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

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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), pentyleneetetrazol (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; pentyleneetetrazol; 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 pentyleneetetrazol (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 ⁶⁰Co 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 Casaret (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
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, 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 noxious 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 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). Benzo triazole (internal standard) and acetaminol were added to the serum samples and the supernatant was chromatographed. To determine the PTZ brain concentration, one hemisphere was homogenized in acetaminol, 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-