INTRAOSSEOUS TEMPERATURE DURING AUTOCLAVING

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One method of reconstruction in limb salvage surgery for bone tumours is wide resection, extracorporeal devitalisation of the excised segment by autoclaving, and reimplantation of the segment. We have studied the changes in temperature in the medullary cavity, the head, the medial condyle and lateral condyle of calf femora during autoclaving at 134°C in two different autoclaves.

There were impressive differences of temperature at different sites. The most unfavourable position was the lateral condyle, which consists mainly of cancellous bone: a short programme of 11 minutes produced a lowest temperature in the series of only 45°C, which may not be sufficient to kill all tumour cells.

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The reimplantation of tumour-bearing bone segments after wide resection and extracorporeal autoclaving is a reconstruction technique which has recently become popular (Johnston et al 1983; Ewers and Wangerin 1986; Harrington et al 1986; Sijbrandij 1986; Böhm 1991; Lauritzen et al 1991; Freiberg, Saltzman and Smith 1992; Harrington 1992).

Killing of all tumour cells is an essential condition for this method. To ensure their death it is necessary not only for the temperature to be high enough but the duration of heating is also important. The inductive capacity and mechanical strength of bone, however, are considerably reduced by a higher temperature and longer heating time (Köhler, Kreicbergs and Strömberg 1986; Kreicbergs and Köhler 1989; Früh et al 1990; Inokuchi et al 1991; Knaepler et al 1992; Bernstein, Vannini and Ochsner 1993).

The objective of treatment is therefore a thermal dose which guarantees devitalisation of tumour tissue but does not impair the biological quality of the reimplant more than necessary. The temperature and heating time of 76 published cases are given in Table I. There are no data available about the real thermal doses, because detailed intraosseous thermometry was not performed.

We have analysed the changes in temperature at different positions within the bone during autoclaving. We used calf femora to examine whether the rapid intraosseous rise of temperature measured in small bones (Kreicbergs and Köhler 1989; Inokuchi et al 1991) is similar to that in large bones of human dimensions.

MATERIAL AND METHODS

We autoclaved 80 calf femora; 40 were used in preliminary tests to ensure optimal experimental conditions and 40 were used in the main experiment. The anatomical data of the latter group are given in Table II.

We used negative temperature coefficient (NTC)-thermists made of polycrystalline mixed-oxide ceramics for thermometry. To obtain as representative an overview as possible of the course of temperature at different positions, these were positioned at the following points (Fig. 1): interior of the autoclave (a); medullary cavity (m); head of the femur (cf); medial condyle (cm); and lateral condyle (cl).

The intraosseous thermists were positioned in 3 mm drill holes at a depth of 30 mm. To prevent increased heat conduction through the drill holes these were filled and insulated using silicone paste.

The measurements were fed by an analogue-digital converter to a computer. Temperatures were measured simultaneously and registered every 3.5 seconds at all the thermists.

We used two dissimilar autoclaves. The major autoclave (Sterilisator Typ 669/1 AST-FD-PC-J3; Aigner, Aigner Medizin-Apparate GmbH, Martinsried, Germany) uses a pre-vacuum process. After the defined vacuum has been reached, steam with excess pressure streams inside the autoclave so that an interior temperature of 134°C is reached within one minute. After this has been obtained, the effective sterilisation time was ten minutes. After that period the excess pressure is released and the temperature is reduced to 100°C. The total time of the sterilisation programme was 11 minutes, but the autoclave remained closed so that the total measurement period was 15 minutes (Fig. 2).
Table I. The reported details of autoclaving and oncological results of 76 patients treated by resection of the tumour-bearing bone segment, autoclaving of the segment, and reimplantation of the devitalised bone.

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of cases</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Local or systemic recurrence</th>
<th>Follow-up (yr; mean, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orell (1937)</td>
<td>1</td>
<td>100</td>
<td>15 to 20</td>
<td>0</td>
<td>1.3</td>
</tr>
<tr>
<td>Thompson and Steggall (1956)</td>
<td>1</td>
<td>?</td>
<td>?</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Evans et al (1969)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harding (1957, 1971)</td>
<td>2</td>
<td>?</td>
<td>?</td>
<td>0</td>
<td>10 (3 to 17)</td>
</tr>
<tr>
<td>Smith and Simon (1975)</td>
<td>8</td>
<td>135</td>
<td>12 to 15</td>
<td>1 local</td>
<td>15.6 (5 to 24)</td>
</tr>
<tr>
<td>Smith and Struhl (1988)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niederdellmann et al (1982)</td>
<td>1</td>
<td>134</td>
<td>20</td>
<td>1 systemic</td>
<td>0.7</td>
</tr>
<tr>
<td>Ewers and Wangerin (1986)</td>
<td>1</td>
<td>140</td>
<td>20</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>Harrington et al (1986)</td>
<td>42</td>
<td>135</td>
<td>7 to 10</td>
<td>2 local and 2 systemic</td>
<td>4.8 (2.8 to 25)</td>
</tr>
<tr>
<td>Sijbrandj (1986)</td>
<td>4</td>
<td>120</td>
<td>15</td>
<td>0</td>
<td>7.5 (6 to 11)</td>
</tr>
<tr>
<td>Lauritsen et al (1991)</td>
<td>13</td>
<td>120</td>
<td>20</td>
<td>0</td>
<td>? (&gt; 1)</td>
</tr>
<tr>
<td>Freiberg et al (1992)</td>
<td>1</td>
<td>135</td>
<td>12</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Harrington (1992)</td>
<td>4*</td>
<td>135</td>
<td>10</td>
<td>2 systemic</td>
<td>8.1 (5 to 11)</td>
</tr>
</tbody>
</table>

* two of the four cases were published in 1986

Fig. 1
Calf femora. The position of the thermistors is shown on the sectioned femur (arrows).

Fig. 2
Temperature curves from the thermistor in the middle of the lateral condyle in series A1. The minimum temperature curve as well as the mean and the maximum are recorded. The minimum temperature was only 45°C at the end of the autoclaving programme and 56°C at the end of the measuring period.
Table II. Anatomical data of 40 calf femora. For comparison the human anthropometric mediolateral bicondylar width is given

<table>
<thead>
<tr>
<th></th>
<th>Mean ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>1390 ± 132</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>322 ± 9</td>
</tr>
<tr>
<td>Diameter of diaphysis (mm)</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>Diameter of femoral head (mm)</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>Ventrodorsal diameter of medial condyle (mm)</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>Ventrodorsal diameter of lateral condyle (mm)</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>Mediolateral bicondylar width (mm)</td>
<td>196 ± 5</td>
</tr>
<tr>
<td>Human mediolateral bicondylar width (mm): male, 18 to 59 years (Flügel et al 1986)</td>
<td>96 ± 6</td>
</tr>
</tbody>
</table>

Table III. Details of the four series of measurements on calf femora

<table>
<thead>
<tr>
<th>Series</th>
<th>A1</th>
<th>A2</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclave</td>
<td>Aigner</td>
<td>Aigner</td>
<td>Melag</td>
<td>Melag</td>
</tr>
<tr>
<td>Medullary cavity</td>
<td>Closed</td>
<td>Reamed</td>
<td>Closed</td>
<td>Reamed</td>
</tr>
<tr>
<td>Number</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>134</td>
<td>134</td>
<td>134</td>
<td>134</td>
</tr>
<tr>
<td>Autoclaving time at 134°C (min)</td>
<td>10</td>
<td>10</td>
<td>15 to 18</td>
<td>15 to 18</td>
</tr>
<tr>
<td>Period of programme (min)</td>
<td>11</td>
<td>11</td>
<td>30*</td>
<td>30*</td>
</tr>
<tr>
<td>Measuring period (min)</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Number of thermistors</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

* the Melag autoclave has no regularly installed autoclaving programme

For the small steam-autoclave (Melag, Melag Apparate GmbH, Berlin, Germany) we also used a programme which reached 134°C. The measuring time was 30 minutes. In this apparatus the desired temperature is achieved only after 12 to 15 minutes and the effective sterilisation time varied between 15 and 18 minutes.

We carried out two series of experiments with each of the autoclaves. In the series A1 (Aigner autoclave) and M1 (Melag autoclave) the calf femora were left intact. In the A2 and M2 series the medullary canals of the femora were opened by longitudinal drilling and reaming to 12 mm. In these experiments the temperature in the medullary cavity of the reamed femora was identical to that in the autoclave, so did not specifically measure it (Table III).

RESULTS

The effect of heating on the devitalisation of tumour cells is a function of temperature and time. The results of each series of measurements were therefore recorded graphically for each thermistor position. We also recorded the mean temperatures and the curves of the minimum and the maximum temperatures.

The temperature curves showed impressive differences depending on the position of the thermistor. In the medullary cavity the temperature increased quickly and was close to that of the autoclave, but when the thermistor was placed in the middle of a thick part of cancellous bone, for example in the lateral condyle, the temperature increased very slowly (Fig. 2). For clarity, we show temperature recordings at intervals of one minute.

The temperatures reached and their duration (Table IV), permitted the following conclusions:

1) The autoclave temperature of 134°C was not reached at any of the intraosseous thermistors.
2) There were considerable differences between the different sites. In all four series of measurements there was the same distribution of temperature: medullary canal > femoral head > medial condyle > lateral condyle.
3) All the thermistors were in a phase of increasing temperature until the end of the experiment, except those in the intramedullary canal.
4) Additional drilling and reaming of the medullary canal produced no significant increase in end temperatures.
5) The lowest curve of temperature (series A1 for the lateral condyle) showed an end temperature of 56°C. At the end of a regular 11-minute programme with a 10-minute autoclaving period at 134°C the minimum temperature in the
middle of the lateral condyle was only 45°C.

DISCUSSION

If treatment of bone tumours is to be effective and to produce a cure it is essential to remove all tumour tissue. Extracorporeal devitalisation and reimplantation requires both a safe margin of resection and also definite destruction of all tumour cells.

Friedgood (1928) was the first to show that Walker rat sarcoma cells were killed after heat treatment at 44°C for 30 minutes and many studies have since shown that the thermal doses for different tumour cell lines were on the same scale (Pincus and Fischer 1931; Johnson 1940; Selawry, Goldstein and McCormick 1957; Auersperg 1966; Rivard 1984; Inokuchi et al 1991) (Table V). Because of the exponential effect of increasing temperatures above 43°C it has been shown that the time required for an isoeffect must be decreased by a factor of 2 when the temperature is elevated by 1°C (Suit and Shwayder 1974; Dewey 1984). The minimum exposure times according to this formula are shown in Table V. Certain devitalisation of tumour tissue requires as an absolute minimum two minutes at 60°C or 0.5 minutes at 65°C.

The safe resection of bone tumours has been the subject of numerous investigations (Enneking, Spanier and Goodman 1980; Campanacci and Laus 1980; Kotz et al 1989) but as yet no doubts have been expressed about autoclaving as a safe method for devitalising bone tumours (Enneking and Flynn 1969).

Köhler et al (1986) demonstrated the rapid penetration of heat into the diaphyseal bones of rabbits during autoclaving at 121°C for 20 minutes and at 131°C for two minutes, respectively, and concluded that the method can be used safely for uniform and complete sterilisation of entire specimens. Knaepler et al (1992) measured heat-conduction curves of human cancellous bone by thermon incubation in a water-bath at 80°C and showed that 60°C could be reached after 140 seconds using a thickness of 15 mm. With a thickness of 30 mm a period of 520 seconds was necessary to reach 60°C. Taking these data as a basis, a treatment of 11 minutes in a water-bath at 80°C or autoclaving at 130°C using a short programme would be sufficient for safe devitalisation. Similar findings were reported by Inokuchi et al (1991).

Clinically, the situation is somewhat different. Neither human cancellous bone bars, rabbit diaphyseal bone segments, nor porcine tarsal bones are realistic test objects. In our experiments, we used complete long bones, which are much more realistic clinical examples (Freiberg et al 1992). The calf femur seemed to be most suitable because it is large and has extensive cancellous areas in the condyles which give the most unfavourable conditions. The large width of the condylar region in the calf femur (Flügel, Greil and Sommer 1986) allowed us to assume that the measured temperatures will be more unfavourable in the calf femur than in the human femur.

The final temperatures in the medullary cavities were always higher than 100°C in the most unfavourable case (series A1) but an end temperature of only 56°C was attained in the lateral condyle after 15 minutes (Fig. 2), and of only 45°C after the routine 11-minute autoclaving programme. There is no doubt that this latter temperature is not high enough to kill tumour cells (Inokuchi et al 1991). Therefore, under unfavourable conditions, there must be a high risk of local recurrence after using autoclaving programmes shorter than 15 minutes at 134°C.

The drilling and reaming of the medullary canal did not produce significantly higher temperatures, although the scraping out of metaphyseal and epiphyseal cancellous bone would certainly have resulted in higher minimum temperatures but diminished stability.

The death of tumour cells could be ensured by using long autoclaving programmes with high temperatures, but Früh et al (1990) showed a considerable biomechanical deterioration with a prolonged autoclaving time. The inductive capacity of bone is destroyed to a large extent after autoclaving (Burwell 1966; Urist and Hernandez 1974; Kreigers and Köhler 1989), and osteoinductivity is also reduced with increasing temperatures and with increasing times (Bernstein et al 1993). The increase of temperature between 80°C and 134°C is reported to reduce healing.
(Knaepler et al. 1992). We recommend a minimum effective autoclaving time of 15 minutes at 134°C when heating large long bones to devitalise tumour cells.

Allografts are sometimes autoclaved to prevent the transfer of viral and bacterial infections (Wagner and Pesch 1989). One reason for this is to avoid the comprehensive multiphasic screening and the numerous and expensive laboratory tests necessary for safe bone banking (Buck and Malinin 1994). Our results indicate that disinfection or even sterilisation of massive bone allografts is not guaranteed after a 15-minute programme at 134°C.

Thermobiological research has shown that it is not necessary to heat to 100°C to kill tumour cells, but more research is needed to determine the minimum exposure time at lower temperatures which will guarantee devitalisation of tumour cells.

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


