*Discussion.* Cyclic GMP has been shown to be increased by aggregatory agents such as thrombin and collagen, but this elevation may represent a feedback control of aggregation. Indeed, several agents that stimulate cyclic GMP formation inhibit platelet reactivity. Smooth muscle relaxants like sodium nitroprusside and nitric oxide have been reported to elevate platelet cyclic GMP levels, effectively preventing or reversing platelet aggregation[2]. The mechanism underlying this effect is still under discussion.

AD6 is an experimental drug with various activities on the cardiovascular system and inhibits, dose-dependently, platelet aggregation induced by several stimuli[7,8]. Among the mechanisms involved in platelet aggregation, an AD6 action on cyclic AMP levels through a cyclic AMP PDE inhibition is unlikely. In fact, Prosdocimi et al. showed that the drug did not modify cyclic AMP accumulation induced by prostacyclin[7]. On the other hand, the present study indicates that AD6 elevates the cyclic GMP level significantly in washed human platelets. We have obtained a similar increase after treatment with dipyridamole. These findings were supported by results from enzymatic analyses which showed that AD6 selectively inhibited cyclic GMP PDE. Thus, AD6-induced elevation of platelet cyclic GMP seems to be related to the inhibition of cyclic GMP hydrolysis, although other effects cannot be ruled out. It has been proposed recently that cyclic GMP may prevent phospholipase C activation, resulting in a reduced formation of $IP_3$ and in a suppression of calcium mobilization[4].

It has been suggested that antiaggregating properties of AD6 are likely to be related to an inhibition of the release of arachidonic acid[15]. It is interesting to compare the action of AD6 to mepacrine, a phospholipase $A_2$ inhibitor. This drug blocks arachidonate release in platelets, increasing at the same time the level of cyclic GMP, and it has been proposed that the increase in the cyclic GMP level may be partially related to the mepacrine-induced change in arachidonate metabolism[16–18].

Our findings that AD6 inhibits cyclic GMP phosphodiesterase and elevates platelet cyclic GMP levels could be important in understanding the action of AD6 in intact platelets.

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0014-4754/88/030226-03$1.50 + 0.20/0


## Enhancement of the cytogenetic efficacy of the antitumor agent bleomycin by the calcium and calmodulin antagonist fendiline

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*Received 9 November 1987; accepted 1 December 1987*

*Summary.* The induction of chromosome aberrations (dicentric and ring chromosomes) in human lymphocytes by the antitumor agent bleomycin is synergistically enhanced when bleomycin is applied together with the calcium antagonist fendiline (Sensit®).
*Key words.* Bleomycin; calcium antagonist; calmodulin antagonist; chromosome aberrations; comutagenesis; cytostatics; fendiline; lymphocytes; verapamil.

Recently we have shown that the calcium antagonist verapamil (Isoptin®) enhances synergistically the cytogenetic efficacy of the antitumor agents bleomycin and peplomycin[1]. In these in vitro studies the yield of chromosome aberrations (dicentric and ring chromosomes) induced in human lymphocytes was drastically increased when verapamil was applied together with the antitumor agent. Verapamil alone exhibited no mutagenic effect. Besides this demonstration that verapamil is a comutagen our results appear to be interesting for two other reasons: not only the *cytogenetic* efficacy of bleomycin and peplomycin is enhanced synergistically by verapamil[1], but also the *cytotoxic* efficacy (cell killing activity) of various antitumor agents including bleomycin[2–6]. Furthermore, mitotic index reduction induced by bleomycin and peplomycin is synergistically increased by verapamil[7].

We were interested to see whether the cytogenetic efficacy of bleomycin is also potentiated by another calcium antagonist, fendiline (Sensit®). This substance is an aliphatic amine, namely N-(3,3-diphenylpropyl)-α-methylbenzyl-aminehydrochloride and exhibits besides calcium-antagonistic also calmodulin-antagonistic properties[8].

Human peripheral blood of a healthy adult female donor was incubated either with bleomycin alone or in combination with fendiline. 1.5 ml of heparinized blood (10 units sodium heparin/ml) were mixed with 8.5 ml prewarmed medium McCoy 5a (37 °C) containing bleomycin at concentrations of 100 and 300 µg/ml, either without fendiline or with 10, 25 or 50 µg/ml fendiline. The cells were incubated for 1 h in sterile tubes at 37 °C in the dark. They were then washed in prewarmed medium (McCoy 5a) and centrifuged for 10 min at 100 × g. This procedure was repeated 3 times. After the supernatant had been removed with a pipette the remaining cell pellet (about 1 ml) was divided into 2 parts. To each part 5 ml culture medium was added. The culture medium consisted of medium McCoy 5a, 20% fetal calf serum, 5 units/ml sodium heparin, 0.1 mg/ml streptomycin, 100 units/ml penicillin, and 0.15 ml phytohemagglutinin M. The cultures were incubated for 48 h at 37 °C in the dark. After 45 h, colcemid (0.33 µg/ml) was added. Slide preparations were made by standard methods. Each metaphase was analyzed by two observers for the presence of dicentrics, polycentrics, and ring chromosomes. Only metaphases with 46 centromeres were used. Polycentrics were scored as dicentrics (number of centromeres − 1 = number of dicentrics). Both the table and the figure show that the yield of dicentrics and rings is increased when fendiline has been added to bleomycin, fendiline alone producing no (or at most very few) chomosome aberrations. Because of their higher absolute yields, the results obtained for dicentrics are more clearcut than those obtained for rings. Therefore in the figure only the dicentric results are represented. The synergistic effect exerted by fendiline roughly increases with increasing fendiline concentration (fig.).

These results resemble those obtained for verapamil, the other calcium antagonist tested by us[1]. Hence fendiline, like verapamil, acts as a comutagen. Theoretically, this comutagenesis might originate either from a promotion of the formation of bleomycin-induced chromosome breaks or from an inhibition of 'repair' (concretely: restitution of chromosome breaks) or from an increase of the bleomycin concentration in the cell.

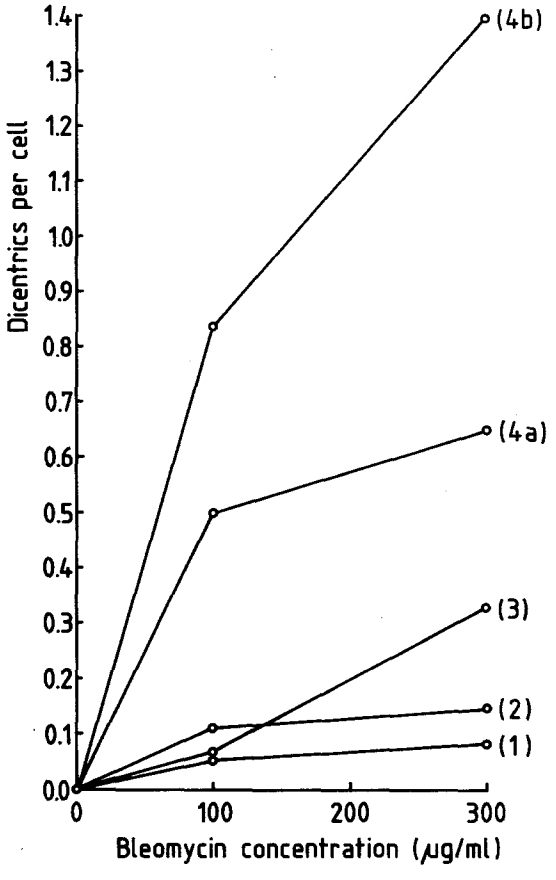
The latter interpretation is used by many[2-6], though not all[9-11] authors to explain the potentiation by verapamil of the cytotoxicity of various anti-cancer drugs, like bleomycin, adriamycin, and vincristine. On the basis of extensive in vitro experiments it is assumed that verapamil inhibits the efflux of the antitumor agent from the cell and that, therefore, the agent accumulates within the cell. Because drug resistance is a very important topic in cancer treatment research today[12], the observation made in those experiments that the acquired resistance of tumor cells against the action of antitumor drugs can be circumvented by the application of verapamil, is especially important. Even in a quite unrelated field, the killing of the malaria parasite *Plasmodium falciparum* with chloroquine, verapamil has been applied successfully to reverse chloroquine resistance in two chloroquine-resistant *Plasmodium falciparum* clones; the effect was possibly due to verapamil inhibiting the efflux of chloroquine from the parasite[13].

Verapamil increases synergistically the cytotoxic effect of bleomycin and other antitumor agents[2-6]. As we have shown[1], this synergism is accompanied by a potentiation of the *cytogenetic* efficacy of bleomycin and peplomycin by verapamil. In order to see whether such a parallelism also exists for fendiline, it would be desirable for experiments on the influence of fendiline on the cytotoxic efficacy of bleomycin (and other antitumor agents) to be performed. We do not yet know whether such studies on verapamil and fendiline will help in developing ways to circumvent acquired anticancer drug resistance in tumor patients. Nevertheless, the following study is of interest. In a recent clinical trial

Potentiation of the yield of bleomycin induced chromosome aberrations by fendiline

| Bleomycin concentration (µg/ml) | Fendiline concentration (µg/ml) | Dicentrics per cell | Rings[a] per cell |
|---|---|---|---|
| 0 | 0 | 0.000 (0/498) | 0.000 (0/498) |
| | 10 | 0.005 (1/209) | 0.000 (0/209) |
| | 25 | 0.000 (0/119) | 0.000 (0/119) |
| | 50 | 0.004 (1/276) | 0.004 (1/276) |
| 100 | 0 | 0.052 (31/590) | 0.007 (4/590) |
| | 10 | 0.111 (26/235) | 0.021 (5/235) |
| | 25 | 0.068 (7/103) | 0.049 (5/103) |
| | 50[b] | 0.840 /110/131) | 0.137 (18/131) |
| | 50[c] | 0.496 (62/125) | 0.008 (1/125) |
| 300 | 0 | 0.083 (42/509) | 0.008 (4/509) |
| | 10 | 0.148 (45/304) | 0.039 (12/304) |
| | 25 | 0.328 (38/116) | 0.043 (5/116) |
| | 50[b] | 1.393 (78/56) | 0.214 (12/56) |
| | 50[c] | 0.649 (74/114) | 0.053 (6/114) |

[a] Sum of centric and acentric rings. [b] Results of experiment BI 20, [c] results of experiment BI 23. These results have been represented separately because of heterogeneity. (The results of the individual experiments belonging to the other fendiline concentrations have been combined, because the $\chi^2$ test revealed homogeneity.)




Dependence of the number of dicentric chromosomes per cell on the concentration of bleomycin and fendiline, respectively. Fendiline concentrations: (1) = 0 µg/ml, (2) = 10 µg/ml, (3) = 25 µg/ml, (4a) and (4b) = 50 µg/ml. For the fendiline concentration 50 µg/ml two curves, (4a) and (4b), are obtained, for the following reason: The results of the two experiments performed for 100 µg/ml bleomycin plus 50 µg/ml fendiline and the results of the two experiments performed for 300 µg/ml bleomycin plus 50 µg/ml fendiline differ significantly from each other according to the $\chi^2$ test, possibly due to different bleomycin charges used in the two experiments. These results, therefore, had to be presented separately.

eight patients with refractory ovarian cancer were treated with adriamycin plus verapamil [14]. As a consequence of that study it was proposed by the authors that verapamil should be replaced by 'less cardiotoxic calcium channel blockers, since the dose of the modifying agent appears to be a critical factor and the toxicity of some of the currently available drugs, such as verapamil, may not permit the necessary plasma concentrations (of verapamil) to be achieved'. Fendiline might be a candidate as a substitute for verapamil in such clinical trials, because 'the clinical profile of fendiline is characterized . . . by an absence of any adverse cardiac effects' [15].

Acknowledgments. We thank Heinrich Mack Nachf., Illertissen (Germany) for supplying the bleomycin and the Thiemann Arzneimittel GmbH, Waltrop (Germany) for supplying the fendiline (Sensit®). We thank Dr D. Tobüren, Thiemann Arzneimittel GmbH, Waltrop, for valuable discussions.

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0014-4754/88/030228-03$1.50 + 0.20/0


## Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis

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*Received 4 January 1988; accepted 18 January 1988*

*Summary.* The honeybee hive product, propolis, is a folk medicine employed for treating various ailments. Many important pharmaceutical properties have been ascribed to propolis, including anti-inflammatory, antiviral, immunostimulatory and carcinostatic activities. Propolis extracts have provided an active component identified as caffeic acid phenethyl ester (CAPE), which was readily prepared in one step. Differential cytotoxicity has been observed in normal rat/human versus transformed rat/human melanoma and breast carcinoma cell lines in the presence of CAPE.
*Key words.* Honeybee hive; propolis; caffeic acid ester; cytoxicity.

The popular folk medicine, propolis [1], is alleged to exhibit a broad spectrum of activities including antibiotic, anti-inflammatory and tumor growth arrest; some of the observed biological activities may be traced to identified chemical constituents such as caffeic acid [2] which is antimicrobial and anti-inflammatory [3]. Ethyl ether extracts of propolis had previously been demonstrated to be cytostatic to KB and HeLa cell lines [4] but the components responsible for this interesting activity were not defined. Guided by an $Ltk^-$ cell growth inhibition assay, we have isolated and characterized one such biologically active component which was readily synthesized in large quantities for further investigation into its cytostatic properties.

Propolis, a gift of Mr Chaim Kalman (Bee Farm-Honey, Israel), was collected from hives located on the Carmel Mountains. It was received in the form of hard, brown lumps (~ 2 cm in diameter) which were chopped, extracted with 80% EtOH/$H_2O$ (1.5 l, 2 d), suction-filtered and evaporated in vacuo to yield a golden brown solid. This extract displayed cytostatic activity in $Ltk^-$ cells at 50 mg/ml. The EtOH extract was dissolved in 80% MeOH/$H_2O$ (400 ml) and sequentially extracted with hexane (6 × 80 ml), toluene (4 × 80 ml), and EtOAc (4 × 100 ml). All organic layers were dried, evaporated and submitted for $Ltk^-$ testing (along with the residue from the aqueous layer). The EtOAc extract exhibited at least twice the cytostatic activity of other fractions (100% inhibition at 65 mg/ml). Subsequent purifications of the EtOAc extract by preparative TLC (7% i-PrOH/$CH_2Cl_2$ then 4% i-PrOH/$CH_2Cl_2$) yielded two increasingly active fractions, with the latter exhibiting 100% $Ltk^-$ inhibition at 40 mg/ml.

Reversed phase HPLC separation of this latter fraction (IBM-C18, 10 × 250 mm, 5 µm; MeOH/MeCN/THF/$H_2O$ – 25:35:3.5:36.5; 1.8 ml/min; 213 nm detection) yielded a pure compound (retention time = 16.8 min; $SiO_2$-TLC: 4% i-PrOH/$CH_2Cl_2$, Rf = 0.25, 366 nm illumination – blue fluorescence) with the following $Ltk^-$ cytostatic activities (% inhibition in parentheses) – 10 mg/ml (20%), 20 mg/ml (80%), 30 mg/ml (95%); values are semiquantitative since only minute quantities were available (see fig. 2 for more quantitative values with synthetic sample).

The structure of this active component was determined as being caffeic acid phenethyl ester (**1**, CAPE) from data shown in figure 1. Acid-catalyzed (p-toluene sulfonic acid) esterification of caffeic acid (CA) with phenethyl alcohol (molar ratios 1:15) in benzene (refluxing, 3–4 days, water removed by Dean-Stark trap) was the simplest synthetic route to CAPE. Following work-up, excess phenethyl alcohol was removed by Kugelröhr distillation (60 °C, < 0.1 mm Hg) to give pure CAPE, mp 126–128 °C, needles (benzene or $H_2O$), 40% yield. All properties of natural and synthetic CAPE were identical.

Cytostatic activities of synthetic CAPE were tested by observing its effect on the number of different cell types in