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IMMUNOREGULATION OF INTERLEUKIN-2 BY GLUCOCORTICOIDS AND THEIR EFFECT ON MITOGEN INDUCED PERIPHERAL BLOOD LYMPHOCYTE TRANSFORMATION IN ADULT MALE RATS

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SUMMARY

Adult male rats were subcutaneously injected with dexamethasone at a dose of 1mg/kg B.w. for five doses. Another group of adult male rats were subjected to elevated ambient temperature and relative humidity for two and four days. The obtained results indicated that the dexamethasone treated group exhibited elevations in plasma levels of dexamethasone and decreased plasma levels of corticosterone. Interleukin-2 (IL-2) as well as peripheral blood lymphocyte transformation showed significant decreases than control group. Elevated ambient temperature and relative humidity for two and four days resulted in a significant elevation in the plasma corticosterone, while both IL-2 and the percent of responding lymphocytes to transformation was significantly decreased. It could be concluded that the stress response in adult male rats involves a series of events including impairment of immune function suggesting that interleukins may play an important role in this phenomenon.

INTRODUCTION

Glucocorticoids have been categorized according to the predominant action on intermediary metabolism and inhibition of inflammation (Slone et al., 1983). They exert a wide of effects on tissue maturation and metabolism (Claus et al., 1996). They are considered the main effector of hypothalamopituitary-adrenal (HPA) axis in re-establishing homeostatic status in peripheral tissues (Makino et al., 1996) and adaptation to stress which evokes a variety of biological responses including activation of HPA axis and synthesis of a panel of stress response proteins at cellular levels (Bauskin et al., 1991).

Because of the different action of glucocorticoids in animals and human, they have proven to be difficult to study, as yet, their exact mode of action in expressing the immune response is not

fully understood, although some aspects have been now elucidated as a result of recent invitro and invivo studies.

T-lymphocytes appear to be more sensitive than B cells to suppression of invitro growth by glucocorticoids (Beck and Browning, 1983) but it is possible that this may reflect the relative sensitivity of different lymphokines and monokines. Evidence that the accessory cells may be the major target for glucocorticoid is provided by the observation that lymphocytes in culture are more sensitive to glucocorticoids during the initial period of culture when interleukins are being generated than at later period when the accessory cells have a more subervient role (Neifeld and Tormy, 1979). Dexamethasone is a potent synthetic glucocorticoid widely used in the treatment of a variety of physiological disorders including allergic, cutaneous, circulatory and intestinal disorders (Toutain et al., 1983). It has 25 times more anti-inflammatory activity than cortisol with little or low mineralocorticoid activity (Slone te al., 1983). The duration of its anti-inflammatory action has been siad to be similar to its immuno suppressive effect. There are considerable evidence demonstrating that glucocorticoids are essential agents in stress induced impairment of immune function (Saperstein et al., 1992) by either activation of anti-inflammatory genes and/or repression of inflammatory genes which may prevent overshoot of inflammation (Makino et al., 1996). Recent reports suggested a complex interplay between neuro-endocrine and immune system (Amado et al., 1995). Activated cells of the immune system release soluble factors called cytokines which play extremely important roles in the communication network that links inducer and effector cells (Saperstein et al., 1992). From these cytokines, interleukin-2 (IL-2) which plays a crucial rolen immune system operation secreted by helper T-lymphocytes and it is an important regulatory cytokine for proliferation of killer T-lymphocytes which destroy antigens (Blecha and Paker, 1986). IL-2 is glycoprotein in nature with a molecular weight of 15000 Dalton. Its receptors can be present in the pituitary cell. (Arzt et al., 1993). The intrinsic production of these interleukines by the pituitary cells of different species was also described (Karanth and McCann, 1991). They are participated in anterior pietuitary cell growth regulation and considered as an interface between the brain, endocrine and immune systems which may add new insight to the endocrine-immune functional interaction (Arzt et al., 1993).

To ascertain the relationship between 1L-2 and corticosteroid in mediating immune suppression, the present study was undertaken in a trial to clarify this interaction by monitoring plasma levels of corticosterone, IL-2 and mitogen induced peripheral blood lymphocyte transformation in response to exogenous synthetic glucocorticoid (dexamethasone) or stimulation of endogenous corticosterone by exposure of the adult male rats to elevated ambient temperature.

MATERIALS AND METHODS

I. Animals :

48 adult male albino rats weighing 200-250gm

were maintained on artificial photoperiod regime of 14hr. light and 10hr darkness and allowed free acess to food and water.

2.Experimental protoicol :

Group I (8 animals): They were s/c injected with 0.5ml saline every other day for 5 doses and considered as control for group II.

Group II (8 animals): They were s/c injected with 1mg dexamethasone sodium phosphate obtained from Butler Co. (Columbus, OH) for use in animals. (dissolved in 0.5ml saline/kg body weight every other day for 5 doses) (Venkatesan et al., 1996).

Group III (16 animals): They were maintained in thermostatically controlled chamber at a temperature 22± 2°C and a relative humidity of 41-46% for four days. This group was considered as control for group IV.

group IV (16 animals): They were kept in an isolated thermostically controlled chamber and subjected to elevated ambient temperature of 40 ± 1.2°C and a relative humidity of 72-78% for 4 days (Hoar, 1983).

In animsls of group III and IV rectal temperature was measured twice daily by means of thermometer (37.2±0.8°C for group III and 39.1± 0.6°C for group IV) while the relative humidity was measured by Igrometro A fibra sintetica, Spige/Germany. Elevated environmental temperature elevates the body temperature but within few weeks the physiological constants are back to normal constants (Ingram and Mount, 1975).

3-Sampling:

1. The samples were taken between 0900 and 1200 a.m.
2. Animals from group I and II were killed 2 says after the last injection.
3. 8 animals from each of group III (control) and IV (experimental) were killed after 2 days and considered as IIIa & IVa where as the rest of animals from the same groups (8) were killed after 4 days and considered as IIIb & IVb.
4. Blood samples were received in clean dry heparinized tubes, and divided into two parts, the first part was centrifuged and the plasma was separated and stored at -20°C until assayed while the second part was used for lymphocyte transformation test.

4- Analytical methods:

A- Interleukin -2 (IL-2) assay

IL-2 was quantified in the plasma using Enzyme Linked Immunosorbent Assay (ELISA) kit for quantification of IL-2 obtained from Genzyme Immunobiologicals, Cambridge USA. The interest kit is solid phase enzyme immunoassay employing the multiple antibody sandwitch principle . A 96-well microtitre plates (precoated with monoclonal anti-m IL-2) was used to capture m IL-2 present in standard and test samples. Standard curve was plotted using point to point interpolation. The analytical steps were performed according to

Kuziel and Greene (1990).

B- Dexamethasone:

Was determined using high performance liquid chromatographic (HPLC) method described by Alvineric and Toutain (1982).

C- Corticosterone:

Was estimated in the plasma using rat corticosterone-H^3 kit procedure for the radioimmunassay of rat plasma obtained from ICN Biomedicals Inc. Diagnotic Division, California. The procedures were discribed in the enclosed leafle

5- Measurement of pre-S phase cell cycle kinetics from volume spectroscopy measurements by mitogen induced peripheral lymphocyte transformation test:

Mononuclear cells were isolated from the pooled heparinized blood of normal adult male rats by density gradient centrifugation over ficol-Hypaque and washed three times in RPMI-1640 medium, buffered at pH 7.3with Hepes (0.01µl) and supplemented with L-glutamine (200 mM), penicillin (200-IU/ml) and streptomycin (100 µg/ml).

The mononuclear cell suspension were cultured in round bottomed microtitre plates, each well containing $2X10^5$ cells in 100 µl medium, 25 µl tested plasma and 50µl mitogen phytohemagglutinin (PHA) used at optimal stimulating concentration. The plates , were sealed with adhesive tape and incubated at 37°C.

The cells were harvested at 21-24 hours for volume spectroscopy. Lymphocytes were resuspended by repeated mixing using a Pasteur piette as described by Gibbs et al. (1979). Briefly the contents of culture wells were measured in a coulter - counter with channylser (1000 multichannel-analyser. The volume profiles were analysed by computer using a mathematical model that allows the assessment of number of cell responding to mitogen and the growth rate of cell it assumes that a certain proportion (P) of stimulated and transformed cells do respond that the other (I-P) remain unchanged in volume during culture. The cells which do so were at a rate which is linerly proportioned to their current size.

6- Statistical analysis:

The obtained data were calculated and subjected to statistical analysis using student "t" test according to Sandecor and Cochram (1969).

The effects of s/c dexamethasone injection on the plasma levels of dexamethasone, corticosterone and IL-2 in adult male rats as well as the percent of responding mitogen induced peripheral blood lymphocytes are illustrated in table (1) and Fig. (1 and 2). It is evident that s/c dexamethasone injection and decrease in plasma corticosterone ($P<0.01$) while the plasma levels of IL-2 and the percent of responding cells showed a significant ($P<0.05$) and a highly significant ($P< 0.01$) decrease respectively. The results presented in table (2) and Fig. (3 and 4) revealed that elevated

RESULTS

Table (1): Plasma levels of dexanethasone, corticosterone, interleukine-2 (IL-2) and percent of responding mitogen induced peripheral blood lymphocytes (P) in the control (Group I) and dexamethasone treated (Group II) adult male rats.

	Group (I)	Group (II)
Dexamethasone (ng/ml)	@	197.83
Corticosterone (ng/ml)	228.02 ± 22.33	99.07 ± 10.06**
IL-2 (Pg/ml)	1.85 ± 0.11	1.04 ± 0.27 *
% of responding cells (P)	80.21 ± 0.45	62.32 ± 0.52 ***

* Significant at P<0.05

** Significant at P<0.01.

*** Significant at P<0.001.

@ : Below the basal level of detection.

Table (2): Plasma levels of d corticosterone, interleukine-2 (IL-2) and percent of responding mitogen induced peripheral blood lymphocytes (P) in the control and experimental adult male rats exposed to elevated ambient temperature for two (Group IIIa & IVa) and four (Group III b & IVb) days.

	Group (IIaI)	Group (IVa)	Group (IIIb)	Group (IVb)
Corticosterone (ng/ml)	259.94 ± 24.53	5.11.57±33.08*	275.12 ± 28.27	645.21±35.99**
IL-2 (Pg/ml)	2.10± 0.15	1.36 ± 0.16**	1.99 ± 0.13	1.15 ± 0.10**
% of responding cells (P)	79.88 ± 0.99	59.20 ± 0.98**	82.91 ± 0.84	38.22 ± 0.89 ***

* Significant at P<0.05

** Significant at P<0.01.

*** Significant at P<0.001.

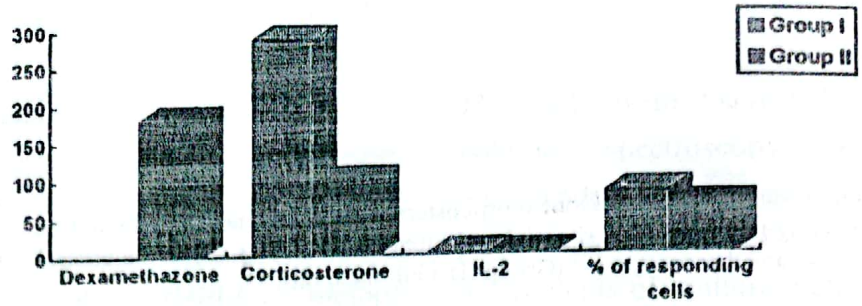

Fig. (1) Histogram representing the plasma levels of dexamethaxone, corticosterone, interleukine-2 (IL-2) and the percent of responding mitogen induced peripheral lymphocyte transformation in the control (Group I) and dexamethaxone treated (Group II) adult male rats.

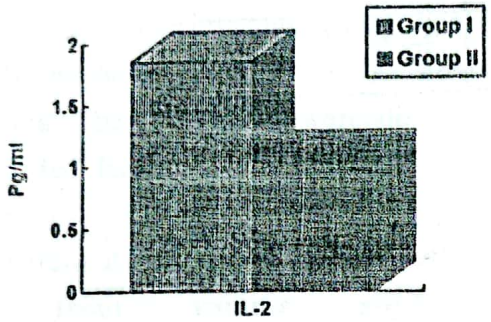

Fig. (2) Histogram representing the plasma levels of interleukine-2 (IL-2) in the control (Group I) and dexamethaxone treated (Group II) adult male rats.

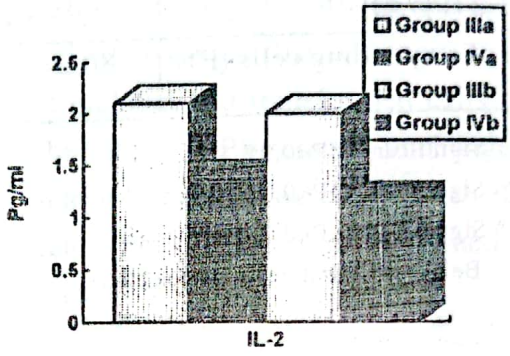

Fig. (4) Histogram representing the plasma levels of interleukine-2 (IL-2) in the control and experimental adult male rats exposed to elevated ambient temperature for two (groups IIIa & IVa) and four (groups IIIb & IVb) days.

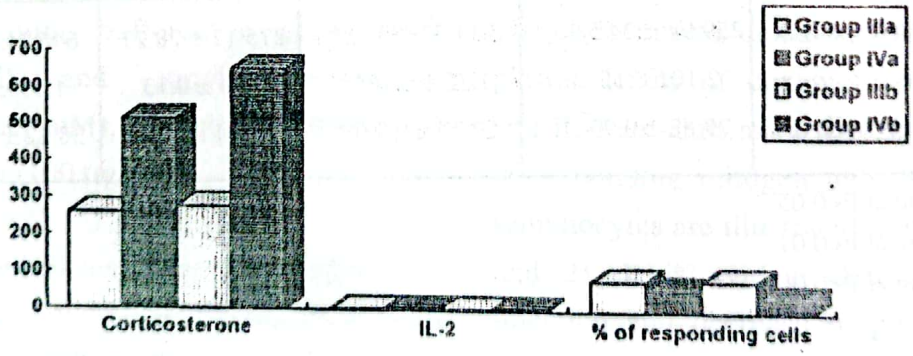

Fig. (3) Histogram representing the plasma levels of corticosterone, interleukine-2 (IL-2) and the percent of responding mitogen induced peripheral lymphocyte transformation in control and experimental adult male rats exposed to elevated ambient temperature for two (groups IIIa & IVa) and four (groups IIIb & IVb) days.

ambient temperature in male rats raised the plasma corticosterone both after two and four days of exposure while significant decrease in the plasma levels of IL-2 after two days ($P< 0.05$) and after four days ($P < 0.01$) were noticed . The percent of responding cells showed also highly significant decrease after exposure to elevated ambient temperature.

DISCUSSION

The suppressive effect of dexamethasone on the polasma level of corticosterone reported in this study confirm the previous findings of Toutain et al. (1983) and Heiss et al. (1996) in which the plasma levels of corticosterone decreased to low or non detectable levels. They suggested that the duration of suppression was much longer and dose dependent (approx 10 days after 0.1mg/kg and 1 month after 1mg/kg) and the duration of antiinflammatory action of synthetic glucocorticoids has been said to be similar to the duration of their suppressive effect. However, the inhibitory influence of dexamethasone on IL-2 production in the present study is in agreement with Gillis et al. (1979a,b) and Larsson (1980) who reported that synthetic glucocorticoids may block the production of IL-1 and IL-2 but neither the generation IL-2 receptors on stimulated cells nor the intrinsic lymphocyte growth mechanism were affected.

On the other hand, the animals subjected to elevated ambient temperature and relative humidity exhibited elevated plasma corticosterone level which inturn were associated with highly significant decreases in plasma IL-2 such results coinside with that reported by Ingram and Mount (1975). Higher levels of corticosterone (700 ng/ml) were reported by Li et al. (1997) in the plasma of male rats. Costas et al. (1996) concluded that the elevated glucocorticoids inhibit both cytokine gene expression and pleiotropic action on targer cells acting as immunosuppressive and anti-inflammatory agent against overreactions of the immune system as well as autogressive response. In accordance, Blecha and Baker (1986) found decreased generated IL-2 in bovine lymphocyte culture at cortisol concentrations attainable in stressed cattle due to impairment of all parameters of lymphocyte activity as proliferation, mixed lymphocyte reaction and suppression of blastogenesis. Furthermore, corticosteroid therapy in patients with active pulmonary sarcoidosis (alveolar accumulation of activated helper T lymphocytes that are spontaneously releasing IL-2 and porliferating at an enhanced rate) was associated with suppression of persistence of IL-2 mRNA transcript in lung T cells and concomitantly with a marked reduction in spontaneous release of large amount of IL-2 (Pinkston et al., 1987). Also Ruscett and Gallo (1981) suggested that corticosteroids inhibit proliferation of activated antigen and mitogen driven lymphocyte invitro. In the presentstudy both exogenous glucocorticoid adminstration or induced their elevation by elevated ambient temperature and relative humidity resulted in

inhibition of PHA induced lymphocyte stimulation by reducing the number of stimulated cells (late G1 phase) and slowing the volume growth rate during the first 24 hours. The cells remained in early G1 phase of cell cycle before production of IL-2 Bettens et al. (1984) and Hussein et al., (1987) attributed this reduction to insufficient RNA synthesis for proliferation. Furthermore, Costas et al., (1996) reported that cytokines enhance the negative control of its own biological action by inducing glucocorticoid secretion which act as a safeguard in preventing cytokine deleterious affects, since glucocorticoids inturn inhibit cytokine synthesis thus prevent the immune system from overreacting.

In conclusion, glucocorticoids either exogeneous or induced by environmental stressors may lower the immune reaction in rats both by lowering the percent of peripheral blood transformation and the altering IL-2 production by, the helper T cells. This suggests that immune system can interact in a dramatic fashion with the neuroendocrine system. Further investigations are necessary to define the mechanism of hormonal sensitization, a phenomenon that may have an important clinical and theoretical implication.

REFERENCES

Alvenirie, M. and Toutain, PL. (1982): Simultaneous detrmination of corticosterone, cortisol, and dexamethasonee in dog plasma by high perlormane liquid chromatography using a radial compresson system . J. Pharm. Sci. 7: 816-818.

Amado, J.; Lopez-Espadas, F.; Vazque-Barquero, A.; Salas, E.; Riancho, J. A.; Lapez-Cordovilla, J. J., and Garcia-Unzueta (1995): Blood levels of cytokines in brain of dead patients: Relationship with circulating hormone and acute phase reactant. Metabolism. 44 (6): 812-816.

Arzt, E.; Buric, R.; Stelzer, G.; Stalla, J.; Saner, J.; Renner, U. and Stalla, G. K. (1993): Interleukin involvement in anterior pituitary cell growth regulation Effect of IL. 2 adn. IL.6. Endocrinology, 132: 459-467.

Bauskin, A. R.; Aikalay, I.; and Ben-Neriah, Y. (1991): Redox regulation of a protein tyrocin kinase in the endoplasmic reticulum. Cell. 66: 685-696.

Beck. J. S. and Browning. M. CK. (1983): Immunosuppression with glucocorticoid-a possible immunological explanation for inter patient variation in sensetivity: discussion paper. J Royal Society of Medlcine, 76: 473.

Bettens, F.; Kristensen, F.; Walker, C.; Bomard, G. D. and Deweck, A. C. (1984): Lymphokines regulation of activated (G1) lymphocytes. 11 Glucocorticoid and anti-Tac induced inhibition of human T. lymphocyte proliferation. J. Immunol. 132: 261.

Blecha, F.; and Baker, P. (1986): Effect of control invitro and invivo on production of bovine interleukin 2. Am. J. Vet. Res. 47 (4): 841-845.

Claus, R.; Raab, S.; and Dehnhard, K. M. (1996): Glucocorticoid receptors in the pig intestinal tract and muscle tissue. J. Vet. Med. A. 43: 553-560.

Costas, M.; Trapp, T.; Pereda, M.; Sauer, J.; Rupprecht, R.; Nahmod, V.; reul, M.; Holboer, F.; and Arzt, E. (1996): Molecular and functional evidence for invitro cytokine enhancement of human and murine target cell sensitivity to glucocorticoids. J. Clin. Invest. 95 (6): 1409-1416.

Gibbs, J. H.; Brown, R. A.; Robertson, A. J.; Potts, R. C. and Beck, J. S. (1979): A new method of testing for mitogen induced lymphocyte stimulation: Measurement of percentage of growing cells and some aspects of their cell kinetics with an electronic particle counter. J. Immunol. Meth, 25, 147.

Gillis, S.; Crabtrtee, G. R.; and Smith, K. A. (1979a): Glucocorticoid induced inhibition of T cell growth factor production. 1-The effect on mitogen induced lymphocyte proliferation. J. Immunol. 123:1624.

Gillis, S.; Crabtree, G. R.; and Smith, K. A. (1979b): Glucorticoid induced inhibition of T cell growth factor production II-The effect on the invitro generation of cytolytic T cells. J. Immunol. 123:1632.

Heiss, J.; Popavassiliou, E.; Merril, M.; Neiman, L.; Knightly, J.; Waldbridge, S.; Edward, N.; and Oldfied, E. (1996): Mechanism of dexamethasone suppression of brain tumor-associated vascular pemeability in rats. The Journal of Clin. Invest. 98 (6): 1400-1408.

Hoar, W. S. (1983): Temperature "Compensation through acclimation and acclimatization" In General and Comparative Physiology. Third ed. pp. 689-693.

Hussein, Y, M.; Kerr, M. A. and Beck, J. S. (1987): The mechanism of action of the factor in leprosy serum which inhibits the growth of mitogen stimulated normal human lymphocytes. Immunol. 61. 317.

Ingram, D.; and Mount, E. (1975): Man and Animals in Hot Environments. Springer-Verlag, Heidelberg. PP. 185.

Karanth, S. and McCann, S. M. (1991): Anterior pituitary hormone control by interleukin 2. Proc. Natl Acad. Sci. USA. 88: 2961-2965.

Kuziel and Greene (1990): J. Invest. Dermatol. 94: 275. Cited in ELISA kit for quantification of IL-2 Genzyme Immunobiologicals.

Larsson, E. L. (1980): Cyclosporin A and dexamethasone suppressor T. cells responses by selectively acting at distinct sites of triggering process. J. Immunol. 124, 2828.

Li, Y. H.; Fan, J. and Lang, C. H. (1997): Differential role of glucocorticoids in mediating endotoxin-induced changes in IGF-1 and IGFBP-1. Am. J. Physiol. R 1990-R 1997.

Makino, Y. Okamoto, K.; Yoshikawa, N.; Aoshima, M.; Hirota, K.; Yodoi, J.; Umesono, K.; Makino, I. aned Tanaka, H. (1996): Thioredoxin: a Redox-regulating cellular cofactor for glucocorticoid hormone action. J Clin. Invest. 98 (II) 2469-2477.

Neifeld, J. P. and Tormy, D. C. (1979): Effect of steroid Hormones on phytohemagglutinin stimulated human peripheral blood lymphocytes. Transplantation, 27, 309.

Pinkston, P., Saltini, C.; Muller-Quernheim. J. and Crystal, R. (1987): Corticosteroid therapy suppresses spontaneous interleukin 2 release and spontaneous proliferation of lung T lymphocytes of patients with active pulmonary sarcoidosis. The Journal of Immunity 755-760.

Ruscetti, F. W. and Gallo, R. C. (1981): Human T-lymphocyte growth factor: regulation and function of T-lymphocytes. Blood 57:379.

Saperstein, A.; Brand, H.; Audhya, T.; Nabriski, D.; Hutchinson, B.; Rosenzweing, S. and Hollander, C. (1992): Interleukin-1 B mediates stress-induced immunosuppression via corticotropin-releasing factor. Endocrinology 130: 152-158.

Slone, D. E.; Purohit, R. C.; Ganjam, V. K. and Lowe, J. L. (1983): Sodium retension and cortisol (Hydrocortisone) suppression caused by dexamethasone and triamcinolone in equids. Am. J. Vet. Res. 44 (4) 280-283.

Snedecor, G. W. and Cochran, W. (1969): Statistical Methods: 6th ed. Iowa State. Univ. Press. Ames. Iowa. USA.

Toutain, P. L.; Alvinerie, M. and Ruckebusah, Y. (1983): Pharmacokinetics of dexamethasone and its effect on adrenal gland function in the dog. Am. J. Vet. Res. 44 (2) 212-217.

Venkatesan, N.; Lim, J.; Bouch, G.; Marciano, D. and Davidson, M. (1996): Dexamethasone-induced impariment in skeletal muscel glucose transport is not reversed by inhibition of free fatty acid oxidation. Metabolism. 45 (1) 92-100.