Pergamon

European Journal of Cancer Vol. 32A, No. 5, pp. 877–882, 1996

Printed in Great Britain
0959-8049/96 $15.00+0.00

S0959-8049(96)00011-1

Original Paper

Antiproliferative Effect of Silybin on Gynaecological Malignancies: Synergism with Cisplatin and Doxorubicin

G. Scambia,[1] R. De Vincenzo,[1] F.O. Ranelletti,[2] P. Benedetti Panici,[1] G. Ferrandina,[1] G. D'Agostino,[1] A. Fattorossi,[1] E. Bombardelli[3] and S. Mancuso[1]

Departments of [1]Gynaecology and [2]Histology, Catholic University, Largo F. Vito 1, 00168 Rome, Italy; and [3]Indena, Milan, Italy

The aim of this study was to test the antiproliferative activity of silybin, a flavonoid, on human ovarian and breast cancer cell lines. Since flavonoids are thought to act through Type II oestrogen binding sites (Type II EBS), silybin binding to Type II EBS was also examined. Silybin, used in concentrations from 0.1 to 20 μM, exerted a dose-dependent growth inhibitory effect on OVCA 433, A2780 parental and drug-resistant ovarian cancer cells, and MCF-7 doxorubicin (DOX)-resistant breast cancer cells (IC_{50} = 4.8–24 μM). Both L and D diastereoisomers of silybin were effective in inhibiting A2780 WT cell growth (IC_{50} = 14 and 20 μM, respectively). Flow cytometry revealed that silybin decreased the percentage of cells in the S and G2-M phases of the cell cycle with a concomitant increase in cells in the G0-G1 phase. Silybin was able to compete with [^{3}H]E_2 for nuclear but not cytosolic Type II EBS. Its affinity parallels its efficacy in inhibiting cell proliferation. Furthermore, silybin (0.1 and 1 μM) potentiates the effect of cisplatin (CDDP) (0.1–1 μg/ml) in inhibiting A2780 WT and CDDP-resistant cell growth. Similar results were obtained on MCF-7 DOX-resistant cells when silybin (0.1 μM) was associated with doxorubicin (0.1–10 μg/ml). As assessed by the Berembaum isobole method, the effect of silybin–CDDP and silybin–DOX combinations results in a synergistic action. Using the 'stem cell assay' described by Hamburger and Salmon [*Science* 1977, 197, 461–463], we found that silybin exerted a dose-dependent inhibition of clonogenic efficiency of cells derived from three ovarian tumours (IC_{50} = 7.4, 4 and 6.4 μM, respectively). Since CDDP and DOX are the two most commonly used drugs for gynaecological tumours, the clinical application of silybin is currently under investigation in our institute.

Key words: silybin, ovarian and breast cancer, synergism with CDDP and DOX
Eur J Cancer, Vol. 32A, No. 5, pp. 877–882, 1996

INTRODUCTION

FLAVONOIDS ARE an important family of compounds widely distributed among plants and fruits and, therefore, present in the daily human diet. They have a broad pharmacological profile which includes antilipoperoxidant [1] and anti-inflammatory [2] properties, and the ability to exert anticancer [3] and chemopreventive activities [4].

In particular, the flavonoid, quercetin, has a strong inhibitory effect on the growth of several human cancer cell lines [5–8] and enhances the antiproliferative action of cisplatin (CDDP) both *in vitro* [9] and *in vivo* [10].

In spite of this wide spectrum of pharmacological properties, the main limitation of flavonoids is their low bioavailability as unaltered compounds, since after oral administration, they undergo a rapid glucuronidation and are inactivated [11].

However, it has recently been demonstrated that, when complexed with phosphatidylcholine, the flavonoid, silybin (the main flavolignan constituent of silymarin, widely used as an effective antihepatotoxic agent [12]), crosses the intestinal barrier with greater ease [13].

These findings prompted us to test the antiproliferative activity of silybin on several established cell lines obtained from human ovarian and breast cancers, and investigate whether it can synergise the inhibitory action of CDDP and doxorubicin (DOX), the most effective drugs in the treatment of gynaecological malignancies. Moreover, since an involve-

Correspondence to S. Mancuso.
Received 14 Aug. 1995; accepted 22 Nov. 1995.

ment of the binding interaction with the so-called type II oestrogen binding sites (Type II EBS) has been suggested [6, 7] to explain the antiproliferative action of flavonoids, the possibility that silybin could bind Type II EBS was also investigated.

MATERIALS AND METHODS

Drugs and chemicals

Silybin (2-[2, 3-dihydro-3- (4-hydroxy-3-methoxyphenyl)-2-(hydroxy methyl)-1, 4-benzodioxin-6-y1]-2, 3-dihydro-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one), Dehydro-silybin, Silydianin and Silymarin were purchased from Indena Co. (Milan, Italy). Quercetin (3, 3′, 4′, 5, 7-pentahydroxy-flavone) was purchased from Aldrich (Steinhein, Germany). The compounds were added from an ethanol–DMSO solution (9:1, v/v) and the control cells were treated with the same amount of vehicle alone. The final vehicle concentration in the culture medium never exceeded 1% (v/v) both in control and treated samples. CDDP (II) and DOX, in distilled water, were used at concentrations varying from 0.1 to 1 and from 0.1 to 10 μg/ml, respectively.

Cell culture

The human ovarian carcinoma cell line A2780 was kindly provided by Dr R.F. Ozols (NCI, Bethesda, Maryland, U.S.A.). Both parental (A2780 WT) and A2780 CDDP-resistant cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 U/ml antibiotics and 0.3 μg/ml glutamine. Resistance was induced in our laboratory by continuous exposure of A2780 cells to increasing scalar doses of CDDP (range 0.1–0.6 μg/ml). Cells were trypsinised every 4 days and plated at a density of 6×10^4 cells/ml. As revealed by a radioligand, whole cell assay previously described [8], both cell lines express Type II EBS, but not oestrogen receptors (ER) (unpublished data).

Human ovarian carcinoma cell line OVCA 433 was kindly provided by Dr B.A. Littlefield (Yale University, Newhaven, Connecticut, U.S.A.).

The breast cancer cell line, MCF-7 DOXr, selected as previously described [8], was kindly provided by Dr Kenneth H. Cowan (NCI, Bethesda, Maryland, U.S.A.). Both cell lines were grown in monolayer culture in Minimum Essential Medium (MEM) supplemented with 10% FCS and 200 U/ml penicillin. Cells were trypsinised weekly and plated at a density of 8×10^4 cells/ml.

All cells were incubated at 37°C under 5% CO_2–95% air in a high humidity atmosphere.

Both OVCA 433 and MCF-7 DOXr cells express Type II EBS, as previously reported [7, 8].

Growth experiments

For growth experiments, cells were plated in six-well flat bottom plates (Falcon 3046, Becton Dickinson, Lincoln Park, New Jersey, U.S.A.) at a density of 4×10^4 cells/ml (8333 cells/cm^2) in the appropriate medium as described above. After 24 h, the medium was replaced with fresh medium containing the compounds to be tested. Quadruplicate haemocytometer counts of triplicate culture dishes were performed after three days of exposure. Results are expressed as percentage of variation as compared to vehicle treated cells.

Cell cycle analysis

A2780 cells were plated in 75 cm^2 tissue culture flasks at a concentration of 1×10^5 cells/ml in RPMI 1640 supplemented as above. Twenty-four hours after plating, the medium was replaced with fresh medium containing silybin or vehicle alone. After 24 h, cells were harvested and centrifuged. Aliquots of 5×10^5 cells/ml were resuspended with 500 μl freshly prepared DNA-staining solution (50 μg/ml propidium iodide, 1–2 mg/ml (50–75 kU/mg) RNase A and 0.1% Nonidet P40 in PBS), incubated for 30 min at room temperature and analysed immediately. Flow cytometric analysis was performed with an Ortho Cytoron flow cytometer (Werthwood, Massachusetts, U.S.A.) as previously described [14].

Evaluation of drug interaction

Synergy experiments were carried out as previously reported [9], and the extent of the effect of the combined treatment was then analysed by the isobole method [15] for a combination of drugs A and B, applying the equation: $Ac/Ae + Bc/Be = D$, where Ac and Bc correspond to concentrations of drugs used in the combination treatment, Ae and Be correspond to concentrations of drugs able to produce alone the same magnitude of effect. If D (combination index) <1, the effect of the combination was synergistic, while if $D = 1$ or $D > 1$, the effect was additive or antagonistic, respectively. Drug potentiation was calculated as the amount of the drug used alone to give the same effect as the drug in combination divided by the amount of the drug used in the combination.

Culture assay for colony forming cells

Tissue samples were obtained from 3 patients with advanced ovarian cancers. Tumour specimens obtained immediately after surgery were processed for the clonogenic assay as described elsewhere [9]. The cell concentration was adjusted to 2×10^5 ml for the clonogenic assay, performed as described by Hamburger and Salmon [16]. Various concentrations of the drugs to be tested were added to both 0.5% agar (underlayer) and 0.3% agar-containing cells. Cultures were incubated at 37°C in a 7.5% CO_2 humidified atmosphere. The colonies (collections of 30 or more cells) appeared 10–20 days after plating.

Measurement of cytosolic Type II EBS by ^{3}H-oestradiol exchange

Tissue specimens obtained from a pool of three advanced serous ovarian carcinomas were homogenised in TE buffer (10 mM Tris; 1.5 mM EDTA; pH 7.4) (wet tissue/buffer: 100 mg/ml) by the Ultra-Turrax homogeniser (4°C). The homogenate was centrifuged (800g for 20 min) to obtain the low-speed supernatant and crude nuclear pellet. The supernatant was ultracentrifuged (105000g for 60 min) and the resulting cytosolic fraction was diluted to 0.2–0.4 mg protein/ml. Protein determination was performed by the method of Bradford [17]. An aliquot of the cytosol was assayed for Type II EBS by the hydroxylapatite (HAP) exchange method described by Markaverich and associates [18]. Radioactivity of the samples was determined by liquid scintillation spectrometry in a beta-counter (Packard 1900 TR). Saturation analysis was performed by incubating the HAP–cytosol suspension with increasing (2.5–50 nM) concentrations of [^{3}H]-E_2 with or without a 300-fold molar excess of quercetin, and analysed by the Scatchard method [19]. Results were expressed as fmol/mg of cytosolic protein.

Measurement of nuclear Type II EBS by sucrose pad nuclear exchange assay

Nuclear Type II EBS were analysed by the sucrose pad assay, as described by Syne and associates [20]. For saturation

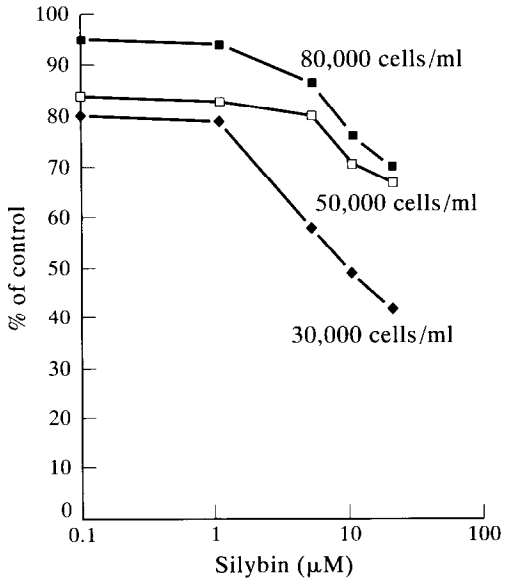

Figure 1. Antiproliferative effect of different concentrations of silybin on A2780 WT cells, plated at different densities. Each value is the mean of three experiments performed in triplicate. Standard deviations were less than 10%.

analysis, the purified nuclear pellet was incubated with increasing concentrations (2.5–50 nM) of [^{3}H]-E_2 with or without a 300-fold molar excess of quercetin, for 60 min at 30°C. Radioactivity of the extracts was determined as described above. DNA content was determined by the method of Burton [21]. Results were expressed as fmol/mg DNA.

RESULTS

Figure 1 shows the effects of silybin on ER-negative, Type II EBS positive A2780 WT ovarian cancer cells. The inhibition of cell growth was dose dependent, and more evident when cells were plated at low density, thus the growth experiments were carried out at a density of 30000 cells/ml (6250 cells/cm^2).

Silybin, used in concentrations from 0.1 to 20 μM, exerted a dose-dependent growth inhibitory effect on A2780 parental and drug-resistant cancer cells, OVCA 433 and MCF-7 DOXr cancer cells. The concentration giving 50% inhibition of cell growth (IC_{50}) varied from 4.8 to 24 μM (Table 1). Furthermore, we demonstrated that both L and D diastereoisomers of silybin were effective in inhibiting A2780 WT cell growth with an IC_{50} of 11 and 18 μM, respectively (data not shown).

We also tested the effect of the flavolignans, dehydrosilybin, silydianin (a constituent of silymarin) and silymarin, on A2780 WT cell growth, and the order of their potency, in terms of growth inhibition, after a 72-h exposure, was dehydrosilybin > silydianin = silybin > silymarin (IC_{50} = 2.88, 12, 12, >20 μM, respectively).

In order to obtain some insight into the cell kinetics modifications induced by silybin, its effect on the A2780 WT cell cycle was analysed by flow cytometry (Figure 2). Results revealed that silybin (10 μM) decreased the percentage of

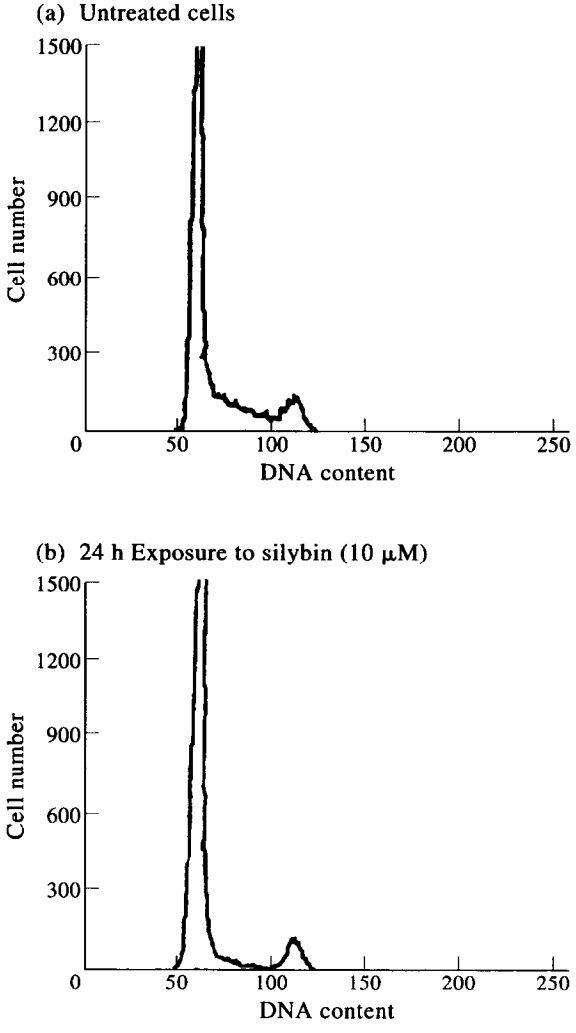

Figure 2. Effect of silybin on A2780 WT cell cycle. (a) Phase G1: 58.7%, S: 31%, G2-M: 10.3%. (b) Phase G1: 70.5%, S: 20.9%, G2-M: 8.6%. The above shows one of four experiments performed.

Table 1. Antiproliferative effect of silybin on cancer cell lines

Cell lines	Number of experiments*	Cell type	Exposure time (h)	IC_{50} (μM)†
OVCA-433	3	Ovarian cancer	72	4.8
A2780 WT	8	Ovarian cancer wild type	72	12
A2780 CDDP	5	Ovarian cancer CDDP-resistant	72	14
MCF-7 DOXr	5	Breast cancer DOX-resistant	72	24

* Each experiment was performed in triplicate. † Concentration giving 50% inhibition of cell growth.

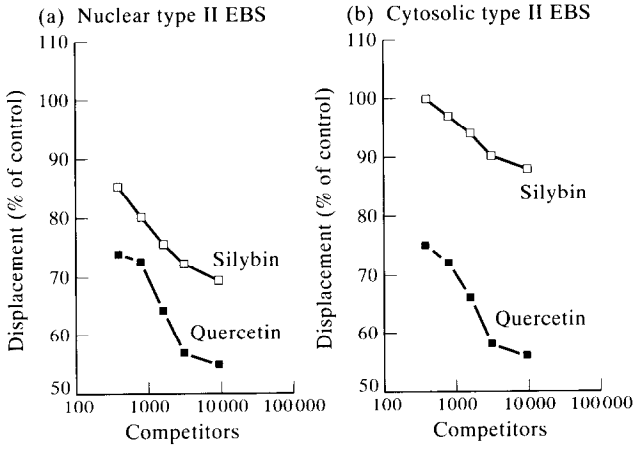

Figure 3. Competition of silybin and quercetin for nuclear and cytosolic Type II EBS in ovarian tumour samples. Silybin and quercetin were used at concentrations varying from 10 to 9000 nM. Results are expressed as the percentage of ^3H-E_2 bound in the absence (100%) or presence of competitors. Each value is the mean of three experiments performed in triplicate. Standard deviations were less than 10%.

cells in the S and G2-M phases of the cell cycle with a concomitant increase in the G0–G1 phase.

Since the antiproliferative effect of flavonoids has been thought to be mediated by the interaction with the Type II EBS [6, 7], we tested the ability of silybin to bind to these receptors. In Figure 3, the competition curves of quercetin and silybin for Type II EBS are shown. Quercetin was able to compete with [^{3}H]-E_2 for both nuclear (Figure 3a) and cytosolic Type II EBS (Figure 3b), whereas silybin competed only for nuclear Type II EBS (Figure 3a).

We also tested the ability of silybin to potentiate the antiproliferative effect of CDDP and DOX, which are commonly used in the treatment of gynaecological malignancies. CDDP (0.1–1 μg/ml) proved to be more effective in inhibiting A2780 WT cell growth when associated with silybin (0.1 and 1 μM) than when used on its own (Figure 4a). Similar results were obtained for A2780 CDDP-resistant cells with the combination silybin–CDDP (data not shown). Figure 4b shows that silybin (0.1 μM) also enhances the antiproliferative effect of DOX (0.1–10 μg/ml) on MCF-7 DOXr cells. As assessed by the Berembaum isobole method, the effect of silybin–CDDP and silybin–DOX combinations was due to a synergistic action, in that the combination indexes were less than 1 in

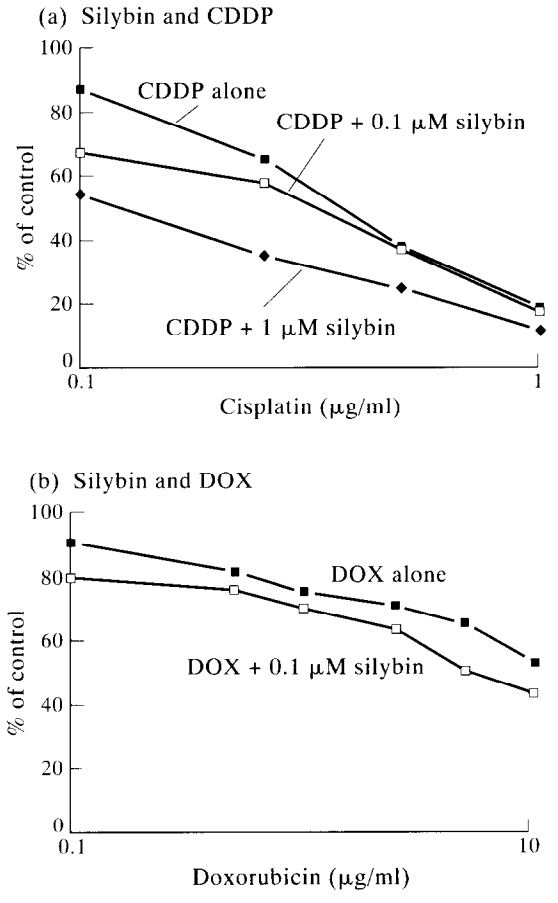

Figure 4. Combined effect of silybin with CDDP (0.1–1 μg/ml) on A2780 WT cells, or with DOX (0.1–10 μg/ml) on MCF-7 DOXr cells. Each point is the mean of three experiments performed in triplicate. Standard deviations were less than 10%.

A2780 WT (Table 2), A2780 CDDP-resistant (data not shown) and MCF-7 DOXr (Table 3) cell lines.

The antiproliferative activity of silybin was exerted not only on established cell lines, but also on cells obtained from primary human tumours. Using the 'stem cell assay' described by Hamburger and Salmon [16], we found that silybin exerted a dose-dependent inhibition of clonogenic efficiency of cells derived from ovarian cancers (Table 4). The IC_{50} for cases 1, 2 and 3, were 7.4, 4 and 6.4 μM, respectively.

Table 2. Synergistic antiproliferative combination of CDDP and silybin on A2780 WT cells

CDDP (μg/ml) (*Ac*)	Silybin (μM) (*Bc*)	% of control growth	CDDP (μg/ml) (*Ae*)	Silybin (μM) (*Be*)	Combination index*
0.1	0.1	67	0.23	2.9	0.46
0.25	0.1	58	0.31	6.4	0.81
0.5	0.1	37	0.54	>50	<0.92
1	0.1	18	> 1	>50	<1.00
0.1	1	54	0.34	8.2	0.41
0.25	1	35	0.56	>50	<0.46
0.5	1	25	0.82	>50	<0.62
1	1	12	> 1	>50	<1.02

* Calculated by the isobole method [15].

Table 3. Synergistic antiproliferative combination of DOX and silybin on MCF-7 DOXr cells

DOX (μg/ml) (*Ac*)	Silybin (μM) (*Bc*)	% of control growth	DOX (μg/ml) (*Ae*)	Silybin (μM) (*Be*)	Combination index*
0.1	0.1	80	0.6	0.1	1.16
0.5	0.1	76	0.9	0.3	0.77
1	0.1	69	2.5	2.2	0.44
2.5	0.1	62	5.4	7	0.51
5	0.1	47	>10	35	<0.50
10	0.1	39	>10	>50	<1.00

* Calculated by the isobole method [15].

Table 4. Effect of silybin on the clonogenic efficiency of cells derived from primary ovarian tumours

	Number of colonies (% of control)		
Treatment	Case 1	Case 2	Case 3
None	139 (100.0)	135 (100.0)	160 (100.0)
Silybin 0.1 μM	121 (87.0)	109 (80.7)	136 (85.0)
Silybin 1 μM	109 (78.4)	89 (65.9)	116 (72.5)
Silybin 5 μM	84 (60.4)	64 (47.4)	88 (55.0)
Silybin 10 μM	60 (43.1)	50 (37.0)	67 (41.8)
IC_{50} (μM)	7.4	4.0	6.4

DISCUSSION

Our *in vitro* results show that, in the range 0.1–20 μM, silybin displays a dose-dependent antiproliferative action on sensitive and drug resistant established tumour cell lines and on cells obtained from primary malignant tumours. It has been previously reported [11] that the concentrations used in our study can be reached by oral administration, are effective and non-toxic *in vivo* [22]. These results are also in keeping with the demonstration that silymarin, in the same range of concentrations effective in our *in vitro* experiments, suppresses the ability of several tumour promoters to increase ornithine decarboxylase (ODC) activity and ODC mRNA expression *in vivo* [23].

In view of the potential clinical applications, the demonstration that silybin is synergistic with two of the drugs most commonly used in the therapy of gynaecological cancers, namely CDDP and DOX, would appear to be particularly interesing.

The synergistic antiproliferative effect of silybin and CDDP, could depend on the fact that silybin recruits cells in G0–G1 phases of the cell cycle, when cells are more sensitive to the action of CDDP [24].

As far as the synergism between silybin and DOX is concerned, it may be explained by the possibility that, like other flavonoids [25], silybin inhibits expression and function of the p170 glycoprotein.

Type II EBS are present in all the established cancer cell lines ([7, 8]and unpublished data) and in the primary ovarian cancers examined by us. In the latter, silybin bound to nuclear Type II EBS with a lower affinity than quercetin and was less effective than quercetin in inhibiting tumour cell proliferation. This correlation between the affinity for Type II EBS and the antiproliferative effect, together with previous data reported for other flavonoids tested [26], suggests that the inhibitory action of silybin can be mediated by an interaction with nuclear Type II EBS. Unlike quercetin, silybin binds to nuclear, but not cytosolic Type II EBS. However, it has been reported that only nuclear Type II EBS mediate the growth inhibitory effects of flavonoids [6], although the mechanism has still to be understood.

In conclusion, the data reported here, together with the low toxicity and the good bioavailability [13] of silybin, may constitute the basis for the introduction of this compound into the treatment of gynaecological cancers, either alone or in combination with other cytotoxic drugs. At present, a phase I study using silybin in patients with advanced ovarian cancer is in progress in our institution.

1. Bosisio E, Benelli C, Pirola O. Effect of the flavanolignans of Silybum marianum on lipid peroxidation in rat liver microsomes and freshly isolated hepatocytes. *Pharmacol Res* 1992, **25**, 147–154.
2. Abad MJ, Bermejo P, Villar A, Valverde S. Anti-inflammatory activity in two flavonoids from "Tanace microphillum". *J Nat Prod* 1993, **56**, 1164–1167.
3. Verma A, Johnson JA, Gould MN, Tanner MA. Inhibition of 7, 12-dimethylbenz (a) anthracene and N-nitrosomethylurea induced rat mammary cancer by dietary flavonol quercetin. *Cancer Res* 1988, **48**, 5754–5758.
4. Cassady JM. Natural products as a source of potential cancer chemotherapeutic and chemopreventive agents. *J Nat Prod* 1990, **53**, 23–41.
5. Larocca LM, Piantelli M, Leone G, *et al.* Type II oestrogen binding sites in acute lymphoid and non-lymphoid leukemias. Growth inhibitory effect of oestrogens and flavonoids. *Br J Haematol* 1990, **75**, 489–495.
6. Markaverich BM, Roberts RR, Alejandro MA, Johnnan GA, Middledich BS, Clark GM. Bioflavonoid interaction with rat uterine type II binding sites and cell growth inhibition. *J Steroid Biochem* 1988, **30**, 71–78.
7. Scambia G, Ranelletti FO, Benedetti-Panici P, *et al.* Inhibitory

effect of quercetin on OVCA 433 cells and presence of type II oestrogen binding sites in primary ovarian tumours and cultured cells. *Br J Cancer* 1990, **62**, 942–946.
8. Scambia G, Ranelletti FO, Benedetti-Panici P, *et al.* Quercetin inhibits the growth of a multidrug-resistant estrogen receptor negative MCF-7 human breast cancer cell line expressing type II estrogen binding sites. *Cancer Chemother Pharmacol* 1991, **28**, 255–258.
9. Scambia G, Ranelletti FO, Benedetti Panici P, *et al.* Inhibitory effect of quercetin on primary ovarian and endometrial cancers and synergistic activity with cis-diamminedichloroplatinum (II). *Gynecol Oncol* 1992, **45**, 13–19.
10. Hofman J, Fiebig HH, Winterhalter BR, Berger DP, Grunicke H. Enhancement of the antiproliferative effect of cis-diamminedichloroplatinum by quercetin. *Int J Cancer* 1990, **45**, 536–539.
11. Gugler R, Lochik M, Dengler MJ. Disposition of Quercetin in man after single oral and intravenous doses. *Eur J Clin Pharmacol* 1975, **9**, 229–235.
12. Ferenci P, Dragosics B, Dittrich H, *et al.* Randomized controlled trial of silymarin treatment in patients with cirrhosis of liver. *J Hepatol* 1989, **9**, 105–113.
13. Moscarella S, Giusti A, Marra F, *et al.* Therapeutic and antilipoperoxidant effects of Silybin-phosphatidylcholine complex in chronic liver disease: preliminary results. *Curr Ther Res* 1993, **53**, 98–102.
14. Sica G, Dell'Acqua G, Iacopino F, *et al.* Androgen receptors and hormone sensitivity of a human prostatic cancer cell line (PC-3) are modulated by natural β-interferon. *Urol Res* 1994, **22**, 36–38.
15. Berenbaum MC. Criteria for analyzing interaction between biologically active agents. *Adv Cancer Res* 1981, **25**, 269–335.
16. Hamburger AW, Salmon SE. Primary bioassay of human stem cell. *Science* 1977, **197**, 461–463.
17. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976, **72**, 248–254.
18. Markaverich BM, Adams NR, Roberts RR, Alejandro M, Clark JH. Cytosol Type II sites in the rat uterus: interaction with an endogenous ligand. *J Steroid Biochem* 1987, **28**, 599–608.
19. Scatcard G. The attraction of protein for small molecules and ions. *Ann NY Acad Sci* 1949, **51**, 660–672.
20. Syne JS, Markaverich BM, Clark JH, Panko WB. Estrogen binding sites in the nucleus of normal and malignant human tissue: characteristics of the multiple nuclear binding sites. *Cancer Res* 1982, **42**, 4449–4454.
21. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1976, **62**, 315–317.
22. Yugarani T, Tan BKH, Teh M, Das NP. Effects of poliphenolic natural products on the lipid profiles of rat fed high fat diet. *Lipids* 1992, **27**, 171–176.
23. Agarwal R, Katiyar SK, Lundgren DW, Mukhtar H. Inhibitory effect of silymarin, an antihepatotoxic flavonoid, on 12-O-tetradecanoylphorbol-13-acetate-induced epidermal ornithine decarboxylase activity and mRNA in SENCAR mice. *Carcinogenesis* 1994, **15**, 1099–1103.
24. Fraval HNA, Roberts JJ. G1-phase Chinese hamster V79-379A cells are inherently more sensitive to platinum bound to their DNA than mid S phase or asynchronously treated cells. *Biochem Pharmacol* 1979, **28**, 1575–1580.
25. Scambia G, Ranelletti FO, Benedetti-Panici P, *et al.* Quercetin potentiates the effect of Adriamycin in a multidrug-resistant MCF-7 human breast cancer cell line: P-glycoprotein as a possible target. *Cancer Chemother Pharmacol* 1994, **34**, 459–464.
26. De Vincenzo R, Scambia G, Benedetti Panici P, *et al.* Effect of synthetic and naturally occurring chalchones on ovarian cancer cell growth: structure–activity relationship. *Anticancer Drug Des* 1995, **10**, 481–490.

Acknowledgement—R. De Vincenzo is recipient of a fellowship from the Italian Association for Cancer Research (A.I.R.C.).