

JNI 02071

Benzodiazepine anesthesia in humans modulates the interleukin-1 β , tumor necrosis factor- α and interleukin-6 responses of blood monocytes

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(Received 7 May 1991)

(Revised, received 11 June 1991)

(Accepted 11 June 1991)

Key words: Monocyte; Midazolam; Benzodiazepine; Interleukin-1; Tumor necrosis factor; Interleukin-6

Summary

The influence of sedative and anxiolytic benzodiazepines on human monocyte function was assessed in 11 patients undergoing anesthesia prior to control endoscopy of the urinary tract. A single i.v. injection of 0.08 mg/kg midazolam induced a marked and delayed inhibition of the lipopolysaccharide-induced production of interleukin-1 β , tumor necrosis factor- α and interleukin-6 by monocytes isolated from peripheral blood. Corticosteroids were not responsible for the observed immunosuppression. These studies demonstrate that, when administered in man, benzodiazepines markedly alter the capacity of monocytes to synthesize major mediators of the host inflammatory response.

Introduction

The hypothesis of an interaction of benzodiazepines with the immune system was based on the reported impact of stress and anxiety on immune function (Khansari et al., 1990). It was supported by the detection on macrophages (Zavala et al., 1984) of benzodiazepine receptors of the peripheral type (Sprengel et al., 1989; Riond et al., 1991) (distinct from the GABA-associated neuronal receptor) and the subsequent

demonstrations that the corresponding benzodiazepines modulated the respiratory burst capacity of P388D1 macrophagic cell line (Zavala and Lenfant, 1987) and induced chemotactic activity in human monocytes (Ruff et al., 1985). Implication of the benzodiazepine receptor in phagocyte activation was further supported by (i) the evidence of an altered expression of this receptor in the NADPH-oxidase defective neutrophils of patients with X-linked chronic granulomatous disease (X-CGD) (Zavala et al., 1990a); (ii) its restoration to normal values in monocyte-derived macrophages from X-CGD patients undergoing a therapeutic trial with interferon- γ (Zavala et al., 1990b); (iii) the fact that a monoclonal antibody against peripheral benzodiazepine receptor

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activates the NADPH-oxidase of human neutrophils (Zavala et al., 1991)

We recently reported (Zavala et al., 1990c) that in vivo treatment of mice with benzodiazepines significantly inhibits phagocyte oxidative metabolism and production of interleukin-1, tumor necrosis factor and interleukin-6. This activity was exerted by non-anxiolytic peripheral type ligands, but also by the so-called mixed type ligands which, like diazepam (Valium) display both psychoactive and immunopharmacological properties.

The widespread use of mixed type benzodiazepines in humans, as molecules endowed with anxiolytic, sedative, anticonvulsant and myorelaxant properties, prompted us to investigate their influence on human immunological parameters, and more particularly on macrophage/monocyte functions. We performed a clinical study demonstrating the influence of anesthesia with a mixed type benzodiazepine molecule, midazolam, on human monokine production capacity.

Material and methods

Patients and anesthesia protocol

Eleven male patients (mean age 65 ± 3 years) undergoing control endoscopic examination of the urinary tract (performed as day-case procedure) and receiving no medical treatment (particularly no anti-inflammatory or psychotropic drugs) for at least 3 months before examination, were anesthetized with an i.v. injection of 0.08 mg/kg midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]-benzodiazepine, Hypnovel, Roche, Paris, France). With their informed consent, at t_0 (9 a.m.), i.e. just before midazolam injection, and 1 h (t_1 , 10 a.m.) and 6 h (t_6 , 3 p.m.) after injection, blood samples were collected in heparinized tubes (Liquemine, Roche, Paris, France).

Cell preparation and LPS induction of cytokine production

Mononuclear cells were isolated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. 5×10^4 cells per well were placed on 96-well tissue culture dishes (Falcon

3072, Becton-Dickinson, New Jersey, NJ, U.S.A.) in the absence of serum for 1 h. After adherence, the wells were carefully washed and replaced with RPMI-1640 medium (Gibco, Paisley, Scotland, U.K.) supplemented with 100 IU/ml penicillin, 100 μ g/ml streptomycin, 2 mM L-glutamine (Gibco) and 5% heat-inactivated fetal calf serum (FCS, Flow Laboratories, Irvine, Scotland, U.K.). Adherent cells (approximately 90% monocytes) were cultured at 37°C in a humidified atmosphere with 5% CO₂ in the presence or the absence of 10 μ g/ml lipopolysaccharide (LPS, *Escherichia coli* 055:B5, Sigma Chemical Company, St. Louis, MO, U.S.A.). After 20 h incubation, supernatants containing the secreted cytokines were collected and stored at -70°C until assay.

Measurement of LPS-induced cytokines

Secreted interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) productions were measured with specific enzyme-linked immunosorbent assay (ELISA) kits (Cistron Biotechnology, Pine Brook, NS, U.S.A., for IL-1 β and TNF- α ; Quantikine, R&D Systems, Minneapolis, MN, U.S.A., for IL-6). Each ELISA was shown to be specific for the respective cytokine. The lowest detectable concentration was 15–20 pg/ml for each cytokine. In some cases, biological activity of the cytokines was also assessed as previously described (Zavala et al., 1990c).

Measurement of plasma cortisol values

Plasma samples were prepared from these blood samples at all selected times and their cortisol content was measured using a specific ELISA (Immunotech, Marseilles, France). The lowest detectable concentration was 1.8 ng/ml of cortisol and cross-reactivity with other products was less than 10%. Normal plasmatic cortisol concentrations measured with this ELISA vary from 90.5–235 ng/ml in the morning to 18–101 ng/ml in the evening.

Statistical analysis

Statistical significance was assessed by paired Student's *t*-test.

Results

Monocytes of patients undergoing anesthesia with midazolam demonstrated a significant inhibition of the LPS-induced production of IL-1 β (-57% , $p < 0.001$), TNF- α (-45% , $p < 0.001$) and IL-6 (-58% , $p < 0.001$), when comparing t_0 to t_6 (Fig. 1A). Similar results were obtained when monocytes were activated with $1 \mu\text{g/ml}$ LPS and when measuring cytokine levels by their biological activity (not shown). Interestingly, the kinetics of this inhibition was distinct depending on the cytokine considered: while mean IL-1 β and IL-6 production capacities were only very moderately altered at t_1 (-33% for IL-1 β , -15% for IL-6), mean TNF- α production capacity was already inhibited by 40% at t_1 ($p < 0.001$) and showed no further change between t_1 and t_6 . At t_6 , six of 11 patients already tended to recover the level of t_0 production capacity for TNF- α . For IL-1 β production capacity, three patients reached their lowest level at t_1 , while for IL-6, t_6 levels were clearly decreased in nine out of 11 patients, when compared to t_1 levels. At t_6 , no patient had yet recovered his initial IL-1 β and IL-6 production capacity. When considering the whole group of patients, the decrease in cytokine production capacity between t_0 and t_6 was correlated for IL-1 and IL-6 ($r = 0.62$, $p < 0.05$), but not for TNF- α in relation with either IL-1 β or IL-6.

In the absence of LPS, isolated monocytes did not produce measurable amounts of any of the three cytokines. Furthermore, no measurable cytokine levels (IL-1 β , IL-6, TNF- α) were detectable in plasma at all sampling times considered.

Blood cell counts and relative percentages were not modified at all three times of the protocol (data not shown).

We also examined in control individuals receiving no injection whether a similar variation in the capacity of LPS-induced monokine production could result from circadian rhythm. Six control individuals did not show any change in their LPS-induced production of either IL-1 β , TNF- α or IL-6 (Fig. 1B) by monocytes purified from blood samples taken according to the same timing protocol as for the anesthetized patients.

The possibility that the observed immunosup-

pression was due to corticosteroid production resulting from the stress of endoscopy or possibly induced by benzodiazepine injection was checked by measuring plasma levels of cortisol. As shown in Fig. 2, cortisol levels of all 11 patients varied from 145 ± 11 at t_0 (9 a.m.) to 134 ± 7 at t_1 (10 a.m.) and 61 ± 6 at t_6 (3 p.m.), therefore following the well-established circadian decrease illustrated by our six control individuals (157 ± 3 at t_0 , 137 ± 29 at t_1 and 64 ± 10 at t_6).

Discussion

Evidence has accumulated that benzodiazepines interact with the immune system and more particularly with phagocytic cells. In the present study, we investigated the effect of anesthesia with a benzodiazepine, midazolam, on monokine production capacity in patients under control urinary tract endoscopic examination. The choice of this drug and of this anesthetic procedure is justified by the kinetic properties of midazolam (Kanto, 1985) which is a relatively short-acting sedative drug, without accumulation, and is well tolerated. An interesting feature of midazolam is the fact that its plasmatic half-life time is short ($t_{1/2} = 2$ h) and unaltered by age (Kanto, 1985). Since at t_6 , it has been shown (Kanto, 1985) that almost no measurable level of the drug remains in the circulation, this time point was taken as a final blood sampling time.

Besides the fact that our patients were not under medication for at least 3 months before endoscopic examination, they can be considered as representative of a homogeneous population of normal subjects since: (i) no circulating cytokines were detected in their plasma and no spontaneous production of cytokine was observed upon culture of their monocytes in the absence of LPS, thus demonstrating no sign of activation or of pro-inflammatory situation; (ii) the LPS-induced cytokine production at t_0 was within normal values, when compared to control subjects; (iii) only patients whose endoscopic examination did not detect any inflammatory lesion or tumor were included in our protocol.

I.v. injection of 0.08 mg/kg midazolam induced a significant inhibition of the capacity of

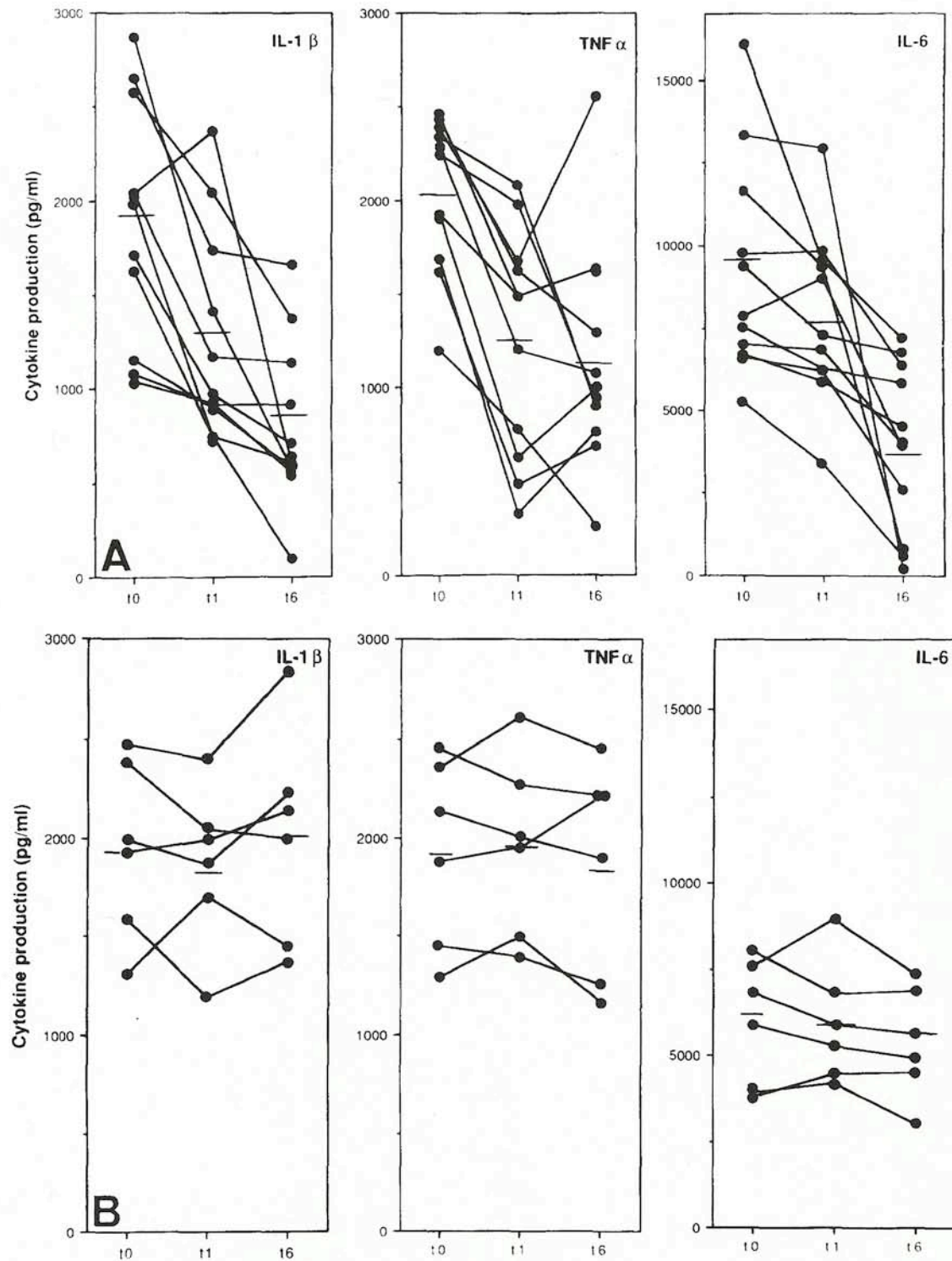


Fig. 1. Effect of midazolam treatment on LPS-induced monokine production. ELISA measurements of LPS-induced productions of IL-1 β , TNF- α and IL-6 by peripheral blood monocytes isolated from 11 patients (A) before (t_0), 1 h (t_1) and 6 h (t_6) after i.v. injection of midazolam (0.08 mg/kg) and from six controls (B) at the same sampling times. Horizontal bars represent mean group values.

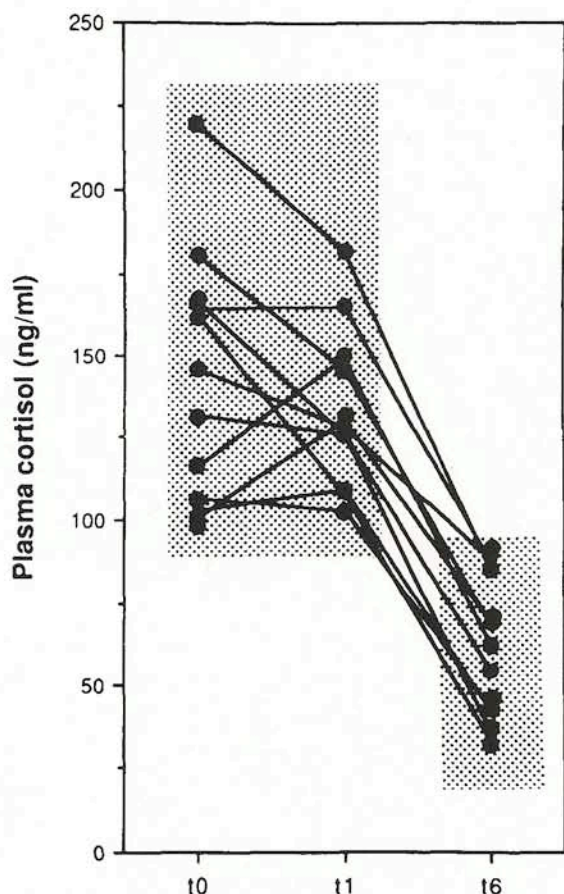


Fig. 2. Midazolam injection does not affect plasma cortisol levels. Plasma cortisol levels of patients before (t_0), 1 h (t_1) and 6 h (t_6) after midazolam i.v. injection. Shaded portions indicate normal range of cortisol values in the morning and in the evening.

peripheral blood monocytes of anesthetized patients to produce IL-1 β , TNF- α and IL-6 in response to LPS 10 μ g/ml. In most patients, this inhibition was a delayed phenomenon, with LPS-induced IL-1 β and IL-6 production capacities being more significantly affected at t_6 than at t_1 . The earlier inhibition observed at t_1 for TNF- α production capacity as compared to other cytokines is in good keeping with the known characteristics of the induction of TNF- α production, as compared to IL-1 β and IL-6. Indeed, upon endotoxin injection in animals and in human volunteers (Beutler et al., 1985; Michie et al., 1988; Zuckerman et al., 1989; Fong and Lowry, 1990), TNF- α is produced in plasma as a transient and

early peak while IL-1 β and IL-6 appear later and remain for a longer period in the circulation. Furthermore, the correlation in the decrease of IL-1 β and IL-6 production capacities upon midazolam injection coincides with the observed better correlations between IL-1 β and IL-6 production, as compared to TNF- α in control cells (Schindler et al., 1990) as well as in a clinical situation such as hemodialysis (Herbelin et al., 1991).

The delay in establishing the inhibition, far longer than, on the one hand, the time needed for an almost immediate interaction of benzodiazepines with their receptors on phagocytes (Zavala et al., 1984) and, on the other hand, the plasmatic half-life time of midazolam (Kanto, 1985), suggests that the injection of midazolam triggers some cellular or molecular suppressive activity. Our results demonstrate that this suppressive mechanism is not accounted for by corticosteroid (Waage et al., 1988; Zuckerman et al., 1989) production. Cortisol plasma levels followed the expected circadian decrease. It further appears from our data in six healthy blood donors that cortisol values varying within the normal circadian range do not modulate LPS-induced cytokine release of isolated monocytes. These results are in good keeping with our previous results obtained in mice (Zavala et al., 1990c) which demonstrated that i.p. injection of peripheral- and mixed-type benzodiazepines at 0.1 mg/kg and 1 mg/kg exerted a marked, delayed, prolonged and systemic inhibition of monokine production by LPS-induced macrophages. Interestingly, participation of T cells in the immunosuppressive mechanism could be excluded since nude mice responded similarly to benzodiazepine treatment. Therefore, the possible involvement of T cell-derived IL-4, described (Essner et al., 1989; Hart et al., 1989) as inhibitor of IL-1 β and TNF- α production by human monocytes, appears most unlikely in the present case. This is further supported by our observation that 1% and 2% plasma collected at t_6 from a given patient were devoid of inhibitory activity on monocytes collected from the same patient at t_0 (data not shown). A previous report did not detect significant suppressive activity of midazolam on human T cell-related functions (Mallmann et al., 1988).

The influence of endoscopy itself could not be ruled out in our protocol since no control could be envisaged in the total absence of anesthesia. Nevertheless, it should be noted that it was not experienced as a stressful or pro-inflammatory event by patients, since (i) the measured plasma cortisol levels at all three times were well within normal references; (ii) no inflammatory cytokine was detectable in plasma; (iii) there was no spontaneous release of any of the cytokine tested by isolated monocytes cultured in the absence of LPS.

Our previous results had demonstrated that benzodiazepines interact with the immune system and more particularly with macrophage-monocyte functions (Zavala et al., 1984; Zavala and Lenfant, 1987). Interestingly, mixed-type benzodiazepines, among which figure the well-known diazepam (Valium) and midazolam (Kanto, 1985), widely used as sedative-hypnotic anesthetic, display both psychoactive properties and immunoregulatory capacities. The present results demonstrate that an immunosuppression of monocyte functions is observed in patients after a single low-dose injection of midazolam, a common medical procedure. These findings should be kept in mind when discussing the still obscure mechanisms of the immunodepression frequently observed in the early post-operative period. Furthermore, taking into account the wide prescription in humans of benzodiazepines, it should also be interesting to investigate their influence on immune functions when chronically administered.

The cytokines that are affected by *in vivo* treatment with benzodiazepines are implicated in host defense mechanisms against infection and tumor (Fong and Lowry, 1990; Michie et al., 1988; Hirano et al., 1990; Platanius and Vogelzang, 1990). Most interestingly, IL-1 β , TNF- α and IL-6 represent major mediators in inflammatory reactions and are involved in many clinical situations (Hirano et al., 1990; Platanius and Vogelzang, 1990). Our data disclose the property of benzodiazepines to inhibit in humans the production of all three cytokines and thus suggest that their use as anti-inflammatory compounds may be envisaged.

Acknowledgments

The authors are grateful to Françoise Tresset for technical assistance. We thank Doreen Broneer for revising the manuscript.

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