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Original Article

Effect of Hydration on Barrier Performance of Third-Degree Burn Eschar

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Abstract

Infection is the primary source of mortality in burn patients. One of the main treatment methods of burn wound infections is topical antimicrobial therapy, in which drugs have to permeate a dead tissue called eschar. Unfortunately, most antimicrobial agents can not permeate eschar in therapeutic levels. Surprisingly, permeation properties of this barrier and effects of chemical or environmental conditions on it, including hydration level which is the subject of the present investigation, is not thoroughly studied as yet.

Here, permeation of silver sulfadiazine (SSD), the most frequently used topical agent in burn management, from its' 0.6 mg/ml solution through human third-degree burn eschar was studied in vitro at different hydration levels of fully-hydrated, semi-hydrated and dry eschar. The experiments were performed at 32°C, using Franz-type diffution cells. Hydration level was adjusted by controlling the contact condition of eschar tissue with an aqueous medium.

Results showed that hydration can severely affect permeation of SSD through the burn eschar. Permeation of SSD through fully-hydrated tissue was about 20 times more than that of semi-hydrated samples. Permeation of SSD through dry eschar was initially (up to 3 h) more than those of semi- or fully hydrated tissues, but it ceased and reached a plateau at this time point, while for the other systems continued and became more than that of the dry eschar at later stages. The cumulative amount of drug permeated through the fully-hydrated tissue in 8 h was about 30 times more than that of the dry eschar.

Our results showed that hydration can clearly improve permeation of SSD and possibly other drugs through third-degree burn eschar. A property which could easily change during patient management, e.g., by covering, washing, or application of occlusive formulations.

Keywords: Burn Eschar; Wound; Permeation; Hydration; Enhancement; Silver sulfadiazine.

Introduction

Burn wound infection is the primary source of morbidity and mortality in burn patients. Burn injuries occur in every age group and both sexes. Depth of burn injury depends on the burning agent, temperature and length of exposure to the burning agent. The equilibrium temperature for skin is approximately 44°C. This temperature can be tolerated for up to 6 h without burning (1).

Time-temperature relationship in the depth and degree of damage to skin is very important in the physiopathology of burn. As temperature increases, various enzyme systems begin to

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malfunction and early denaturation of proteins occurs. The early denaturation described above gives way to severe alteration of proteins, a process referred to as coagulation. This phenomenon involves destruction of all levels of protein architecture. New aberrant bounds are formed, creating macromolecules not resembling the original structures. This condition may be reached immediately in cases where skin is exposed to high temperatures (third-degree burn). In this case, cell necrosis is universal and complete, usually starting at the skin surface where the heat energy is most directly received and then extending downward. This zone is called the zone of coagulation and is the first of three zones of burn injury (2, 3).

Zone of coagulation comprises the initial burn eschar. Therefore, initial eschar is formed 3-24 h post -burn. During this period, adhesion of platelets and leukocytes on the surface of injured endothelial cells, local thrombosis and aggregation and degranulation of platelets, induce blood clothing and formation of a fibrin plug or eschar in the coagulation zone (2, 3).

Lying deep and peripheral to the zone of coagulation is a zone of lesser injury, where most cells are initially viable. Here, circulation becomes progressively impaired leading to cessation of blood flow, hence it is termed the zone of stasis. The development of ischemia within this zone is devastating to already compromised cells. Necrosis follows and in severe injury converts the zone of stasis to essentially dead eschar. Impairment of blood flow ensues within a couple of hours in more severe burn areas and is delayed for up to 16-24 hrs in less severe regions (2, 3).

Therefore, the initial eschar tissue is formed 3-24 hrs post burn and during several days, conversion of stasis zone to dead eschar tissue result in the formation of a thick avascular structure consisting of coagulative necrosis of the epidermis, as well as variable degree of dermal and subcutaneous fat necrosis.

This devitalized tissue, the burn eschar, is avascular and consists of denatured protein and cellular debris and provides an ideal environment for the growth of microorganisms. The burnt tissue is sterile immediately after burn. But, in the absence of effective antimicrobial agents, Gram-positive microorganisms grow and after 3 to 7 days are superseded by Gram-negative species (4, 5).

Infection is one of the most important and fatal complications in burn patients. Microorganisms may colonize in the burn wound, proliferate on the eschar tissue, progress in depth and initiate systemic sepsis (6**,** 7). Prevention and treatment of burn wound sepsis include surgical debridemant and topical and systemic antimicrobial therapy. Surgical debridemant, besides being difficult, has a lot of risk factors for patients and therefore topical antimicrobial therapy is very crucial in the management of burn patients. The burn wound is avascular and drug diffusion from the perfused wound margins into the avascular area is not that effective and therefore, systemically administered antimicrobial agents can not usually provide therapeutic levels in the infected area (4, 8).

Although topical antimicrobial therapy has been used for centuries and is now considered as one of the main methods for burn treatment, little is known about the permeation properties of burn eschar and its' barrier performance towards permeation of drugs and also the effects of different conditions on these properties. As discussed above, the burn eschar is completely different from the normal skin and, therefore, normal skin data could not be used for, or extrapolated to, the burn eschar. It has been decided in our laboratory to characterize the permeability of third-degree burn eschar, including the effect of hydration on its' permeation, which is the subject of the present paper study. Hydration usually changes permeation of drugs through biological barriers, such as skin. In skin, water due to its' polar nature, interacts with polar head groups of the stratum corneum bilayers and upsetting the packing at the polar plane and creates a more fluid domain which promotes the diffusion of the drug through skin (9). Water also interacts with intercellular proteins in the stratum corneum and relaxes their fibers and therefore, increases the permeability (9). Eschar is composed of proteins and lipids and its' level of hydration can possibly be affected by wound dressings, as well as topical formulations. However, there is no report on the effect of hydration on

permeation of drugs through the burn eschar.

Silver sulfadiazine, which is one of the main topical antimicrobial agents used in the treatment of different stages of burn wound infection, and in fact the first drug of choice for this purpose, was used as the penetrant in this investigation. This drug has an excellent spectrum of activity, with low toxicity and minimal pain (5). To our knowledge, there is no data available in the literature on the permeation of this drug through third-degree burn eschar**.**

Experimental

Materials

Silver sulfadiazine was purchased from Argenol (Spain**)**. Acetonitrile (HPLC grade) and phosphoric acid (liquid) were obtained from Merck (Germany). Third- degree burn eschar was obtained from Motahari Burn Center (Tehran, Iran).

Drug analysis

A HPLC method was developed for analysis of silver sulfadiazine, based on the USP method (10), This method employed the Merck HPLC system with C8 column (length, 15 cm, 4.6 mm Id, 5µ) and UV detector at 254nm. A water: acetonitrile: phosphoric acid (900:99:1) mixture at a flow rate of 1.2 ml/min was used as the mobile phase**.**

HPLC method validation

To validated the above-mentioned HPLC method, different concentrations of silver sulfadiazine in a mixture of water:acetonitrile: phosphoric acid (82:16:2) were prepared and injected into the HPLC system and the area under the curve of the peaks obtained were measured and analyzed for the limit of quantification, recovery and finally interday and intraday variations.

Permeation studies

Preparation of eschar

Large eschar samples were obtained from 6 donors, 4 male (mean age of 35 ± 10 years) and 2 female (mean age of 31 ± 7 years) patients 20-27 days post burn at the time of surgical debridemant. The cause of burning in all patients was flame and only the samples from abdominal and leg regions were collected for this study. These considerations minimize the permeability variations. Samples were stored at -20°C until use. This temperature does not damage the eschar tissue (11). Before each experiment, eschar samples were thawed, initially in a refrigerator for 24 h and then for another 3 h at room temperature. Samples were then washed with water and measured for thickness at room temperature (11). The samples were then cut into smaller pieces suitable for permeation studies. Eschar samples used for this study showed a thickness of 0.15 ± 0.018 cm (mean ± SD, n = 9).

General permeation procedure

Home-made Franz-type diffusion cells with an effective diffusion area of approximately 5 cm^2 were used for permeation studies. Eschar samples were placed between donor and receptor chambers of the cells, while the epidermal side faced the donor compartment. After an initial pretreatment phase of about 24 h, to control the level of eschar hydration (see the following section), the receptor chamber was washed with water and then filled with 30 ml of fresh receptor phase. 5ml of the drug solution (donor phase) was then placed in the donor chamber and this time point was considered as time zero. The receptor chamber was stirred magnetically, using a multi-station stirring system at 100 rpm. 1 ml samples were taken from the receptor solution at designated time intervals and replaced with the same amount of fresh receptor solution. Serial samples were collected from the receptor phase for 32 h and their drug contents determined. Cumulative amount of permeated drug was plotted against time and permeation flux calculated from the slope of the linear part of the graph. The differences between permeation fluxes of different systems were analyzed statistically with two-tailed t-test. Experiments were performed at 32°C, which is the skin surface temperature (12).

Hydration level

To evaluate the effect of hydration on permeability of eschar towards silver sulfadiazine, three hydration levels of fully-

hydrated, semi-hydrated and dry eschar tissues were used. These hydration levels were obtained by controlling the contents of receptor and donor chambers of the diffusion cells in the 24 h-pretreatment phase described above while the eschar was mounted on the diffusion cells. Fully- hydrated samples were prepared by placing water in both donor (5ml) and receptor (30ml) chambers throughout the pretreatment phase. For semi-hydrated samples, 30 ml water was placed in each receptor chamber, while the donor chambers were kept empty. In case of the dry eschar samples there was no water within donor or receptor chambers, during the pretreatment phase. During the pretreatment phase, cells were refrigerated for 23 h, followed by keeping at room temperature for 1 h.

Receptor and donor phases

The water:acetonitrils:phosphoric acid (82:16:2) mixture was used as the receptor phase. Silver sulfadiazine has a suitable solubility in the receptor phase (10). The solubility of silver sulfadiazine in this receptor phase was found to be 2.21 ± 0.09 mg/ml (mean ± SD, n= 4). Our results show that this receptor phase provides a perfect sink condition for permeation of silver sulfadiazine through the eschar tissue, under the present conditions. The same solvent mixture containing 0.6 mg/ml silver sulfadiazine was used as the donor phase. Our preliminary experiments show that the above-used solvent mixture dose not affects the permeation of silver sulfadiazine through the eschar tissue.

Results and Discussion

HPLC method validation

Figure 1 shows a sample silver sulfadiazine HPLC chromatogram. Results (Figure 2) show a linear relationship (R^2 = 0.9996) between the area under the curve and concentration in the range of 0.015- 0.3 mg/ml.

Limit of quantification of this method was measured to be 12 ng/ml. Recovery percentage of this method was greater than 99%. Recovery percentage is an evidence for accuracy. Interday and intraday studies revealed good repeatability of the method used. Interday RSDs were in the range of 1.0-1.9% and intraday RSDs were in the range of 0.95-2.0%. Therefore this method was considered to be valid for silver sulfadiazine assay.

Silver sulfadiazine permeation through eschar

The amount of silver sulfadiazine permeated through fully-hydrated, semi-hydrated and dry eschar samples are provided in Table 1 and the corresponding permeation profiles are shown in Figures 3-5.

Results show that the cumulative amount of

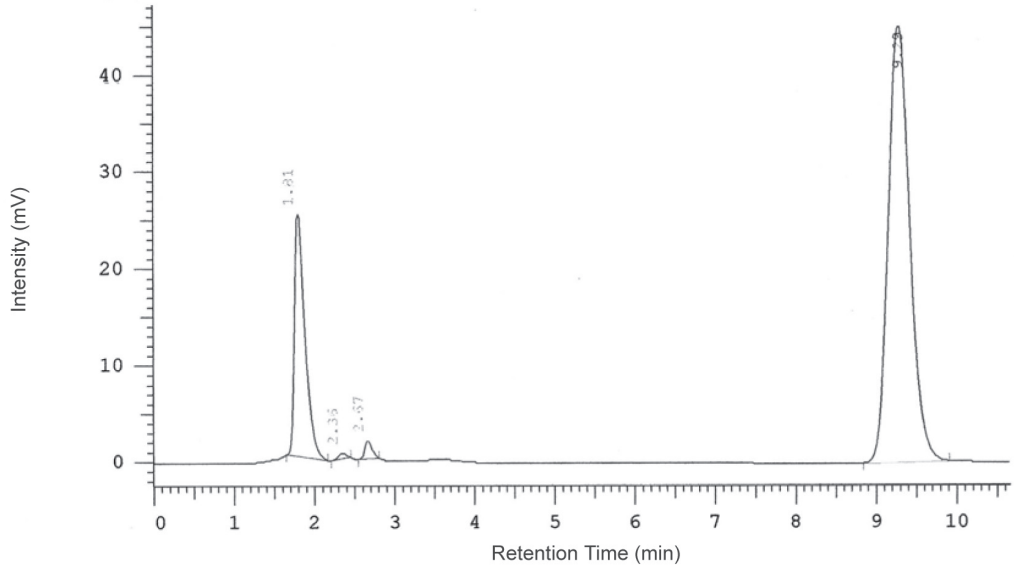

Figure 1. Sample HPLC chromatogram of the receptor phase, showing the permeated silver sulfadiazine through burn eschar (blank retention time = 1.4-2.5 min, silver sulfadiazine retention time = 9.29 min).

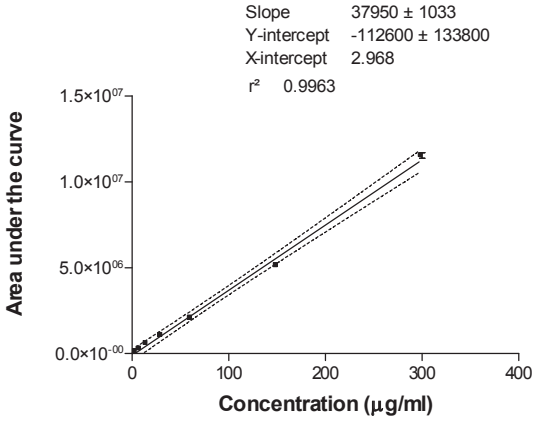

Figure 2. Standard curve of silver sulfadiazine assay, constructed from the HPLC method (mean ± SD, n = 4).

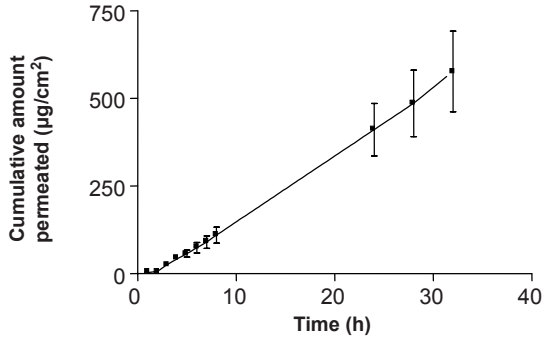

Figure 3. Cumulative amount of silver sulfadiazine permeated through fully-hydrated buran eschar tissue at 32ºC (mean ± SD, n=3).

silver sulfadiazine permeated through dry eschar is initially (up to 3 hrs) more than those of semi- or fully-hydrated systems (Table 1). After this time, permeation through dry eschar ceases and reaches a plateau (Figure 5), while still continues in the other two systems (compare Figures 3 and 5). This might show that the dry eschar tissue has microscopic cracks which could be closed during hydration. The cracks, which are possible in dry tissues, provide permeable pathways (possibly channels filled with surrounding medium) and, after closure, e.g. due to hydration, permeation ceases. The results of the other two systems (Figures 3 and 4) show that higher hydration levels will eventually overcome this problem by providing more permeable pathways in the hydrated systems. No such a study has been reported for the burn eschar. However, it has been shown that intact stratum corneum shows higher permeability in very dry conditions (water contents less than 10 %) than that seen within the normal tissue (13). The same system (intact stratum corneum) also shows higher permeability in much hydrated systems (14, 15). Our data show that the same phenomenon also happens in the burn eschar.

Semi-hydrated eschar showed a biphasic permeation profile (Figure 4). This system was hydrated only from the receptor side during the pretreatment phase. Therefore, the system is not fully-hydrated and its' hydration level, and therefore its' permeability, can change during the experiment, which could be the reason behind observing the biphasic system. Fully-hydrated system shows one linear phase (Figure 3) and reveals that the system is in its' equilibrium state with the surrounding environment.

Table 2 provides permeation fluxes of silver sulfadiazine through different systems. The permeation flux through the first phase of the semi-hydrated system is twice that of the 2 second phase (Table 2 and Figure 4) and shows that a higher level of hydration that occurs during the experiment from the donor phase (as discussed above) can close the pathways and therefore reduce the permeability of the system. Comparison of the fully-hydrated and semi-hydrated systems shows that after these stages, greater hydration levels result in higher permeabilities. Permeation flux of silver sulfadiazine through fully-hydrated system is 20 times more than that of the second phase of semi-hydrated sample and 10 times that of it's 1

Table 1. Cumulative amount of silver sulfadiazinc (µ/cm2) permeated through eschar sampels at different hydration levels at 32°C (mean±SD, n=3).

Time (h)	Fully-hydrated	Semi-hydrated	Dry
1	3.6 ± 0.96	5.6 ± 1.2	22.2 ± 5.2
2	6.0 ± 1.5	7.7 ± 1.4	28.1 ± 5.6
3	26 ± 6.0	9.8 ± 2.2	29.1± 5.4
4	42 ± 7.0	12 ± 2.8	29.3 ± 6.0
5	57 ± 10	14 ± 3.4	29.5 ± 6.8
6	74 ± 15	16 ± 3.5	29.7 ± 7.9
7	90 ±18	18 ± 3.3	29.4 ± 6.6
8	110 ± 23	21 ± 4.0	29.0 ± 6.5
24	411 ± 75	31.8 ± 7.0	NS
28	486 ± 95	36 ± 8.1	NS
32	577 ± 115	40 ± 8.2	NS

NS: not studied because of the permeation plateau

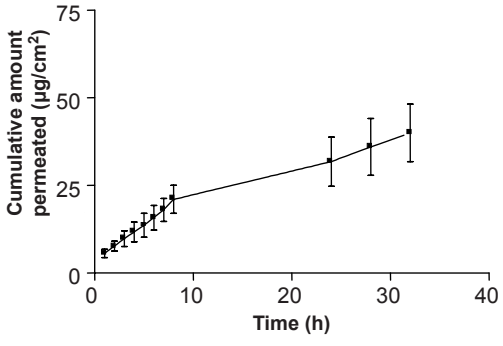

Figure 4. Cumulative amount of silver sulfadiazine permeated through semi-hydrated burn eschar tissue at 32ºC (mean ± SD, n=3).

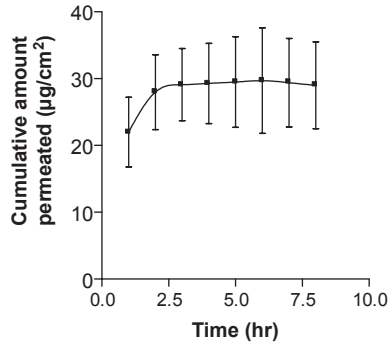

Figure 5. Cumulative amount of silver sulfadiazine permeated through dry burn eschar at 32ºC (mean ± SD, n=3).

first phase.

Permeability of dry and semi-hydrated systems did not reach that of fully-hydrated eschar even after hydration during the course of the experiment. Perhaps the activity of water within the donor phases, during the experiment, is not high enough to change dry or semi-hydrated eschar samples to fully-hydrated systems.

The present data show that permeability of eschar is very sensitive to its' level of hydration. Hydration of eschar or prolonged exposure to water might open the compact structure of eschar tissue and at the same time hydration of dry eschar can close the microscopic cracks of the tissue and, therefore, depending on the situation, increased hydration level can result in both an increased or decreased permeation.

These results are in agreement with those reported for permeation of drugs through normal human stratum corneum (14), although these membranes are structurally different. Water interacts with proteins which are present in both the eschar tissue and the stratum corneum. This interaction is said to be one of the main reasons behind the increased permeability of the stratum corneum (14, 15). Water also interacts with polar groups of stratum corneum's lipid bilayers and disorders packing at the polar plane and improves diffusion of drug through the barrier (14-15), a mechanism that might still be important in the burn eschar. These results show that permeation of drugs through burn eschar could be severely increased by full hydration of the tissue, a process that is easily achievable in burn patients by covering the eschar with occlusive dressings.

These results show that hydration promotes eschar permeation to a large extent. During the burn treatment different conditions, such as dressing with occlusive vehicles, wet dressings and so on, might be imposed on the system, might change the hydration of the system, all of which can change its' permeability. Should this change be towards permeability reduction, it could lead to problems in the treatment. On the other hand, this method could be used to improve permeation, as well as an important enhancement method. The type of the vehicle used can also change the efficacy, through hydration. There are not such studies in the literature on permeation of drugs through burn eschars. Further studies are in progress in our laboratory to investigate different aspects of permeation of drugs through burn eschar.

Table 2. Permeation of silver sulfadiazine through burn eschar tissues with different hydration levels (mean ± SD, n=3).

Hydration level	Flux (µg cm^{-2} hr^{-1})	R^2*	Flux ratio**	P- value***
Full-hydrated	19.06 ± 0.19	0.999	1.0	
Semi-hydrated (Phase 1)	1.92 ± 0.054	0.996	9.9	< 0.0001
Semi-hydrated (Phase 2)	0.96 ± 0.014	0.998	19.8	< 0.0001

Linear regression analysis of cumulative amount permeated vs. time profile.

Comparison with the fully-hydrated system.

** Two- tailed t-test analysis

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