Ethanol treatment of tendon allografts: a potential HIV inactivating procedure

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Summary. *The penetration rate of ethanol in human tendons was studied in order to define the conditions which were necessary to achieve an inactivating concentration against the Human Immunodeficiency Virus (HIV) within the tendon. The rate of alcohol penetration was found to be slow and did not differ with different types of tendons. An average ethanol concentration of 14% (v/v) was measured in human tendons after they had been immersed for 2 h in 70% (v/v) ethanol, and a concentration of 19% (v/v) was reached after 3 h. Ethanol immersion of human tendons may represent an additional safety procedure in inactivating the HIV virus provided the duration is at least 3 h.*

Résumé. *La pénétration de l'éthanol dans les allogreffes tendineuses humaines a été étudiée pour établir les conditions nécessaires à l'obtention d'une concentration inactivatrice pour le HIV. La pénétration tendineuse de l'éthanol est assez lente et ne diffère pas selon les différents types de tendons. Une concentration moyenne de 14% (v/v) a été mesurée après une immersion de 2 heures dans une solution d'éthanol 70% (v/v), tandis qu'une concentration de 19% (v/v) est obtenue après 3 heures. Une immersion d'une durée minimale de 3 heures est donc nécessaire si une inactivation virale du HIV par l'alcool est envisagée.*

Introduction

Tendon allografts are in great demand for use in the repair of knee cruciate ligaments. A constant fear,

however, associated with the use of human material, is the potential transmission of viral disease such as HIV and hepatitis [5, 9, 14]. It is significant that the HIV virus is located not only in the bone marrow but also within the cortical bone and the tendon substance [5, 15].

It is possible to reduce the risk of disease transmission to a very low level; several guidelines for donor selection have been published [10, 1] and each selected donor is extensively screened using different biological assays [2, 4]. It has been calculated that the risk of HIV transmission by tissue and organ donors may be 10-fold higher than in blood donors [12]. The probability of HIV transmission by blood was estimated by the Belgian Red Cross Society as 5/million in 1993 and at 1/million in 1996 [16]. According to 1994 data from the Belgian Hygiene and Epidemiology Institute [8] the probability of HIV transmission by a tissue donor is 44/million. The window period used in this evaluation was 42 days. After the introduction of the third-generation double antigen sandwich assays for anti-HIV, the infectious window period was shortened to 22 days [12], thus reducing the probability of transmission by a factor of 2. The window period may be further shortened by applying the genome amplification tests for HIV-DNA and the detection of HIV p24 antigen. This may reduce the window period by a further 11 days. Applying all these assays as a standard screening of donors, the probability of HIV transmission would be 11/million.

In order to decrease such occurrence, it has been proposed to process the donated tissue, as various chemical agents, including ethanol, have been shown to inactivate the HIV virus in vitro [14, 17, 18, 20]. Simonds et al. reported an instance where alcohol-processed tissues from a HIV seronegative but infected donor were implanted and did not transmit the disease [22].

Table 1. Variation of ethanol concentration in tendons with time of exposure (35 samples)

	30 min	1 h	2 h	3 h	16 h	24 h
Achille's tendon (vol %)	10.5	16	25	38	55	70
<i>P</i>		<0.05	<0.05			

The aim of our work was to verify whether or not ethanol could penetrate a human tendon, such as the patellar or Achilles tendons, and if a virucidal concentration could be obtained in this material.

Materials and methods

Tendon procurement

A hundred and thirty different tendon allografts, notably tibialis anterior, Achilles and patellar tendon were obtained from refrigerated cadavers between 6 and 12 h post mortem. Age and sex and concomitant pathology were not taken into consideration except for any bone or joint diseases or related conditions. The soft tissues around the tendons were removed and each of them was cut into pieces of 5 cm³ (5×1×1 cm) and weighed before being tested.

Measurement of ethanol

Ethanol was used at a concentration of 70% (v/v) in association with 0.02% chlorhexidine, as in clinical practice. In part of the investigations, 97% (v/v) ethanol was also used. After immersion in ethanol (3 ml/g of tendon) for different periods of time, the tendons were dried and weighed. They were then cut into smaller pieces (3×3×3 mm), and placed in an air-tight receptacle containing distilled water for an 18-h maceration.

Ethanol penetration in thick tendons was investigated by dissecting the inner one-third layer (core) from the outer two-third layers (surface). Measurements were performed in both

the cores and the surfaces. Tissue samples were centrifuged and ethanol concentration was measured in 100 µL of the supernatant by gas liquid chromatography (GLC) associated with a flame ionisation detection (FID).

Results

The variations in ethanol concentration in tendons according to the time of exposure are listed in Table 1. A concentration of 35% (v/v) was obtained after 2 h of immersion. After 3 h, the concentration rate was 38% (v/v). Twenty-four hours were necessary to reach the initial concentration of the solution of 70% (v/v). Statistical analysis showed a significant increase in concentration between 1 and 2 h and between 2 and 3 h ($P<0.05$).

The absorption rate of ethanol by different tendons (tibialis anterior, Achilles or patellar) did not differ significantly after 1 h of immersion. The average ethanol concentration was 15.5% (v/v) in tibialis anterior tendons, 16% (v/v) in Achilles tendons and 14.5% (v/v) in patellar tendons (Table 2).

The thickness of the tissue had a significant influence on the concentration of ethanol which was achieved. Using the 70% (v/v) solution, 7.6% (v/v) and 18.2% of ethanol were measured respectively in the core and in the surface layer after one-hour of immersion. After 2 h, a concentration of 14.5% (v/v) was reached in the core layer of the tissue while 27.8% (v/v) was present in the surface layer (Table 3.1) ($P<0.05$). In order to improve the penetration rate, the 70% ethanol was replaced by 97% (v/v). Table 3.2 shows that there was no significant difference between the concentration of ethanol in tendon with 70% (v/v) ethanol, the time of immersion being 1 h or 1½ h.

Using an immersion volume of 20 ml/g of tissue instead of 3 ml/g tissue, the ethanol concentration ob-

Table 2. Ethanol concentrations after 1 h exposure in different types of human tendons (15 samples)

	Mean	SD	<i>P</i>
Tibialis anterior tendon (vol %)	15.48	4.43	NS
Achilles tendon (vol %)	16.4	6.47	NS
Patellar tendon (vol %)	14.48	3.73	NS

Table 3. Ethanol concentrations in Achilles tendons

	Mean	SD		Mean	SD	<i>P</i>
3.1 Ethanol penetration rate between core and surface of the tendon (20 samples)						
Core 1 h	7.6	2.1	Surface 1 h	18.2	10.7	NS
Core 2 h	14.5	3.4	Surface 2 h	27.8	11.7	<0.05
3.2 Influence of the concentration of ethanol solutions (30 samples)						
Ethanol 70 % (v/v) 1 h	16.4	6.4	Ethanol 97% (v/v) 1 h	18.5	6.3	NS
Ethanol 70% (v/v) 1½ h	25.2	6.4	Ethanol 97% (v/v) 1½ h	27.9	2.6	NS
3.3 Influence of the ratio between weight of the tendon and the volume of ethanol (30 samples)						
Tendon 3 mL/g 1 h	16.4	6.4	Tendon 20 ml/g 1 h	21.7	4.1	<0.01

tained after one hour was 21.6% (v/v); this value was significantly higher than the 16.4% (v/v) obtained with the smaller immersion volume (Table 3.3).

Discussion

We combined chlorhexidine 0.02% with alcohol 70% (v/v) because of its bactericidal properties but also for the virucidal effect on HIV [20]. The clinical use of such protocol for human tendons for more than six years has not been associated with side effects such as synovitis or brittleness of the grafts and represent evidence of the excellent tolerance of allografts which are comparable with autografts [6, 23].

Experience with reported viral transmission through implanted tissue allografts shows that only non-processed tissues have contaminated the recipients [7, 22]. According to current knowledge, there has been no report of a virus transmitted through a bone or a tendon that has been treated with ethanol. Ethanol is able to penetrate a thick tendon through a concentration gradient.

Taking into account the lowest concentration observed, about 3 h will be required to reach 20% (v/v) in the deepest part of a large human tendon. As this concentration inactivates the HIV virus in vitro, a human tendon should be exposed to ethanol 70% (v/v) for at least 3 h if a truly efficient safety measure is to be added [20, 21].

Among means to increase the ethanol concentration, the use of a larger volume of solution was found to be effective. [20]. This virucidal concentration will only be obtained after three hours in the deepest layer of the tendon. Consequently, any additional safety procedure for HIV with ethanol should have a duration of at least 3 h.

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