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Reduced wound healing capacity in alcohol abusers – reversibility after withdrawal

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Abstract

Background Alcohol abusers have increased risk of wound complications following surgical procedures, however the development of complications is reduced after preoperative withdrawal from alcohol. Therefore the aim of the study was to evaluate wound healing at alcohol abuse and after withdrawal.

Methods In total 16 alcohol abusers were included and tested. Nine abusers were able to abstain from alcohol and were retested after 8 weeks of abstinence. No patients had clinical or biochemical signs of hepatic or renal disease.

Collagen and total protein accumulation in wound granulation tissue were evaluated from the deposited amount of hydroxyproline and proline in two subcutaneously implanted polytetrafluoroetylene tubes.

Results The amount of proline and total protein increased significantly after 8 weeks of abstinence, median 81.3 nmol/mm (inter-quartile range: 77.1-92.9) versus 69.3 nmol/mm (68.5-76.3), $p < 0.05$, and 632 nmol/mm (505-1,127) versus 571 nmol/mm (544-831), $p < 0.05$, respectively. There was no significant change of hydroxyproline.

Conclusion This study showed a change in the protein level of the wound healing process among alcohol abusers, which seemed reversible after withdrawal.

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Introduction

Alcohol abusers have three to four times increased postoperative morbidity after surgical procedures (1-3). Especially wound complications are often seen (1-2). However, the knowledge of impact of alcohol on wound healing is sparse.

Wound healing is a dynamic process characterised by clot inflammation, local inflammatory response and deposition of mature collagen and non-collagenous proteins by fibroblasts. The amino acid proline is found in all proteins deposited in the granulation matrix of a wound. Hydroxylation at proline in the procollagen molecules precedes the formation at triple helical mature collagen molecule, which is especially rich in hydroproline (4). The healing process is influenced by many factors, including alcohol (5).

A standard wound healing model consists of subcutaneously inserted tubes of expanded polytetrafluoroetylene (ePTFE) with a 90-120 micro-millimetre pore size, which allows ingrowths of inflammatory cells and fibroblasts. After removal, the subcutaneous accumulation of hydroxyproline, proline and total protein is measurable (6). In this model, the accumulated collagen in the ePTFE tube has been found to correlate with the tensile strength at experimental wounds (7).

The aim of the study was to evaluate wound healing at alcohol abuse and after withdrawal.

Material

After informed consent, sixteen alcoholic outpatients (12 men and 4 women) were included for evaluation of wound healing capacity, when they entered the Alcohol Unit for treatment of alcohol abuse. The patients had been drinking 240 g (median) ethanol daily (range 72-1,020) for at least three months before inclusion. They were all in relatively good physical condition; however, two patients suffered from chronic bronchitis in mild to moderate degree, and one from non-insulin dependent diabetes mellitus. None of the abusers had clinical or biochemical signs of hepatitis or hepatic cirrhosis.

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Seven men and two women were re-examined for wound healing capacity after eight weeks of total abstinence; the treatment comprehended supervised disulfiram 800 mg twice weekly, chlordiazepoxide 50-100 mg daily according to withdrawal symptoms, and behaviouristic support). The other patients had relapsed before eight weeks and were therefore not included in the re-evaluation. The characteristics are given in Table I.

Table I Characteristics of all alcohol abusers and of the alcohol abusers who remained abstinent for eight weeks (median and range)

	Alcohol abusers n=16	Abstinent patients n=9
Women/men	4 / 12	2 / 7
Age (yrs)	45 (36-51)	45 (40-48)
Smoking (cigarettes per day)	22 (0-40)	20 (0-40)
Body mass index (kg/m^2)	23 (19-29)	23 (20-28)
PN (%)[1]	29 (20-45)	34 (10-45)
Haemoglobin (mmol/litre)	8.9 (7.2-11.8)	8.9 (7.6-11.4)
Albumin (mol/litre)	624 (527-711)	613 (550-874)
Bilirubin (U/l)	12 (7-19)	10 (7-13)
Creatinine (micromole/litre)	75 (62-90)	71 (59-1 09)
CRP (nmol/litre)[2]	<95 (<95 - 474)	< 95

[1] Prognostic nutrition index (8), [2] Lower detection limit is 95 nmol/litre

Methods

A paired design was used. Two ePTFE tubes (International Polymeer Engineering inc., Tempe, Arizona, USA), 6 cm length, 12 mm inner diameter, 2.4 mm outer diameter, 90—120 micrometers pore size were inserted subcutaneously in the upper arm under local anaesthesia as described previously (6). The tubes were removed ten days after implantation.

High-Performance Liquid Chromatography (HPLC-assay): 3 cm of the middle part of the ePTFE was delipized in acetone and diethyl ether and dried. The length of each section was measured before hydrolysis for 24 hours in concentrated hydrochloric acid and liquefied phenol at 114°C. The samples were prepared for chromatographic analysis after re-dissolving the dried hydrolysates. The samples were evaporated to dryness and re-dried with triethylamine for removal of traces from hydrochloric acid and reacted with phenylisothiocyanate.

The resulting phenylthiocarbamyl derivatives from the amino acids were re-dissolved in sample diluents and analysed in a system using a Hypersil 6DB C18 column analyser (Shandon, Runcorn, Chesire, UK) with increasing concentrations from acetonitrile in an acetate buffer pH 5.70. The UV-absorbency of the eluate from the column was monitored at a wavelength of 254 nm using instruments from Waters (Milford, MA, USA). Calibration curves for the individual amino acids were constructed from equal analysis of standard samples from the "Amino Acid Standard H" (Pierce, Rockford IL USA) and L-4-hydroxyproline (Merck, Darmstadt, Germany).

Based on all the amino acids measured in the assays, the amounts of hydroxyproline, proline and total protein, were calculated from the chromatograms by averaging the results from two injections of the same hydrolysate, and the contents were expressed as the amount per mm at dry delipidised ePTFE tubes.

Prior to wounding procedure venous blood was sampled for routine analysis of haemoglobin (1-12, Bayer, New York, USA), electrolytes and liver enzymes (SMAG, Bayer, New York, USA). The local Scientific Ethical Committee (No KA 93062) approved the study. The Wilcoxon test was used for statistical analyses of paired samples, and the Mann-Whitney test for unpaired results. A level of 5% was chosen for statistical significance.

Results

The characteristics of the nine abstinent patients, who were reinvestigated after alcohol withdrawal, did not differ significantly from the seven, who were only investigated once.

The proline, hydroxyproline and total protein measurements are given in Table 2 and Figure 1.

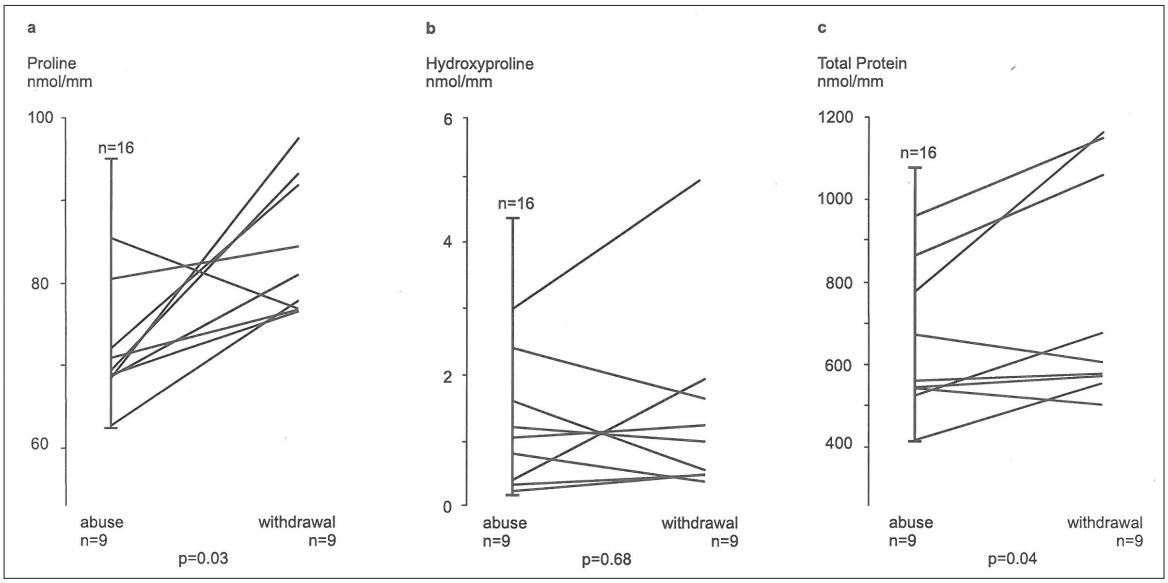
There were no complications in relation to the implanted material.

Table 2 The proline, hydroxyproline and total protein content in experimental wounds (Median and range)

	Alcohol abusers all patients.	**Alcohol abusers** with follow-up.	**Alcohol abusers** with follow-up.
	n=16	Before abstinence from drinking. n=9	After 8 weeks withdraw from drinking alcohol. n=9
Proline (nmol/l)	72.0 (62.7-95.5)	69.3 (62.8-85.5)	81.3 (76.9-98.0)
Hydroxyproline (nmol/l)	1.1 (0.3-4.4)	1.1 (0.3-3.0)	1.1 (0.4-7.8)
Total Protein (nmol/l)	651 (425-1,080)	571 (425-971)	632 (526-1,186)

Median and range

Figure 1



1a: Accumulated proline in the nine alcohol abusers before and after eight weeks at abstinence. * $p < 0.05$.

1b: Accumulated hydroxyproline in the nine alcohol abusers before and after eight weeks of abstinence.

1c: Accumulated total protein in the nine alcohol abusers before and after eight weeks at abstinence. * $p < 0.05$.

Discussion

We found the slowed healing process in alcohol abusers to be associated with decreased amount of proline as well as total protein. The process normalises after abstinence from alcohol.

The mechanism of reduced synthesis and/or secretion of proline and total protein in the wounds of alcohol abusers are unknown. Ethanol has a direct toxic effect on the ultra structure and function of mitochondria as well as endoplasmatic reticulum (9). Disperse reduction of protein synthesis and cell atrophy follows (7;10), as it is often reflected in alcohol-induced skeletal and cardiac myopathy (11-12).

Alcohol-induced suppression of the immune capacity may also be of importance. Mobilisation, adhesion and signal transduction across the cell membrane of the inflammatory cells relevant for wound healing are reduced (12), and probably followed by a delay of the inflammatory phase in the healing process.

The reversibility of proline deposition in wounds after abstinence from alcohol may be parallel to the myopathy and immune-suppression, which normalise after three and two months of abstinence, respectively (12;14-15).

There are other potential factors to delay the healing process in the alcohol abusers of the actual study: malnutrition, dehydration and illness (16-19). Alcoholism may be associated with malnutrition, and though the patients in the present study were not malnourished, reduced concentration of minerals, vitamins and oxygen-derived free radicals cannot be excluded, and they may all be of importance for a normal healing process. Ethanol blocks the anti-diuretic hormone leading to dehydration in alcohol abusers, which was however not found in the abusers of the actual study, who all had normal biochemical values. Likewise there was no difference between the groups concerning illness evaluated by history and C reactive protein.

Smoking has been reported to depress accumulation of collagen, but not proline or total protein, in the ePTFE wound healing model by inducing hypoxia, which leads to reduction of the oxygen-demanding conversion from proline to hydroproline (20). The accumulation of collagen did not change during abstinence in our alcohol groups, nor did the smoking habits in the test period.

The constant production of collagen in this study is in agreement with in vitro experiments demonstrating that addition of clinical concentrations of alcohol to fibroblast cultures fails to inhibit the basal collagen synthesis (21). In contrast, the response to transforming growth factor beta (TGF-beta) was significantly reduced (21), corresponding to the direct binding of ethanol on membrane proteins followed by disturbance of signalling be-

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tween the cells (22). The influence of combined abstinence from both alcohol and tobacco may be a relevant subject of a future study.

The alcohol abusers stopped drinking when they underwent the first wounding procedure, and may therefore have developed symptoms of withdrawal, characterised by an overactive endocrine stress response, including hypercortisolaemia (23). Other authors have reported reduced collagen deposition secondary to treatment with corticosteroid (24), but similar to a transient endocrine over-activity during withdrawal, a single dose of intravenous prednisolon was not associated with changes in collagen accumulation in wound (25).

Low collagen concentration also follows increased regeneration, which is further characterised by simultaneously increased non-collagen protein (26). We did not find that combination, thus excluding an increased regeneration as an explanation of our results.

Major surgery reduces the collagen and thereby the strength of the surgical wound (27). Our results add to the pathogenesis of the severely increased wound complications after surgery in alcohol abusers (1-3). Furthermore, the results suggest that two months of preoperative soberness may reduce the wound complications postoperatively, which could be relevant in the risk reduction for elective surgery.

Limitations

The small number of included persons is a limitation to this study. The data should be confirmed with larger samples.

Conclusion

In conclusion, the wound healing capacity is slowed in alcohol abusers; however, the healing reverses to normal after eight weeks of abstinence.

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Competing interests: None declared

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