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RESEARCH ARTICLE

THE USE OF NATURAL ADMINISTRATION TECHNIQUE AS AN ALTERNATIVE TO OROGASTRIC GAVAGE PREVENTS UNDESIRABLE CHANGES IN THE RESULTS OF ACTH PLASMA AND ANTIBODIES DUE TO STRESS IN RATS

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ABSTRACT

Orogastric gavage is a common laboratory method in toxicology and pharmacokinetic studies, where it is commonly used for daily dosing of rats, mice, rabbits, and monkeys. This work performed on Wistar rats showed that the repeated use of this technique, unlike the natural administration directly from the syringe to be treated with a dose of 1 ml/kg of the placebo (NaCl 0 9%), triggers in rats undesirable stress for the success of the experiment expressed by a significant increase in ACTH levels that cause immuno suppression of detected antibodies.

INTRODUCTION

Virtually, all animals are exposed to a wide range of potential stressors during their lifetimes. These stressors can be a result of environmental perturbations or social interactions. Regardless of the type of stressor, prolonged exposure to stress can have profound, long-lasting effects on physiology and behavior (Stefanski*et al*, 1998). These changes are mediated by specific neuroendocrine mechanisms, including the activation of the hypothalamo-pituitary-adrenal (HPA) axis (Sgoifo*et al*, 1996).Increased HPA activity in response to stress, and the resultant release of adrenal glucocorticoids, can exert substantial effects on physiology, including brain remodeling (Stefanski*et al*, 1998; McEwen*et al*, 1998; McEwen*et al*, 2000; Fuchs*et al*, 2002) and immune modulation (Fleshner*et al*, 1989; Avitsur*et al*, 2002).

Orogastric gavage is a common laboratory method in toxicology and pharmacokinetic studies, where it is commonly used for daily dosing of rats, mice, rabbits, and monkeys (Murphy*et al*, 2001; Nickerson*et al*, 1994; Alban *et al*, 2001).Gavage involves the physical stresses of handling and restraint, insertion of a rigid metal or flexible plastic tube from mouth to stomach, and stomach distension. Other potential stressors include possible elevations of heart rate, blood pressure, and glucocorticoid concentrations that persist for 30 to 60 min or more following the event, suggesting that despite their routine use in laboratory studies, these procedures are acutely stressful for animals (Brown*et al*, 1974).

Sharp *et al* noted, Care should be exercised in dismissing a procedure as non-stressful just because it is simple or routine. Being present when these procedures are being conducted on other animals also significantly elevates physiologic parameters indicative of stress, at least in rats, mice and monkeys. Both rats and mice produce and respond to signals and odors associated with stressful laboratory procedures (Fox*et al*, 1986; Beynen*et al*, 1992).

Because pain and fear, and resulting stress and distress, may introduce confounding variability to scientific data, these states have the potential to lessen the reliability of animal studies. Scientists are well aware of this, and some have warned of the hazards of disregarding stress effects, including those arising from laboratory routines as the orogastric gavage (Mason*et al*, 1968; Roberts*et al*, 1995; Brenner*et al*, 1990). Despite this, the negative effects of pain, stress and distress and their influence on study outcome are either not reported or underreported in published scientific papers (Reinhardt*et al*, 2000). What is clear, however, are that handling effects can significantly alter an animal's immune status either enhancing or compromising and could have important methodological implications (Moynihan*et al*, 1990).

In order to elucidate this aspect, this work focuses on the existing complex between the gavage technique and plasma distribution of antibodiesandof ACTH which aims to show the negative impact of such technology on the quality of the obtained immune results and the importance of maintaining a positive contact through the development of passive administration techniques on rats. This is the case of natural administration directly from the syringe which eliminates every stress that effects scientific experimentation.

MATERIALS AND METHODS

Animals and housing

Twenty-four (24) male Wistar rats obtained from Pasteur Institute (Algiers, Algeria) were housed in transparent cages at a constant temperature (23 ± 1 °C) with a 12 h/12 h light/dark cycle (lights on at 07:30 a.m.). Rats had access to standard

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rodents chow and tap water ad libitum. Weighing 230–250 g at the beginning of the experiment, the animals were weighed daily before any other experimental procedure in order to calculate the 24 h-body weight gains. The study protocol was carried out according to the NIH revised Guidelines for the Care and Use of Laboratory Animals (no. 80–23, 1996).

Study protocol

The twenty-four ratswere divided into three groups of eight (n = 8). The control group and naturally administeredoneunderwent a week of training receiving 2 ml of 5% sugar solution directly from the syringe, then treated temporarily with the gavage group for a period of one month with the vehicle of NaCl 0.9% (1 ml/kg) or placebo three times a day by direct administration and gavage using dry gavage needle for thegavagegroup. The rats were then sacrificed by decapitation under mild anesthetic diethyl ether and the blood collection was carried out in ethylenediaminetetraacetic acid (EDTA) tubes.While 3 ml of blood was transferred into a plain tube and allowed to clot.

Blood containing plain tubes were centrifuged for 15 minutes in cold centrifuge machine (Model 5810R; Eppendorf, Germany). Centrifuge temperature was adjusted at 4°C and speed at 4000 rpm. After cold centrifugation, serum was pipetted out. Approximately 1.5 ml of serum was obtained from each blood sample, transferred to serum tubes (Eppendorf, Germany).Estimation of immunoglobulin A, E, G and M levels were done by enzyme linked immunosorbent assay (ELISA) using immunoperoxidase assay kits of Immunology Consultants Laboratory Inc. U.S.A.(Caplan *et al*, 1979).To measure the plasma levels of the ACTH hormone, ELISA tests were performed as directed by the manufacturers. The kit for ACTH assay was obtained from Phoenix Pharmaceuticals Inc. Burlingame, USA.

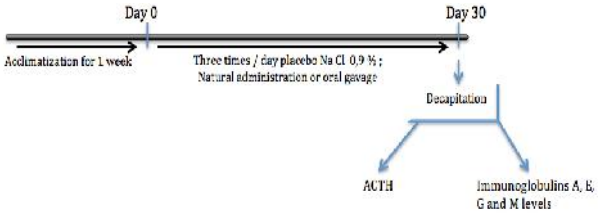

Figure 1 Scheme of experimental procedure

Statistical analysis of results

All data are expressed as the mean±SEM (Standard Error of the Mean). All groups showed normal distributions, so a parametric statistical method; one way analysis of variance (ANOVA) followed by Tukey post hoc test, was used for multiple comparisons. The value of $p<0.05$ or less was considered as the significant difference. Data were analyzed using MINITAB (Minitab® 13.31).

Table 1 Comparison of serum immunoglobulin levels between rats treated with placebo and healthy control group.

Groups / Parameters	Control	Gavage	Natural administration
IgA (ng/ml)	107,06 ± 13,81	82,35 ±7,31b	101,66 ± 16,9 β
IgE (ng/ml)	21,11 ± 0,95	17,46 ± 0,65 b	20,95 ± 2,35 β
IgM (ng/ml)	748,48 + 44,36	678,68 + 11,65 b	744, 33 + 27,78 β
IgG (ng/ml)	620 ± 40,31	562 ± 9,15 b	616 ± 21,90 β

RESULTS

The results are expressed as mean ± SEM. [b] $P< 0.01$ vs control;[β] $P <0.01$ gavage vs natural administration.

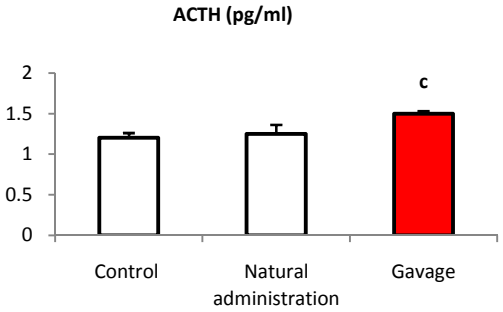

Figure 2 Plasma levels of ACTH in rats treated with placebo (0.9% NaCl) by gavage and natural routes. The results are expressed as mean ± SEM. [c] $P< 0.001$ vscontrol;[γ] $P <0.001$ gavage vs natural administration.

The comparison of immunoglobulin levels between the three groups has been presented in Table 1. Immunoglobulin levels weresignificantly decreased in rats exposed to chronic gavage compared to healthy rats and the natural administration group ($p < 0.01$).The results of one month's gavage show a significant increase in plasma level of ACTH compared to control and natural administration group ($p <0.001$).

DISCUSSION

Stress induced by highly invasive procedures used in some animal studies is well recognized by regulatory authorities and Institutional Animal Care and Use Committees. Animals used in laboratory research and testing are also regularly subjected to routine maintenance or monitoring procedures, such as personnel entering the animal housing room, cage movement and cleaning, body weight collection, physical examination, injections, and collection of blood or other tissues. Because these procedures may be considered incidental in nature, their effects on laboratory animal well-being may be over-looked by ethical review committees, whose task is to try to reduce the potential for animal pain and suffering.

This study performed on Wistar rats aims to demonstrate the nature of human contact influence with the experimental model on the clarity of immuno-endocorine results; Mainly ACTH and antibodies in the case of oral administration of placebo (0.9% NaCl). This choice specifically focused on the rates of blood antibodies is justified by the known complex between the Handling linked systematically to the gavage techniqueand the immune system response demonstrates evidence of both depressed and enhanced effects due to handling accordingto larger body of literature that reports various effects on immunity (e.g., tumor growth, susceptibility to infectious disease) in response to handling (Moynihan *et al*, 1990; Aarstad *et al*, 1992).

The significant decrease in plasma levels of antibodies obtained in our results in rats force-fed with placebo does not explainhow Handling may affect the health and well-being of animals, particularly rats, used in laboratory research. What is clear, however, is that handling effects can significantly alter an animal's immune status either by enhancing or compromising, and could have important methodological implications.For this reason, the development of new techniques for passive administration, such as accustoming the animal to take its dose of treatment directly from the syringe, can be a very good alternative

to preserve the natural levels of antibodies. This was demonstrated in the results of the batch naturally given the NaCl 0.9%.Moreover, adopting such approach in an experimental procedure prevents the animal from the physical stress of gavage triggered by therestraint and insertion of a rigid metal in the mouth. This lead to a rise in heartbeats, blood pressure and activation of the HPA axis reported in this study by a significant increase of ACTH levels in the bloodof theforce-fed group compared to the naturally administered group. According to (Brown *et al*, 1974), this phenomenon can persist 30 to 60 min after the event.

Other scientific works about rats administered water, Tween 80 (a non-toxic surfactant), and various food-grade oils, both the vehicle and the volume influenced stress responses (Brown*et al*, 2000). Despite prestudy acclimation to daily restraint and insertion of a dry gavage needle, rats administered corn, sesame, soybean, or peanut oil as vehicles demonstrated a dose-dependent increase in serum corticosterone levels over a 24-h period.Besides this, the immune system is a complex network of cells, proteins, tissues, and organs that work together to protect the body against infectious diseases or other insults. Stressincluding that resulting from gastric gavage has been documented to produce a profound effect on the immune system. It influences hormones that bind specific receptors on the membrane or in the cytoplasm of cells of the immune system, including various cells that participate in the production of antibodies. Experimental studies report heterogenous findings in relation to stress and the immune mechanisms(Hitti*et al*, 2008).long-term stress can have a detrimental effect on the body that may lead to serious disease and debilitation (Porterfield *et al*, 2011).

Stress can alter antibody production which corroborates perfectly the results obtained in this process protocol where chronic gavage in rats caused an activation of the stress axis (HPA), which results inan increased release of ACTH. These ones can bethe cause of significant depression of the antibodies in these rats. On the contrary, keeping a soft and friendly contact with these animals by administering the vehicle directly reduces the negative complications associated with stress. Furthermore, preventing depression of antibodiesmeans to maintain the results at the same state as the control rats. This fact adds more credibility to the results of a scientific experiment.

Finally, this work has shown the influence of chronic gavage and repeated Handling on the rates of ACTH and antibodies. This affects adversely the data in a scientific experiment, in which the importance of the approaches using passive techniques, and including natural Administration to prevent the generation of unwanted stress.

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