J. Pharm. Pharmacol. 1987, 39: 323–324
Communicated August 4, 1986

Tamoxifen inhibits 5-lipoxygenase in human polymorphonuclear leucocytes

I. A. TAVARES*, I. F. STAMFORD, A. BENNETT, *Department of Surgery, King's College Hospital School of Medicine and Dentistry, The Rayne Institute, 123 Coldharbour Lane, London SE5 9NV, UK*

Breast cancer patient survival is increased by tamoxifen, and we therefore need to understand how this drug exerts its effect. We describe a novel action of tamoxifen, the inhibition of LTB_4 and 5-HETE production from [^{14}C]-arachidonic acid by human polymorphonuclear leucocytes.

Tamoxifen is an important anticancer drug since it increases the survival of breast cancer patients by 20% over a 6-year period (Nolvadex Adjuvant Trial Organisation 1985). Although tamoxifen is thought to act via oestrogen receptors, it helps some patients with oestrogen receptor-negative mammary carcinomas. Thus one or more other mechanisms may be involved. Substantial amounts of prostaglandins and lipoxygenase products can be formed by human mammary carcinomas (Stamford et al 1983), and both these classes of substances may contribute to cancer (Powles et al 1982). Tamoxifen inhibits prostaglandin formation by human breast cancers in-vitro (Ritchie 1980), and we now report that it inhibits 5-lipoxygenase in human polymorphonuclear leucocytes.

Materials and methods

Human polymorphonuclear leucocytes (PMNs) were studied because they are readily available. They form mainly leukotrienes from arachidonic acid (Salmon et al 1985), and are present in malignant tumours. The PMNs in venous blood from 6 healthy male volunteers (age 25–40 years) were separated as described by English & Anderson (1974), using a Ficoll-Paque gradient with Histopaque-1119 and 1077 (Sigma Chemical Company UK). Aliquots were then incubated (5×10^5 PMNs in 900 μL) for 15 min at 37 °C with or without tamoxifen citrate (ICI) at final concentrations of 1, 10 and 100 μg mL^{-1} (equivalent to 0·66, 6·6 and 66 μg mL^{-1} tamoxifen). The drug was first dissolved in ethanol (10 mg mL^{-1}) and then diluted in phosphate-buffered saline. After incubation with [^{14}C]arachidonic acid (Radiochemical Centre, Amersham, 0·1 μCi, 1·7 nM) and the calcium ionophore A23187 (Sigma Chemical Company, 1 μg) in a final volume of 1 mL for an additional 5 min, the metabolic activity was terminated by adding 1 mL of 1% formic acid in methanol.

The [^{14}C]arachidonic acid products were extracted into diethyl ether and separated by thin layer chromatography on silica gel plates (Eastman Kodak Ltd, UK) using the organic phase of ethyl acetate–hexane–acetic acid–water (112 : 48 : 24 : 120). After autoradiography (Kodak NS-2T film, 10 day exposure), the metabolites were characterized by comparing their chromatographic mobilities with 5-hydroxy-eicosatetraenoic acid (5-HETE; The Upjohn Company, USA) and leukotriene B_4 (LTB_4; ICI), and quantified by liquid scintillation counting.

Results

PMNs stimulated by the calcium ionophore A23187 converted [^{14}C]arachidonic acid into substances that co-chromatographed with LTB_4 and 5-HETE. The effects of tamoxifen citrate, the cyclooxygenase inhibitor, flurbiprofen, and the lipoxygenase/cyclo oxygenase inhibitor, BW755C (3-amino-1-[(*m*-trifluoromethyl)-phenyl]-2-pyrazoline), on lipoxygenase product accumulation are shown in Table 1. None of the drugs had an effect at 1 μg mL^{-1}

Table 1. Effect of drugs on LTB_4 and 5-HETE accumulation in incubates of human polymorphonuclear leucocytes. All the drugs at the highest concentration reduced the yields of the lipoxygenase products, and BW755C was effective at 10 μg mL^{-1}. The results are expressed as a % of control (mean ± s.e.m.), n = 6.

Drugs μg mL^{-1}	LTB_4	5-HETE
Tamoxifen citrate		
1	106·3 ± 25·9	100·6 ± 12·5
10	90·2 ± 13·1	85·4 ± 6·9
100	37·7 ± 4·6 a	40·5 ± 5·3 b
Flurbiprofen		
1	102·6 ± 24·9	100·4 ± 7·8
10	85·7 ± 10·4	93·5 ± 4·2
100	60·3 ± 17·4 a	55·4 ± 11·7 a
BW755C		
1	103·0 ± 26·8	95·4 ± 6·7
10	63·2 ± 14·6 a	58·2 ± 11·1 a
100	23·8 ± 7·1 a	26·7 ± 4·9 b

P values, a < 0·05; b < 0·005 (Student's *t*-test for paired data).

At 10 μg mL^{-1} BW755C there was a reduction of about 40% ($P < 0·05$), whereas at this concentration the other drugs showed only a weak tendency to inhibit. However, at 100 μg mL^{-1} all the drugs caused a

* Correspondence.

substantial reduction, the order of potency being BW755C > tamoxifen > flurbiprofen.

Discussion

The human peripheral blood PMNs stimulated with the calcium ionophore A23187 converted [^{14}C]arachidonic acid to LTB_4 and 5-HETE; this conversion was concentration-relatedly inhibited by the lipoxygenase/cyclooxygenase inhibitor BW755C (Salmon et al 1983). At the highest concentration (100 $\mu g\,mL^{-1}$) tamoxifen citrate and flurbiprofen also caused substantial inhibition, and a similar weak trend was seen with 10 $\mu g\,mL^{-1}$. Daniel et al (1981) found a mean tumour content of 25·1 (range 5·4–117) ng tamoxifen mg^{-1} protein in patients taking 40 mg tamoxifen daily. Patterson et al (1982) converted the mean value to 6·7 μM (2·5 $\mu g\,mL^{-1}$) by assuming a 10% content of protein in the tumours, and on the same basis the top of the range would be 11·7 $\mu g\,mL^{-1}$ tamoxifen (equivalent to 17·7 $\mu g\,mL^{-1}$ tamoxifen citrate). Thus an effect on 5-lipoxygenase may occur at therapeutically relevant concentrations. Furthermore, preliminary evidence indicates that the 5-lipoxygenase in a cell-free system is more sensitive than human PMNs to tamoxifen. This may be due to the rather high resistance of human PMNs to drugs that modify eicosanoid synthesis (Tavares et al 1986). It remains to be seen whether inhibition of 5-lipoxygenase contributes to the beneficial effect of tamoxifen in breast cancer.

We thank the CRC and MRC for support.

REFERENCES

Daniel, P., Gaskell, S. J., Bishop, H., Campbell, C., Nicholson, R. I. (1981) Eur. J. Cancer 17: 1183–1189

English, D., Anderson, B. R. (1974) J. Immunol. Methods 5: 249–254

Nolvadex Adjuvant Trial Organisation (1985) Lancet i: 836–840

Patterson, J., Furr, B., Wakeling, A., Battersby, L. (1982) Breast Cancer Research and Treatment 2: 363–374

Powles, T. J., Bockman, R. S., Honn, K. V., Ramwell, P. (1982) Prostaglandins and Cancer: First International Conference, Alan R. Liss, New York

Ritchie, G. A. F. (1980) In Henningsen, B., Lindner, F. Steichele, C. (eds) Endocrine Treatment of Breast Cancer, Springer Verlag, Heidelberg, pp 96–101

Salmon, J. A., Simmons, P. M., Moncada, S. (1983) J. Pharm. Pharmacol. 35: 808–813

Salmon, J. A., Tilling, L. C., Moncada, S. (1985) Prostaglandins 29: 363–375

Stamford, I. F., Carroll, M. A., Civier, A., Hensby, C. N., Bennett A. (1983) J. Pharm. Pharmacol. 35: 48–49

Tavares, I. A., Sergis, A. N., Berry, H., Bennett, A. (1986) Prog. Lipid Res. 25: 577–578

J. Pharm. Pharmacol. 1987, 39: 324–326
Communicated October 24, 1986

Potentiation of contractile response and increase in tissue sodium content induced by aconitine in the guinea-pig vas deferens

YASUSHI OHIZUMI*, AKIKO KAJIWARA, *Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan*

Aconitine potentiated the contractile response of the guinea-pig vas deferens and increased the tissue Na and Ca content. These effects were abolished in the presence of tetrodotoxin. These results suggest that aconitine causes an increasing Na^+ permeability of the smooth muscle membrane to increase Ca^{2+} availability and thus induces potentiation.

In Japan and China, *Aconitum* root has been used to treat symptoms such as pain, paralysis, atonia and coldness of extremities and one of the alkaloids from the root, aconitine, is known to produce an arrhythmia of the heart (Scherf & Terranova 1949). Electrophysiological studies have revealed that aconitine-induced arrhythmia is due to a delay in the repolarization phase of the action potential (Matsuda et al 1959; Schmidt 1960). In frog skeletal muscles, aconitine causes bursts of repetitive firing which are prevented by tetrodotoxin (TTX) (Ellis & Bryant 1973). Aconitine has been shown to induce an increase in the membrane permeability of the squid giant axon (Herzog et al 1964) and motor nerve fibres (Schmidt & Schmitt 1974) to Na^+. Mesaconitine, one of the related compounds of aconitine has been found to cause a marked contraction of the guinea-pig isolated vas deferens (Sato et al 1979) and ileum (Sato et al 1980) mediated through neurotransmitter release. However, the direct actions of these alkaloids on smooth muscles have not yet been studied. In this paper, we report the first evidence of the direct action of aconitine on smooth muscle using guinea-pig vas deferens.

*Correspondence.

Methods

Male guinea-pigs, 250 to 350 g, were used. The method of preparing the tissue and the technique for measurement of contractions were performed as described