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Therapeutic efficacy of hydrophilic gels of α-tocopherol and tretinoin in skin ulcers induced by adriamycin hydrochloride

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Abstract

α-Tocopherol, tretinoin and ascorbic acid can act as biological antioxidants. Hydrophilic gels of these drugs were prepared, alone or in combination, and their therapeutic efficacy on ulcers induced by adriamycin hydrochloride in experimental animals was studied. The qualitative study considered the four phases observed and clearly defined: ulcer, scab, crater and cicatrix. The quantitative study yielded results showing statistically significant differences between the animals treated with tretinoin and those receiving α-tocopherol. Tretinoin was not shown to be effective in reducing either the size of the lesion or the time of disappearance of the acute period with respect to control; moreover, the ulcers were even larger than those of control. α-Tocopherol administered in semisolid vehicles protects the epidermal cells from free radical attack, as indicated by the reduction in lesions induced by the promoter (adriamycin hydrochloride). At the same time it may accelerate epidermal regeneration. This double activity — protection and regeneration — is potentiated by the presence of ascorbic acid in the formulations, as shown by the smaller size of the ulcers and the shorter duration of the acute effects.

Keywords: Ascorbic acid; α-Tocopherol; Tretinoin; Adriamycin hydrochloride; Hydrophilic gel; Free radical; Skin

1. Introduction

α-Tocopherol (E) is the predominant and most biologically and antioxidant-active form of vitamin E (Skinner and Parkhurst 1970; Hoffmann-La Roche, 1984; Ball, 1988; US Pharmacopoeia, 1995). Its reactivity with free radicals (FR) is considered its main biochemical function (Sies, 1989), based on the inhibition of certain enzymes, such as glutathione peroxidase, superoxide dismutase, catalase (Bieri et al., 1983; Simon-Schnab and Koeppe, 1983; Hoffman-La Roche 1984; Furuse, 1987; Frankel, 1989;) and 5-lipoxygenase (Sies, 1989). It protects the cell from FR damage result-

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ing from normal metabolism or induced by environmental agents and medicaments (Bieri et al., 1983; Simon-Schnab and Koeppe, 1983; Hoffmann-La Roche, 1984; Furuse, 1987; Frankel, 1989; Gardner, 1989; Vigo et al., 1992a, b). E is present mainly in cell membranes, and acts as an antioxidant by breaking chains, neutralizing the FR that are produced above all in fats. Thus hydroperoxide formation is inhibited, or at least reduced (Tappel, 1972; Hoffmann-La Roche, 1984). In general, vitamin E plays an important role in maintaining the stability and integrity of cell membranes (Vanderveen and Vanderveen, 1987; Thomas et al., 1989). Therefore, the E integrated in the lipid-protein structure of the membranes has a protective antioxidant and scavenger action (Hosta, 1981; Niki, 1987).

Tretinoin (T) is the *trans* isomeric form of retinoic acid. It can be an antioxidant and an FR inhibitor. Its use not always well justifiable, although there is evidence that its topical application may protect the skin from the effects of actinic rays and other radiations. Moreover, administrated by whatever systemic route, it can act against the carcinogenesis resulting from radiation or other carcinogens. It is not known if it has any physiological function, although some authors consider it to be the active form of vitamin A in the skin (Bonhomme et al., 1990). T plays a critical role in the control of the terminal differentiation of keratinocytes (Noji et al., 1989), inhibiting their differentiation (Regnier and Darmon, 1989) while stimulating their growth (Voorhees, 1990). It affects the process of keratinization, so that the corneous cells cannot unite strongly one to another (Mills and Kligman, 1982). Thus the epidermal response to topical T can be explained by the natural biochemical mechanisms taking place in the keratinocytes (Weiss et al., 1988). In contrast to the action mechanism of E, that of T is not completely known. Of the different suggestions, perhaps that of antioxidant action is the most determinant of its effect in photo-aging, as this results from a high exposure to solar UV radiations one of the most important promoters of FR.

L-ascorbic acid (**AA**), monodehydroascorbic acid (**MDHAA**) and dehydroascorbic acid (**DHAA**) constitute the redox system vitamin C. This has the property of acting as an antioxidant (Tolbert 1986; Vanderveen and Vanderveen, 1987; Frankel, 1989; Honnegger et al., 1989; Nagy and Degrell 1989; Irache and Vega, 1990) and as a co-antioxidant by interacting with vitamin E (Doba et al., 1985; Sies 1989), protecting cells from diverse injuries caused by oxidation (Henning, 1991). Thus in aerobic organisms AA protects the biological tissues against active oxygen species and simple oxygen (Tolbert, 1986). It is therefore a scavenger of oxygen (Frankel, 1989) and FR (Tolbert, 1986; Frankel, 1989; Honegger et al., 1989; Nagy and Degrell, 1989), reacting directly with these to form the stable intermediate radical (MDHAA) (Seib, 1986). It can also act as synergist (Frankel, 1989), potentiating the action of other antioxidants (Bright-See, 1983a); as blocker in the formation of nitrosamines — so preventing cancer (Bright-See, 1983a, b; Lohmann, 1987; Tannenbaum, 1989, Harman, 1993); as metabolic regulator intervening in the synthesis of collagen, in the absorption and mobilization of iron (Tolbert, 1986; Sitren, 1987; Vanderveen and Vanderveen, 1987; Hallberg et al., 1989; Harman, 1993; Reynolds, 1993b), in the metabolism of copper and aminoacids, and as cofactor of mixed-function oxidases (Tolbert, 1986; Reynolds, 1993a).

This led us to wonder whether the proven efficacy of E, T and AA might be equally apt when they were administered topically in semisolid vehicles.

The present work is a pharmacotherapeutic study of E and T administered topically in gelled hydrophilic vehicles. It demonstrates their therapeutic efficacy against lesions induced in experimental animals. At the same time, the effect of AA in the potentiation of the therapeutic effects of these drugs is determined.

2. Materials and methods

2.1. The semisolid preparations

Hydrophilic gels were used as dermatological bases (Vigo, 1993), prepared with the components indicated in Table 1.

Table 1
Components of the semisolid preparations prepared

	gel G	gel AA	gel E	gel EAA	gel T	gel TAA
Carbomer® 940 (%)	1	1	1	1	1	1
Ethanol (96%) (ml)	15	15	15	15	15	15
Triethanolamine (ml)	3	3	3	3	3	3
Distilled water (ml)	85	85	85	85	85	85
Ascorbic acid (%)	-	0.1	-	0.1	-	0.1
α-Tocopherol (%)	-	-	2.5	2.5	-	-
Tretinoin (%)	-	-	-	-	0.025	0.025

Gel G was prepared with Carbomer® 940 (Acofarma, Barcelona, Spain) following the usual technique. **Gels AA, E, EAA, T** and **TAA** were obtained by dissolving the corresponding substances, AA (Acofarma, Barcelona, Spain), E (*dl*-α-tocopherol, Merck, Darmstadt, Germany) or T (all-*trans* retinoic acid. Merck, Darmstadt, Germany), alone or with the antioxidant AA, in the alcohol (Merck, Darmstadt, Germany) followed by incorporation to the rest of the formula with the aid of a magnetic stirrer (SBS Mod. A-06).

Prior to the preparation of gel G, the amount of TEA (Acofarma, Barcelona, Spain) necessary to neutralize the gels (BF Goodrich, 1981) was determined. The pH required was around 5.6, ideal for the stability of AA (Deritter, 1982).

Once the different formulations had been prepared, they were left in repose for 24 h in small-volume plastic containers, protected from the light and at ambient temperature.

2.2. *Promoter of free radicals: adriamycin*

Anticancer therapy includes the anthracyclines (Simó-Camps et al., 1977; Janssen et al., 1985; Calabresi and Chabner, 1990). The best known is adriamycin (ADM) or doxorubicin (Handschumacher, 1990; Harvey, 1990; Reynolds, 1993a). This is made up of a tetracyclic chromophore joined by a glucoside bond to an amino sugar, daunosamine (Harvey, 1990). It has quinone and hydroquinone remains in adjacent rings, allowing it to function as an electron acceptor and donor (Handschumacher, 1990).

ADM is able to penetrate between the bases of DNA, interfering with its functions, and affecting not so much the synthesis of this, but of RNA. DNA division is related with the generation of FR. ADM reacts with mitochondrial cytochrome *P*-450 reductase in the presence of NADPH to form intermediate semiquinone radicals, which in turn can react with oxygen to produce O_2^-. These can generate both H_2O_2 and OH^-, which are highly destructive for the cell (Kappus and Sies, 1981; Svingen et al., 1981b; Speth et al., 1988; Handschumacher, 1990; Harvey, 1990; Vigo et al., 1992a; Vigo et al., 1992b).

One of the cell structures most damaged by FR is the cell membrane, by the processes of lipoperoxidation (Pascoe and Reed, 1987; Handschumacher, 1990; Harvey, 1990).

Intravenous administration (among others) may induce signs of local irritation (Harvey, 1990), cell damage (Svingen et al., 1981b) and tissue necrosis in man (Svingen et al., 1981a), above all if there is extravasation (Speth et al., 1988; Handschumacher, 1990; Harvey, 1990) or if erythematous striations appear near the administration site (adriamycin reddening).

Svingen et al., 1981a, b and Nobbs and Barr, 1983 claim that if the skin cytotoxicity caused by ADM is due to FR, it may be possible to prevent the effects of accidental extravasation using the FR scavengers E and dimethylsulphoxide. Dorr and Alberts, 1983 and Odukoya et al., 1984 indicate that these scavengers reduce the skin ulcerations produced by ADM in pig and rat.

In this assay, ADM (adriamycin hydrochloride, Farmiblastina®, Farmitalia Carlo Erba S.A.) was administered intradermally to all the animals with a syringe of 1 ml and needle of calibre 25 G.

Following prior studies of Lucero, 1989; Lucero et al., 1993, 0.05 mg of ADM was the amount chosen to produce an adequate dermal lesion for this study. ADM was injected as 0.025 ml of physiological saline solution.

2.3. Animals

Female Wistar rats, 9 months old and approximately 380 g in weight, were used. They were all fed on artificial fodder (Pamlab®) and water ad libitum, and kept in a room maintained at a temperature of 25 ± 1°C.

The back of each animal was shaved with an electric razor (Kuno Moser GMBH·D-7731) 24 h before beginning the assay, so that the skin was in the best possible condition. At the end of this period, the animal was anaesthetized with 0.04 ml of ketamine hydrochloride (100 mg/ml, Ketolar®, Parke Davis) i.m.

The total number of animals (42) was divided into seven groups of six: the *control group* comprised six rats that received only ADM on one side of their back; *groups G, AA, E, EAA, T and TAA* comprised the groups of six rats treated with the respective gels, on one side of their back, prior to the injection of ADM.

Each group, except the control, received one application/day of the respective gel on two consecutive days prior to the injection of ADM. Treatment was continued, with the different gels, immediately before and after this injection and for the following 5 days. Each animal received a total of nine doses of 0.2 g/dose over a skin surface of 11 cm^2.

2.4. Lesions

Each animal was observed macroscopically 36 days after beginning the assay. Given the possible existence of observational bias, this study was carried out double-blind.

The most significant macroscopic observations found were ulcer, scab, crater and cicatrix. All evaluations were qualitative, except in the first case, where additionally the diameters of the ulcer were measured with a calibrator normally used for such measurements.

3. Results

The FR theory, among others, explains the basic causes of certain cellular and tissue degenerative states (Del Maestro, 1980; Masoro, 1989; Sies, 1989; Vigo et al., 1992a, b). These reactive species have a very brief life span (tenths of a second), so it has not been possible to isolate them (Pryor, 1976). Consequently, to work with them, it is necessary to use promoter species.

Of the many substances that give rise to FR, ADM was chosen as reacting with mitochondrial cytochrome *P*-450 reductase and with oxygen, to produce the anionic radicals superoxide, hydrogen peroxide and hydroxyl — highly destructive for cells (Kappus and Sies, 1981; Pascoe and Reed, 1987; Speth et al., 1988; Handschumacher, 1990; Reynolds, 1993a).

These theories on radicals are the basis for demonstrating if the two active principles studied, E and T, have protective or regenerative actions in vivo on lesions induced by ADM when administered in a gelled semisolid vehicle.

It was necessary to establish a model of treatment to be followed with the different active principles. The results obtained in a study of diffusion in vitro (Vigo, 1993) were used for this purpose.

The treatment was the same for T and E, independently of their formulation composition. Given the nature of the lesion-causing agent, and to ensure sufficient amounts of active principle, it was thought appropriate to administer the formulations prior to injuring the tissue. Treatment was maintained until the appearance of ulcers (the moment when ADM lost its activity).

In this investigation, two studies have been made: one qualitative (the type of lesion) and the other quantitative (its size).

In all the animals, in the acute phase, an ulcer was observed, usually appearing on the 4th day after injection of ADM and which was associated with chemically-induced tissue necrosis. The existence of the ulcer was very brief, disappearing between the 6th and 7th days. Later, the appearance of a fibrin scab was detected covering the ulcer, which was related with the beginning of the tissue regeneration process, still within the acute

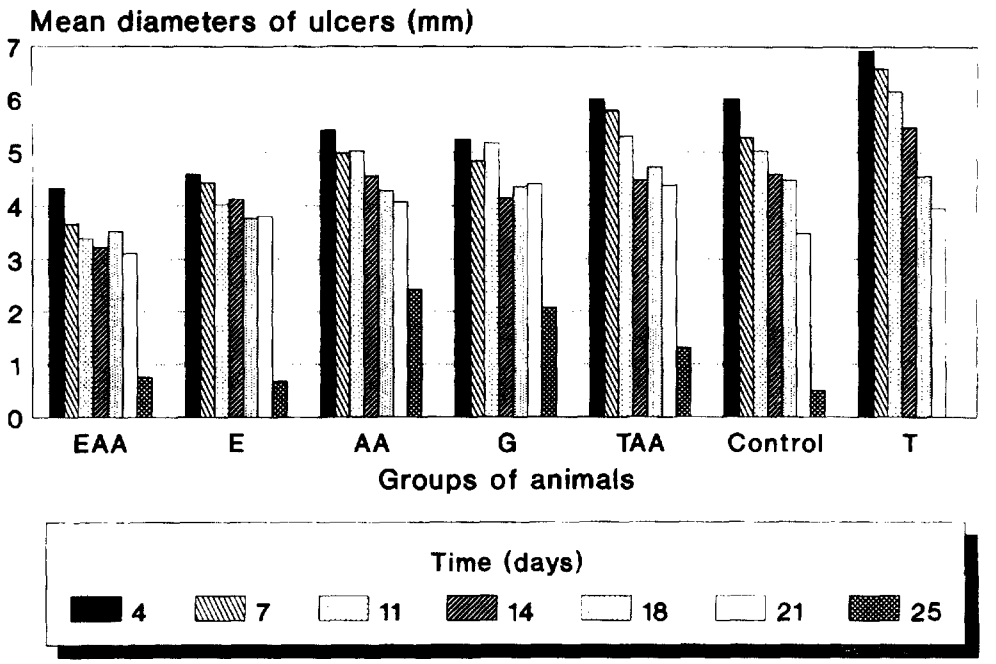

Fig. 1. Qualitative study: evolution of the lesions for the groups of animals and times indicated.

phase. In this phase, inflammatory and erythematous phenomena predominated, but tended to disappear slowly with the appearance of the scab. A second phase of the reparative process was the consolidation of cicatrization, beginning with the disappearance of the scab and the formation of a clean, non-ulcerous crater at the site of the tissue necrosis. In this matter, differences were observed in the different groups of rats. Nonetheless, complete cicatrization was detected between days 32 and 36, independently of the treatment undergone by the animals.

Observation of the evolution of the lesions showed that they had the same characteristics but were of different intensity. At the same time, differences in the consolidation of cicatrization were detected. This last aspect was taken as a criterion for the comparative qualitative study via the profiles of disappearance of acute effect, reflecting the tendency of the different groups of animals to consolidate cicatrization.

Fig. 1 shows the profiles of the Control group and of the animals receiving treatment with the semisolid preparations G, AA, E, EAA, T, TAA. It can be seen that group EAA shows a smaller lesion and remission in a shorter time than the other groups. The results for group E are similar. In contrast, the groups treated with T and TAA present large lesions that are even larger than those in the control group.

From this qualitative study, firstly the size of the lesion obtained after injection of ADM was estimated, and its remission with time was determined. Table 2, giving the mean diameters of these lesions, shows that for any group of animals the size of the lesion (ulcer, scab or cicatrix) decreases with time. In addition, the differences between the different groups are shown as a function of the semisolid preparation received.

These results were studied statistically by analysis of variance. Table 3 shows the most important parameters when comparing the evolution with time of the diameter of the lesion for each group. This shows statistically significant differences for all the groups except E and EAA; that is, in only these two cases is the size of the lesion independent of the time from administration of the lesion-causing agent.

Table 2
Mean diameters (± SD) of ulcer (mm) for the groups of animals and times indicated

Groups	Time (days)						
	4	7	11	14	18	21	36
Control	6.0(± 0.55)	5.3(± 0.50)	5.0(± 0.45)	4.6(± 0.80)	4.5(± 0.75)	3.5(± 0.35)	1.4(± 0.79)
G	5.3(± 0.42)	4.8(± 0.41)	5.2(± 0.71)	4.1(± 0.37)	4.3(± 0.62)	4.4(± 0.82)	2.2(± 1.60)
AA	5.4(± 0.38)	5.0(± 0.28)	5.0(± 0.79)	4.5(± 0.47)	4.3(± 0.45)	4.1(± 0.63)	1.3(± 0.69)
E	4.6(± 1.16)	4.4(± 0.78)	4.0(± 0.99)	4.1(± 0.93)	3.8(± 0.98)	3.8(± 1.01)	1.7(± 0.71)
EAA	4.3(± 0.82)	3.7(± 1.03)	3.4(± 0.89)	3.2(± 0.73)	3.5(± 0.52)	3.1(± 0.64)	1.0(± 0.46)
T	6.9(± 0.80)	6.6(± 0.58)	6.2(± 0.91)	5.5(± 0.60)	4.6(± 0.50)	3.9(± 1.22)	1.5(± 0.54)
TAA	6.0(± 1.52)	5.8(± 0.78)	5.3(± 1.02)	4.5(± 0.85)	4.7(± 1.01)	4.4(± 0.78)	1.7(± 0.71)

G, carbomer gel; AA, L-ascorbic acid gel; E, α-tocopherol gel; EAA, α-tocopherol gel with ascorbic acid;T, tretinoin gel; TAA, tretinoin gel with ascorbic acid.

Table 3
Statistical parameters of the analysis of variance carried out with the diameters of ulcer for each group of animals as a function of time

Groups	Source	DF	Sum of squares	Mean square	*F*	Prob.
Control	Factor	5	21.5558	4.3112	12.47	<0.0001
	Error	30	10.3717	0.3457		
	Total	35	31.9275			
G	Factor	5	6.4255	1.2851	3.79	0.0088
	Error	30	10.1700	0.3390		
	Total	35	16.5956			
AA	Factor	5	7.7956	1.5591	5.62	0.0009
	Error	30	8.3267	0.2776		
	Total	35	16.1222			
E	Factor	5	3.2433	0.6487	0.67	0.6486
	Error	30	29.0067	0.9669		
	Total	35	32.2500			
EAA	Factor	5	5.7458	1.1492	1.84	0.1348
	Error	30	18.7217	0.6241		
	Total	35	24.4675			
T	Factor	5	41.0747	8.2149	12.62	<0.0001
	Error	30	19.5350	0.6512		
	Total	35	60.6097			
TAA	Factor	5	14.1422	2.8284	2.69	0.0403
	Error	30	31.5933	1.0531		
	Total	35	45.7356			

G, carbomer gel; AA, L-ascorbic acid gel; E, α-tocopherol gel; EAA, α-tocopherol gel with ascorbic acid;T, tretinoin gel; TAA, tretinoin gel with ascorbic acid.

In the light of these results and with the aim of comparing the different groups, analysis of variance was carried out for the phases detected in the qualitative study. That is, the acute period on the 4th day, the disappearance of this period between the 7th and 25th days, and complete cicatrization by the 36th day.

The statistical parameters for the first phase and Fisher's test are shown in Table 4. The results indicate the existence of significance; that is, the diameters of the ulcers of the seven groups of animals assayed are different. Fisher's test shows that there is a difference with respect to control only for groups E and EAA; at the same time

Table 4
Statistical parameters of the analysis of variance and Fisher's test carried out with all the groups on day 4

Source	DF	Sum of squares		Mean square		F	Prob.
Factor	6	28.6667		4.7778		6.01	0.0002
Error	35	27.8333		0.7952			
Total	41	56.5000					
Fisher's test ($\alpha = 0.05$)							
Groups	Control	G	AA	E	EAA	T	TAA
Control	-	-	-	S	S	-	-
G	-	-	-	-	-	S	-
AA	-	-	-	-	S	S	-
E	S	-	-	-	-	S	S
EAA	S	-	S	-	-	S	S
T	-	S	S	S	S	-	-
TAA	-	-	-	S	S	-	-

G, carbomer gel; AA, L-ascorbic acid gel; E, α-tocopherol gel; EAA, α-tocopherol gel with ascorbic acid;T, tretinoin gel; TAA, tretinoin gel with ascorbic acid.

groups E and EAA are different with respect to T and TAA. There is no significant difference between group G and the groups receiving E, though there is for group T.

The second phase of study was the disappearance of acute effects, whose mean times are shown in Table 5. Given the numerical difference, analysis of variance and Fisher's test were carried out. The results are shown in Table 6. It can be seen that there are statistically significant differences between all the groups assayed. From the latter test, only the significant difference between group G and EAA is important because of its contradiction with the results obtained in the previous phase.

Table 5
Mean times (± SD) of disappearance (d) of acute phenomena for the groups indicated

Groups	× ± SD
Control	16.67 ± 2.16
G	24.33 ± 1.63
AA	18.83 ± 8.16
E	19.00 ± 5.29
EAA	13.00 ± 7.67
T	18.67 ± 2.16
TAA	19.50 ± 5.17

G, carbomer gel; AA, L-ascorbic acid gel; E, α-tocopherol gel; EAA, α-tocopherol gel with ascorbic acid;T, tretinoin gel; TAA = tretinoin gel with ascorbic acid.

Lastly, consilidation of the cicatrices was detected after 36 days, when their diameters were measured. The results are shown in Table 2. Analysis of variance (Table 7) indicated no statistically significant difference. This is probably due to the small size of the cicatrices, making their exact measurement difficult. Therefore these were not used to compare remission of the lesions as a function of time. It should be borne in mind that appearance of the cicatrix produced tissue retraction phenomena at its edges, resulting in — because of the size of the lesion that had induced it — a great similarity in final diameter in all the animals.

4. Discussion

The results indicate that the semisolid preparations with active principles did not show the same therapeutic effect. They can be divided into two large groups: those formulated with T and those with E.

Treatment with T showed that its antioxidant action did not reduce either the size of the lesion or the time of disappearance of the acute period, with respect to the control. Moreover, the ulcers induced in these animals were large, even larger than those of the control. This has led to the idea that T has an epidermis regeneration action, re-

Table 6
Statistical parameters of the analysis of variance and Fisher's test between the times of disappearance of the acute phenomena of all the groups

Source	DF	Sum of squares		Mean square		F	Prob.
Factor	6	413.9526		68.9921		2.51	0.0396
Error	35	960.3333		27.4381			
Total	41	1374.2860					
Fisher's test ($\alpha = 0.05$)							
Groups	Control	G	AA	E	EAA	T	TAA
Control	-	S	-	-	-	-	-
G	S	-	-	-	S	-	-
AA	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-
EAA	-	S	-	-	-	-	S
T	-	-	-	-	-	-	-
TAA	-	-	-	-	S	-	-

G, carbomer gel; AA, L-ascorbic acid gel; E, α-tocopherol gel; EAA, α-tocopherol gel with ascorbic acid;T, tretinoin gel; TAA, tretinoin gel with ascorbic acid.

Table 7
Statistical parameters of the analysis of variance carried out between all the groups at the time of complete cicatrization (day 36)

Source	DF	Sum of squares	Mean square	F	Prob.
Factor	6	4.9724	0.8287	1.12	0.3683
Error	35	25.7874	0.7368		
Total	41	30.7598			

ducing the corneous layer (Kaidbey et al., 1975) and increasing the shedding efficiency (Mont and Caputo, 1990), so improving the architecture of the dermis and the consistency or firmness of the skin (Bailly et al., 1990; Berardesca et al., 1990). In contrast, protection of the skin against damage induced by FR was not observed in our studies, so it does not seem that it has a scavenger effect in free radical reactions. However, a characteristic of the treatments with T is the presence of an inflammatory infiltration of tissue irritation (Schiltz et al., 1986) that may be responsible for the increase in acute effects of the lesion induced by ADM.

The presence of AA in the preparations with T does not modify the therapeutic response of T despite the results obtained in a study of diffusion in vitro through biological membranes, where it was observed that AA aided the penetration of T into the epidermis (Vigo, 1993).

Treatment with E resulted, from the beginning of these assays, in reduced diameter of the lesions induced by the intradermal administration of ADM. This phenomenon had already been demonstrated using other topical formulations (Lucero et al., 1993) or using other lesion-causing agents, in particular ionizing radiations (Lucero et al., 1994). The present work has gone more deeply into this aspect, modifying the composition of the formulas. Thus, from a physicochemical point of view, these hydrophilic gels protect E against oxidation by environmental agents. At the same time, a combination of AA and E results in a better chemical stability (Vigo, 1993) and (more importantly) a greater therapeutic efficacy of E, possibly due to the higher amount of E penetrating the epidermis in the presence of AA, as shown by the studies of diffusion in vitro (Vigo, 1993).

It is clear from the results of these assays that the lesions in the animals of group EAA are smaller than in the other groups and that the duration of acute effects is also shorter. The same is not true for group E in which, although the

ulcers are the same size, the acute phase ends later.

It can thus be deduced that E protects the epidermal cells from the attack of FR; at the same time and given that the lesion is smaller, the consolidation of cicatrization, and therefore epidermal regeneration, take place sooner. These effects — protective and regenerative — are potentiated by the presence of AA in the formulation, as this permits the recovery of therapeutic activity, acting jointly with the glutathione enzyme system (Hosta, 1981; Furuse, 1987; Sies, 1989).

5. Conclusions

The results obtained indicate that tretinoin, in any of its formulations, has not been shown to be effective in reducing either the size of the lesion or the time of disappearance of the acute period with respect to control. Moreover, the ulcers induced were even larger than those in control. This effect is not modified by the presence of ascorbic acid.

α-Tocopherol administered in gelled vehicles protects the epidermal cells from the attack of free radicals, as indicated by the reduction of the lesions induced by the promoter adriamycin, at the same time that it may accelerate epidermal regeneration. This double activity — protection and regeneration — is potentiated by the presence of ascorbic acid in the formulations (as shown by the smaller size of the ulcers). This prevents oxidation of the active principle, at the same time achieving the recovery of its therapeutic activity, shown by the shorter duration of the acute effects.

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