Placenta (1991), 12, 285–288

Antibacterial Properties of Human Amniotic Membranes

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Paper accepted 14.2.1991

SUMMARY

Amniotic membranes are widely used in a multitude of surgical applications and have been shown to reduce bacterial counts and promote healing in infected wounds. Antibacterial properties of amniotic fluid are well documented and the presence of many potentially antibacterial factors has been demonstrated. No such factors have yet been found in amniotic membranes. We have applied a direct disc-diffusion susceptibility test to try to establish the possible existence of such a factor. Amniotic membranes did not inhibit five bacterial species when tested at 3×10^6 and 3×10^8 colony forming units/ml. However, complete growth inhibition of all organisms was seen immediately under the amniotic membrane discs. These results support the hypothesis that the antimicrobial effect of amniotic membranes in vitro is due to their close adherence to the wound surface.

INTRODUCTION

Since 1910, human amniotic membranes have been used in a multitude of surgical applications. Temporary wound dressing, mainly for burns (Colocho et al, 1974; Trelford and Trelford-Sauder, 1979), treatment of leg ulcers (Trelford and Trelford-Sauder, 1979; Ward and Bennet, 1984), skin loss in Stevens-Johnsons disease (Prasad, Feller and Thomson, 1986), pelvic and vaginal surgery (Tancer, Katz and Perez-Verdidiano, 1979; Gannaway, Barry and Trelford, 1984), and otolaryngologic and head and neck surgery (Zohar et al, 1987; Talmi, Finkelstein and Zohar, 1990) are some of the more common uses reported.

'Amnion' and 'amniotic membranes' are terms interchangeably used in the literature. We define amnion as the membrane separated from the chorion, and amniotic membrane as a general name encompassing both amnion and chorion.

Antigenicity of the human amnion appears to be extremely low (Trelford and Trelford-Sauder, 1979; Ackle, Adinolfi and Welsh, 1981), and it has also been demonstrated both

0143–4004/91/030285 + 04 $05.00/0

clinically and experimentally to be able to decrease bacterial growth (Robson and Krizek, 1973; Rao and Chandrasekharam, 1981; Robson, Samburg and Krizek (1972)). Rao and Chandrasekharam (1981) noted control of infection in patients with full-thickness burns treated with amnion. They have also experimentally treated burn areas in rabbits with placement of bovine amnion. Cultures from the amnion covering half the wound were sterile, while the control area showed coagulase-positive staphylococcus or Pseudomonas pyocyanea organisms. Robson and Krizek (1973) inoculated full-thickness burn areas in rats with 10^8 pseudomonas organisms. One-third of the burn area was then covered with amnion, one-third with human split-thickness skin, and one-third was left open as control. All three methods of treating the infected burn were effective in decreasing the bacterial count in the sub-eschar tissue. The degree of decrease was significantly greater with amnion.

We have also subjectively noted marked improvement in local infection in patients with head and neck denuded areas after flap necrosis (Zohar et al, 1987) and have set out to directly evaluate the possible effect of amniotic membranes on bacterial growth.

MATERIALS AND METHODS

Fresh amniotic membranes were aseptically obtained from caesarean sections in 20 seronegative mothers (Syphilis, hepatitis B, toxoplasmosis, AIDS) with a mean age of 28 (range 19–38 years). Placentae were not taken in cases where the mothers received antibiotic treatment 30 days previous to delivery or when this fact could not be assured. The membranes were removed en-bloc from the placenta, rinsed in sterile saline solution to remove blood and debris, and partially separated under sterile conditions. A 10×10-cm piece of amniotic membrane (AM) and a similar piece of amniotic membrane unseparated from the chorion (AMC) were removed. As a control membrane we have used Omiderm™ (OD), which is a synthetic, inert, thin, transparent polyurethane-based membrane (Omikron Scientific Ltd., Rehovot, Israel). The film is inelastic when dry, but becomes highly flexible after becoming wet, and is of similar texture to that of amnion (Behar et al, 1986).

Discs of 1-cm diameter were cut from all membranes and were immediately put in sterile isotonic saline solution until placed on the Petri dishes as described. The discs were stored in 4°C until placed on the Petri dishes no later than 12 h after harvesting (mean 7 h).

The effect on growth of five types of bacteria was tested: coagulase-positive staphylococcus, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*.

The AM, AMC, and OD discs were placed on Mueller-Hinton agar plates after first being seeded with the bacteria strains tested. For staphylococci growth, 5 per cent defibrinized sheep blood was added to the medium. Twenty cultures were tested for each organism at every inoculum concentrate, i.e. a total of 200. The inoculums were of 3×10^6 and of 3×10^8 organisms/ml. One series of five specimens/organism/inoculum concentrate (50 total) was done with the discs placed on the seeded Petri plates immediately after harvesting, without prior rinsing and without a period of storage. One disc of each membrane tested was placed in every Petri dish. The cultures were incubated at 37°C for 18 h. Results were assessed 24 and 48 h following incubation.

RESULTS

'Light' to semiconfluent growth was seen in the 3×10^6 inoculum and semiconfluent in the 3×10^8 inoculum. Bacterial growth inhibition in the form of a 'halo' around the AM, AMC

and OD discs was not seen in any of the Petri dishes of all placentae tested. Thus, no difference was seen between the 3×10^6 and 3×10^8 inoculi or between the stored or fresh AM and AMC. In five cases, a halo was seen around the AMC discs in a variety of organisms and placenta. This halo did not represent decreased or increased organism growth but rather a subtle change in the colouring of the agar media. No such phenomenom was observed in any of the dishes where AM was placed immediately after harvest. Bacterial growth was completely inhibited under all discs of AM, AMC, and OD and inhibition was limited to the exact disc area.

DISCUSSION

Antibacterial properties of amniotic fluid (AF) are well documented. Galask and Synder (1970) demonstrated that AF contains lysozyme, transferrin, 7S immunoglobulin, and β_{1c}/β_{1a} globulin, all factors which can play an antimicrobial role. AF inhibited growth of all bacteria tested by Thadepalli, Bach and Davidson (1978) and by Appelbaum, Shulman and Chambers (1980). Burleson and Eiseman (1972a) studying the mechanisms of antibacterial effect of biologic dressings did not show interference in any way with bacterial growth by the substances tested by them. Rubin and Bongiovi (1971) stated that human skin possesses bactericidal substances in its biological make-up such as lysozyme and certain fatty acids, but could not, however, demonstrate bacterial inhibitory activity of split-thickness human skin in vitro when measured by a disc-sensitivity technique.

Robson and Krizek's (1973) studies already described, and also our experiments, have failed to demonstrate the presence of an inherent antibacterial factor of either AM or AMC. Complete inhibition of growth was noted only under the discs, with no halo of inhibited bacterial growth seen.

Although lysozyme, allantoin, transferrin, peroxidase, and many other factors have been implicated in AF activity against bacterial growth, no such activity could be demonstrated in AM and AMC. Not only that, but the same direct contact bacterial-inhibitory effect could be seen in use of the control synthetic membranes and another hypothesis is needed to explain the documented antibacterial effect of AM and AMC.

The intimate adherence of the membranes to the wound bed may well be the dominant factor in this inhibitory action. Burleson and Eiseman (1972b) demonstrated that skin dressings that did not adhere to the wound were of no antibacterial benefit. In Robson and Krizek's work (1973), human skin decreased bacterial count by 29 per cent while the better adhering AMC decreased the count in 58 per cent. Robson, Samburg and Krizek (1972) have shown in another comparative study that AM were found to be equal to isograft and superior to both allograft and xenograft skin in decreasing bacterial levels in full thickness skin defects in rats. As vascularization of skin dressing, viability of the skin, and inherent immunologic properties of biologic dressings have been eliminated as of causal importance (Burleson and Eiseman, 1972b), the close contact of the dressing to the wound surface seems to play a major role. Fibrin accumulating beneath the skin and connecting the elastin in the skin to the elastin in the wound granulations is perhaps a bonding factor. This elastin-fibrin bond seems to be important in the adherance of biologic dressing to infected granulation tissue, assuring a close contact which is essential for wound sterilization (Burleson and Eiseman, 1972b). The role of such a bond in the AM and AMC is doubtful and is not present in the synthetic membrane. Close contact of these membranes due to their nature is, however, reported by the authors discussing their clinical and experimental application.

Our results did not demonstrate a difference between the inhibitory effect on bacterial growth of AM, AMC, and OD. In the five cases where a 'halo' was observed, bacterial counts inside its area were similar to those of the medium and we suppose that they appeared due to some diffusion of saline from the AMC into the agar plate and are of no significance.

The close adherence and superior bonding characteristics of amniotic membranes compared to skin grafts account perhaps for their more prominent antibacterial effect. The complete inhibition of growth on all bacteria tested by all discs support the assumption that the intimate contact by the membranes and wound bed is the dominant cause of their antibacterial effect. In vivo, this bonding may allow the host's own defence mechanisms to deal with the infection.

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