Local treatment with phenol is often used after intrallesional excision of chondroblastomas and giant-cell tumours which involve bone near joints, and has been shown to reduce the rate of recurrence. The ideal concentration of phenol is uncertain, but may be important because of the high rate of absorption and toxicity. We have studied the effectiveness of different concentrations on standard sarcoma cell lines.

Our results suggest that a 5% solution of phenol is effective against dispersed single cells, and that higher concentrations give no significant advantage, but create problems due to lack of homogeneous mixing, temperature and safety.

This procedure is repeated several times with careful coverage of the whole exposed area. The aim is that the phenol solution eliminates the remaining tumour cells by non-specific coagulation necrosis. This has been shown to reduce the rate of recurrence as compared with surgical resection alone (Schiller et al 1989; Szendrői 1992), but the minimum concentration of phenol which can induce maximal tumour cell death has not been established.

We exposed established human sarcoma cell lines to various concentrations of phenol in tissue culture to determine the dose-response relationship.

**MATERIALS AND METHODS**

Bone tumours such as chondroblastoma and giant-cell tumour may occur in the metaphyses and epiphyses, commonly at the shoulder, hip and knee. Complete block resection of these tumours is usually not feasible because this would damage the function of the adjacent joint. An intrallesional procedure is therefore indicated (Enneking 1983). The tissues are usually friable and when tumour cells are left in the bone cavity or the operative field there is recurrence in 20% to 55% of cases (Schiller et al 1989; Carnesal 1992; Szendrői 1992). These cartilaginous tumours continue to grow locally and may spread along the neurovascular bundle, making amputation inevitable (Carnesal 1992). One therapeutic approach after intrallesional excision and curettage is the instillation of a solution of phenol.

Cells in an exponential growth phase were washed with phosphate-buffered saline (PBS), trypsinised with 0.25% trypsin/EDTA for 2 minutes at 37°C, counted and seeded as a single-cell suspension in 25 cm² plastic flasks at a density of 250×10³ cells. After 24 hours, medium containing 5.0%, 7.5% or 10% phenol was added for 60 seconds. The supernatant was then aspirated and fresh phenol solution added for an additional 60 seconds. After three 60-second cycles of phenol, the phenol solution was aspirated from the flasks, the contents were washed twice with PBS and fresh medium was added. Control flasks without phenol were treated identically with medium alone.

After a total incubation time of 168 hours the cells were trypsinised and counted. Three parallel flasks were used for each concentration of phenol and all the experiments were repeated twice and the mean values calculated. The results are reported as survival fractions, the number of cells found in the treated flasks divided by the average number in the control flasks.

**RESULTS**

The cell count after the three cycles of incubation for one minute with 5% phenol solution showed a survival fraction
of 7% (Fig. 1). The increase in concentration of the phenol solution to 7.5% and 10% in vitro was associated with only minimal gain in antitumour activity, to 6.5% and 3.75% respectively.

**DISCUSSION**

The reported concentration of phenol solution used for this purpose varies from 5% (Carnesal 1992) to 75% (Szendrői 1992). The mutual solubility of water and phenol is limited because both fluids are incompletely miscible. If phenol and water are shaken together a separation into phases occurs with a specifically heavier layer of 92% phenol and 8% water and above it one of 92% water and 8% phenol. If the mixture is warmed, the mutual solubility increases until at 66°C only one phase exists (Hill and Malisoff 1926). The use of a 75% solution (7.5 g to 100 ml water) at 56°C (Szendrői 1992) does not solve this problem.

An additional risk from the use of high concentrations of phenol solution is its general toxicity: the lethal dose is 1 g and the biological tolerance value for occupational exposure is 300 mg phenol per litre of urine (Greim and Lehnert 1990).

We aimed to establish the concentration of phenol which gave maximal antitumour activity when applied locally to dispersed single cells from a chondroblastoma or giant-cell tumour. It was not possible to establish permanent cell lines in vitro from these semimalignant tumours, and we therefore used cell lines from malignant soft-tissue sarcomas. These may differ in their growth kinetics from slower growing, differentiated tissues but seem suitable to evaluate the effect of phenol since this kills cells by coagulation necrosis, an effect which is independent of growth kinetics. We considered that it was of some practical importance to establish the lowest concentration of phenol which would exert an adequate antitumour effect.

Our results suggest that a concentration of 5% is satisfactory, but the limitations of our assay system must be realised. We used monolayer cultures and it can therefore be assumed that a 5% solution of phenol is effective against dispersed single cells. More experiments are required to determine whether higher concentrations achieve a deeper penetration through several cell layers; these should use tumour spheroids or multilayer cultures. Until the results of these tests are available a 5% solution appears to be effective and offers advantages in terms of safety and practical handling. The increased risks of a high concentration showed no significant advantage.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

**REFERENCES**


