



## Topical tamoxifen – a potential therapeutic regime in treating excessive dermal scarring?

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**SUMMARY.** Abnormal dermal scarring which affects a large number of people is aesthetically disfiguring and can be functionally disabling. Existing medical and surgical strategies to prevent or to treat scars are frequently disappointing and more effective therapies are needed. Tamoxifen, which has been used extensively in the treatment of breast cancer over the last 20 years has recently been shown to inhibit the proliferation of fibroblasts cultured from keloid biopsies. Successful treatment of retroperitoneal fibrosis and desmoid tumours with tamoxifen has also been reported. We have investigated the potential of tamoxifen as an inhibitor of wound contraction, using fibroblast-populated collagen lattices as an *in vitro* model. From these studies we postulate that tamoxifen may have potential clinical significance in the treatment of abnormal scarring.

Normal adult human skin fibroblasts were embedded within type I collagen, then medium either with or without addition of tamoxifen was added to the collagen lattices. Lattice diameters were measured at intervals to assess the influence of tamoxifen on the lattice contraction. The reversibility of the inhibitory effect of tamoxifen on lattice contraction was investigated by 'washing out the tamoxifen' at different time-points. To visualise changes in the morphology of fibroblasts MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was added to the lattices.

Tamoxifen at 1 and 5  $\mu\text{M}$  had no significant influence on lattice contraction but higher concentrations of 50 and 100  $\mu\text{M}$  completely inhibited contraction. At intermediate concentrations from 10 to 20  $\mu\text{M}$  the degree of lattice contraction was dose-dependent. The reversibility of the inhibition was both dose- and time-dependent. Both the inhibition of contraction and the reversibility of inhibition appeared to correlate with changes in fibroblast morphology. The dose- and time-dependent inhibition of contraction by fibroblasts suggests that tamoxifen could be investigated as a novel potential therapeutic agent in treating abnormal dermal scarring.

Abnormal cutaneous scarring, such as hypertrophic and keloid scars and contracture following burn injury can be aesthetically disfiguring and severely disabling and, unfortunately, existing treatments are often unsuccessful. Recently, it has been suggested that tamoxifen (1-[p-Dimethylaminoethoxyphenyl]-1,2-diphenyl-1-butene), which has been used extensively in the treatment of breast cancer over the last 20 years, might be a novel means by which keloids could be treated.<sup>1</sup> This was based on *in vitro* studies demonstrating that tamoxifen inhibited the proliferation of keloid fibroblasts and their rate of collagen synthesis. It has also been reported that benign tumours such as desmoids and retroperitoneal fibroses have been treated successfully with tamoxifen.<sup>2-6</sup> The effects of tamoxifen include altered RNA transcription, decreased cellular proliferation, delay or arrest of the cells in the G<sub>1</sub> phase of the cell cycle and interference with multiple growth factors such as TGF- $\beta$  (transforming growth factor- $\beta$ ) and IGF (insulin growth factor).<sup>1</sup>

We have studied the effects of tamoxifen on normal human dermal fibroblast proliferation in monolayer culture to determine whether these would be similar to effects on keloid fibroblasts. However, a three-dimensional lattice of type I collagen containing human skin fibroblasts resembles the living dermis more closely than does a cell monolayer, and contraction of this type of collagen lattice by fibroblasts has been used as

an *in vitro* model for connective-tissue contraction in wound healing.<sup>7,8</sup> In this early report we present the results of *in vitro* studies using the collagen lattice model to examine further the potential effects of tamoxifen on wound contracture.

### Materials and methods

#### Cell culture

Adult human fibroblasts from breast skin were thawed from frozen stock, and maintained as monolayers in DMEM (Dulbecco's Modified Eagle's Medium) with 10% (v/v) FCS (Foetal Calf Serum). Cells from passages 3 to 7 were used for experiments.

#### Tamoxifen solution

Stock solutions of tamoxifen (Sigma) 9 mM were prepared by dissolution in 100% ethanol, and stored at 4°C. The stock solution was diluted in culture medium immediately before use in experiments.

#### Cell proliferation

Adult human dermal fibroblasts were seeded in 96-well plates at 10<sup>3</sup> cells/well in medium with 10%

FCS. After 24 h the medium was changed to 0.4% FCS for 48 h to make the cells quiescent. Further growth in either 0.4% FCS or 10% FCS containing various concentrations of tamoxifen was monitored using the MTT assay. In this assay the mitochondria of living cells reduce a yellow solution of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to a blue formazan and the colour is eluted and the absorbance at 570 nm is measured on a spectrophotometer.<sup>9</sup>

#### Preparation of collagen lattices

Type I collagen was extracted from rat tail tendons<sup>10</sup> and the collagen stock solution was diluted to a working solution of 1.6 mg/ml on the day before the experiment. Cells were trypsinised, resuspended in medium and counted using a hemocytometer. A collagen/cell suspension was prepared<sup>11</sup> and 2 ml of the suspension were dispensed to 32 mm petri dishes. Final concentrations were 0.8 mg/ml of collagen, 4.7% FCS with a cell density of  $6.7 \times 10^4$  cells/ml. The lattices were incubated for 10 min at 37°C and then 1 ml of medium either with or without tamoxifen was added to the collagen lattices. Medium containing a volume of ethanol equivalent to that in the maximum dose of tamoxifen was added to one set of lattices. The gels were immediately released from the sides and bottom of the petri dish with a spatula and this was considered as zero time. The reversibility of the effect of tamoxifen was determined by the substitution of normal medium for tamoxifen-containing medium after various periods of time. Lattice diameters were measured by placing dishes on transparent graph paper and using a fibre optic lamp to optimise the visibility of the gel edges. Two diameters at right angles were measured and the mean diameter used to calculate the area and then the percentage of initial lattice area.

Some small thinner gels were prepared<sup>12</sup> by putting 0.4 ml of collagen-fibroblast suspension into 24-well plates and adding 0.2 ml of medium, and these were used for determining cell viability.

#### Visualisation of living cells in lattices

At various time points, 100 µl of a stock solution of MTT (5 mg/ml in PBS) was added to one of each set of small gels. After 1–1.5 h the gels were transferred to a microscope slide, covered with a coverslip and photos taken with a Leitz Diavert inverted microscope and camera.

## Results

#### Cell proliferation

A single dose of tamoxifen at concentrations of 10 µM or higher in 0.4% FCS killed normal human dermal fibroblasts in monolayer within 24 h. At lower concentrations the degree of toxicity was dose-dependent, with an  $IC_{50}$  of 3.9 µM for prolonged exposure (9 days). In an experiment using tamoxifen in medium with 10% FCS the  $IC_{50}$  was 12.5 µM indicating that

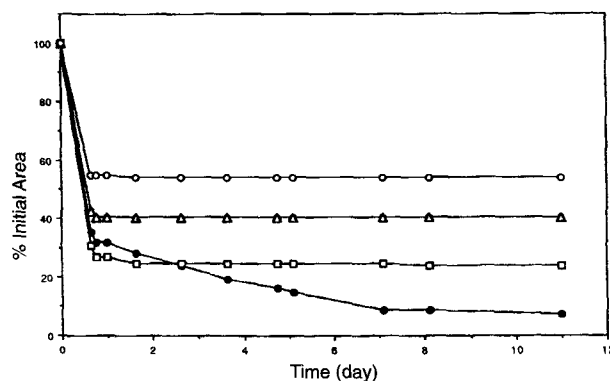
the serum was conferring some protection against the toxic action of tamoxifen. The MTT response in 10 µM tamoxifen in this higher concentration of FCS was 62% of controls at 24 h. However, the fibroblasts continued to proliferate with the absorbance on day 9 being 230% of that on day 1. The ratio of the response in 10 µM tamoxifen remained at approximately 60% of controls (monolayer data not shown).

#### Collagen lattice contraction

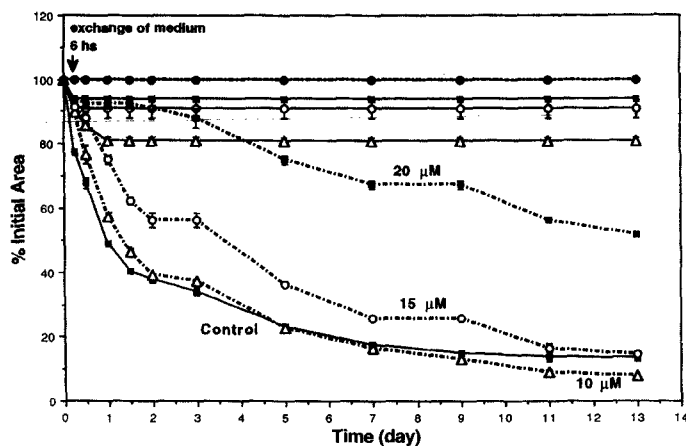
Tamoxifen at 1 µM and 5 µM had no significant effect on lattice contraction, whereas concentrations of 50 µM and 100 µM completely inhibited contraction (data not shown). By 14 h, inhibition of contraction was evident in 15 µM and 20 µM tamoxifen (Fig. 1) and by 40 h contraction was less than in controls by 12% and 27% respectively. There was no further contraction for the remaining 9 days of the experiment, while the controls continued to contract steadily till day 7 so that inhibition by then was 33% and 48% respectively in 15 µM and 20 µM tamoxifen. In 10 µM tamoxifen contraction was slightly greater than in controls for the first 2 days, but contraction then ceased so that by day 7 it was 16% less than in controls. The effect of a volume of vehicle equivalent to that contained in a 50 µM tamoxifen solution was negligible (data not shown).

#### Reversibility of the inhibition of collagen lattice contraction by tamoxifen

The reversibility of the inhibition of lattice contraction by tamoxifen was both dose-dependent (Fig. 2) and time-dependent (Fig. 3). Tamoxifen was washed out of the lattices at intervals from 6 h to 3 days. Inhibition for 3 days in tamoxifen proved to be irreversible (data not shown). When tamoxifen was washed out after 6 h lattices in 10 µM began to contract immediately at the same rate as controls, while those in 15 µM contracted more slowly than controls,

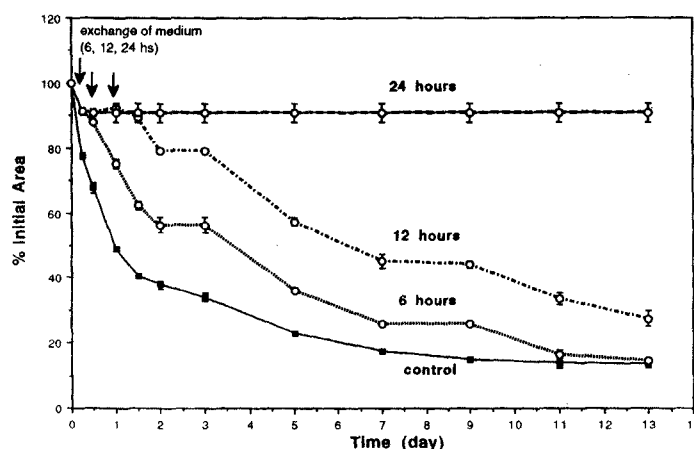


**Figure 1**—Effect of tamoxifen on lattice contraction by human skin fibroblasts. Human skin fibroblasts, P4. Each point is a mean ( $\pm$  SEM) from three lattices. Where error bars for the SEM are not visible, it is because they are too small relative to the size of the symbol.  
 ●—DMEM/10%FCS □—TAM 10 µM ▲—TAM 15 µM  
 ○—TAM 20 µM



**Figure 2**—Dose-dependence of the reversibility of inhibition of collagen lattice contraction by tamoxifen. Human breast skin fibroblasts, P7. Dotted lines show resumed contraction of the lattices when the tamoxifen was washed out after 6 hours. Each point is a mean ( $\pm$  SEM) from three lattices.

■— DMEM/10%FCS    △— TAM 10  $\mu$ M    ○— TAM 15  $\mu$ M    □— TAM 20  $\mu$ M    △— TAM 10  $\mu$ M  
 ○— TAM 15  $\mu$ M    □— TAM 20  $\mu$ M    ●— TAM 30  $\mu$ M    ●— TAM 30  $\mu$ M



**Figure 3**—Time-dependence of the reversibility of inhibition of collagen lattice contraction by 15  $\mu$ M tamoxifen. Human adult breast skin fibroblasts, P7. Dotted lines show resumed contraction of the lattices when the tamoxifen was washed out at different times. Each point is a mean ( $\pm$  SEM) from three lattices.

■— DMEM/10%FCS    ○— TAM 15  $\mu$ M, 6 hours    ○— TAM 15  $\mu$ M, 12 hours  
 ○— TAM 15  $\mu$ M, 24 hours

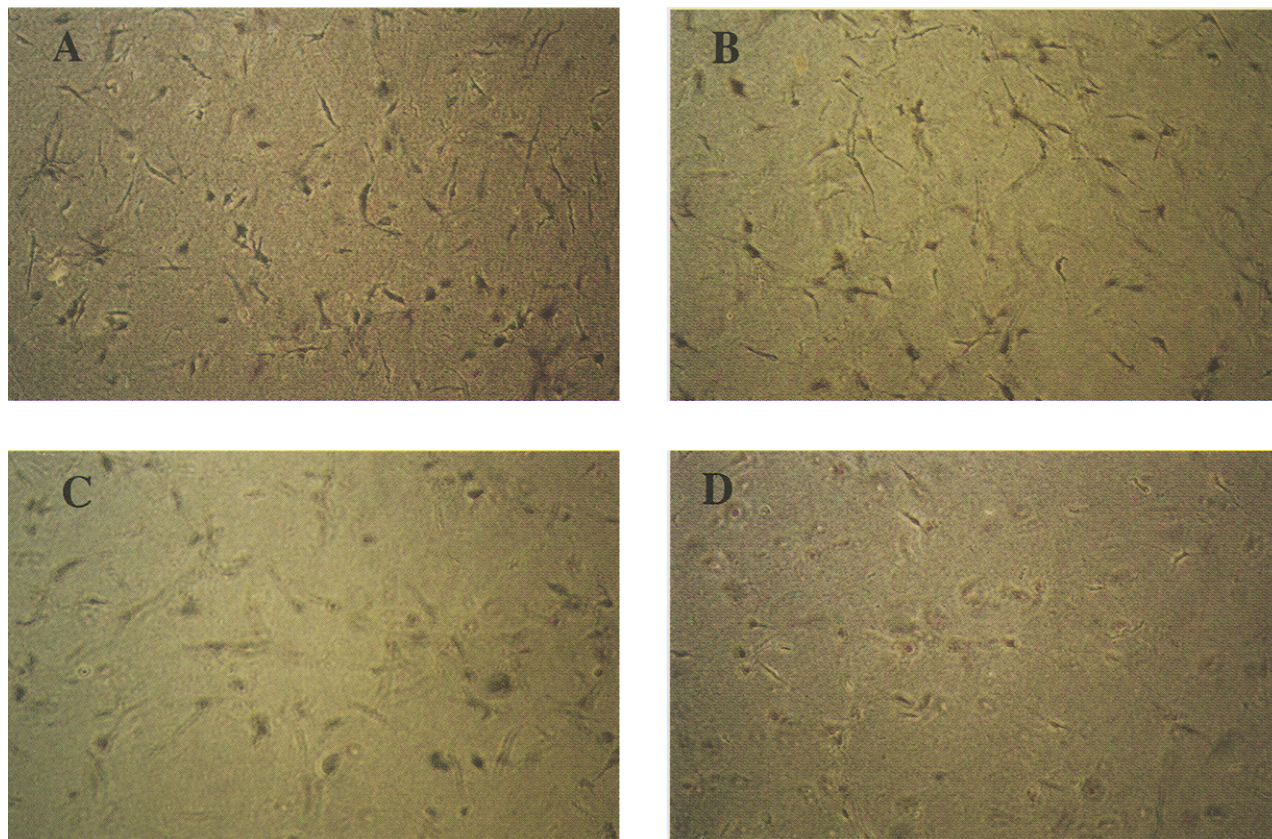
but decreased to the same size by day 11 (Fig. 2). Lattices in 20  $\mu$ M tamoxifen only resumed contraction 2 days after the washing out and contraction was still 38% less than controls by day 13, the last time point measured. In 30  $\mu$ M tamoxifen the inhibition of contraction was completely irreversible after only 6 h.

In the initial experiment on reversibility after 24 h, the inhibition of contraction in 10  $\mu$ M and 15  $\mu$ M tamoxifen did not appear to be due to cell death as shown by microphotographs (Fig. 4 A–C). The tamoxifen was washed out of certain gels in each set after 24 h and gel contraction resumed. In 20  $\mu$ M some cells were still alive at 24 h (Fig. 4D), but there was not enough recovery for contraction to resume after washout. A further experiment was designed to investigate the reversibility of inhibition both at 24 hours and at earlier time points and the

time-dependency for 15  $\mu$ M tamoxifen is shown in Figure 3. When tamoxifen was washed out after 6 h, contraction was observable after a delay of a further 10 h and the area closely approached that of controls by day 12. When tamoxifen was washed out after 12 h, it was a further 48 h before contraction was resumed and by day 12 the area was still less than that of controls. However, in contrast to the earlier experiment, when tamoxifen was in the gels for 24 h the inhibition by 15  $\mu$ M tamoxifen was irreversible.

#### Morphology

Fibroblasts in lattices in control medium began to elongate and put out processes after a few hours. This change to elongate morphology is essential to enable the cells to gather and compact the collagen.<sup>13</sup> Similar



**Figure 4** Morphology of human dermal fibroblasts in fibroblast-populated collagen lattices at 24 h. Morphology monitored under light microscope (X 100). Cells were stained with MTT to improve visualisation of live cells. 100  $\mu$ l of MTT (5 mg/ml in PBS) was added to the gels and after 1 to 1.5 hours photographs were taken. (A) control; (B) 10  $\mu$ M TAM; (C) 15  $\mu$ M TAM; (d) 20  $\mu$ M TAM. In 10  $\mu$ M (B) and 15  $\mu$ M (C) cells were stained blue and many are elongated and have processes similar to controls (A). In 20  $\mu$ M TAM(D) some cells are blue, a few have short processes, but most are rounded.

fibroblast morphology to that in control lattices was observed in lattices cultured with low tamoxifen concentrations of 1, 5, and 10  $\mu$ M. However, in lattices cultured with tamoxifen at concentrations higher than 10  $\mu$ M, fibroblast morphology appeared abnormal. In 15 and 20  $\mu$ M tamoxifen cells had fewer and shorter processes than controls, with some cells remaining rounded. In 50 and 100  $\mu$ M tamoxifen, all cells in the lattices appeared completely rounded. In one experiment in which the 15  $\mu$ M tamoxifen was washed out after 24 h, a change in the morphology with cells developing more cytoplasmic processes correlated with resumed lattice contraction (Fig. 5). In the experiments where cells in 15  $\mu$ M tamoxifen remained rounded when the drug was washed out there was no resumption of contraction.

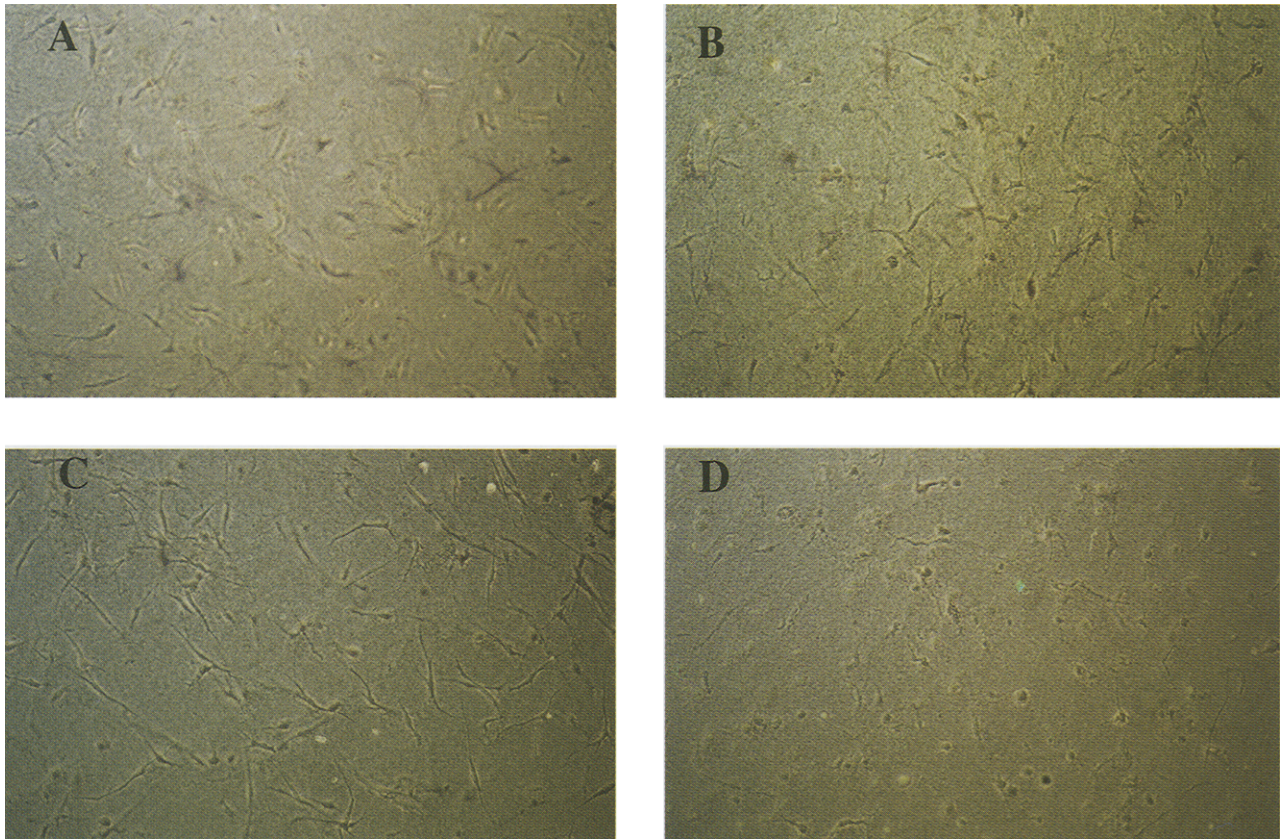
## Discussion

Hypertrophic and keloid scars are characterised by abnormal proliferation of fibroblasts and overproduction of collagen.<sup>14</sup> It has been reported that tamoxifen inhibits the proliferation of keloid fibroblasts and their collagen synthesis in monolayer culture<sup>1</sup> and our investigations have demonstrated similar effects on the proliferation of normal human dermal fibroblasts. Our studies have also shown that tamoxifen inhibits the contraction of collagen lattices by fibroblasts in a

dose-dependent manner. Variations in cytotoxicity levels are some times observed between monolayer and three-dimensional cell culture systems. The cytotoxic concentrations for our normal dermal fibroblasts in monolayer culture were of the same order of magnitude as those of Mancoll et al for keloid fibroblasts,<sup>1</sup> although dependent on the percentage of FCS in the medium. MacNeil<sup>15</sup> suggested that the availability of drugs for interaction with cells was much less if the drugs were able to bind to serum protein or to collagen-coated dishes rather than to the cell. In the lattice system the concentration of collagen was considerably higher than in a coated dish. MacNeil's group had found, as we did, that the potency of tamoxifen was less in the presence of serum. The increased concentration for toxicity in gels as opposed to monolayer culture also occurred in our minoxidil study,<sup>12</sup> though the difference was greater than for tamoxifen.

In the three-dimensional collagen lattice model, tamoxifen at 50  $\mu$ M caused complete inhibition of lattice contraction. In assessing agents that impair wound contraction it is important to determine that the drug is not simply inhibiting contraction by killing the cells and, because of this, studies looking at reversibility are mandatory. The inhibition of contraction by tamoxifen could be reversed to different degrees depending on both the tamoxifen concentration and the time of washing out the drug. It is not





**Figure 5** The morphology of fibroblasts in FPCL at 3 days, after 'washout' of tamoxifen at 24 h (X 100). (A) controls without tamoxifen; In 10  $\mu$ M (B) and 15  $\mu$ M (C) tamoxifen for 24 h fibroblasts have elongated and extended processes and regained close to normal morphology. D0 in 20  $\mu$ M tamoxifen the fibroblasts have died, even though the tamoxifen was washed out.

clear whether the delay of 2 days before resumed contraction after 6 h in 20  $\mu$ M tamoxifen was due to a recovery in cell number or to recovery in the metabolism of the cells, expression of fibronectin or some other factor. Normal primate skin fibroblasts do not usually proliferate within collagen lattices in the first three or four days.<sup>16,17</sup> The reversibility of lattice contraction following washout of 15  $\mu$ M tamoxifen at 24 h was less consistent than for 10  $\mu$ M and further studies of intermediate concentrations will be undertaken.

The *in vitro* results indicate that tamoxifen might be of use *in vivo* to control the remodelling of the extracellular matrix in abnormal scarring. These findings have potential clinical significance. Doses of tamoxifen similar to those used in breast cancer, (10 mg bid) have been shown to cause regression of desmoid tumour.<sup>24</sup> Tamoxifen is generally a well-tolerated drug even when therapy has continued long-term.<sup>18</sup> In the case of desmoid tumours, oral tamoxifen had to be maintained and cessation of the drug led to recurrence. Safe and relevant doses of tamoxifen could be determined for clinical use in cases of scarring.

Oral administration of tamoxifen is usually given at either 10 mg or 20 mg twice daily. In one study of 14 patients, tamoxifen therapy of 20–40 mg daily for up to 3.5 years was generally shown to produce levels of 0.3–0.6  $\mu$ molar in the serum (100–200 ng/ml) where the drug is 98% bound to albumin.<sup>19</sup> In patients that had only been on therapy for up to 4 months levels

were lower, about 0.06  $\mu$ molar. This serum level was shown to represent only a very small proportion of the body load of tamoxifen with resultant levels in the tissues, measured at autopsy, being 10- to 60-fold higher than in serum. In three cases, Lien<sup>19</sup> measured levels of tamoxifen and its metabolites in the skin and in the subcutaneous fat as well as in other organs. Concentrations of tamoxifen in the skin were up to nine times higher than in the serum, while in the subcutaneous fat they were up to sixteen times higher. Accumulation of the desmethyltamoxifen metabolite in the skin was up to 7 times higher than in serum in one patient, but not in the others. It was suggested that adipose tissue may act as a reservoir compartment for the drug. In one patient, significant amounts of tamoxifen and several metabolites were found in some tissues 14 months after tamoxifen withdrawal.

Although oral tamoxifen is generally well-tolerated, there are side-effects in some women, including an incidence of 6% ocular toxicity in one study,<sup>20</sup> but this was mainly reversible on withdrawal of the drug. However, there appears to have been little work done on the topical application of tamoxifen. In a small randomised study (5–6 patients per treatment group) Pujol<sup>21</sup> investigated the distribution of 4-hydroxy-tamoxifen after percutaneous gel administration of that metabolite and found that levels in plasma, normal and tumour breast tissue were not as high as levels following oral administration of tamoxifen. Relating the oral dose of tamoxifen and the percutaneous doses

of the metabolite gel was not simple, and in addition, intra-group variation in concentrations was high and the metabolite 4-hydroxy-N-desmethyltamoxifen was not measured. In Lien's investigations,<sup>19</sup> in which levels of skin were measured after oral tamoxifen in three patients, no 4-hydroxytamoxifen could be detected in two patients while in the other the amount of 4-hydroxy-N-desmethyltamoxifen was 8 times higher and -N-desmethyltamoxifen was 56 times higher than that of 4-hydroxytamoxifen and was equivalent to the amount of tamoxifen detected. Various factors may influence the proportions of tamoxifen metabolites, thus the rate of hydroxylation in the liver could be increased by administration of other drugs although the effect was eight times stronger in rat than in human tissue.<sup>22</sup>

A chlorinated tamoxifen analogue, toremifene, has also been used for cancer treatment and for treating desmoids<sup>4</sup> and has been investigated in a topical methylcellulose formulation in the opossum melanoma model study.<sup>23</sup> It was found that, after administration of 0.5–1 mg/day, concentrations in the skin, 1200 nmoles/g, were 500 times higher than in other tissues, including the eyes, and the drug was only detectable in the plasma in 1 animal out of 6. Moderate amounts of metabolites were found.

Circulating growth factors, mechanical stress, and hormones are important factors in the formation of hypertrophic scar and keloid.<sup>24</sup> It was the suggestion that the growth of desmoids, which occur mainly in women of childbearing-age, may be stimulated by oestrogens that led Kinzbrunner to use an anti-oestrogen to treat desmoids. Tamoxifen has been shown to be an effective treatment for several desmoid cases<sup>24</sup> and also for retroperitoneal fibrosis.<sup>5,6</sup>

Tamoxifen was originally thought to inhibit cell growth by competitive binding to the oestrogen receptor, but it has been shown to inhibit the growth of some oestrogen-negative breast cancer cell lines. Clinically, up to 30% of oestrogen-negative breast cancer patients respond to tamoxifen therapy. Desmoids, which are mesenchymal tumours, are also low in oestrogen receptors. Later studies have shown tamoxifen to have many other effects such as the alteration of metabolic pathways by inhibition (ornithine decarboxylase, cholesterol synthesis, microsomal lipid peroxidation, protein kinase C); the reduction of IGF-1 availability in the circulation and tissues; the upregulation of TGF- $\beta$  production in fetal fibroblasts in culture and in breast tissue of cancer patients.<sup>25</sup>

TGF- $\beta$ 1 is known to play a major role in the excessive production of extracellular matrix leading to fibroses and, like other fibroses, keloids are also characterised by overproliferation of fibroblasts and overproduction of collagen and fibronectin. Mancoll<sup>1</sup> emphasised that such overproduction is considered to be due in part to an increased response to TGF- $\beta$  and that inhibition of TGF- $\beta$  can decrease collagen production. The response of fibroblasts to TGF- $\beta$  may be abrogated by drugs such as glucocorticoids which decrease the secretion of the growth factor and prevent the increase in collagen production.<sup>26</sup> Colletta et al had shown that tamoxifen at 500 nM increased the secretion of active TGF- $\beta$  in fetal fibroblasts grown in monolayer.<sup>27</sup>

In contrast to this effect, Mancoll et al have shown that a single dose of tamoxifen at a higher concentration of 10  $\mu$ M decreases the production of TGF- $\beta$  by keloid fibroblasts by 49% in monolayer culture.<sup>28</sup> In extrapolating possible *in vitro* effects from one cell system to another or one set of conditions to another, it is important to be aware of the many different factors that may affect the outcome. For example, when Clark et al<sup>29</sup> investigated the effect of exogenous TGF- $\beta$  on the stimulation of proliferation, they found that the stimulatory effect of TGF- $\beta$  on DNA synthesis operated only for postconfluent cells and in serum-rich medium. Their medium also contained ascorbic acid which stimulates collagen synthesis. There is also variation in the number of TGF- $\beta$  receptors expressed by different fibroblast types and these have been shown to be higher in fibroblasts from hypertrophic scars and granulation tissue than in unaffected skin.<sup>30</sup> Processes operating in cells cultured in monolayer and responses to growth factors may be very different from those in cells cultured in a three-dimensional matrix.<sup>8,31</sup>

The mitogenic effect of TGF- $\beta$  can also be abrogated by disruption of the secreted fibronectin matrix, but not by disruption of the collagen matrix.<sup>29</sup> This could be another possible mechanism by which tamoxifen might influence fibrosis as both the drug and its hydroxy- and desmethyl-metabolites have been shown to inhibit the interaction of melanoma cells with fibronectin and also with other ECM proteins with IC<sub>50</sub> from 11 to 30  $\mu$ M.<sup>15,32</sup>

Much of the work investigating the effects of tamoxifen on cells *in vitro* has been in the field of cancer research looking at the effects of the drug on cell proliferation and on its potential to inhibit metastasis. The invasion process leading to metastasis requires the attachment of the cancer cells to the extracellular matrix and their movement through it. There is a parallel with some of the processes involved in gel contraction, in which the fibroblasts must first attach to the collagen fibres and then move through the lattice, compacting it.<sup>33</sup> In their study of the effects of tamoxifen and its main metabolites (N-Desmethyltamoxifen and 4-Hydroxytamoxifen) on cancer cell attachment to various extracellular matrices, MacNeil et al<sup>15</sup> showed that at 10  $\mu$ M all three drugs completely inhibited calmodulin activity in a cell-free enzyme assay (with an IC<sub>50</sub> of 2  $\mu$ M) and at 25  $\mu$ M decreased cell attachment to collagen I when added up to 3 h after initial attachment of cells, but not when added after 6 h or more. In the presence of 10% FCS a concentration of 40  $\mu$ M was required for the equivalent effect. In the same experiments the effect of tamoxifen was similar to the specific calmodulin antagonist W7 although the latter was slightly more potent and could detach some cells even after 6 h. This kind of effect could be even more evident in a three-dimensional collagen lattice system where the attachments of fibroblasts to collagen are believed to be via a fibronectin-collagen complex and must take place on all surfaces of the cell, not just underneath. Lattice contraction is also a calcium-calmodulin-dependent process.<sup>34</sup> Ehrlich showed that W7 inhibited the contraction of collagen lattices at concentrations of 6  $\mu$ M while the calcium antagonists verapamil and nifedipine at 10  $\mu$ M

caused 40% inhibition of contraction. The latter compounds have also been shown to inhibit collagen synthesis and, in addition, nifedipine at 10  $\mu\text{M}$  inhibited sulphated glycosaminoglycan production. The first clinical use of intralesional verapamil to improve hypertrophic scarring was reported by Lee<sup>24</sup> in 1994, with doses to achieve 100 to 500  $\mu\text{M}$  in the scar giving considerable improvement in three out of five patients representative of the 30 treated at the time.

In normal wound healing the transition of granulation tissue to scar is marked by a clear decrease in cellularity, with apoptotic cells being removed by phagocytosis either by macrophages or neighbouring cells.<sup>35</sup> In mature keloids the centre of the lesion is avascular, but the lesion-normal dermis interface contains abundant lymphocytes and proliferating fibroblasts, as well as evidence of cells undergoing apoptosis and others undergoing necrosis.<sup>36</sup> Hypertrophic scars differ in containing nodules comprised of high density cells and collagen. Steroids have also been shown to induce apoptosis and cells damaged by these or other drugs might be expected to be eliminated by phagocytosis.

Some of the alterations in metabolic pathways cited by Gagliardi<sup>25</sup> can be ruled out as possible mechanisms for the effect of tamoxifen on lattice contraction. For example, modulation of protein kinase C has been shown to have little effect on the attachment of cells to matrix<sup>32</sup> or on lattice contraction.<sup>34</sup> However, Gagliardi considered it to be a possible mechanism in the inhibition of cell growth.<sup>25</sup>

To our knowledge this is the first report of the effects of tamoxifen on collagen lattice contraction. We have shown that it inhibits contraction at concentrations achieved by oral clinical doses in tissues *in vivo*. From the preliminary results, further investigations are warranted, which would include determination of viability by removing fibroblasts from the lattice and counting them, and determining the mechanisms of the effect of tamoxifen on both normal dermal fibroblasts and hypertrophic scar and keloid fibroblasts cultured in a three-dimensional matrix. Fibroblasts from young active hypertrophic scars have been shown to contract collagen lattices faster than normal fibroblast<sup>37</sup> and we plan to investigate the effect of tamoxifen on such lattice contraction.

## References

- Mancoll JS, McCauley RL, Phillips LG. The inhibitory effect of tamoxifen on keloid fibroblasts. *Surgical Forum* 1996; 47: 718-20.
- Kinzbrunner B, Ritter S, Domingo J, Rosenthal CJ. Remission of rapidly growing desmoid tumors after tamoxifen therapy. *Cancer* 1983; 52: 2201-4.
- Procter H, Singh L, Baum M, Brinkley D. Response of multicentric desmoid tumours to tamoxifen. *Br J Surg* 1987; 74: 401.
- Brooks MD, Ebbs SR, Colletta AA, Baum M. Desmoid tumours treated with triphenylethylenes. *Eur J Cancer* 1992; 28: 1014-8.
- Clark CP, Vanderpool D, Preskitt JT. The response of retroperitoneal fibrosis to tamoxifen. *Surgery* 1991; 109: 502-6.
- Owens LV, Cance WG, Huth JF. Retroperitoneal fibrosis treated with tamoxifen. *Am Surg* 1995; 61: 842-4.
- Adams LW, Priestley GC. Contraction of collagen lattices by skin fibroblasts: drug-induced changes. *Arch Dermatol Res* 1988; 280: 114-8.
- Dubertret L, Coulomb B. Dermo-Epidermal Interactions, Wound Healing and Psoriasis. In T.J. Ryan (Ed.), *Beyond Occlusion: Dermatology Proceedings* (Int. Congress Symp. Series, No. 137.R). London: Society of Medicine, 1988; 19.
- Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J Immunol Methods* 1989; 119: 203-10.
- Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential *in vitro*. *Proc Natl Acad Sci USA* 1979; 76: 1274-8.
- Montesano R, Orci L. Transforming growth factor- $\beta$  stimulates collagen-matrix contraction by fibroblasts: implications for wound healing. *Proc Natl Acad Sci USA* 1988; 85: 4894-7.
- Hughes MA, Cherry G, Dawber RPR, Ryan TJ. Minoxidil-induced changes in the contraction of collagen lattices by human skin fibroblasts. *Plast Reconstr Surg* 1992; 89: 722-30.
- Delvoye P, Nusgens B, Lapière CM. The capacity of retracting a collagen matrix is lost by dermatosparatic skin fibroblasts. *J Invest Dermatol* 1983; 81: 267-70.
- Linares HA, Larson DL. Proteoglycans and collagenase in hypertrophic scar formation. *Plast Reconstr Surg* 1978; 62: 589-93.
- MacNeil S, Wagner M, Kirkham PR, et al. Inhibition of melanoma cell/matrix interaction by tamoxifen. *Melanoma Res* 1993; 3: 67-74.
- Greco RM, Erlich HP. Differences in cell division and thymidine incorporation with rat and primate fibroblasts in collagen lattices. *Tissue and Cell* 1992; 24: 843-51.
- Parish JL, Hughes MA, Cherry GW, Ferguson DJP. The effect of minoxidil analogues and metabolites on the contraction of collagen lattices by human skin fibroblasts. *Br J Plastic Surg* 1995; 48: 154-60.
- Jordan VC. Overview from the International Conference on Long-Term Tamoxifen Therapy for Breast Cancer. *J Natl Cancer Inst* 1992; 84: 231-4.
- Lien EA, Solheim E, Ueland PM. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. *Cancer Res* 1991; 51: 4837-44.
- Pavlidis NA, Petris C, Briassoulis E, et al. Clear evidence that long-term, low-dose tamoxifen treatment can induce ocular toxicity. A prospective study of 63 patients. *Cancer* 1992; 69: 2961-4.
- Pujol H, Girault J, Rouanet P, Fournier S, Freuer J, Simony J, et al. Phase 1 study of percutaneous 4-hydroxytamoxifen with analyses of 4-hydroxy-tamoxifen concentrations in breast cancer and normal breast tissue. *Cancer Chemother Pharmacol* 1995; 36: 493-8.
- Kupfer D, Mani C, Lee CA, Rifkind AB. Induction of tamoxifen-4-hydroxylation by 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), b-naphthoflavone (b NF) and phenobarbital (PB) in avian liver: identification of P450 TCDD<sub>AA</sub> as catalyst of 4-hydroxylation induced by TCDD and bNF. *Cancer Res* 1994; 54: 3140-4.
- Maenpaa J, Dooley T, Wurz G, et al. Topical toremifene: a new approach for cutaneous melanoma? *Cancer Chemother Pharmacol* 1993; 32: 392-5.
- Lee RC, Doong H, Jellema AF. The response of burn scars to intralesional verapamil. Report of five cases. *Arch Surg* 1994; 129: 107-11.
- Gagliardi AR, Henig B, Collins DC. Antiestrogens inhibit endothelial cell growth stimulated by angiogenic growth factors. *Anticancer Res* 1996; 16: 1101-6.
- Parrelli JM, Meisler N, Cutroneo KR. Abrogation of the fibrotic effect of transforming growth factor  $\beta$  in dermal wound healing. *Wound Rep Reg* 1997; 5: 136-40.
- Colletta AA, Wakefield LM, Howell FV, et al. Anti-oestrogens induce the secretion of active transforming growth factor beta from human fetal fibroblasts. *Br J Cancer* 1990; 62: 405-9.
- Mancoll JS, Chau D, Munger J, et al. Tamoxifen downregulates TGF- $\beta$  production by keloid fibroblasts. *Surgical Forum* 1997; 48: 704-7.
- Clark RAF, McCoy GA, Folkvord JM, McPherson JM. TGF- $\beta$ 1 stimulates cultured human fibroblasts to proliferate and produce tissue-like fibroplasia: a fibronectin matrix-dependent event. *J Cell Physiol* 1997; 170: 69-80.

30. Schmid P, Itin P, Cherry G, Bi C, Cox DA. Enhanced Expression of Transforming Growth factor- $\beta$  Type I and Type II receptors in wound granulation tissue and hypertrophic scar. *Am J Pathol* 1998; 152: 485-93.
31. Sato M, Ishikawa O, Abe M, Miyachi Y. Opposite effects of tumour necrosis factor- $\alpha$  on type I and III collagen gene expression by human dermal fibroblasts in monolayer and three-dimensional cultures. *Br J Dermatol* 1998; 138: 118-21.
32. MacNeil S, Wagner M, Rennie IG. Investigation of the role of signal transduction in attachment of ocular melanoma cells to matrix proteins: inhibition of attachment by calmodulin antagonists including tamoxifen. *Clin Exp Metastasis* 1994; 12: 375-84.
33. Ehrlich HP, Rajaratnam JBM. Cell locomotion forces versus cell contraction forces for collagen lattice contraction: an in vitro model of wound contraction. *Tissue Cell* 1990; 22: 407-17.
34. Ehrlich HP, Buttle DJ, Bernanke DH. Physiological variables affecting collagen lattice contraction by human dermal fibroblasts. *Exp Mol Pathol* 1989; 50: 220-9.
35. Desmoulière A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am J Pathol* 1995; 146: 56-66.
36. Appleton I, Brown NJ, Willoughby DA. Apoptosis, Necrosis and Proliferation: Possible implication in the etiology of keloids. *Am J Pathol* 1996; 149: 1441-7.
37. Garner WL, Rittenberg T, Ehrlich HP, et al. Hypertrophic scar fibroblasts accelerate collagen gel contraction. *Wound Rep Reg* 1995; 3: 185-91.

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