Dimethyl sulfoxide and oxidative stress on cultures of human keratinocytes

Ivone da Silva Duarte MD PhD, Alfredo Gragnani MD PhD, Lydia Masako Ferreira MD PhD

I da Silva Duarte, A Gragnani, LM Ferreira. Dimethyl sulfoxide and oxidative stress on cultures of human keratinocytes. Can J Plast Surg 2004;12(1):13-16.

OBJECTIVES: The aim of the present study was to examine the protective action of the antioxidant dimethyl sulfoxide (DMSO) against the oxidative stress on keratinocyte cultures caused by glucose deprivation and hypoxia, using the concentration of malonyl dialdehyde existing in the cell culture as an indicator of the oxidative stress level.
METHODS: Eighty flasks with cultured human keratinocytes in a confluent layer were divided into eight groups, including the following: culture medium with and without glucose, culture medium with and without the addition of DMSO, culture medium subjected and not subjected to hypoxia, and culture medium with a combination of these factors.
RESULTS AND CONCLUSIONS: The statistical analysis of the results showed that DMSO proved to be an effective agent against the oxidative stress on cultures of keratinocytes under the experimental conditions studied.

Key Words: *Culture of cells; Dimethyl sulfoxide; Free radicals; Human keratinocytes; Oxidative stress*

Le diméthylsulfoxyde contre le stress oxydatif dans des cultures de kératinocytes humains

OBJECTIFS : La présente étude se penchait sur la protection conférée par l'antioxydant diméthylsulfoxyde (DMSO) contre le stress oxydatif causé par le déficit en glucose et l'hypoxie dans des cultures de kératinocytes avec, comme indicateur du degré de stress oxydatif, la concentration de malonyldialdéhyde (MDA) dans la culture cellulaire.
MÉTHODE : Quatre-vingt flacons renfermant des cultures de kératinocytes humains en couches confluentes, ont été divisés en huit groupes : milieu de culture avec et sans glucose, avec et sans DMSO ajouté, soumis ou non à l'hypoxie et combinaisons de ces facteurs.
RÉSULTATS ET CONCLUSIONS : L'analyse statistique des résultats a confirmé l'efficacité du DMSO contre le stress oxydatif dans des cultures des kératinocytes à l'échelle expérimentale.

The culture of keratinocytes was a significant advancement in the treatment of skin loss, because a small skin fragment can be expanded in the laboratory to about 5000 times in three to four weeks (1); this could potentially provide the patient with a considerable amount of epithelial surface. However, the use of this laboratory-grown epithelium to treat patients has not produced the expected results (2).

Several studies contributed to formulating a culture medium that stimulated the proliferation of keratinocytes (3-7). However, only a few studies in the literature report the use of substances that may protect laboratory-grown keratinocytes from damaging factors.

There are many studies on the role of free radicals in the mechanisms of ischemic tissue damage. Long periods of anoxia result in changes in the microcirculation in skin flaps, resulting in endothelial edema, increased permeability and ensuing migration of the intravascular fluid and, finally, vascular block and necrosis (8).

Free radicals combine among themselves in various ways; furthermore, they are cytotoxic (9) and take part in chain reactions that lead to tissue necrosis, unless they are eliminated by antioxidants (10,11).

Because the mechanisms of cellular death associated with free radicals involve lipid peroxidation (9,12), the activity level of free radicals can be indirectly assessed in a simple and reliable way by determining the concentration of malonyl dialdehyde (MDA) in the culture medium. MDA is a low weight aldehyde containing three carbon atoms, which is the stable final product of the lipid peroxidation (9,13).

In 1866, Alexander Saytzeff discovered dimethyl sulfoxide (DMSO), a substance that protected human tissues. The chemical and pharmacological properties of DMSO, as well as its use in microbiology, immunology and dermatology, were described in detail in 1964, 98 years after its discovery (14).

DMSO is a nonenzymatic antioxidant that interacts with the hydroxyl group on any substance (15-18). It passes through organic membranes and is able to carry along other substances to which it is combined. It enhances the power of drugs that are used in combination, and lessens their unwanted effects (14).

Before its antioxidant properties were described DMSO was used as anti-inflammatory, especially in rheumatic pathologies such as amyloidosis and scleroderma.

Plastic Surgery Division, Surgery Department, Universidade Federal de Sao Paulo, Sao Paulo, Brazil
Correspondence and reprints: Dr Ivone da Silva Duarte, rua Xingú, 175, ap92, Bairro Valparaíso, Santo André, Sao Paulo, Brazil, CEP 09050-060. Telephone 55-11-4426-1138, e-mail ivone@ivoneduarte.med.br

TABLE 1
Experimental and control groups

Without DMSO		With DMSO	
Control (n)	1 with glucose	Control (n)	5 with glucose
	2 with glucose + hypoxia		6 with glucose + hypoxia
Experimental (n)	3 without glucose	Experimental (n)	7 without glucose
	4 without glucose + hypoxia		8 without glucose + hypoxia

Note: Each group in the table comprised 10 flasks, with keratinocyte culture making up a total of 80 flasks. DMSO Dimethyl sulfoxide

Several experiments with skin flaps (19), egg cell cultures (20), hepatocytes (21-24), macrophages (25), fibroblasts (5) and keratinocytes from the cornea (26) were performed under the action of DMSO at 1% to 4% concentrations. These studies reported the influence of DMSO on cellular proliferation and differentiation.

The objective of the present study was to assess the oxidative stress on cultures of keratinocytes undergoing glucose deprivation and hypoxia. DMSO was added to some of these cultures and the level of oxidative stress was assessed by determining the concentration of MDA present in the culture medium.

EXPERIMENTAL PROCEDURES

Cell culture

Human keratinocytes from posthectomy skin scraps, normally disposed off, were cultured according to the Green protocol of 1979 (27), which was revised in 1985 (28). This protocol was introduced to our laboratory by Gragnani et al (29) in 2002.

DMSO

The keratinocytes were seeded in 80 flasks with 25 cm^3 capacity and cultured to a confluent layer. The cultures for control were then randomly taken and half of them were treated with DMSO, produced by Sigma Laboratories (USA). The DMSO (99.9% concentration) was diluted to 2% with miliQ H_2O. Both control groups received regular (glucose-containing) culture medium (Table 1).

Likewise, one-half of the experimental cultures were treated with DMSO; however, the culture medium used in the experimental group did not contain glucose.

The above preparations were carried out simultaneously on the same day, and the experiment began when glucose deprivation was established. During the experiment the cultures were kept in a humid, 5% CO_2 concentration incubator.

Hypoxia

Twenty-four hours after the establishment of glucose deprivation, one-half of the experimental cultures were subject to 30 min of gaseous hypoxia outside of the incubator and on a shaker (30). The control group for hypoxia was also taken out of the incubator and put on an identical shaker.

At the end of the 30-min hypoxia, experimental and control groups were put back into the incubator where all the cultures remained for a further 24 h, for a total of 48 h, marking the endpoint of the experiment.

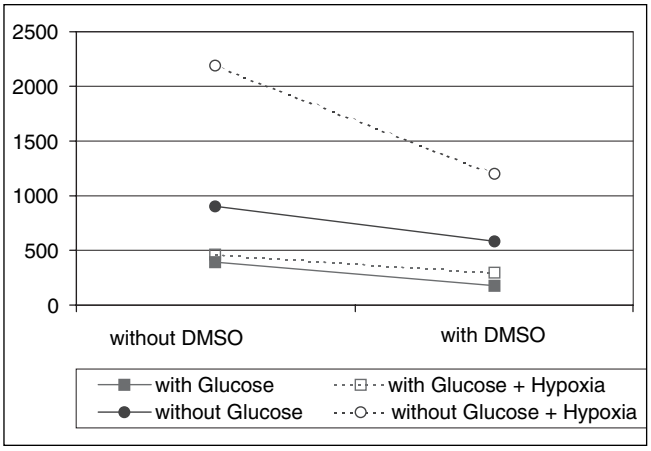

Figure 1) *Concentration levels of cellular malonyl dialdehyde. DMSO Dimethyl sulfoxide*

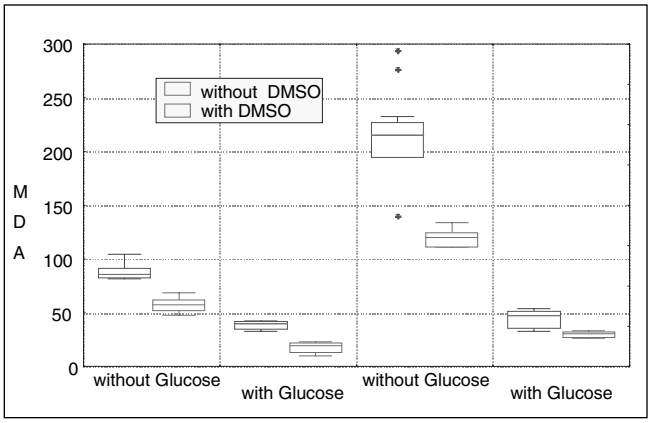

Figure 2) *Concentration levels of cellular malonyl dialdehyde (MDA). DMSO Dimethyl sulfoxide*

Oxidative stress assessment

Samples (1 mL each) of cellular material from the culture groups were collected at start and endpoints of the experiment and were prepared for the determination of MDA concentration by a tiobarbituric acid modified method (13). Because the initial concentration values of MDA were similar for all groups, these values are not discussed in this report.

RESULTS

Figures 1 to 3 show the final concentrations of MDA for the various adverse experimental conditions. They show that MDA levels were consistent with the level of stress to which the various experimental groups were subjected. They also show the protective action of the DMSO, shown by the similar concentrations of MDA in the experimental groups treated with DMSO and their controls.

DISCUSSION

As the cells incur damage resulting from glucose deprivation, the mechanisms associated with oxidative stress – among them lipid peroxidation – are established and the production of MDA is accelerated.

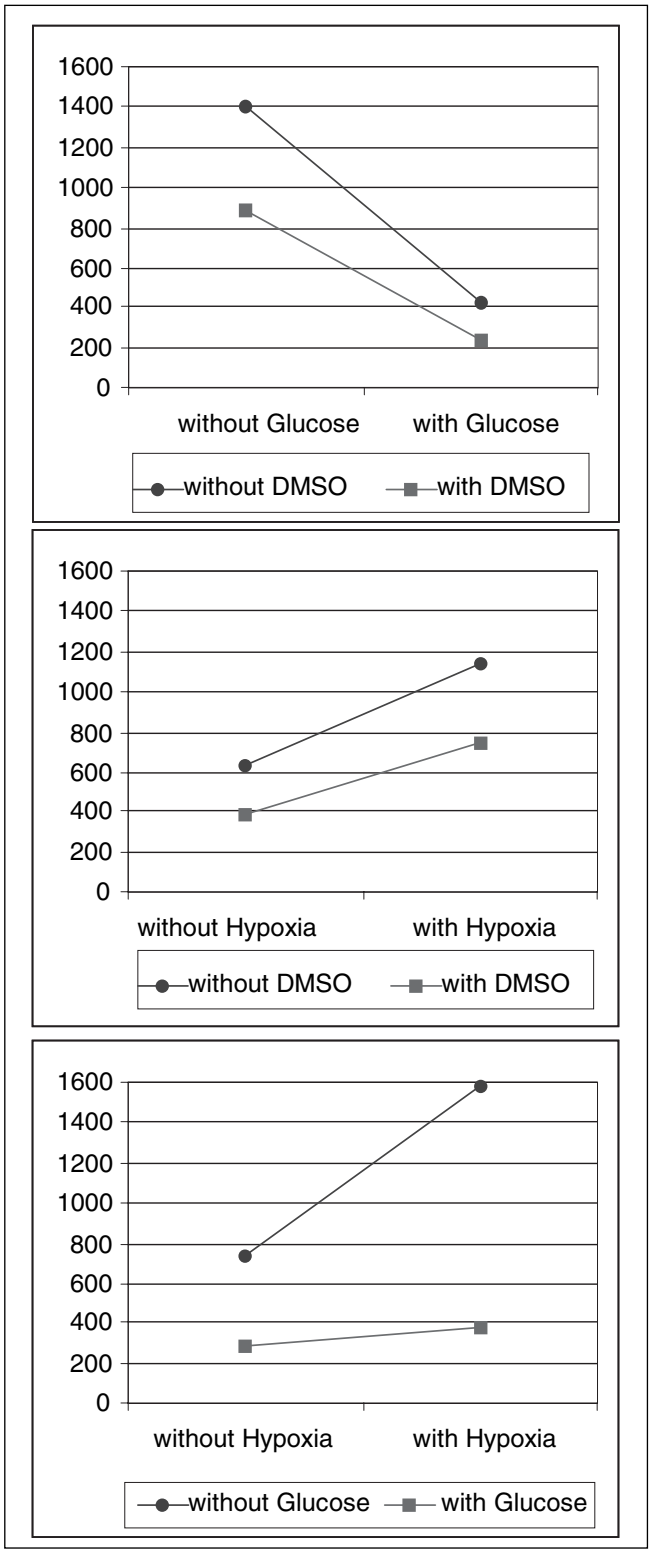

Figure 3) *Graphs of interaction. Y-axis: Concentration levels of cellular malonyl dialdehyde. DMSO Dimethyl sulfoxide*

MDA levels were recorded only at the end of the experiment – 48 h of glucose deprivation and for two experimental groups an additional adverse condition of 30 min of hypoxia between start and endpoints – because the events created experimentally are similar to the mechanisms of an ischemic event followed by reperfusion.

The mean values of the concentrations of MDA for the four experimental and four control groups are shown in Figures 1, 2 and 3 and indicate that DMSO keeps the levels of MDA production down.

Figure 1 gives the final MDA concentrations for all groups and underscores the difference between the groups without the addition of DMSO, whether experimental or control, and their corresponding groups treated with DMSO. It should be noted that the biggest difference of MDA levels between experimental cultures and controls was registered for the most adverse condition: glucose deprivation plus hypoxia.

Figure 3 is subdivided into three subgraphs, which are graphs of interaction. The first two subgraphs compare the levels of MDA in cultures treated and not treated with DMSO according to the restrictions to which the cultures were subjected (glucose deprivation or hypoxia.) These graphs reveal, that in both restrictive conditions, the addition of DMSO resulted in lower levels of MDA, and that this reduction was roughly proportional to the level of aggression due to each restriction.

The third graph compares the effect of glucose deprivation and hypoxia insult alone and in combination in cultures that did not receive DMSO. It should be noted that glucose deprivation resulted in a significant increase in the production of MDA. Moreover, hypoxia followed by reoxygenation has a more deleterious effect on cultures deprived of glucose than on those regularly supplied with it. This is represented by the much steeper inclination of the line that represents the cultures that were subject to glucose deprivation. If the combined effect of glucose deprivation and hypoxia were the same for cultures with and without restriction of glucose, two parallel or slightly nonparallel lines would be expected.

CONCLUSIONS

The present study revealed the different activity levels of the radical hydroxyl producing different cell death rates for different adverse conditions by evaluating changes in MDA concentrations. These radicals produce less damage in the occurrence of hypoxia than when glucose is deprived. Furthermore, the combination of these two conditions make the radical hydroxyl much more active than one might expect. Maintaining an adequate supply of glucose then becomes vital to avoid cell necrosis.

The present study also demonstrated that DMSO at 2% added to the culture medium blocked the activity of the hydroxyl group, especially when the two experimental adverse conditions are imposed simultaneously. These findings suggest that DMSO and other antioxidant agents could have an important role in plastic surgery, by preventing postoperative complications due to a possible inadequate supply of blood through microcirculation in the area being operated on.

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