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DIMETHYL SULFOXIDE INDUCES ACUTE PHASE RESPONSE IN RAT

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We have studied the ability of DMSO to induce the acute-phase response (APR) in rats. The injection of DMSO to laboratory rats caused a rapid increase of the plasma corticosterone concentration. It peaked 2 h after DMSO application and was 300% higher relative to the control. By 12 h, the corticosterone concentration returned to the basal value. The elevated corticosterone concentration induced an increase of acute phase proteins (APP) in sera 24 h after DMSO administration. At 24 h after DMSO administration the relative serum concentration of cysteine proteinase inhibitor (CPI) increased about 710%, α_1-acid glycoprotein (AGP) 630%, α_2-macroglobulin (MG) 510%, γ fibrinogen (Fb) 420%, haptoglobin (Hp) 280%, whereas the relative concentration of albumin, a "negative" APP, decreased to 93% in relation to the corresponding control values. Such changes of APP and corticosterone concentrations after DMSO treatment are comparable to those observed during the APR induced by other irritants. On the basis of these findings we concluded

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that administration of DMSO induces the APR in rats. Therefore, we suggest a more cautious use of DMSO in clinical purposes.

Key words: DMSO, acute-phase reactants, corticosterone, inflammation.

INTRODUCTION

DMSO or $(CH_3)_2SO$ is an amphipathic molecule with a polar domain and two apolar groups that contribute towards its solubility in both aqueous and organic media (SANTOS *et al.*, 2003). DMSO is a commonly available product with a wide variety of non medical uses. In industry, it has been used as a chemical solvent, especially for insecticide solvation (JACOB and HERSCHLER, 1986). In laboratory research, it is often used as a cryopreservative for cultured cells (SMITH *et al.*, 1998). One of the properties of DMSO is that it is absorbed very rapidly through the skin and cell membranes, carryng along almost anything else (particularly low molecular weight molecules) dissolved in it that would not otherwise be able to cross those barriers (JACOB and HERSCHLER, 1986). Intravenous and oral administrations of DMSO allow it to penetrate rapidly into vascular and non-vascular tissues in the body. As a possible cytotoxic agent, DMSO has been studied in human tumor cell lines and in human tumor model systems in animals, and in each case, DMSO demonstrated no activity. As a possible tumor differentiating agent DMSO was found to be active in mouse and human leukemic cell cultures, but it did not improve survival in animals implanted with human tumor cells (JACOB and HERSCHLER, 1986).

DMSO is taken orally for relief of symptoms of interstitial cystitis, a painful chronic bladder disorder. Its popular use among athletes, people with arthritis and others have stemmed from claims that topical DMSO reduces pain, decreases swelling, and promotes healing of injured tissue. In laboratory studies a variety of primary pharmacological actions of DMSO have been documented such as anti-inflammatory activity and analgesia (JACOB and HERSCHLER, 1986; EVANS *et al.*, 1993). But, there are some controversies about its mechanisms of action. Some autors reported possible damaging effects to the liver, kidneys, toxic effects to the lens of the eye, and to efficient free radical scavenging (GONSKYI *et al.*, 1991). There is evidence that points to anticholinesterase effects of DMSO (SCHREIBER and SLAPKE, 1990; PESTRONK *et al.*, 1985). Previously described effects of organophosphorus cholinesterase inhibitors on APP expression in rat liver (ŠEVALJEVIĆ *et al.*, 1990; 1992) suggest a possible role of DMSO in the induction of the APR.

The acute phase response (APR) is an adaptive metabolic reaction of mammals to acute tissue injury aimed at preservation of homeostasis from the harmfull effects of tissue damage. A variety of stressors such as infections, physical or chemical injury can induce the APR (ROTHENBURGER *et al.*, 1999; ŠEVALJEVIĆ *et al.*, 1989; IVANOVIĆ-MATIĆ and POZNANOVIĆ, 1996). The activation of tissue macrophages or blood monocytes leads to the production of soluble mediators of inflammation that ultimately serve to mobilize the metabolic

response of the whole organism (BAUMANN and GAULDIE, 1994; GABAY and KUSHNER, 1999; KOJ, 1998). A key event that occurs during the primary inflammatory reaction is the release of factors from damaged cells which stimulate the production of cytokines in macrophages, which in turn initiate secondary reactions that lead to the secretion of adrenocorticotropic hormone (ACTH) in the pituitary gland. The realeased ACTH in turn simulates the adrenal glands to secrete glucocorticoid hormones. Glucocorticoids modulate energy metabolism, the immune system and in combination with cytokines promote the synthesis of the so-called acute phase proteins (APP) or reactants in the liver (STREETZ *et al.*, 2001). The APP have a variety of activities, from those contributing to the host's defense either by directly neutralizing inflammatory agents and helping to minimize the extent of local tissue damage, to those participating in tissue repair and regeneration. In contrast to the APP, the concentrations of plasma albumin and transferrin fall during the APR and the term "negative AP reactants" has been ascribed to these proteins (KUSHNER, 1982).

Assuming that the propensity of a compound to promote the synthesis of APP is a comperatively sensitive indicator of its abillity to behave as a noxious agent, our aim was to establish whether the application of DMSO leads to a desturbance of homeostasis that promotes the APR. The time course of changes in the serum levels of corticosterone and selected APP after DMSO administration were examined. Such a study contributes towards a better understanding of the effects of DMSO. This is particularly important in light of its many uses.

MATERIALS AND METHODS

Ten week old male Wistar rats weighing between 200-300 g were used. One group of rats received interperitoneal injections (1 mg/g body weight) of DMSO. The control group received saline. The rats were kept at constant temperature, humidity and dark/light intervals and killed at the indicated times.

Blood samples for corticosterone determination were taken from the abdominal aorta of ether anaesthetized rats at 14:00 h. The concentration was determined as described (HODGES and SADOW, 1967). The corticosterone concentration was expressed in μg/100 ml plasma.

The serum from turpentine-treated rats was used for the preparation of antibodies against APP. Rats were killed 24 h after the induction of the APR by turpentine injection. The sera (150 μl) were diluted with saline (350 μl), mixed with complete FREUND'S adjuvant (1:1) and subcutaneously injected into rabbits. Over a period of 2 weeks, 3-5 more injections were given with incomplete Freund's adjuvant. Crossed immunoelectrophoresis of rat serum proteins was performed according to the described procedure (WEEKE, 1970). After the optimal antigen-antibody concentration ratios were determined, the established (same) polyspecific antigen concentration was used throughout in all of the subsequent experiments. The areas under the obtained immunoprecipitation peaks were quantified and the relative percent increase in concentration of AGP, CPI, Hp and MG expressed

relative to the respective control peak area that was assumed to be 100% (ŠEVALJEVIĆ *et al.* 1989). The gels were stained with Coomassie Blue.

Al was determined after the precipitation of globulins with polyethylene glycol (GAMBALL, 1971). Fb was determined as described by KOJ and MCFARLANE (1968). The changes in their concentrations were expressed as percentages with respect to the control values (100%).

RESULTS AND DISCUSSION

The increase in concentration of circulating APP is a marked feature of the systemic stress reaction (ŠEVALJEVIĆ *et al.*, 1989). In the present paper we examined whether DMSO administration could bring about an early increase of corticosterone concentration in the blood and the subsequent increase of synthesis and rise in circulating APP concentrations. Temporal changes of serum corticosterone concentrations after treatment with DMSO are shown in Fig. 1. Corticosterone peaked 2 h after the administration of DMSO and was about 300% higher than in control serum. Four h after treatment the corticosterone concentrations remained 260% higher in DMSO-treated rats. By 12 h after administration the corticosterone concentrations approached the basal values. Thus, we concluded that DMSO exerted an effect on corticosterone induction.

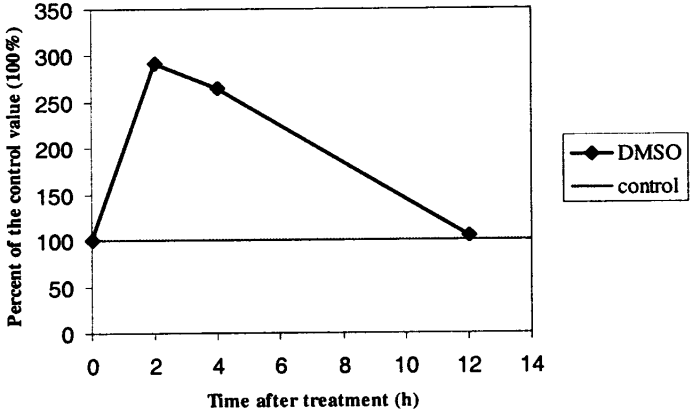

Fig. 1. - Change of serum corticosterone concentrations after DMSO administration. Rats were divided into two groups according to the administered agents: one group received DMSO (mg/g s. c.), and the second control group received saline

Corticosterone either directly stimulates, or by enhancing the effects of interleukin-6 and 1 type cytokines, acts synergistically on the expression of APP genes (BAUMANN and GAULDIE 1994; SCOTTE *et al.* 1996). In order to determine whether the increased corticosterone concentration affected changes in the concentration of APP in the circulation we performed crossed immunoelectrophoretic analysis after DMSO intoxication. During the APR the plasma APP

level is known to increase with time, reaching a maximum by the end of the 1st day and declining after the 2nd day (Kushner *et al.*, 1982). The APR is a nonspecific reaction of an organism to stress, and different inducers of the APR invariably elicit a comparable temporal course of APP production. Therefore, we decided to analyse sera obtained 24 h after DMSO intoxication (KUSHNER, 1982; RUMINY *et al.*, 2001).

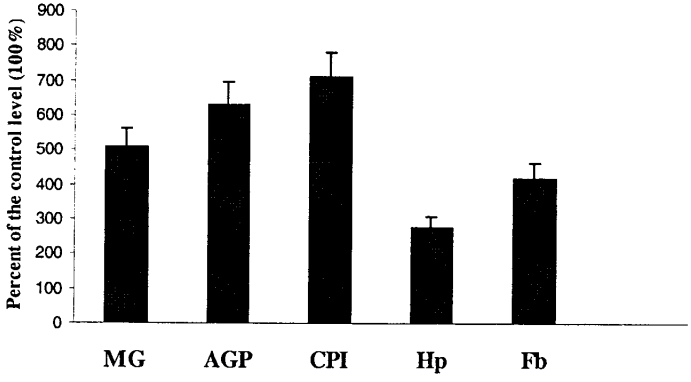

Fig. 2. - Summary of DMSO-induced changes of relative APP concentrations in the serum. MG, AGP, CPI and Hp were identified by crossed immunoelectrophoresis, the areas under each immune-precipitated peak were measured. DMSO-induced alterations are expressed as the percent change relative to the respective control values (100%). The values are means ± S.E. from three to five separate experiments

Quantification of the electrophoregrams of APP (Fig. 2) in sera isolated 24 h after DMSO treatment revealed that the relative concentrations of MG, AGP, CPI and Hp were 510%, 630%, 710% and 280% higher than corrensponding control values. Real concentrations of Fb and Al in sera of control and DMSO treated rats (measured as described in the Methods section) are shown on Fig. 3. The relative concentrations of Fb were 420% greater in the sera of DMSO-treated rats compered to the control rats. The negative AP reactant Al decreased to 93% of control level in DMSO-treated rats. In respect to the magnitude and timing of the increase of the serum APP levels, the effect of DMSO was thus comparable to that of other APR inducers.

The results presented here show that DMSO is capable of eliciting the APR. Qualitative and quantitative patterns of plasma proteins depend on the type of injury. Although the plasma concentration of many APP increases, it does not increase uniformly under different stress conditions (GABAY and KUSHNER, 1999). In DMSO-intoxicated rats we observed that CPI is a major APP. The hepatic APR is elicited through interactions of liver cells with certain released mediators (ENGLER, 1995). Those which determine the level of individual APP have been identified as cytokines, whereas glucocorticoids were recognized as cofactors that enable the full induction of APP. The irritant-related differences in individual APP levels could be viewed as the reflection of nature and composition of released

cytokines. The assumed injury specific variations in the APP composition imply specific and mostly unknown functions of each protein in recovery (BAUMANN, *et al.*, 1988). The benefits of a preferential synthesis of CPI in the rats exposed to DMSO have yet to be assessed.

On the basis of these findings we concluded that administration of DMSO induces the classic APR in rats. Therefore, we suggest a more careful use of DMSO.

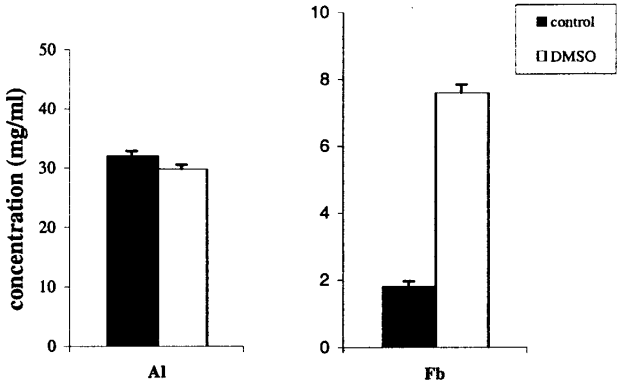

Fig. 3. - The concentrations of Al and Fb were determined as described in the Methods section. The diagrams show the concentrations (mg/ml) of Al and Fb determined in the sera of control and DMSO-treated rats. The results are means from three separate experiments.

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PRIMENA DIMETIL SULFOKSIDA NA AKUTNO FAZNI ODGOVOR KOD PACOVA

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Izvod

U ovom radu praćena je sposobnost dimetil sulfoksida (DMSO) da izazove akutno fazni odgovor (AFO), seriju metaboličkih procesa u cilju re-uspostavljanja narušene homeostaze, kod pacova. Nakon tretiranja pacova dimetil sulfoksidom dolazi do naglog skoka koncentracije kortikosterona u serumu životinja. Tako, već 2h nakon tretmana relativna koncentracija kortikosterona je 300% povećana u odnosu na kontrolu. Na tako visokom nivou se održava i u narednim satima i tek nakon 12h se vraća na bazalni nivo. Ovo povišenje nivoa kortikosterona se odražava na povećanje koncentracije akutno faznih proteina (AFP) u serumu pacova 24h nakon trovanja dimetil sulfoksidom. U tom vremenskom intervalu relativna koncentracija cistein proteinaznog inhibitora (CPI) je povećana oko 710%, α_1-kiselog glikoproteina (AGP) 630%, α_2-makroglobulina (MG) 510%, γ fibrinogena (Fb) 420%, i haptoglobina (Hp) 280% u odnosu na kontrolu (100%). Istovremeno, relativna koncentracija Al, kao negativnog reaktanta, iznosi 93% u odnosu na kontrolnu vrednost. Ovakve promene u koncentraciji kortikosterona i AFP nakon tretmana dimetil sulfoksidom se mogu uporediti sa onima koje su opisane nakon indukcije AFO drugim izazivačima. Na bazi ovih rezultata smo zaključili da dimetil sulfoksid izaziva AFO, zbog čega preporučujemo njegovu daleko oprezniju primenu u kliničke svrhe.

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