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SPECIAL REPORT

Lens opacification by antioestrogens: tamoxifen vs ICI 182,780

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The antioestrogen, tamoxifen, blocks volume-regulated chloride channels and reduces transparency in bovine lenses maintained *in vitro*. In contrast to tamoxifen, the sterodial antioestrogen, ICI 182780, did not block volume-regulated chloride currents in three cultured cell lines and required 10 fold higher concentration to induce significant opacification of bovine lenses maintained *in vitro*. These data suggest that ocular toxic side effects will be minimized by use of the steroidal (ICI 182780) rather than nonsteroidal antioestrogens (tamoxifen).

Keywords: Antioestrogens; tamoxifen; ICI 182,780; chloride channels; lens; cataracts

Introduction Non-steroidal antioestrogens such as tamoxifen are being evaluated for their efficacy as prophylactic agents in women at high risk of breast cancer (Defriend *et al.*, 1994). The incidence of side-effects of tamoxifen treatment is low and appears to be related to its partial steroid activity. However, ocular toxicity has been described in patients on long-term, low-dose treatment (Pavlidis *et al.*, 1992; Gerner, 1992). Tamoxifen has also been shown to cause cataract formation in rats (Furr & Jordan,1984).

Lens clarity depends on the maintenance of adequate cell hydration, an effect that is related to the movement of chloride ions. Removal of extracellular chloride or addition of chloride channel blockers both result in lens opacification (Zhang *et al.*, 1994). Chloride channels in the lens fibre cells have certain electrophysiological and pharmacological characteristics in common with volume-activated chloride channels found in most mammalian cells: notably both are sensitive to blockade by extracellular tamoxifen (Valverde *et al.*, 1993; Zhang & Jacob, 1994). We have suggested that tamoxifen is able to cause cataract by blocking chloride channels in lens fibre cells (Zhang *et al.*, 1994).

In contrast to triphenylethylene compounds such as tamoxifen which are structurally unrelated to oestrogen and possess partial agonist activity, ICI 182,780 and ICI 164380 are structural analogues of oestrogen of greater potency but less agonist activity. ICI 182,780 has been proposed as an alter-

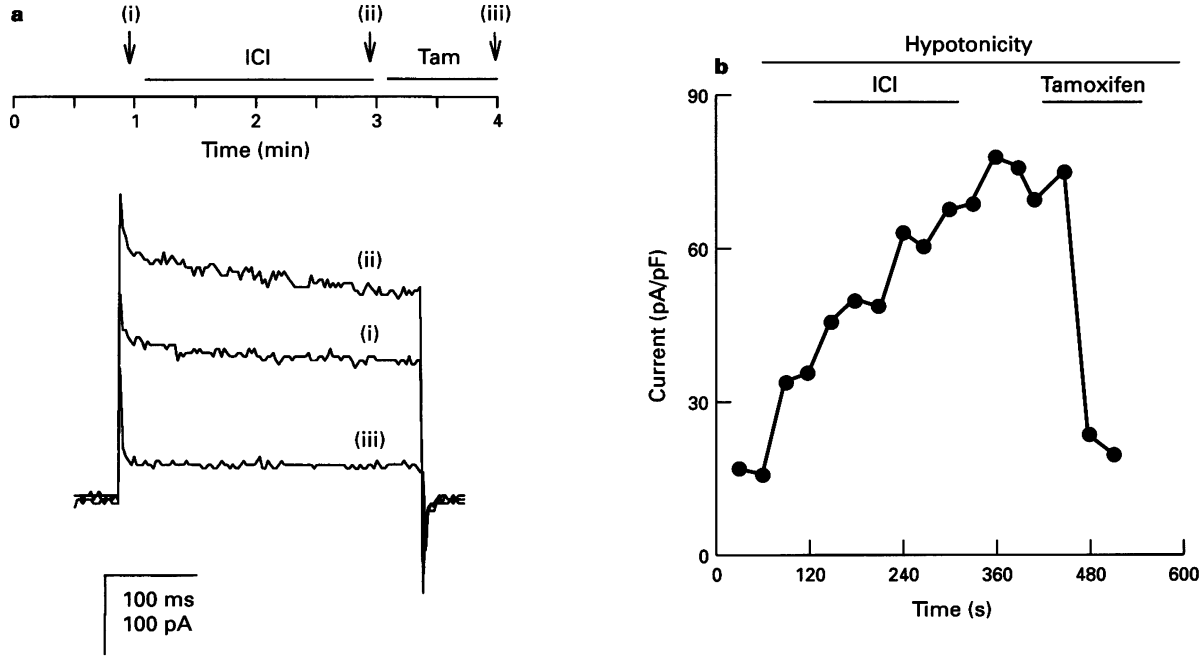

Figure 1 (a) Representative volume-activated chloride currents elicited from a C1300 neuroblastoma cell in response to +80 mV pulses from 0 mV holding potential. Currents were recorded sequentially during exposure to a hypotonic bathing solution for approximately 4 min. Traces shown are taken as indicated (i) after 1 min in hypotonic bathing solution; (ii) 2 min in hypotonic solution containing 30 μM ICI 182,780 and (iii) 1 min in hypotonic solution containing 10 μM tamoxifen. Note the increase in currents in response to hypotonic challenge was not altered by 30 μM ICI 182,780. The osmotic gradient between the bathing solution and the pipette (intracellular) solution was 20 mOsm. (b) Time course of action of 30 μM ICI 182,780 and 10 μM tamoxifen on volume-activated chloride currents in a C1300 cell as indicated above the figure. The currents (measured at +40 mV) are expressed according to cell size (16 pF).

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native to tamoxifen treatment (Wakeling, 1993). We have compared their ability to block volume-activated chloride channels and cause lens opacification *in vitro*.

Methods and Results Details of the bovine lens culture *in vitro*, measurement of lens transparency and whole cell patch clamp experiments are described elsewhere (Zhang *et al.*, 1994).

In isotonic bathing solution (300 ± 10 mOsm) no significant whole cell chloride currents were observed in three cultured cell lines: T84 colonic carcinoma, HeLa cervical carcinoma and C1300 neuroblastoma cells but following exposure to hypotonic solutions (250 ± 10 mOsm) volume-activated chloride currents with outward rectification and current inactivation at depolarizing potentials were elicited from all three cell types (as described previously by many workers). The currents were blocked by >90% within several minutes following exposure to extracellular 10 μM tamoxifen (maximal inhibitory concentration, see Zhang *et al.*, 1994) in all three cell types ($n = 5$ for each cell type. See Figure 1). In contrast, the pure antioestrogen ICI 182,780 at concentrations up to 30 μM (5, 10 and 30 μM; $n \geqslant 3$) had no detectable inhibitory effect in any of the three cell types (Figure 1). The related antioestrogen ICI 164384 also had no inhibitory effect at 30 μM in HeLa cells and T84 cells (data not shown).

These results were mirrored in their effect on lens transparency (Figure 2). In bovine lenses maintained in organ culture for 86 h, tamoxifen reduced the transparency in a dose-dependent manner from 5 μM to 100 μM whereas ICI 182780 was only able to induce opacification at concentrations greater than 10 μM and with markedly reduced potency (Figure 2).

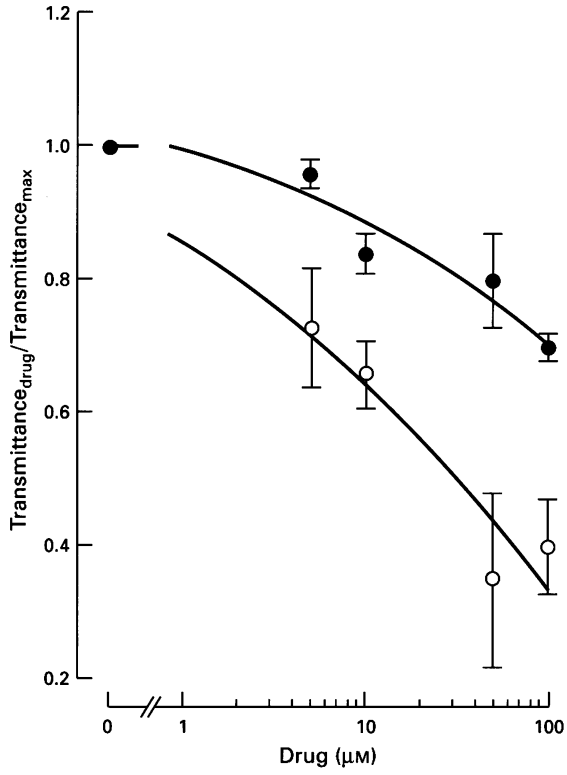

Figure 2 Lens transparency was recorded and expressed as a ratio of transmittance measured in the absence and presence of drug (tamoxifen (○) or ICI 182780 (●)). The slope of the fitted lines, using second-order regression, were −0.17 ± 0.03 and −0.07 ± 0.03 for tamoxifen and ICI 182780 respectively. Values represent the mean ± s.e. of three lenses under each condition.

Discussion The results obtained on volume-activated chloride channels may be used as a predictive marker of the effect of antioestrogens on lens fibre cell chloride channels as it is not possible to examine the lens chloride channels directly by whole-cell patch clamp because the fibre cells are up to 1 cm long and tend to fragment during enzymatic dissociation.

The pure antioestrogen, ICI 182,780, did not block volume-activated chloride channels in three different cell types at concentrations up to 30 μM whereas tamoxifen caused maximal inhibition at 10 μM. These results corresponded with their effects on lens opacification at low concentrations. Compared with tamoxifen, ICI 182,780 had a reduced propensity to cause opacification of the lens. At the higher concentrations tested, some loss of transparency was seen with ICI 182,780 but the degree of opacification induced by tamoxifen was greater at all points. At 5 μM, a concentration comparable to that obtained in patients on high-dose tamoxifen treatment, tamoxifen was able to induce significant opacification of the lens whilst ICI 182,780 had no detectable effect.

These data indicate that the effects of tamoxifen on channel blocking and lens opacification are independent of its antioestrogenic activity and indicate that the use of ICI 182,780 in place of non-steroid antagonists such as tamoxifen is likely to minimize the development of cataract and other side effects unrelated to their antioestrogen activity.

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