ATP-de-

pendent and is also associated with a stimulation of the ATPase activity in MRP-containing GLC4/ADR vesicles. This stimulation followed Michaelis-Menten kinetics. The determined V_{max} for DNP-SG-stimulated ATP hydrolysis was 6.6 nmol/mg protein/min. In similarly prepared GLC4/ADR vesicles, we measured a V_{max} for DNP-SG uptake of 0.56 nmol/mg protein/min. This would imply a ratio of ATP hydrolysis per DNP-SG molecule transported of 12:1. In this context, two remarks should be made. First, it should be mentioned that measurements of the effect of P-glycoprotein substrates on Pgp-associated ATPase activity have shown that such a quantitative comparison may differ largely between studies [35]. Secondly, in our experiments, measurement of ATPase activity included all membrane fragments, whereas in uptake studies only inside-out vesicles contribute to the uptake of DNP-SG.

Further evidence for the MRP specificity of the DNP-SG-mediated ATPase activity was the inhibition by benzbromarone. This compound has been reported to inhibit MRP-mediated transport of drugs [28]. Finally, the observations that oxidised glutathione stimulated ATPase activity of GLC4/ADR membranes, whereas vinblastine (in the absence of GSH) did not (see also [36]), were also consistent with MRP being an ABC transporter with the transport of organic anions coupled to its ATPase activity.

The stimulating effect of three of the four tested (iso)flavonoids (genistein, kaempferol and flavopiridol) on the ATPase activity of GLC4/ADR and the inhibition of this effect by benzbromarone, gives further evidence that the inhibition of MRP-mediated transport of anticancer drugs by these compounds is due to a direct interaction with MRP. Not only the inhibition of the ATPase activity, but also the competitive inhibition by genistein of daunorubicin efflux [19] supports the idea of MRP-mediated transport of certain (iso)flavonoids.

Interestingly, the only isoflavonoid tested here that had no effect, i.e. genistin, was shown to have no inhibitory effect on daunorubicin transport in MRP-overexpressing tumour cells [37]. Although further structure–activity studies are required to explain why genistin has no influence on the ATPase activity, the presence of a glucose moiety (see Fig. 4) may play a role.

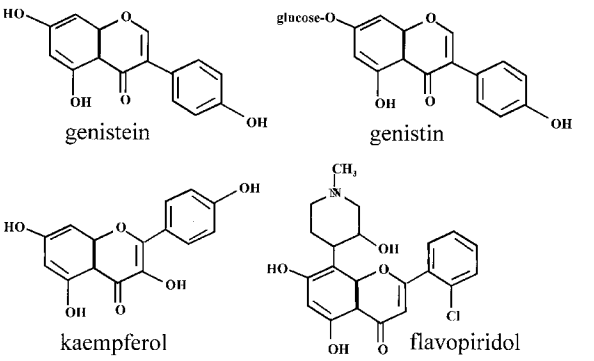

Fig. 4. Structural formulas of the (iso)flavonoids used in this study.

The fact that the stimulation of the ATPase activity by several (iso)flavonoids was seen without addition of GSH or DNP-SG, indicates that these compounds do not need to be metabolised in order to interact with MRP. Whether the effect caused by the (iso)flavonoids in the presence of DNP-SG was an additive of DNP-SG and a (iso)flavonoid, or an increase of only the (iso)flavonoid-MRP interaction in the presence of DNP-SG, is not clear. Therefore, it cannot be excluded that the glutathione group of DNP-SG may play a role in the action or the transport of the (iso)flavonoids.

In conclusion, the stimulation of MRP-associated ATPase activity by (iso)flavonoids, the inhibition of this effect by benzbromarone, as well as the previously described competitive inhibition of daunorubicin transport by genistein [19], support the hypothesis that certain (iso)flavonoids interact directly with the substrate-binding site of MRP. Future experiments will further elucidate the biochemical mechanism(s) of this interaction.

Acknowledgements: This study was supported by the Dutch Cancer Society (Grant KWF-VU-95-933).

Table 2
Effect of flavopiridol on ATPase activity of GLC4 and GLC4/ADR membranes and its inhibition by benzbromarone

Cell line	±DNP-SG	Flavopiridol (μM)	Benzbromarone (μM)	%	*P* value (*t* test)
	−	0	0	0	0
	−	50	0	0	0.500
	−	0	10	1	0.457
GLC4	−	50	10	−3	0.239
	+	0	0	4	0.065
	+	50	0	2	0.300
	+	0	10	7	0.280
	+	50	10	6	0.042
	−	0	0	0	0
	−	50	0	11	0.016
	−	0	10	6	0.004
GLC4/ADR	−	50	10	0	0.070
	+	0	0	23	0.003
	+	50	0	37	0.015
	+	0	10	10	0.022
	+	50	10	7	0.031

Data are mean percentages of stimulation of 3 independent experiments using different batches of vesicles. Statistical differences between 0 and 50 μM flavopiridol as well as between corresponding values plus (+) and minus (−) benzbromarone are calculated.

References

[1] Higgins, C.F. (1992) Annu. Rev. Cell Biol. 8, 67–113.
[2] Cole, S.P.C., Downes, H.F. and Slovak, M.L. (1989) Br. J. Cancer 59, 42–46.
[3] Versantvoort, C.H.M., Broxterman, H.J., Pinedo, H.M., Feller, N., Kuiper, C.M. and Lankelma, J. (1992) Cancer Res. 52, 17–23.
[4] Zaman, G.J.R., Flens, M.J., van Leusden, M.R., de Haas, M., Mülder, H.S., Lankelma, J., Pinedo, H.M., Scheper, R.J., Baas, F., Broxterman, H.J. and Borst, P. (1994) Proc. Natl. Acad. Sci. USA 91, 8822–8826.
[5] Lutzky, J., Astor, M.B., Taub, R.N., Baker, M.A., Bhalla, K., Gervasoni, J.E., Rosado, M., Stewart, V., Krishna, S. and Hindenberg, A.A. (1989) Cancer Res. 49, 4120–4125.
[6] Versantvoort, C.H.M., Broxterman, H.J., Bagrij, T., Scheper, R.J. and Twentyman, P.R. (1995) Br. J. Cancer 72, 82–89.
[7] Jedlitschky, G., Leier, I., Buchholz, U., Center, M. and Keppler, D. (1994) Cancer Res. 54, 4833–4836.
[8] Broxterman, H.J., Giaccone, G.D. and Lankelma, J. (1995) Curr. Opin. Oncol. 7, 532–540.
[9] Müller, M., Meijer, C., Zaman, G.J.R., Borst, P., Scheper, R.J., Mulder, N.H., de Vries, E.G.E. and Jansen, P.L.M. (1994) Proc. Natl. Acad. Sci. USA 91, 13033–13037.
[10] Critchfield, J.W., Welsh, C.J., Phang, J.M. and Yeh, G.C. (1994) Biochem. Pharmacol. 48, 1437–1445.
[11] Versantvoort, C.H.M., Schuurhuis, G.J., Pinedo, H.M., Eekman, C.A., Kuiper, C.M., Lankelma, J. and Broxterman, H.J. (1993) Br. J. Cancer 68, 939–946.
[12] Kuehnau, J. (1976) World Rev. Nutr. Diet. 24, 117–191.
[13] Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S.I., Itoh, N., Shibuya, M. and Fukami, Y.J. (1987) J. Biol. Chem. 262, 5592–5595.
[14] Glossmann, H., Presek, P. and Eigenbrot, E. (1981) Nauyn-Schmeideberg Arch. Pharmakol. 317, 100–102.
[15] Srivastava, A.K. (1985) Biochem. Biophys. Res. Comm. 131, 1–5.
[16] Markovits, J., Linassier, C., Fossé, P., Couprie, J., Pierre, J., Jacquemi-Sablon Saucier, J.-M., Le Pecq, J.-B. and Larsen, A.K. (1989) Cancer Res. 49, 5111–5117.
[17] Bors, W. and Saran, M. (1987) Free Rad. Res. Commun. 2, 289–294.
[18] Cotelle, N., Bernier, J.L., Henichart, J.P., Catteau, J.P., Gaydou, E. and Wallet, J.C. (1992) Free Rad. Biol. Med. 13, 211–219.
[19] Versantvoort, C.H.M., Broxterman, H.J., Lankelma, J., Feller, N. and Pinedo, H.M. (1994) Biochem. Pharmacol. 48, 1129–1136.
[20] Paul, S., Belinsky, M.G., Shen, H. and Kruh, G.D. (1996) Biochemistry 35, 13647–13655.
[21] Butler, J., Spielberg, S.P. and Schulman, J.D. (1976) Anal. Biochem. 75, 674–675.
[22] Zijlstra, J.G., de Vries, E.G.E. and Mulder, N.H. (1987) Cancer Res. 47, 1780–1784.
[23] M. Heijn, J.H. Hooijberg, G.L. Scheffer, G. Szabó, H.V. Westerhoff, and J. Lankelma, Biochim. Biophys. Acta, 1997 in press.
[24] Heijn, M., Oude Elferink, R.P.J. and Jansen, P.L.M. (1992) Am. J. Physiol. 262, C104–C110.
[25] Lanzetta, P.A., Alvarez, L.J., Reinach, P.S. and Candia, O.A. (1979) Anal. Biochem. 100, 95–97.
[26] Zaman, G.J.R., Versantvoort, C.H.M., Smit, J.J.M., Eijdems, E.W.H.M., de Haas, M., Smith, A.J., Broxterman, H.J., Mulder, N.H., de Vries, E.G.E., Baas, F. and Borst, P. (1993) Cancer Res. 53, 1747–1750.
[27] Feller, N., Kuiper, C.M., Lankelma, J., Ruhdal, J.K., Scheper, R.J., Pinedo, H.M. and Broxterman, H.J. (1995) Br. J. Cancer 72, 543–549.
[28] Holló, Z., Homolya, L., Hegedüs, T. and Sarkadi, B. (1996) FEBS Lett. 383, 99–104.
[29] Hertog, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B. and Kromhout, D. (1993) Lancet 342, 1007–1011.
[30] Hoffmann, J., Doppler, W., Jakob, A., Maly, K., Posch, K., Uberall, F. and Grunicke, H. (1988) Int. J. Cancer 42, 382–388.
[31] Filgueira de Azevedo Jr., W., Mueller-Dieckmann, H.J., Schulze-Gahmen, U., Worland, P.J., Sausville, E. and Kim, S.H. (1996) Proc. Natl. Acad. Sci. USA 93, 2735–2740.
[32] Losiewicz, M.D., Carlson, B.A., Kaur, G., Sausville, E.A. and Worland, P.J. (1994) Biochem. Biophys. Res. Comm. 201, 589–595.
[33] Carlson, B.A., Dubay, M.M., Sausville, E.A., Brizuela, L. and Worland, P.J. (1996) Cancer Res. 56, 2973–2978.
[34] Drees, M., Dengler, W.A., Roth, T., Abonte, H., Mayo, J., Malspeis, L., Grever, M., Sausville, E.A. and Fiebig, H.H. (1997) Clin. Cancer Res. 3, 273–279.
[35] Regev, R., Eytan, G.D. and Assaraf, Y.G. (1996) J. Biol. Chem. 271, 3172–3178.
[36] Loe, D.W., Almquist, K.C., Deeley, R.G. and Cole, S.P.C. (1996) J. Biol. Chem. 271, 9675–9682.
[37] Versantvoort, C.H.M., Rhodes, T. and Twentyman, P.R. (1996) Br. J. Cancer 74, 1949–1954.