The Effect of Immunosuppression by Total-Body Irradiation on the Pharmacodynamics of Centrally Active Drugs in Rats

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Received August 2, 1993; accepted November 23, 1993

The aim of this investigation was to assess whether immunosuppression induced by total-body irradiation (TBI) affects the pharmacodynamics of centrally acting drugs. Female Sabra rats were exposed to a single dose of gamma irradiation (5.3 Gy). Four days later, when both the cellular and the humoral immune responses were impaired, they received an i.v. infusion of either phenobarbital (0.8 mg/min), ethanol (16.3 mg/min), pentylenetetrazol (PTZ; 0.618 mg/min), or theophylline (as aminophylline; 2 mg/min). The infusion was stopped at the onset of the pharmacologic end point—loss of righting reflex for the depressant agents or maximal seizures for the stimulant drugs—and the concentrations of the neuroactive drugs at that point were determined. In the ethanol experiment, blood samples were also taken upon awakening. The radiation-induced immunosuppression significantly decreased the CNS sensitivity to the depressant action of both phenobarbital and ethanol as indicated by the higher CSF phenobarbital concentrations required to induce sleep in the irradiated rats versus controls (156 ± 4 vs 133 ± 5 mg/L, respectively; $P < 0.05$), and the higher serum ethanol concentrations at the onset and offset of sleep in the immunosuppressed group versus control values (4.6 ± 0.2 and 1.68 ± 0.01 vs 3.79 ± 0.17 and 1.32 ± 0.9 mg/mL, respectively; $P < 0.04$). Exposure to TBI did not alter the pharmacodynamics of the two convulsant drugs (theophylline and PTZ).

KEY WORDS: total-body irradiation; immunosuppression; anesthesia; phenobarbital; ethanol; induced seizures; theophylline; pentylenetetrazol; pharmacodynamics.

INTRODUCTION

Immunosuppression, by either drugs or radiation, is often associated with cancer therapy (1). It also occurs as a result of the treatment of many inflammatory and autoimmune diseases and in organ transplantation (2,3). The aim of the present investigation was to assess whether immunosuppression by total-body irradiation (TBI) affects the concentration–effect relationship of centrally active agents.

Dafny and his colleagues showed (4–7) that experimentally induced immunosuppression by various techniques, including TBI, attenuates the opiate withdrawal syndrome in rats and mice. This effect could be reversed by the administration of viable lymphocytes from the spleen of untreated donors (8). These results suggest that immunosuppression may alter the sensitivity of the central nervous system (CNS) to the pharmacological action of neuroactive drugs. However, the effect of the immunosuppression on the kinetics of action of stimulant and depressant drugs has not yet been studied in detail. This issue is of prime interest because the pharmacodynamics (i.e., relationship between drug concentration and intensity of pharmacological effect) of drugs is an important consideration for individual optimization of pharmacotherapy (9). Furthermore, since many cases of immunosuppression occur in critically ill patients where an individualized dosage of anesthesia is required, this particular investigation is of utmost importance in order to identify if there are major changes in the brain sensitivity to anesthetic agents.

The experimental strategy used to assess the concentration–effect relationship of these drugs was developed previously by Levy and colleagues (10–13). It is based on the sampling of the drug at a site in which the drug concentration reaches a relatively rapid equilibrium with the site of action at the pharmacological end point of interest and, thereby, represents the drug concentration at the biophase. It has been shown previously that the cerebrospinal fluid (CSF) is the suitable sampling site for the investigation of the anesthetic effect of both phenobarbital- and theophylline-induced maximal seizures (10,13). The drug concentration in the CSF (but not in the serum or the whole brain) at the defined pharmacological end point is used, for both drugs, as a pharmacodynamic marker to assess the effect of TBI on the CNS sensitivity to their pharmacologic activity. The CSF drug concentration has an additional advantage since it represents exclusively the free (unbound) concentration. On the other hand, for drugs such as pentylenetetrazol (PTZ) and ethanol that are not bound to serum proteins and distribute very rapidly from the blood to receptor sites in the brain, the drug concentrations in the serum, brain, or CSF, at the onset of the pharmacological end point, are equally suitable to serve as sampling sites for pharmacodynamic evaluation (11,12). In this investigation TBI decreased the CNS sensitivity to the depressant action of phenobarbital and ethanol, while it did not attenuate the seizure threshold in response to theophylline and PTZ.

MATERIALS AND METHODS

Irradiation Protocol. Female Sabra rats were acquired from the Animal Breeding Unit of the Hebrew University–Hadassah Medical School. They were exposed to 5.3 Gy homogeneous total-body gamma irradiation using a ^{60}Co source (Gammacell 220, Atomic Energy of Canada, Ottawa, Canada). The animals were kept for irradiation in a restricted space in the center of the gamma cell in which the field inhomogeneity was less than 5% as determined by sensitive ionization chambers. The duration of exposure was only about 20 sec to prevent unnecessary stress. Control rats were treated similarly but not irradiated.

Rubin and Casarett (14) have shown that immunosuppression (indicated by a marked reduction of the neutrophil levels and a lymphocyte count of less than 3% of the normal

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0724-8741/94/0500-0704$07.00/0

value) is attained 2–3 days following exposure to TBI and is relatively stable for 5 days. Therefore, as in similar investigations (5,8) the influence of TBI-induced immunosuppression on the pharmacodynamics of neuroactive drugs was assessed 4 days following TBI.

Cannulation Technique. Three days after irradiation a cannula was implanted into the right jugular vein (15), under light ether anesthesia. The cannulas were filled with saline solution. A blood sample of 0.3 mL was withdrawn for hematological evaluation with the use of a Coulter counter, Model S-plus (Coulter Electronics, Luton, UK).

During the experimental period, all animals were housed in individual metal cages in a light-controlled room (light from 0700 to 1900 hr) and were allowed free access to food and water.

Pharmacodynamic Experiments. The pharmacodynamic experiments were performed 4 days after irradiation. Behavioral observation and rectal temperature were recorded just before the pharmacodynamic experiments. The general concepts of the experimental strategy utilized in these studies were detailed before (10–13,16). Briefly, the neuroactive drug was infused until onset of the predefined pharmacological end point, either loss of righting reflex (LRR) for depressants or maximal seizures for stimulants. The pharmacodynamic markers were compared between the irradiated and the untreated control groups to assess the effect of TBI on the pharmacodynamics of these drugs. The specific procedures were as follows.

Depressant Drugs. To investigate the effect of TBI on the pharmacodynamics of phenobarbital general anesthesia, a 40 mg/mL sodium phenobarbital solution in water was administered i.v. at a constant rate of 1.2 mL/hr (0.8 mg/min) until the onset of the predefined pharmacological end point, LRR, which was determined without a nociceptive stimulus (i.e., pressure on the tail). Then, the rats were placed under light ether anesthesia and samples of CSF from the cisterna magna, blood (for serum) from the abdominal aorta, and brain (which was stripped of its external vasculature and meninges) were taken in this order and kept frozen at −20°C until their analysis.

The effect of exposure to irradiation on the CNS sensitivity to ethanol anesthetic action was assessed by comparing serum ethanol concentrations, of irradiated and untreated controls, at two stages of anesthesia, which were practically defined as the onset and offset of LRR. The time elapsed between the two end points was also recorded. For these assessments, a solution of 20% (v/v) ethanol in water was infused at a constant rate of 16.3 mg/min until the onset of LRR, when the rats remained motionless when placed on their back on a thermal pad (37°C). At this point, a blood sample of 0.3 mL was withdrawn through the jugular vein cannula (without any further sedation). The rats were left on the thermal pads in the dorsal position until they regained the righting reflex (RRR) and could turn and stand up on four legs. At that time another blood sample was taken from the abdominal aorta under ether anesthesia (similar to the procedure used for the phenobarbital experiment). Throughout the experiment the rats were placed on thermal pads to maintain normal body temperature.

Stimulant Agents. To assess whether sublethal TBI affects seizure thresholds, the convulsant drugs, PTZ (Aldrich Chemical Co., St. Louis, MO) and theophylline (as aminophylline), were infused intravenously until the onset of maximal seizures, which were expressed by tonic flexion of the forelimbs. This was usually accompanied by tonic extension of the hind limbs.

PTZ at a concentration of 18.54 mg/mL in normal saline solution was administered at a constant rate of 0.618 mg/min, while theophylline at concentrations of 100 mg/mL in water was infused at a rate of 2 mg/min. At the onset of maximal seizures the biological samples were taken (under light ether anesthesia in cases where the animals survived the maximal seizure). The samples were taken in the following order: CSF (only in the theophylline experiment), blood (vena cava), and brain.

Analytical Procedure. Phenobarbital concentrations in serum, brain, and CSF were assayed by a high-performance liquid chromatography (HPLC) method (HPLC system and Data System 450, Kontron Instruments, Switzerland), using a slightly modified method of Danhof and Levy (10). Standard curve was linear in the concentration range of 50–400 mg/L ($r > 0.996$).

Ethanol concentrations in the serum were determined with a commercially available kit (No. 332-UV, Sigma Chemical Co.). Standard curve was linear up to 0.16% (w/v; $r = 0.992$).

PTZ concentrations in serum and brain were assayed using the modified HPLC method of Ramzan (17). Benzotriazole (internal standard) and acetonitrile were added to the serum samples and the supernatant was chromatographed. To determine the PTZ brain concentration, one hemisphere was homogenized in acetonitrile, together with the internal standard solution, and the supernatant was chromatographed. Standard curve was linear in the concentration range of 50–250 mg/L ($r > 0.995$).

Concentrations of theophylline in the CSF, serum, and brain were determined by HPLC following extraction with ethylacetate according to a procedure previously described (13). The standard curve was linear in the concentration range of 50–500 mg/L ($r > 0.995$).

Serum urea nitrogen and total serum protein concentrations, as well as the activity of transaminases, were determined with commercially available kits (Nos. 535, 540, and 505 respectively; Sigma Chemical Co.).

Statistical Analysis. The nonparametric Mann–Whitney test was used in all the statistical analyses; a P value <0.05 was considered statistically significant.

RESULTS

Four days after exposure to 5.3-Gy TBI there was no sign of significant change in any of the physiological values tested: body temperature, total protein, serum urea nitrogen, and both alanine and aspartate aminotransferase activity. No signs of abnormality were detected by physical examination or behavioral observation either. The hematological profile of all the animals (summarized in Table I) showed a dramatic reduction in total white blood-cell count, with extremely low lymphocyte and neutrophil counts. Three days after TBI the red blood-cell count, hemoglobin, and hematocrit values were unchanged. The minor elevation in the platelet count was a typical occurrence for this radiation protocol of sub-

Table I. Hematological Indices of Sabra Rats 3 Days Following TBI[a]

Variable	Control	Irradiated
Total white blood cells (10^9/L)	8.8 ± 0.6	1.5 ± 0.1*
Neutrophils (%)[b]	12.5 ± 1.9	0.0*
Lymphocytes (%)[b]	75.1 ± 2.2	0.0*
Red blood cells (10^{12}/L)	7.3 ± 0.3	7.3 ± 0.1
Hemoglobin (g/dL)	14.6 ± 0.6	13.2 ± 1.3
Hematocrit (%)	39.6 ± 1.6	39.2 ± 0.9
Platelets (10^9/L)	318 ± 73	333 ± 40

[a] Results are reported as mean ± SE; $n = 48$.
[b] Percentage of total white blood-cell count.
* Significantly different from control group by Mann–Whitney test, $P < 0.001$.

lethal TBI. The hematological parameters of the irradiated rats in this investigation were in agreement with the literature (14), confirming the effectiveness of the TBI procedure in causing immunosuppression.

Pharmacodynamics of Phenobarbital. Examination of the effect of TBI on the pharmacodynamics of phenobarbital's general anesthetic action showed no difference between the total dose required for the onset of anesthesia in controls and that in irradiated rats (Table II). On the other hand, at the onset of LRR, phenobarbital concentrations in the CSF and serum were higher in irradiated animals than in controls. The same tendency was found for brain phenobarbital concentrations but the differences were not statistically significant.

Pharmacodynamics of Ethanol. As summarized in Table III, TBI significantly elevated the serum ethanol concentrations required to induce the two different stages of anesthesia that were determined in this investigation, i.e., the onset and the offset of LRR. The total dose required to induce LRR was also significantly higher in the irradiated rat group as compared to the control rats. The time interval between onset of LRR and RR, when the rats remained asleep in a dorsal position, was significantly shorter for the rats exposed to TBI than for the untreated controls.

Pharmacodynamics of PTZ. The infusion time and the total PTZ dose required to induce maximal seizures were significantly lower in the irradiated than in the control rats (Table IV). However, no statistically significant differences were noted between PTZ concentrations in the serum and those in the brain of the treated and untreated groups at this pharmacological end point.

Pharmacodynamics of Theophylline. The effects of TBI on the pharmacodynamics of theophylline neurotoxicity are summarized in Table V. There were no significant differences between the total theophylline doses required to induce the maximal seizures in the TBI-treated group and that in the corresponding control group. Similarly, the drug concentrations in serum, brain, and CSF of the two groups, at the onset of maximal seizures, were not statistically different.

Table II. Effect of 5.3-Gy TBI on Phenobarbital Concentrations at the Onset of the LRR[a]

Variable	Control	Irradiated
Infusion time (min)	34.8 ± 0.9	38.1 ± 1.4
Total dose (mg/kg)	140 ± 5	147 ± 6
Serum conc. (mg/L)	260 ± 5	293 ± 9*
Brain conc. (mg/kg)	179 ± 8	200 ± 11
CSF conc. (mg/L)	133 ± 5	156 ± 4*

[a] Results are reported as mean ± SE; $n = 12$.
* Significantly different from control group by Mann–Whitney test, $P < 0.05$.

DISCUSSION

Exposure of rats to 5.3-Gy gamma irradiation (a mild dose that is well below the LD_{50} of TBI in rats—8.5 Gy) selectively affects the immune system, while it induces only negligible effects on other physiological systems such as the gut, liver, and CNS (14,18). Our results corroborated those found in the literature.

The effect of TBI on the immune system is characterized by direct injury of lymphoid tissue, which induces depletion of antibody producing lymphocytes and alteration of the T-lymphocyte population, favoring T-suppressor cells (18). There is also a marked decrease in the neutrophil count. Thus TBI, according to this protocol, ultimately causes immunosuppression by impairment of both the cellular and the humoral immune responses.

The rationale to investigate alterations in the pharmacodynamics of neuroactive drugs ensues from the accumulated evidence revealing a close interrelationship between the CNS and the immune system (4,8). These observations are of special interest since the blood–brain barrier is expected to reduce penetration of circulating lymphocytes and antibodies. Nevertheless, the brain is capable of responding to changes in the immune system, and vice versa.

Four days after TBI of 5.3 Gy, a decrease in the CNS sensitivity to the general anesthetic action of the barbiturate occurred. This was evidenced by the higher CSF and serum phenobarbital concentrations at the onset of anesthesia in the irradiated group in comparison to the control value. Although phenobarbital is not used clinically for general anesthesia, it was selected in these experiments as an anesthetic drug model that has no optical isomers and no CNS depressant activity of its metabolites (10).

The effects of TBI on the pharmacodynamics of ethanol's depressant action were parallel to those found with the use of phenobarbital. Higher serum ethanol concentrations were required to induce sleep in the immunosuppressed rats, and higher ethanol concentrations were also found upon awakening compared to the untreated controls. Although ethanol and phenobarbital share a similar site of inhibitory action in the brain, their mode of action is not identical (19). Still, the data clearly indicate that the effect of TBI on the response of the CNS to both depressant drugs was very similar.

The seizure threshold to chemically induced epileptiform convulsion by PTZ is a commonly used parameter to assess pro- and anticonvulsant activity of various drugs and (patho)physiological conditions. According to the similar PTZ concentrations in both brain and serum in comparison to the corresponding control values, it seems that a moderate dose of TBI does not attenuate the seizure threshold. This outcome is also supported by the fact that the same TBI treatment did not attenuate the brain sensitivity to theoph-

Table III. Effect of 5.3-Gy TBI on Ethanol Serum Concentrations Measured at Onset of LRR and of RRR[a]

Variable	Control	Irradiated
Infusion time (min) to LRR	30.6 ± 1.4	30.9 ± 0.6
Total dose (mg/kg)	2463 ± 87	2735 ± 59*
Serum conc. (mg/mL) at LRR	3.79 ± 0.17	4.6 ± 0.2*
Time for RRR (min)	216 ± 16	187 ± 16*
Serum conc. (mg/mL) at RRR	1.32 ± 0.09	1.68 ± 0.01*

[a] Results are reported as mean ± SE; $n = 12$.
* Significantly different from control group by Mann–Whitney test, $P < 0.04$.

ylline-induced maximal seizures. This was evidenced by the similar CSF theophylline concentrations in the irradiated and nonirradiated groups at onset of maximal seizures.

The fact that TBI in doses that destroy the immune system and also exposure of the brain to direct gamma irradiation do not attenuate the seizure threshold is of clear clinical relevance. For instance, theophylline treatment in patients with radiation-induced immunosuppression does not appear to increase the risk of theophylline's convulsive action.

Our observation that TBI reduces CNS sensitivity to the depressant action of the anesthetic agents (phenobarbital and ethanol), but not to the two convulsant drugs (PTZ and theophylline), could result via several potential mechanisms. Dafny *et al.* have demonstrated that suppression of the immune system following exposure to TBI is the mechanism by which the severity of the typical behavioral parameters of opiate-withdrawal syndrome were reduced. This hypothesis was verified by restoring the immune system with an implant of $2–6 \times 10^8$ splenocytes to irradiated rats, resulting in the reversal of all the radiation-induced changes in the withdrawal syndrome (8). These studies have also confirmed that the modulation of the opiate-withdrawal syndrome was not due to the direct irradiation of the brain itself. The same mechanism in theory could also be relevant to rationalize the findings of the present investigation.

Although the actual mechanism contributing to the observed effect cannot be concluded from the results of the present investigation, some information can be deduced from the discrepancy in the TBI effects on the CNS sensitivity to the depressant and stimulant drugs. The anesthetic action of both barbiturates and ethanol is mediated mainly by their binding to the γ-aminobutyric acid (GABA) receptor–chloride channel complex. It is unlikely that the desensitization effect found here is associated with the GABA receptor since PTZ's stimulatory action is also believed to be mediated by its binding to this receptor (though not to the same specific site) (20). In addition, the higher concentrations of the sedative drugs required to induce LRR are most probably not due to elevated levels of an excitatory endogenous compound(s), since it would also be reflected by lower stimulant concentrations required to induce convulsions. Another sensible interpretation of the overall data could be that the reduced efficacy of the depressant drugs found in this investigation is attributed to changes in the membrane fluidity in the brain due to TBI which is one of the mechanisms of action of ethanol and barbiturates (21). This assumption is supported by the known impact of radiation on the permeability of biological barriers: blood–brain barrier, hematolabyrinth barrier, and hematoophthalmic barrier (22).

The ratio of the dose required to induce the pharmacologic end point in the irradiated to that required in the control animals tends to be about 10% lower than the ratio between the drug concentrations in these groups found at the onset of the pharmacological end point. This effect was found in the case of phenobarbital, ethanol, and PTZ, while for theophylline these ratios were not different (see Tables II–V). This result illustrates the potential effect of TBI on the pharmacokinetics of drugs, which may evolve due to the reduced volume of distribution and rate of drug elimination. This is in accord with previous reports on the effect of TBI on the pharmacokinetics of other drugs; both the volume of distribution and the total clearance of the local anesthetic carbisocaine diminished after a single dose of 5 Gy in rabbits (23). Volume of distribution and total clearance of vincristine and *cis*-platinum also changed in another investigation after TBI (24).

The usefulness of the large database concerning disease effect in pharmacokinetics is severely limited by the lack of information about factors which may alter the pharmacodynamics of the drugs. It is equally important to identify con-

Table IV. Effect of 5.3-Gy TBI on PTZ Concentrations at the Onset of Maximal Seizures[a]

Variable	Control	Irradiated
Infusion time (min)	26.5 ± 1.9	19.3 ± 1.3*
Total dose (mg/kg)	93 ± 7	73 ± 5*
Serum conc. (mg/L)	113 ± 7	106 ± 8
Brain conc. (mg/kg)	77 ± 6	66 ± 5

[a] Results are reported as mean ± SE; $n = 12$.
* Significantly different from control group, $P < 0.03$.

Table V. Effect of 5.3-Gy TBI on Theophylline Concentration at Onset of Maximal Seizures[a]

Variable	Control	Irradiated
Infusion time (min)	23.6 ± 1.6	23.3 ± 1.5
Total dose (mg/kg)	235 ± 16	228 ± 15
Serum conc. (mg/L)	310 ± 4	297 ± 4
Brain conc. (mg/kg)	214 ± 4	202 ± 4
CSF conc. (mg/L)	229 ± 9	213 ± 4

[a] Results are reported as mean ± SE; $n = 12$.

ditions involved in pharmacodynamic alterations as well as those that are not.

The results of this investigation show that TBI decreases the CNS sensitivity to depressant drugs in the range of 15–20%. This magnitude of change may be detectable in the well-controlled animal model investigation but seems to be too small to have any direct clinical impact in the highly variable population of critically ill patients that receive irradiation. It is also evident that the analeptic response is not attenuated in these patients.

ACKNOWLEDGMENTS

Dr. Amnon Hoffman is affiliated with the David R. Bloom Center for Pharmacy. Ms. Dalia Gilhar provided valuable technical assistance.

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