Critical temperature for \textit{in vivo} cryoablation of human prostate cancer in a xenograft model

P.J. COZZI*, J.A. LAWSON*, W.J. LYNCH† and D.L. MORRIS*
*University Department of Surgery and †Department of Urology, The University of New South Wales, The St George Hospital, Kogarah, Sydney, Australia

Objectives To assess the \textit{in vivo} sensitivity of human prostate cancer cells (PC3) to cryoablation and thus define the minimum temperature needed to prevent the recurrence of cancer after percutaneous transperineal radical prostatic cryoablation.

Materials and methods Twenty-five male \textit{nu/nu} mice were inoculated by a subcutaneous injection with \(5 \times 10^5\) PC3 cells. After 3 weeks the tumours (mean area \(60.4\, \text{mm}^2, \text{SEM} 5.7\)) were frozen using a 3 mm cryotherapy probe (LCS 3000 Cryotech UK) to temperatures ranging from \(0^\circ\) to \(-40^\circ\)C.

Introduction Cryotherapy is not new and was probably first used to treat skin tumours by Arnott almost 150 years ago [1]. The advent of insulated probes and more sophisticated machines capable of delivering liquid nitrogen, as well as the use of ultrasonographic techniques [2,3] for controlling the freezing process, has allowed the more widespread application of the technique to treat visceral tumours. Cryotherapy is now widely used for the treatment of primary [4] and secondary liver tumours [5,6] and prostate cancer [7]. Prostate cryosurgery has been used at open operation to treat prostate cancer and has achieved results equivalent to other local treatment modalities, with equally good long-term survival [8–10]. However, the procedure fell into disrepute because of the high incidence of complications, including the formation of fistulae.

The advent of transrectal ultrasound-guided percutaneous radical cryoablation of the prostate by Onik \textit{et al.} [7] has allowed the use of multiple, synchronous, small diameter (3 mm) percutaneous insulated probes to deliver the low temperature and to monitor the size and position of the frozen tissue mass (‘iceball’), with a resultant reduction in the incidence of complications from fistulae. The procedure is monitored using transrectal ultrasonography (TRUS) to confirm the position of the guidewires and subsequently the probes, and to monitor the size of the iceball created and avoid damage to adjacent structures. The rim of the iceball, as seen on TRUS, is an interface between hyperechoic normal prostate and the hypoechoic iceball, and is used to indicate adequate cytodestruction.

The ultrasonographic characteristics of frozen prostate have been described [11] but there has been no attempt to define the sensitivity of prostate cancer to cryoablative therapy, other than in clinical trials. Of particular concern with this technique is the attainment of adequate destruction of the tumour posteriorly, without injuring the rectum.

The aim of the present study was to define the \textit{in vivo} sensitivities of prostate cancer cells in a mouse model to cryotherapy and to define the minimum temperature required to cause adequate cytodestruction and prevent tumour recurrence.

Materials and methods

Mice were injected with a human prostate cancer cell line (PC-3, Peter M’Cullen Institute, Melbourne, Australia) to produce tumours subsequently treated using cryoablation.

PC-3 cells were grown in RPMI-1640 with 10% fetal calf serum, 7.5% sodium bicarbonate and 200 mM of
l-glutamine, in 5% CO₂, until confluent (Gibco BRL, Life Technologies Inc, Grand Island, NY, USA). Twenty-five male 8–10-week-old nu/nu mice (ANSTO, Lucas Heights, Australia) were subcutaneously injected with 5 × 10⁵ cells of PC-3. After 3 weeks the tumour xenografts had grown to a mean area of 60.4 mm² (SEM 5.7). The mice were anaesthetized by an intraperitoneal injection with Hypnorm (100 µL of a 10% solution: Jansen, Pharmacentica) and midazolam 100 µg (Roche Products, Basel, Switzerland). The subcutaneous xenograft was exposed through a skin incision and a 3 mm cryotherapy probe (Cryotech, UK) applied to the tumour to freeze it. The Cryotec 3000 system (Cryotech, UK) was used to deliver the liquid nitrogen to the probe. An insulated thermocouple was placed with the tip at the part of the tumour most remote from the cryoprobe (Fig. 1). The tumours were frozen to temperatures ranging from 0°C to −40°C at this edge, using a single freeze/thaw cycle. The tumours were then thawed and the skin closed over the tumour with a 5–0 absorbable suture. The mice were recovered and actively rewarmed by an infrared lamp. Three mice died from complications caused by anaesthesia.

The bidirectional diameters of the tumours were measured with vernier calipers twice weekly for 3 weeks and the area calculated. Those mice with tumours that grew markedly were killed and the tumours recovered and fixed in 70% ethanol. A standard haematoxylin and eosin stain was used to verify histologically any tumour recurrence. Those mice that developed skin necrosis or growth of tumours during the next 2 months were also killed (Group 1), which comprised 15 mice. At the end of 3 months, seven mice were alive and tumour-free (Group 2).

Fisher’s exact test was used to determine significant differences in the recurrence rates of each tumour group and Student’s t-test was used to determine significant differences in pre-treatment tumour size between the groups.

Results
The tumour temperatures recorded for the 15 mice in Group 1 ranged from 0°C to −15°C. Histological examination confirmed obvious tumour recurrence in eight mice. The other seven mice had histological evidence of necrosis but not of tumour, although this may represent sampling error, as these tumours had grown. The tumour temperatures recorded for the seven mice in Group 2 ranged from −15°C to −40°C.

The pre-treatment mean area of the tumours for all 25 mice measured 3 weeks after inoculation was 60 mm² (SEM 6). Three weeks after cryotherapy the mean tumour area in Group 1 was 97 mm² (SEM 14) and in Group 2 was 32 mm² (SEM 7). Figure 2 shows individual tumour size at 3 weeks for each group. Figure 3 shows the change in mean size with time for the two groups. There was no significant difference between the groups in mean pre-treatment size.

All recurrences were confirmed histologically. No recurrences from a group of seven animals compared with eight recurrences from a group of 15 is marginally significantly different by Fisher’s exact test (P = 0.083).

Discussion
The viability of tumour cells following in vitro freezing has been investigated [12,13] but temperatures required for in vitro cellular destruction are much lower than those which are effective in vivo. In vitro cell death is thought to be secondary to the formation of ice crystals and to osmotic effects [14] but the in vivo cytodestructive effect of single freeze/thaw cycle cryotherapy in a vascular bed such as liver or prostate is thought to be principally caused by ischaemia from the vascular effects of freezing [15]. It is possible that it is easier to destroy prostate cancer in the relatively well-vascularized prostate than in the subcutaneous site of this mouse model.

The results of this study are novel and potentially
the present study suggests that temperatures considerably less than 0°C are required to achieve ablation of human prostate cancer cells.

The present study demonstrates that, in this in vivo model of prostate cryosurgery, the temperature required to prevent tumour recurrence was $< -15°C$. We would caution against using the rim of the iceball seen on TRUS as an indicator of adequate cytodestruction. However, the importance of the present study is not in establishing a difference between a tumour temperature of more or less than $-15°C$, and indeed there were too few samples to determine statistical significance, but is the observation that more than half the tumours frozen to between 0° and $-15°C$ had histological evidence of recurrence. These temperatures are not often reached at the posterior aspect of the prostate in man, where histological recurrences do occur. This may explain the high rate of positive transrectal biopsies after prostate cryosurgery seen in some series.

**Fig. 2.** The effect of temperature on recurrence rates after cryotherapy. Tumour size was recorded 3 weeks after cryosurgery for both groups of mice. Temperature was recorded at the point furthest from the cryoprobe. Dark green, no recurrence. Light red, recurrence.

**Fig. 3.** The mean size of tumours ($\pm$ SEM) for each group, with time (weeks) from the day of cryoablation.

Important. The hyperchoic rim of the iceball seen on TRUS has been shown to be at 0°C [11]. This rim is used to indicate adequate cytodestruction and also to delineate the edge of the iceball to avoid damage to the rectum. Most centres performing transperineal prostate cryoablation have reported disappointing results from post-operative prostate biopsy early in their series and the present study suggests that temperatures considerably less than 0°C are required to achieve ablation of human prostate cancer cells.

The present study demonstrates that, in this in vivo model of prostate cryosurgery, the temperature required to prevent tumour recurrence was $< -15°C$. We would caution against using the rim of the iceball seen on TRUS as an indicator of adequate cytodestruction. However, the importance of the present study is not in establishing a difference between a tumour temperature of more or less than $-15°C$, and indeed there were too few samples to determine statistical significance, but is the observation that more than half the tumours frozen to between 0° and $-15°C$ had histological evidence of recurrence. These temperatures are not often reached at the posterior aspect of the prostate in man, where histological recurrences do occur. This may explain the high rate of positive transrectal biopsies after prostate cryosurgery seen in some series.

**References**


**Authors**
P.J. Cozzi, MB, BS, Research Fellow.
J.A. Lawson, MSc, Research Assistant.

W.J. Lynch, MBBS, FRACS, Consultant Urologist.
D.L. Morris, MBChB, FRCS, MD, PhD, FRACS, Professor of Surgery.
Correspondence: Professor D.L. Morris, University Department of Surgery, The University of New South Wales, The St George Hospital, Belgrave St, Kogarah, Sydney, NSW 2217, Australia.
学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，
提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具