CRYOGENIC DAMAGE TO PERIPHERAL NERVES AND BLOOD VESSELS IN THE RAT

A. R. MARSLAND, S. RAMAMURTHY AND J. BARNES

SUMMARY
The effects of cryogenic injury on femoral blood vessels and nerves were studied in 28 rats. The femoral neurovascular bundles were dissected out bilaterally and the vessels and nerves subjected to cryogenic injury. The rats were sacrificed at intervals of up to 2 weeks. Damage to the nerves and to a number of femoral vessels was severe and included necrosis and thrombus formation. The results indicate that significant vascular damage can occur during cryotherapy, and the possible complications of this should be considered.

The analgesic action of extreme cold on peripheral nervous tissue was first noted by Hippocrates (Jones, 1931) and various investigators have reported on this effect since. Denny-Brown and colleagues (1945) showed that myelin and the axis cylinders of mammalian peripheral nerves are selectively damaged by exposure to cold with the larger myelinated fibres being the most susceptible. Wallerian degeneration was noted, with disintegration of the axons and breakdown of the myelin sheaths. However, there was minimal disruption of the endoneurium and this presumably is the reason why normal sensation eventually returns in the distribution of the cold-damaged peripheral nerve.

Recently, there has been an upsurge in the use of cold-induced nerve damage in the treatment of chronic pain. Lloyd, Barnard and Glynn (1976) have reported its use in 64 patients with intractable pain. Fifty-two obtained relief of pain for a median duration of 11 days and with a range of up to 224 days. Cryoanalgesia is also being used in the treatment, or prevention, of post-thoracotomy pain by freezing the relevant intercostal nerves (Glynn, Lloyd and Barnard, 1980; Katz et al., 1980; Maiwand and Makey, 1981).

Amongst nerves which are accessible for cryoanalgesia are the intercostal nerves, and the femoral and posterior tibial nerves. However, since these nerves have arteries and veins in intimate relationship, it is possible that coincidental damage could occur to these structures while destruction of the peripheral nerve is being undertaken.

The present study was undertaken to determine, first, whether significant damage to vessels adjacent to nerves occurs when cryogenic destruction of the nerves is carried out and, second, to confirm that destruction of the nerves has occurred and to follow the progress of this destruction.

MATERIALS AND METHODS
Twenty-eight Long Evans rats (average weight 220 g) were divided into four groups depending on the proposed time of sacrifice after the experiment. Each group consisted of two control and five experimental rats. All animals were anaesthetized using thiopentone 25 mg kg⁻¹ i.p. The groins were dissected to expose the neurovascular bundle. Further dissection isolated the femoral nerve on the right. This was frozen using three freeze-thaw cycles, namely freezing for 1 min followed by thawing for 1 min, repeated on three occasions. The femoral artery and veins were then subjected to three freeze–thaw cycles on the right. On the left, the neurovascular bundles were subjected to three freeze–thaw cycles without further dissection. In the control rats, a similar procedure was undertaken, but without inflicting cold damage, and in these rats the wounds were closed after 15 min. Temperatures in the tissue under consideration were measured using a thermocouple. The probe used was a Cryoseeker Probe 91720 with a MC 1000 Miniconsole (Cryomedics, Connecticut). This device does not provide a display of actual tip temperature. The groin incisions were closed and the rats returned to their cages. The rats were sacrificed
using lethal doses of thiopentone as follows: group A at 24 h, group B at 48 h; Group C at 1 week and group D at 2 weeks. Following sacrifice the neurovascular bundles were dissected out and fixed in 5% formalin. The specimens were processed for histological examination.

RESULTS

Temperature
The lowest temperature recorded for each freeze–thaw cycle is detailed, for each animal, in table I.

<table>
<thead>
<tr>
<th>Group</th>
<th>Temperature (°C)</th>
<th>Nerve</th>
<th>Left nerve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sacrifice at 24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nerve</td>
<td>-15</td>
<td>-16</td>
<td>-3</td>
</tr>
<tr>
<td>Vessels</td>
<td>-15</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>Sacrifice at 48 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>-12</td>
<td>-17</td>
<td>-18</td>
</tr>
<tr>
<td>Nerve</td>
<td>-13</td>
<td>-10</td>
<td>-8</td>
</tr>
<tr>
<td>Vessels</td>
<td>-21</td>
<td>5</td>
<td>-12</td>
</tr>
<tr>
<td>Sacrifice at 1 week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>-19</td>
<td>-10</td>
<td>-17</td>
</tr>
<tr>
<td>Nerve</td>
<td>-10</td>
<td>7</td>
<td>-12</td>
</tr>
<tr>
<td>Vessels</td>
<td>-17</td>
<td>-2</td>
<td>-9</td>
</tr>
<tr>
<td>Sacrifice at 2 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>-20</td>
<td>-1</td>
<td>-20</td>
</tr>
<tr>
<td>Nerve</td>
<td>-28</td>
<td>12</td>
<td>-28</td>
</tr>
<tr>
<td>Vessels</td>
<td>-26</td>
<td>-21</td>
<td>-24</td>
</tr>
</tbody>
</table>

Damage to femoral vessels
There was no damage to the arteries or veins in any of the control animals. The vessels from the treated animals exhibited histological changes which varied accordingly to the length of time before sacrifice.

In the specimens obtained at 24 h, there was partial necrosis in one artery from the left side. The other nine arteries showed minor changes consisting of vacuolization of the cytoplasm in the smooth muscle cells with acute inflammatory infiltrates. There were no changes in the veins.

In the 48-h specimens, there was partial necrosis in one artery from the left side. All the arteries and veins showed acute inflammatory cellular infiltrates, primarily mononuclear. One vein on the left and two veins on the right showed poorly formed thrombi.

In the 1-week specimens, there was complete necrosis of one artery and minor reversible changes in four arteries on the left. One vein showed poorly formed thrombi. On the right two out of five arteries showed irreversible changes consisting of denudation of both intimal and muscle cells, and the other three showed only minor reversible changes. One vein was necrotic and the other showed poorly formed thrombus.

In the specimens obtained at 2 weeks, on the left, there were necrotic changes in two arteries, complete in one and partial in the other. The remaining three arteries showed minor reversible changes. There was thrombus formation in one artery and one vein. All vessels showed chronic inflammation. On the right, all arteries were viable. One vein had focal necrosis.

Damage to femoral nerves
In the control group 11 of the 16 nerves were intact and viable. Two nerves could not be evaluated because of fixation artefacts. Three nerves had some swelling of the axon and myelin with histiocytic infiltration indicating some degree of damage.

The nerves which were frozen all showed significant swelling of the axon and myelin, but still had intact basement membranes at 24 h. This progressed to increased swelling of axons and myelin with fragmented basement membranes and slight to moderate mononuclear infiltration by 48 h. At 1 week these changes were more marked and by 2 weeks all the nerves showed evidence of irreversible swelling of axons, vacuolation of myelin with disintegrating basement membranes and histiocytic infiltration.

DISCUSSION
The observations that significant damage to neural tissue results from freezing, are in accord with those of other workers (Menz, 1971; Evans, Lloyd and Green, 1981) and will not be discussed further. On the other hand, cold-induced damage to vessels has not been reported previously.

In this study, when the cryoprobe was directly
applied to the vessels, greater damage resulted than when the intact neurovascular bundle was frozen. Nevertheless, considerable damage to the vessels did occur when the intact neurovascular bundle was frozen. In freezing the intact bundle, the probe was preferentially placed on the nerve, but even so it was not uncommon for the vessels to become frozen solid. The fact that less damage occurred when this was done, presumably indicates a large temperature gradient in the vicinity of the cryoprobe. The nature of the damage is important in that it included various degrees of necrosis as well as thrombus formation.

It might have been expected that the blood flow in the vessels would have conducted the cold away from the area and prevented a very low temperature, thus protecting the vessels. During the experiment, however, it was noted frequently that the vessels would become frozen solid—especially on the side where the vessels were frozen when separated from the nerve. As can be seen from the temperatures listed in table I, blood flow did not prevent the attainment of low temperatures.

It could be argued that the vessels being considered are small in relation to the size of the cryoprobe. This was certainly the case and any extrapolation of these results to the human situation must be viewed with this in mind. When the cryoprobe is used at open operation in man (Nelson et al., 1974), even when vessels lie close to the nerve under consideration, it is unlikely that cold of sufficient intensity will spread to affect these vessels. However, when the procedure is being performed percutaneously, it is possible that the cryoprobe could come into direct contact with vessels. In this situation, despite a greater blood flow than that in our study, the possibility exists that at least focal damage could occur to the vessels. Currently an application of cryoanalgesia is in the management of pain following thoracotomy (Evans, 1981; Maiwand and Makey, 1981). Anatomically, these vessels are in intimate relationship to the nerve. From this study it would appear that at least some damage to the vessels could occur during the freezing of the intercostal nerves. Similar damage could occur also in other regions where vessels are in close anatomical proximity to the nerves being frozen. However, it is debatable whether such vascular damage would be of any clinical significance and it may be that this potential complication is of theoretical concern only. The vessels near which cryosurgery is undertaken normally have an adequate collateral supply. Nevertheless, partial necrosis of vessels could lead to delayed rupture and haemorrhage or haemorrhage. It is difficult to conceive of a clinical situation in which this would be of major importance. Minor bruising and temporary discomfort would appear to be the most likely result. Should these complications occur, there is now an experimental model to explain them. However, if the collateral supply is jeopardized by peripheral vascular disease, damage to the vessels in question could be of greater clinical significance.

In conclusion, it has been demonstrated that, in the rat, vascular damage of significant degree can be produced by application of localized cold injury. The implications of this in the human situation require evaluation.

ACKNOWLEDGEMENT

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REFERENCES


Les effets d'une lésion cryogénique sur les vaisseaux et nerfs fémoraux ont été étudiés chez 28 rats. Les paquets vasculonerveux fémoraux ont été disséqués des deux côtés et les vaisseaux et nerfs ont été soumis à une lésion cryogénique. Les rats ont ensuite été sacrifiés à des intervalles allant jusqu'à 2 semaines. Les lésions des nerfs et d'un grand nombre des vaisseaux fémoraux étaient sévères et comportaient nécrose et formation de thrombi. Ces résultats montrent que des lésions vasculaires sévères peuvent survenir au cours de la cryothérapie, et que les complications possibles de ceci devraient être prises en compte.

Lesions provoquées par le froid sur des nerfs et des vaisseaux sanguins périphériques chez le rat

Résumé


Kryoogene Schädigung von peripheren Nerven und Blutgefäßen bei der Ratte

Zusammenfassung

Se estudiaron en 28 ratas los efectos del daño criogénico sobre los nervios y vasos sanguíneos femorales. Los conjuntos neurovasculares femorales se disecaron bilateralmente y los nervios y vasos se sometieron a daño criogénico. Las ratas fueron sacrificadas posteriormente a intervalos máximos de 2 semanas. El daño a los nervios y a un cierto número de vasos femorales fue considerable e incluyó nécrosis y formación de trombos. Los resultados indican que la crioterapia puede producir daños vasculares significativos, por lo que deben considerarse las posibles complicaciones.