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Nutrition of the Intervertebral Disc: Acute Effects of Cigarette Smoking
An experimental animal study
Sten Holm and Alf Nachemson
Department of Orthopaedics, University of Göteborg, Sahlgren Hospital, Göteborg, Sweden

ABSTRACT

We have in the present experimental study investigated the influence of cigarette smoking on some nutritional parameters of the porcine intervertebral disc.

The results from the acute smoking tests show a significant reduction in solute transport. Diffusion of sulphate, oxygen and methyl glucose was reduced by 30-40 per cent. This effect was obtained after exposure to smoke for 20-30 minutes. A smoking period of three hours reduced the transport efficiency to about 50 per cent. The effect of smoking decreased when the exposure ceased. The concentration gradients were close to normal after 2 hours "recovery".

These findings suggest that cigarette smoking not only significantly affects the circulatory system outside the intervertebral disc, where the most pronounced effect is the reduction in solute exchange capacity, but also significantly deteriorates the cellular uptake rate and metabolite production within the disc.

INTRODUCTION

In a large avascular structure such as the intervertebral disc, solute transport occurs predominantly via diffusion (6,11). Therefore, the efficiency of solute delivery from the blood capillaries and the capacity of disc vascular bed and dimensions of the disc system are factors of specific importance. Particularly in the case of large human discs where the balance between nutrient utilization and supply is precarious; any loss in blood vessel contact or reduction in blood flow at the periphery of the disc could lead to nutritional deficiencies and build-up of waste products (6).
Several factors may be of potential risk considering blockage of capillaries or via direct action on the capillary wall inducing constriction and consequently affecting the blood flow (2,4). Such a risk factor is cigarette smoke (9,12).

Smoking has also been regarded to be a potential risk factor for initiating low back pain (3,10). These epidemiological studies evaluated unfortunately only the subjective symptoms of low back pain and not any structural changes; therefore the conclusions of such studies can be controversial (8).

In the present study it was considered important to investigate whether or not smoking could affect transport of nutrients, into the intervertebral disc, and to what extent smoking affects the metabolic properties, since nutrition of the disc critically depends upon an adequate blood vessel supply to its periphery.

MATERIAL AND METHODS

In this study we used the domestic pig (Sus Scrofa) as the experimental animal. Nine female and six male animals were included in the experiments, having an average age and weight of 1.5 (range 1.3-2.0) years and 90 (range 70-120) kg respectively. The animals were prior to surgery sedated via an intramuscular injection of Ketalar (Parke Davis). For general anesthesia we have used Hypnodil (LEO, Sweden) (0.1 mg/kg body weight).

After intubation the animals were ventilated in an Engstroem respirator. An additional pumping system was attached to the respirator so that the smoke could be inhaled (Fig.1). Smoking time for one cigarette was on average 4.5±0.5 minutes.

During the testing time (ranging from 20-30 minutes to 3 hours) blood gases and intradiscal oxygen tensions were measured continuously via oxygen probes introduced into one central artery, vein and in an intervertebral disc in the lumbar region (5,6).

The cigarettes used were each containing 16 mg of tar, 1.2 mg of nicotine and 13 mg of carbon monoxide.

After the smoking period radioactive isotopes (S-35 as sodium sulphate and H-3 as methyl glucose) (Radiochemical Centre, Amersham, England), tetracycline and fluorescein (Sigma Co., Miss. USA) were introduced intravenously. The animals were sacrificed at 15 minutes, 1 hour and 2 hours after the infusion and their spines were thereafter frozen in situ and excised for analysis. Twelve age and weight matched animals served as a control group.
These animals were kept in their cages having free access to fresh air and food.

EXPERIMENTAL SYSTEM AND PROCEDURES

Fig.1. Schematic drawing of the experimental set up. The cigarette was attached to a pumping system which allowed smoke to enter via the air flow from the Engstroem respirator. From blood samples the tracer distribution could be investigated. The continuous oxygen tension measurements were obtained from probes inserted into central arteries, veins and nuclei of the intervertebral disc. Biopsies from relevant areas were collected from in situ frozen spines.

Concentration gradients of solutes and metabolic properties were analysed according to standard procedures (6).

The radioactivity was counted in a Packard-Tri-Carb (3320) liquid scintillation spectrophotometer (Packard Instrument Co.). Correction for quenching was performed by the external standard method.

Standard procedures were used to calculate the mean and standard deviation (S.D.) in the different groups. The Mann-Whitney ranking test was used for comparison between different groups.

RESULTS

When the animals were exposed to smoke the blood vessels reduced their efficiency and there was a considerable constriction.
of the capillaries (Fig.2). These results were deduced from photographs of histological sections after intravenous tetracycline and fluorescein injections. This situation was predominantly present in the central nucleus area; similar effects were also found, but to a lesser extent, in the peripheral area of the annulus fibrosus. Solute transport was significantly reduced already after 20-30 minutes of exposure (5-8 cigarettes).

Fig.2 Nutritional routes in the intervertebral disc. Normally, most of the solutes and metabolites enter the disc via the very central portion of the vertebral end-plate and via the periphery of the annulus fibrosus (5).

Included in the figure are two drawings showing the situation of the capillary bed in the normal situation and after smoke exposure for 3 hours. Drawings were made from photographs of histological sections after intravenous injections of tetracycline and fluorescein.

Transport of sulphate (Table 1), methyl glucose (Table 1) and oxygen (Fig.3) showed similar reduction pattern (18-50 per cent). There was a fairly slow decrease with increasing time after the initial smoking period for all tracers investigated and the decay was not linear. The recovery time after smoke exposure (i.e., the time for the oxygen tension in the disc to reach 95% or more of the initial tension level) was longer for the charged sulphate molecule than for the uncharged methyl glucose and oxygen (Table 2).
Table 1. Transport of inorganic sulphate and methyl glucose into the central part of the disc (nucleus pulposus) was significantly reduced in relation to the control group (p<0.05) after being exposed to smoke for 20-30 minutes and 3 hours respectively. For the inorganic sulphate a significant reduction difference was found between short and long exposure time (p<0.05). Included in this table are also the situations before and after smoking exposure. Transport into the nucleus of the control group was normalized and set to 100%. The recovery period was performed by inhalation of fresh air.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL GROUP</th>
<th>20-30 minutes</th>
<th>3 hours</th>
<th>2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NOT EXPOSED</td>
<td>EXPOSURE</td>
<td>EXPOSURE</td>
<td>RECOVERY</td>
</tr>
<tr>
<td>INORGANIC SULPHATE</td>
<td>100%</td>
<td>79±5%</td>
<td>65±7%</td>
<td>95±3%</td>
</tr>
<tr>
<td></td>
<td>(p&lt;0.05)</td>
<td>(p&lt;0.05)</td>
<td>(N.S.)</td>
<td></td>
</tr>
<tr>
<td>METHYL GLUCOSE</td>
<td>100%</td>
<td>70±8%</td>
<td>50±9%</td>
<td>97±3%</td>
</tr>
<tr>
<td></td>
<td>(p&lt;0.05)</td>
<td>(p&lt;0.05)</td>
<td>(N.S.)</td>
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</table>

Fig. 3 Oxygen tension measurements obtained in the central part of the nucleus pulposus. The reduction was significant after 30 minutes of smoke exposure (p<0.05).

The metabolic properties of the control disc (nucleus pulposus) revealed similar pattern as did the transport properties. The cell reaction acted in an anaerobic direction. Already after 20-30 minutes the oxygen tension decreased, whereas the lactate concentration increased (Fig. 4). The organic sulphate uptake rate was also affected by some 35 ± 12 per cent during the short exposure time. The situation after three hours was even more pronounced (48 ± 15 per cent) although there was no linear
relation with time.

Table 2. Recovery time for different solutes in the nucleus pulposus of the intervertebral disc. Recovery time was defined as the time necessary to reach 95 per cent of the initial (normal) value. Mean values, S.D. and number of discs analysed (N) are given. For the shorter exposure time 8 animals were used whereas for the 3 hrs exposure tests 7 animals were employed.

<table>
<thead>
<tr>
<th>EXPOSURE TIME in minutes</th>
<th>RECOVERY TIME (minutes)</th>
<th>$^{35}$S (sulphate)</th>
<th>$^{3}$H (methyl glucose)</th>
<th>Oxygen (Probe)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>58 ± 11 (N=13)</td>
<td>42 ± 8 (N=12)</td>
<td>37 ± 6 (N=17)</td>
</tr>
<tr>
<td>20 - 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>116 ± 14 (N=16)</td>
<td>93 ± 10 (N=15)</td>
<td>89 ± 10 (N=15)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4 Concentration of lactate in the nucleus pulposus. Already after a smoking period of half an hour, the production and transport of lactate out of the system are not in balance. The accumulation of lactate, after 60 minutes and 3 hours of smoke exposure, is significantly higher in comparison to the control group ($p<0.05$).

DISCUSSION

The capillary network, underneath the hyaline cartilage of the vertebral body, is under normal physiological conditions well developed and covers 80-90 per cent of the contact area between cartilage and the vertebral body (6,11). In animals, which not previously had been exposed to smoke, the blood rheology and metabolite concentrations were affected. These effects were significant already after 20-30 minutes of exposure and increased with time.
The recovery time was not a linear function with exposure time which implies that already a short smoking period requires a relatively long recovery period in order to normalize the solute concentration gradients.

The difference between sulphate and glucose transport suggests that the transport routes are affected differently as methyl glucose penetrated freely into the disc according to the exchange area, whereas the sulphate is excluded by the charged proteoglycans and affected by the metabolic demands (6,7,11). In the nucleus high concentrations of negatively charged proteoglycans are present, which are repelling a negatively charged solute as sulphate but ignoring neutral molecules. Of specific interest was the cell reaction shown by the sulphate uptake. These very quick cellular reactions in the nucleus do indeed indicate that the intervertebral discs are sensitive to an affected rheological surrounding. The concentration of lactate increased constantly over the 3 hour period, a situation which is of specific interest as accumulation of acidic products inevitably leads to an unhealthy cascade of events involving lowering of the Ph, increased activity of proteolytic enzymes, structural breakdown, instability etc. Therefore, the implications for the central disc region are not favoured by time. Furthermore, when blood vessels are closed or constricted, the exchange area will be decreased and the transport of nutrients to the cells as well as disposal of waste products will not be adequate. Disc cells could either die or not be able to meet the functional energy demands required in, for example, matrix production.

We should point out that the results obtained in this investigation only can be extrapolated with extreme precaution to the human situation. The effects were found in animals after a single exposure to smoke and may not represent effects of chronic smoking. Furthermore, in this experimental set up it was not possible to measure the surrounding capillary blood flow. It is therefore not possible to evaluate whether the changes found in the discs are caused merely by a reduced blood flow caused by the nicotine induced vascular constriction or if there may be an additional effect. These findings demonstrate, however, that cigarette smoking affects the circulatory system outside the intervertebral disc as well as cellular uptake rates and metabolite production within the disc. By reducing the transport of substrates into the
disc and waste products out of the system, the inevitable consequence over a longer period of time will be deficient nutrition leading to degenerative metabolic processes, enzymatic degradation, instability and probably to back pain.

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REFERENCES


Adress:

Sten Holm
Department of Orthopaedics
Sahlgren Hospital
413 45 GÖTEBORG
Sweden