Effect of Solcoseryl on Cadaveric Split-Skin Oxygen Consumption during 4°C Storage and in Frozen Biopsies

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Oxygen consumption rate in cadaveric split-skin biopsies was investigated. Biopsies were harvested at different times postmortem and stored at different temperatures in either Solcoseryl (a protein-free bovine hemodialysate) or placebo-containing media. During the first week of storage Solcoseryl had no influence on oxygen consumption. However, in the second and third weeks the oxygen consumption was improved by Solcoseryl.

Within the field of burn surgery, cadaveric split-skin (syn. homograft or allograft) plays a significant role as a temporary skin substitute (9). Yet, the demands placed on cadaveric split-skin now include prolonged survival as a graft on the patient. At least until reharvesting of autograft skin from donor areas can be performed, or until subplaced supermeshed autograft has healed (1, 2), there is a need for a fully functional, long-term wound cover incorporating keratinocytes able to undergo mitosis, and with low intrinsic tissue antigenicity.

As the extent of nonphysiological conditions in cadaveric split-skin may be assumed to be inversely proportional to its viability, it is essential after removing skin from the donor site to reestablish and maintain a normophysiological status as soon and as optimally as possible.

The postmortem life span of donated cadaveric split-skin is limited (7). The initial warm ischemia time from death of the donor until harvest of the tissue is seldom less than 6 hr. Then follows 24-48 hr of cold ischemia time in a tissue bank laboratory, where the tissue is processed and stored at 4°C. The skin is then either grafted or frozen for subsequent use. After grafting, there follows a warm ischemia time of 48-96 hr, i.e., until microcirculation in the graft has been reestablished (4). Therefore, every effort should be made to provide the tissue with viability-enhancing factors before grafting. Solcoseryl, a standardized calf blood hemodialysate free of protein, antigens, and pyrogens, may well provide such factors and thus improve metabolic conditions in the skin.

The present study investigated the effect of Solcoseryl on the viability of necro split-skin biopsies. Viability was expressed as the oxygen consumption per unit time in the tissue.

MATERIAL AND METHODS

Eight cadavers were selected as cadaveric split-skin donors according to standard procedures of the Hvidovre Hospital Tissue Bank. Split-skin was excised at 12 and 18-72 hr postmortem. Cadavers were refrigerated at 4°C from 6 hr postmortem until the two skin harvestings. Excisions were performed with an electric Brown dermatome, set to cut at a depth of 0.16 mm. Immediately after the excision, the split-skin had three 1-cm² biopsies punched out with a steel puncher. One biopsy was kept at 4°C, one biopsy was kept at 22°C, and the third was frozen at a rate of −1°C/min to a final temperature of −80°C after addition of
glycerol to the medium to a final concentration of 10% (v/v). Exactly the same procedure was carried out for the 18- to 72-hr biopsies. Four cadavers were allocated to media containing Solcoseryl, and four cadavers were allocated to media containing a placebo (physiological saline). The trial was double-blinded.

The medium used was Dulbecco's modified Eagle's medium 041-2430 (Gibco Laboratory, Scotland), containing penicillin 500 µg/ml and gentamycin 0.1 mg/ml. There were 20 ml of medium per biopsy. The medium contained 25 mM Hepes buffer maintaining pH between 7.2 and 7.7. No color shift (indicating pH changes) was observed in the study. Media were changed every third day. There was free access of atmospheric air to the media. Before viability recordings, the biopsies and the media were warmed to 38°C. Solcoseryl or placebo was added to the media to a final concentration of 3% (v/v).

The viability of the skin biopsies was recorded by an oxygen-measuring platinum microelectrode (Radiometer, Denmark) according to a previously described method (6). Using this method the rate of oxygen consumption in the biopsy was recorded. Oxygen consumption was recorded daily until Day 7 and then on Days 14 and 21. Frozen biopsies were thawed by immersion in a 37°C water bath on Day 14, and viability was recorded on Days 14 and 21.

STATISTICAL ANALYSIS

The following variables were analyzed:

- Solcoseryl: 0 if basic medium plus Solcoseryl, 1 if basic medium plus placebo
- PM: 0 if skin harvested 12 hr postmortem, 1 if skin harvested 18-72 hr postmortem
- TP: 0 if incubated at 4°C, 1 if incubated at 22°C

A 2 × 3 factorial ANOVA with four replications was performed on a microcomputer by Yate's algorithm (10). One missing value (one biopsy lost) was substituted in the ANOVA by least-squares estimate. Stepwise multiple log-linear least-squares regression was used if linear assumptions satisfied the Von Neumann test for random distribution of residuals along the line of regression and the chi squared test for normality of residuals. For all purposes P < 0.05 was considered statistically significant.

RESULTS

The decline of oxygen consumption in the skin biopsies followed a complex three-phase pattern: (i) an initial delay phase with only minor loss of oxygen consumption capacity (Days 0-2), (ii) an intermediate rapid decline phase (Days 3-6), and (iii) a terminal protracted decline phase (Days 7-21). This decline pattern was seen in all single investigations.

During the initial delay phase, only PM affected oxygen consumption significantly (P < 0.0001) (Fig. 1). Neither Solcoseryl nor TP had any influence on oxygen consumption during this phase (Figs. 2-5).

During the intermediate phase, the de-
CADAVERIC SPLIT-SKIN OXYGEN CONSUMPTION

FIG. 2. Oxygen consumption in biopsies harvested at 12 hr postmortem and stored at 4°C in medium plus Solcoseryl (●) or placebo (○).

FIG. 4. Oxygen consumption in biopsies harvested between 18 and 72 hr postmortem and stored at 4°C in medium plus Solcoseryl (●) or placebo (○).

FIG. 3. Oxygen consumption in biopsies harvested at 12 hr postmortem and stored at 22°C in medium plus Solcoseryl (●) or placebo (○).

FIG. 5. Oxygen consumption in biopsies harvested between 18 and 72 hr postmortem and stored at 22°C in medium plus Solcoseryl (●) or placebo (○).

cline followed an exponential course. The regression was \( \ln(\text{oxygen consumption + 1}) = 5.07 - 0.13 \times \text{TP} - 0.17 \times \text{days} \) (3–6). The coefficient of determination \( R^2 \) was 0.72 (\( P < 0.0001 \)). Addition of the independent factors Solcoseryl or PM did not improve the regression significantly. Thus, only storage temperature (TP) affected oxygen consumption during this phase (Fig. 6). The magnitude of this effect is illustrated by the predicted values of Day 6,

which would then be 56.4 and 49.4 (percentage of Day 0 value) for 4 and 22°C, respectively.

During the terminal phase Solcoseryl consistently improved oxygen consumption (Figs. 2, 5, and 7; Table 1). This effect was significant in the 4 and 22°C groups as well as in the 12- and 18- to 72-hr postmortem groups. Differences in oxygen consumption decline rates (percentage of Day 0 value) in all biopsies stored at 4 or 22°C were only significant on Day 21. Whether the biopsies were harvested at 12 hr or at 18–72 hr postmortem gave oxygen consumption differ-
FIG. 6. Oxygen consumption in all biopsies stored at 4°C (●) or 22°C (○).

ences only in the terminal phase at Day 7 (Table 1).

The analysis of the frozen skin also showed that oxygen consumption was significantly better preserved by addition of Solcoseryl to the medium (P < 0.0001) (Table 2).

DISCUSSION

In the present study, the basic medium was a phosphate-buffered, balanced salt solution which is well known to be superior to physiological saline as a tissue storage medium (3).

The viability recording system was based on the oxygen consumption rate in a defined time period (1 min) at 37°C. This tissue oxygen consumption was measured by a platinum microelectrode in close and controlled contact with the tissue. The system has been described in detail (6).

Solcoseryl, a protein-free bovine hemodialysate, has been described as promoting wound healing, enhancing the proliferation of reversibly damaged fibroblasts, and exerting an oxygen consumption-stimulating effect on guinea pig liver homogenate (5). This stimulation of oxygen consumption was also demonstrated in the present study, but only when the tissue was in a metabolically "exhausted" state. In all biopsies, oxygen consumption during the first week was high and unaffected by Solcoseryl. Later in the investigation period, i.e., after 1 week, Solcoseryl significantly improved oxygen consumption compared to placebo. This finding was evident at different storage temperatures as well as at different postmortem harvesting times. That 4°C storage temperature and early postmortem harvest are connected with high preserved viability is well known, and was clearly confirmed in the present study. Freezing and thawing of skin are also connected with an inevitable viability decline. Addition of Solcoseryl to storage and freezing media resulted in better preservation of the capacity for oxygen consumption after thawing.

The reason for this improved oxygen consumption capacity induced by Solcoseryl, as seen in phase II and III of the skin biopsies, remains unknown. It might be that Solcoseryl provides the same additional viability factors as human or bovine serum. No effect was detectable during the first week of harvesting, possibly because the supply of preexisting metabolic factors in the skin was adequate at this time, so that oxygen consumption could not be further improved by adding more. It may well be that the levels of these factors in the tissue

FIG. 7. Oxygen consumption in all biopsies stored in medium plus Solcoseryl (●) or placebo (○).
TABLE 1
The Effect during the Terminal Phase of Any of the Variables

<table>
<thead>
<tr>
<th></th>
<th>Day 7 Difference (%)</th>
<th>Day 14 Difference (%)</th>
<th>Day 21 Difference (%)</th>
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</thead>
<tbody>
<tr>
<td>Solcoseryl</td>
<td>14.17 &lt;0.0001</td>
<td>19.18 &lt;0.001</td>
<td>20.48 &lt;0.0001</td>
</tr>
<tr>
<td>TP</td>
<td>--- NS</td>
<td>7.98 &lt;0.01</td>
<td>11.22 &lt;0.005</td>
</tr>
<tr>
<td>PM</td>
<td>5.94 &lt;0.05</td>
<td>--- NS</td>
<td>--- NS</td>
</tr>
<tr>
<td>Mean viability (%)</td>
<td>48.11</td>
<td>31.12</td>
<td>19.41</td>
</tr>
</tbody>
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Note. Solcoseryl = Solcoseryl or placebo treatment; TP, storage temperature; PM, time postmortem of skin harvesting on skin viability in percentage of Day 0 values.

decayed after the first week but were replenished by adding Solcoseryl to the medium.

It can be concluded that Solcoseryl has a positive effect on the oxygen consumption rate in skin biopsies, but that this is only detectable in the metabolically “exhausted” stages of the tissue. This may indicate that Solcoseryl provides the tissue with factors which either stabilize cell integrity/function or directly stimulate oxygen consumption.

This three-phase pattern has great resemblance to the structural and metabolic degradation of human skin described by May and Wainwright (8). Their phases were however slightly prolonged (Days 1-10, 11-30, and 31-58). The present oxygen consumption rate dropped to low levels at the end of phase II (Day 7), similar to the metabolic degradation described in phase I (Day 10) in (8).

TABLE 2
Skin Oxygen Consumption Tests on Frozen Biopsies

<table>
<thead>
<tr>
<th></th>
<th>12 hr PM</th>
<th>18-72 hr PM</th>
</tr>
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<tbody>
<tr>
<td>Solcoseryl</td>
<td>Day 14 68.95</td>
<td>88.56</td>
</tr>
<tr>
<td></td>
<td>Day 21 63.69</td>
<td>71.29</td>
</tr>
<tr>
<td>Placebo</td>
<td>Day 14 62.82</td>
<td>59.96</td>
</tr>
<tr>
<td></td>
<td>Day 21 54.66</td>
<td>44.59</td>
</tr>
</tbody>
</table>

Note. Oxygen consumption is expressed in percentage of Day 0 values.

REFERENCES