1 Blum, M.S., and Kearns, C.W., J. econ. Ent. *49* (1956) 862.
2 O'Brien, R.D., Insecticides: Action and Metabolism, 4th edn, p. 121. Academic Press, New York-London 1967.
3 Roth, A.R., and Lindquist, A.W., J. econ. Ent. *46* (1953) 127.
4 Chalfant, R.B., J. econ. Ent. *66* (1973) 339.
5 Normant, B.R., and Chambers, H.W., J. econ. Ent. *63* (1970) 502.
6 DeVries, D.H., and Georghiou, G.P., J. econ. Ent. *72* (1979) 48.
7 Georghiou, G.P., and Atkins, E.L., J. Epicult. Res. *3* (1964) 31.
8 Harris, C.R., Svec, H.J., and Chapman, R.C., J. econ. Ent. *71* (1978) 642.
9 Hirano, M., Appl. ent. Zool. *14* (1979) 404.
10 Yoke, O.P., and Sudderuddin, K.I., Seast Asian J. trop. Med. public Health *6* (1975) 525.
11 Harris, C.R., and Kinoshita, G.B., J. econ. Ent. *70* (1977) 215.
12 Riskallah, M.R., Pestic. Biochem. Physiol. *19* (1983) 184.
13 Abbott, W.S., J. econ. Ent. *18* (1925) 265.
14 Finney, D.J., Probit Analysis, 2nd edn. Cambridge University Press, Cambridge 1952.
15 Riskallah, M.R., Abo-Elghar, M.R., Radwan, H.S.A., Nassar, M.E., and Abd-Elghafar, S.F., Intern. Pest. Control (1983) in press.
16 Narahashi, T., Pestic. Sci. *7* (1976) 267.
17 Gammon, D.W., Pestic. Sci. *9* (1978) 79.
18 Gammon, D.W., and Berkely, A.G., Pestic. Biochem. Physiol. *13* (1980) 53.
19 Osborne, M.P., and Hart, R.J., Pestic. Sci. *10* (1979) 407.

0014-4754/84/020188-03$1.50 + 0.20/0

Calcium dobesilate (Doxium) as a prostaglandin synthetase inhibitor in pregnant human myometrium in vitro

G. Falkay and L. Kovács

Department of Obstetrics and Gynecology, University Medical School, Szeged (Hungary), 27 April 1983

Summary. This comparative study on the effect of calcium dobesilate and indomethacin on prostaglandin biosynthesis was performed on microsomal fractions of pregnant human myometrium. Both drugs inhibited prostaglandin synthesis, indomethacin being more potent. Calcium dobesilate inhibited, in a dose-dependent manner, the synthesis of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TXB_2. Its inhibitory action is comparable to that of etamsylate.

There is a surprisingly large number of compounds known to inhibit the enzymes of the arachidonate metabolic pathway. It was demonstrated in our previous study that etamsylate (diethylammonium-1,4-dihydroxy-3-benzenesulfonate) inhibits in vitro the synthesis of prostaglandins (PGs) in the microsomal fraction of pregnant human myometrium[1].

Calcium dobesilate (calcium 2,5-dihydroxybenzenesulfonate) is also a benzenesulfonate derivative that has proved to be effective in the treatment of chronic venous insufficiency[2–4] and diabetic retinopathy[5–7]. The chemical resemblance of calcium dobesilate to etamsylate has prompted us to undertake a similar study on PG synthesis, making a comparison with indomethacin, which is a commonly used PG synthetase inhibitor.

Materials and methods. Myometrial strips from pregnant women were obtained by excision from the edge of the surgical incision in lower uterine segment caesarian sections. The procedure and the assay of PG-synthetase activity were the same as described in our etamsylate study[1].

The developing-solvent system used in thin layer chromatography for the isolation of PG metabolites after incubation of (1–^{14}C) arachidonic acid with microsomes of pregnant human myometrium allowed the separation of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TXB_2. The incubation media also contained different concentrations of calcium dobesilate (0.01, 0.1, 1.0, 5.0 and 10.0 mM) obtained from OM Laboratories, Geneva, Switzerland. The activity of PG synthetase was calculated as pmol of PG formed per 30 min per mg of protein.

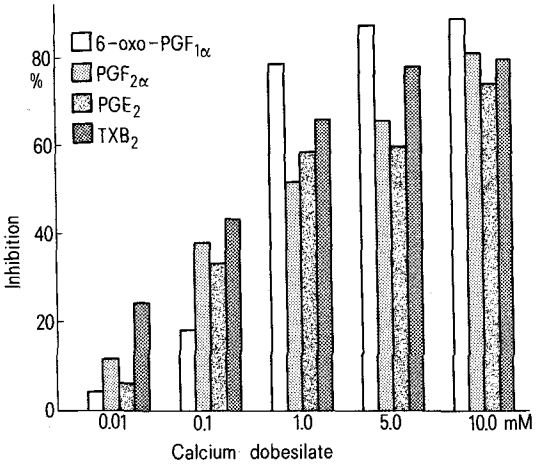

Figure 1. Inhibition of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TXB_2 synthesis during incubation with Ca dobesilate. Each value represents the average of duplicate experiments (variation 4–6%).

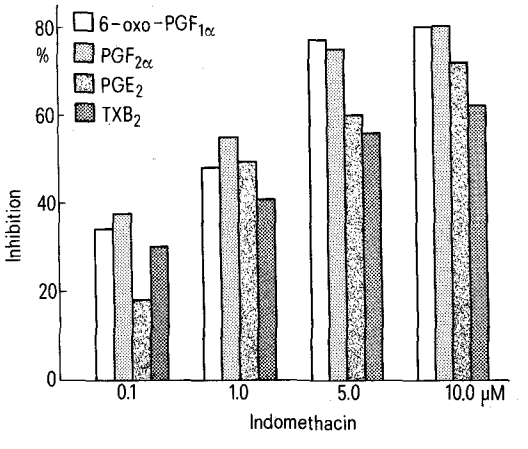

Figure 2. Inhibition of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TXB_2 synthesis during incubation with indomethacin. Each value represents the average of duplicate experiments (variation 4–6%).

Results. Data reported in figure 1 show that calcium dobesilate produced a dose-dependent inhibition of PG synthesis in microsomes of pregnant human myometrium. Figure 2 shows the inhibition of PG synthesis by indomethacin. As with calcium dobesilate, the greater the concentration of indomethacin the greater was the inhibition of PG synthesis. In our assay system, the potency of indomethacin was greater than that of calcium dobesilate, as shown in the table.

Discussion. The above results indicate that calcium dobesilate exerts a potent inhibitory effect on the human pregnant myometrial PG synthesis in vitro. The results of this experiment are more or less comparable to those with etamsylate[1]. Quantitatively the inhibitory potency, expressed in I_{50}-values, for calcium dobesilate (mM) is less than that of indomethacin (μM) (table).

The inhibitory potency of aspirin on PG synthesis tested in vitro on bovine seminal vesicles[8] was of the same order as that of calcium dobesilate. In our experiments the inhibition of PG synthesis, as evaluated by the I_{50}-values for the different arachidonate metabolites, was more marked with calcium dobesilate than with etamsylate.

The findings of the present study are of particular interest in determining the possible therapeutic role of PG synthetase inhibitors like calcium dobesilate, in certain pathological conditions related to various hematological disturbances or microcirculatory disorders.

Inhibition by calcium dobesilate and indomethacin of PG synthesis in microsomes of pregnant human myometrium

PG products	Ca dobesilate I_{50} (mM)	Indomethacin I_{50} (μM)
6-oxo-$PGF_{1\alpha}$	0.87	0.85
$PGF_{2\alpha}$	0.92	0.81
PGE_2	1.01	1.00
TXB_2	0.74	0.91

PG synthesis was measured as described in the text. I_{50} = concentration (mM or μM in final dilution) producing 50% inhibition. I_{50} values were calculated from 10 points of a concentration curve, using regression analysis when the transformations were ln [y/(100-y)] = b lgx + const. for calcium dobesilate, and ln (100-y) = b lgx + const. for indomethacin.

1 Kovács, L., and Falkay, G., Experientia *37* (1981) 1182.
2 Altmann Canestri, E., Leguizamon, O.G., Sanchez, C., Cuccarese, M., and Martin G., Recherche clinique, chirurgicale et pharmacologique dans le traitement de l'insuffisance veineuse par le dobésilate de calcium. Communication to the 6th World Congress of Phlebology, Buenos Aires, 10-15 October 1977.
3 Baricevic, J., Vasa *9* (1980) 240.
4 Schmid, K., and Haase W., Calcium dobesilate (Doxium) in the treatment of chronic venous insufficiency. Result of a multicenter study conducted in FRG, Austria and Switzerland. J.F. Ziegler KG, Remscheid 1980.
5 Binkhorst, P.G., and Van Bijsterveld, O.P., Curr. ther. Res. *20* (1976) 283.
6 Hudomel, J., Nemeth, B., Palfalvy, M., and Farkas, A., Ophthal. Res. *9* (1977) 25.
7 Sevin, R., Traitement au long cours (5-9 ans) de la rétinopathie diabétique avec Doxium. Rapport d'expérimentation, March 1977.
8 Taylor, R.J., and Salata, J.J., Biochem. pharmac. *25* (1976) 2475.

0014-4754/84/020190-02$1.50 + 0.20/0

Free radicals and aflatoxin biosynthesis*

C. Fanelli, A.A. Fabbri, E. Finotti, P. Fasella and S. Passi

Dipartimento di Biologia vegetale, Università di Roma (La Sapienza), Largo Cristina di Svezia 24, I-00165 Roma (Italia); Cattedra di Chimica Biologica, Università di Roma (Tor Vergata), Roma (Italia), and Ospedale S. Gallicano, Via S. Gallicano, Roma (Italia), 23 March 1983

Summary. The addition of some halogenated alkanes (bromotrichloromethane, carbontetrachloride and chloroform) to cultures of *A. parasiticus* and *A. flavus* have shown a high stimulating effect on aflatoxin biosynthesis. When the production of aflatoxin increases during the stimulating effect the peroxidase activity is inhibited.

We have shown previously that compounds with an epoxide ring (cerulenin, tetrahydrocerulenin, methyl 9, 10 epoxystearate and methyl 9,10:12,13 diepoxystearate)[1-3] and lipoperoxides[4,5] promoted aflatoxin production to a remarkable extent when added to cultures of *Aspergillus parasiticus* or *A. flavus.* In addition, on the basis of the results of the aflatoxins analyzed in various non-aged and aged seeds inoculated with *A. parasiticus* and *A. flavus* we thought it probable that the products of the oxidation of unsaturated fatty acids and lipids (lipoperoxides and their breakdown products) played a leading role in the production of aflatoxins.

Recently, the presence of compounds with polysubstrate monoxigenase (PSMO) activity, viz. cytochrome P-450 reductase, has been established in the microsomes of *A. parasiticus*[6]. In this connection we decided to investigate the effects of the addition of some halogenated alkanes, viz. bromotrichloromethane (CCl_3Br), carbontetrachloride (CCl_4) and chloroform ($CHCl_3$) on cultures of *A. parasiticus.* It has been postulated, with good evidence, that the basis of the hepatotoxicity of CCl_3Br and CCl_4 is the peroxidation of lipids of endoplasmic reticulum by the highly reactive trichloromethyl radicals (CCl_3) formed by the interaction with NADPH-cytochrome P-450 system[7,8]. In addition, the amount of peroxidase activity in the mycelia of *A. parasiticus* was studied.

Materials and methods. Aspergillus parasiticus (strain NRRL 2999) was used in this study. In some experiments we also used 2 toxigenic strains of *A. flavus:* strain ATCC 22548 and CF 1 isolated in our Institute from wheat seeds (*Triticum vulgare* var. Manitoba). Stock cultures were maintained on Czapek Dox agar (Difco) supplemented with 5 mg/l $ZnSO_4 \cdot 7\ H_2O$ and 1 mg/l $NA_4MoO_4 \cdot 2\ H_2O$ at 4 °C.

The fungal strains were normally grown on Czapek Dox Broth medium + 5 mg/l $ZnSO_4 \cdot 7\ H_2O$ and 1 mg/l $Na_2MoO_4 \cdot 2\ H_2O$ in 100 ml conical flasks at 30 °C. The inoculum was 10^6 15-day-old conidia from culture grown