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Pharmacokinetics and pharmacodynamics of single-dose triazolam: electroencephalography compared with the Digit-Symbol Substitution Test

David J. Greenblatt, Lu Gan, Jerold S. Harmatz & Richard I. Shader
Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine and Tufts-New England Medical Center, Boston, MA, USA

Correspondence
David J. Greenblatt, MD, Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA.
Tel: +1 617 636 6997
Fax: +1 617 636 6738
E-mail: dj.greenblatt@tufts.edu

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Aims
To investigate whether the electroencephalogram (EEG) directly reflects the CNS effects of benzodiazepines by evaluating the relation of the EEG to plasma drug concentrations and to Digit-Symbol Substitution Test (DSST) scores after a single dose of triazolam, a representative benzodiazepine agonist.

Methods
Thirteen healthy male subjects were given 0.375 mg triazolam or placebo in a double-blind crossover study. Plasma samples were collected during 8 h after dosage. Pharmacodynamic effects were measured by DSST and EEG at corresponding times.

Results
Pharmacokinetic parameters for triazolam were consistent with established values. Compared with placebo, triazolam significantly impaired psychomotor performance on the DSST ($P < 0.001$) and increased beta amplitude on the EEG ($P < 0.002$). DSST and EEG changes both closely tracked changes in plasma concentrations over time. The changes for the two measures were highly correlated with each other ($r = -0.94$, $P < 0.001$) based on aggregate values at individual time points. However, the variations in area under the curve of pharmacodynamic effect *vs.* time (AUC_{effect}) measured by either method did not reflect the variations in plasma AUC across individuals. The individual variability in AUC_{effect} from the EEG was similar to that measured by the DSST.

Conclusions
Both the EEG and the DSST reflect the central benzodiazepine agonist effects of triazolam. Intrinsic variability in both measures is similar.

Introduction

Benzodiazepines are widely prescribed drugs for the treatment of anxiety, insomnia, seizures, alcohol withdrawal, and many other disorders [1–4]. Understanding of the clinical effects of benzodiazepines requires appropriate measures for quantification of their central nervous system (CNS) actions. Methods used to assess pharmacodynamic response include (i) subjective measures, through rating of sedative or antianxiety effects by the subject or by an observer; (ii) semiobjective measures, such as psychomotor tests, memory tests, and critical flicker fusion frequency; (iii) objective measures, such as the electroencephalogram (EEG), saccadic eye movements, postural sway, etc. [5–10].

The subjective and semiobjective measures have limitations, in that they are influenced by practice, adap-

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tation, placebo response, interpretation, fatigue, and motivation. One extensively used measure of this type is the Digit-Symbol Substitution Test (DSST) [5–11]. Objective measures do not have these limitations and are sensitive, continuous and reproducible. Based on quantitative analysis of the EEG, acute administration of benzodiazepine derivatives produces replicable EEG changes that are dependent on plasma concentration [5–7,11]. However, it is not established whether the EEG is an indirect measure of drug effect unrelated to the primary clinical action, or whether the EEG reflects the primary effect of benzodiazepines on the brain.

The present study evaluated the relation of the time-course of the EEG to the DSST after a single dose of a benzodiazepine, and the relation of the EEG or the DSST to plasma drug concentrations. Triazolam, a benzodiazepine derivative having a short half-life [12–14], was used as a pharmacological model. We did not address the question of the mechanism of benzodiazepine effects on the EEG.

Materials and methods

The protocol was approved by the Human Investigation Review Committee serving Tufts-New England Medical Center and Tufts University School of Medicine. Fifteen healthy male subjects participated in the study after giving written informed consent. Two subjects were withdrawn during the trials because of protocol non-compliance. Therefore 13 subjects, aged 20–35 years (nine Caucasian and two African-American), completed the entire study. All were in good health based on medical history, physical examination and routine laboratory tests. The subjects were nonsmokers and not taking any medications.

This was a placebo-controlled, double-blind, single-dose, two-way crossover study. Subjects initially underwent a nonblind 'practice' trial to allow familiarity with the testing procedures, thereby minimizing the effects of practice. Data from this trial were not used in the analyses. The subsequent two trials were under randomized, double-blind conditions. The two medications were placebo and 0.375 mg triazolam, which were identically packaged. The interval between trials was at least one week.

Subjects fasted overnight and had a light liquid breakfast (orange juice) at around 07.00 h on the day of study. They arrived at the Clinical Psychopharmacology Research Unit at approximately 08.00 h and remained fasting until 12.00 h. After 12.00 h, they resumed a normal diet (without grapefruit juice or caffeine-containing foods). A single dose of the medication was given orally with 200 ml of tap water at approximately 09.00 h.

Venous blood samples (8 ml each) were drawn prior to and at 10, 20, 30, 40, 50 min, and 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 7, and 8 h after the drug administration. The blood samples were centrifuged and the plasma was separated and stored at −20 °C until the time of assay.

The DSST was used to assess psychomotor performance [13, 14]. The DSST was administered twice prior to dosing and at times corresponding to blood sampling. A worksheet, on which digits (0–9) were arranged randomly in rows and a code of symbol-for-digit was shown on the top, was presented to a subject at each time point. Each individual was given a different worksheet at each time point throughout the study. Subjects were required to write down as many symbol-for-digit substitutions as possible in 2 min. Scores were the total number of attempted substitutions.

The EEG was recorded using a six-electrode montage, with instrumentation and methodology described previously [5, 13, 15]. At two predosing times and during 8 h postdosing at times corresponding to blood sampling, the EEG was quantified in 4-s epochs for as long as necessary to ensure at least 2 min of artefact-free recording. Subjects were kept awake throughout the study. During the EEG recording, subjects were instructed to relax with their eyes closed. Data were digitized over the power spectrum from 4 to 30 Hz and then fast Fourier-transformed to determine amplitude of the total spectrum (4–30 Hz) and the beta frequency range (13–30 Hz).

Plasma concentrations of triazolam were determined by gas chromatography with electron-capture detection [15]. The detection limit was 0.2 ng ml^{-1}. The intra-assay variance did not exceed 10%, and the interassay variance did not exceed 12%.

Pharmacokinetic parameters for triazolam were determined by nonlinear regression analysis [16]. Data points were fitted by weighted nonlinear regression to a linear sum of two or three exponential terms, consistent with first-order absorption and a one-compartment model (nine subjects) or a two-compartment model (one subject), with incorporation of a lag time prior to the start of first-order absorption [16]. For three subjects, nonlinear regression did not provide an adequate fit of the data points; for these individuals, model-independent analysis was used. Standard pharmacokinetics were used to calculate the elimination half-life ($t_{1/2}$), total area under the plasma concentration curve (AUC), and apparent oral clearance (CL). Also determined were the peak plasma concentration (C_{max}) and the time of peak concentration (T_{max}).

For DSST scores, the two predosing scores were averaged and used as the baseline value. Post-dosing scores

were expressed as the increment or decrement over the mean predose value.

For each EEG recording session, the ratio (in per cent) of beta amplitude divided by total amplitude was calculated. The mean value obtained from the two predose recordings was used as baseline. All values after drug administration were expressed as the increment or decrement over the baseline value.

The area under the effect-*vs.*-time curve (AUC_{effect}) during 8 h after triazolam or placebo administration was calculated using the linear trapezoidal method for both DSST and EEG. The relation of DSST changes and EEG changes were evaluated in two ways. First, mean values of DSST changes at each observed time point were compared with EEG changes at corresponding times. The mean values during the triazolam trial were normalized by subtracting the values associated with placebo at corresponding times. Second, values of AUC_{effect} from the two measures (placebo-normalized) were compared across the 13 subjects.

To evaluate the concentration–effect relation, the mean placebo-normalized changes in DSST and EEG across the 13 subjects at individual time points were plotted against plasma triazolam concentrations at corresponding times. Inspection of the plots indicated that a 'maximum' pharmacodynamic effect was not attained. Accordingly, data points were analysed by nonlinear regression using an exponential model [5, 17]. The equation was: $E = B*C^A + K$, where E is pharmacodynamic effect and C is plasma concentration. Iterated variables were the coefficient B, the exponent A, and a constant K. This model allows inferences regarding the relation between plasma concentration and effect within the observed range of concentrations. However the model does not allow extrapolation to plasma concentrations exceeding this range.

The relation between the plasma AUC and the AUC_{effect} also was evaluated across 13 subjects.

Statistical procedures included Student's *t*-test, linear and nonlinear regression. Pearson product-moment correlation analysis was used to evaluate the relation between EEG effects, DSST effects, and plasma concentrations.

Results

Mean (±SD) pharmacokinetic parameters for triazolam (Figure 1) were: C_{max}, 2.8 ± 0.9 ng ml^{-1} (95% CI: 2.2–3.6); T_{max}, 1.2 ± 0.5 h (95% CI: 0.9–1.6); $t_{1/2}$, 3.4 ± 1.2 h (95% CI: 2.7–4.2); CL, 455 ± 129 ml min^{-1} (95% CI: 374–536). The coefficient of variation (CV) in plasma AUC among the 13 subjects was 28%.

Triazolam, but not placebo, produced a reduction in

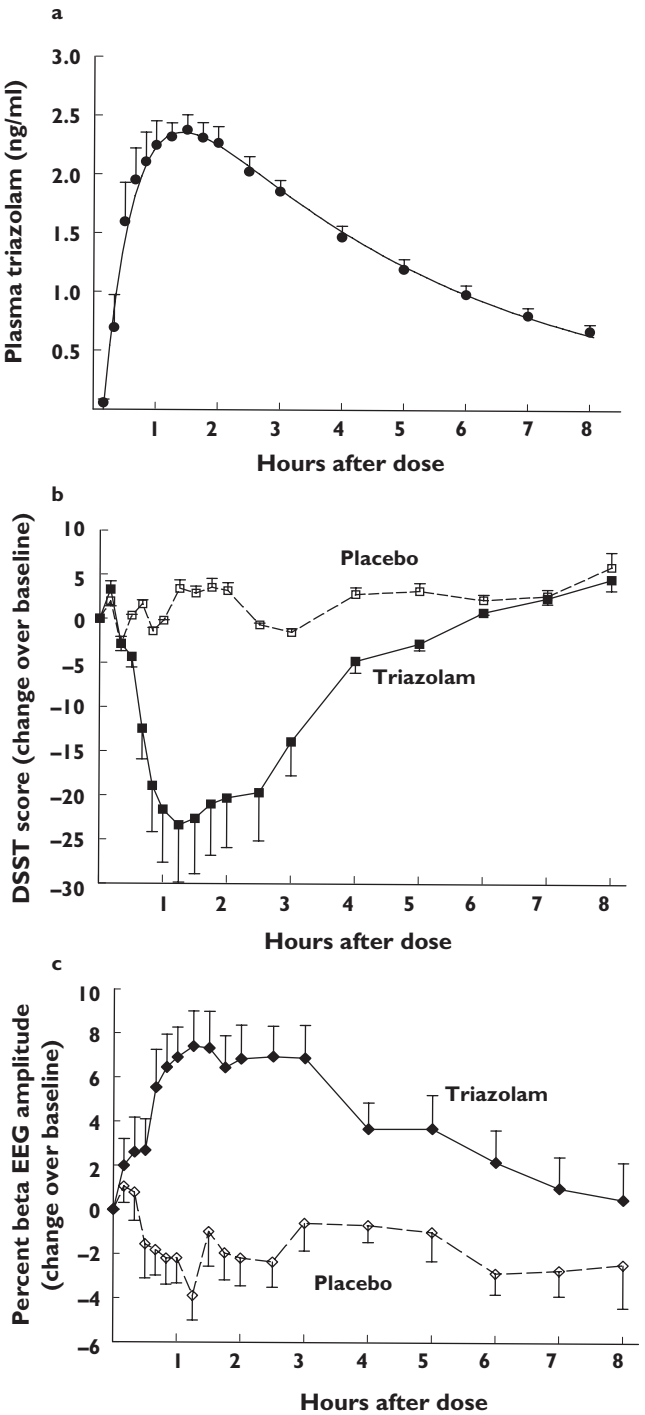

Figure 1

(a) Plasma concentrations of triazolam at corresponding times (mean ± SE, *n* = 13). Line represents the function of best fit based on a linear sum of two exponential terms, modified by a lag time (0.16 h) elapsing prior to the start of absorption; (b) Pharmacodynamic effects of triazolam and placebo at corresponding times (mean ± SE, *n* = 13) for the DSST; (c) Pharmacodynamic effects of triazolam and placebo at corresponding times (mean ± SE, *n* = 13) for the EEG

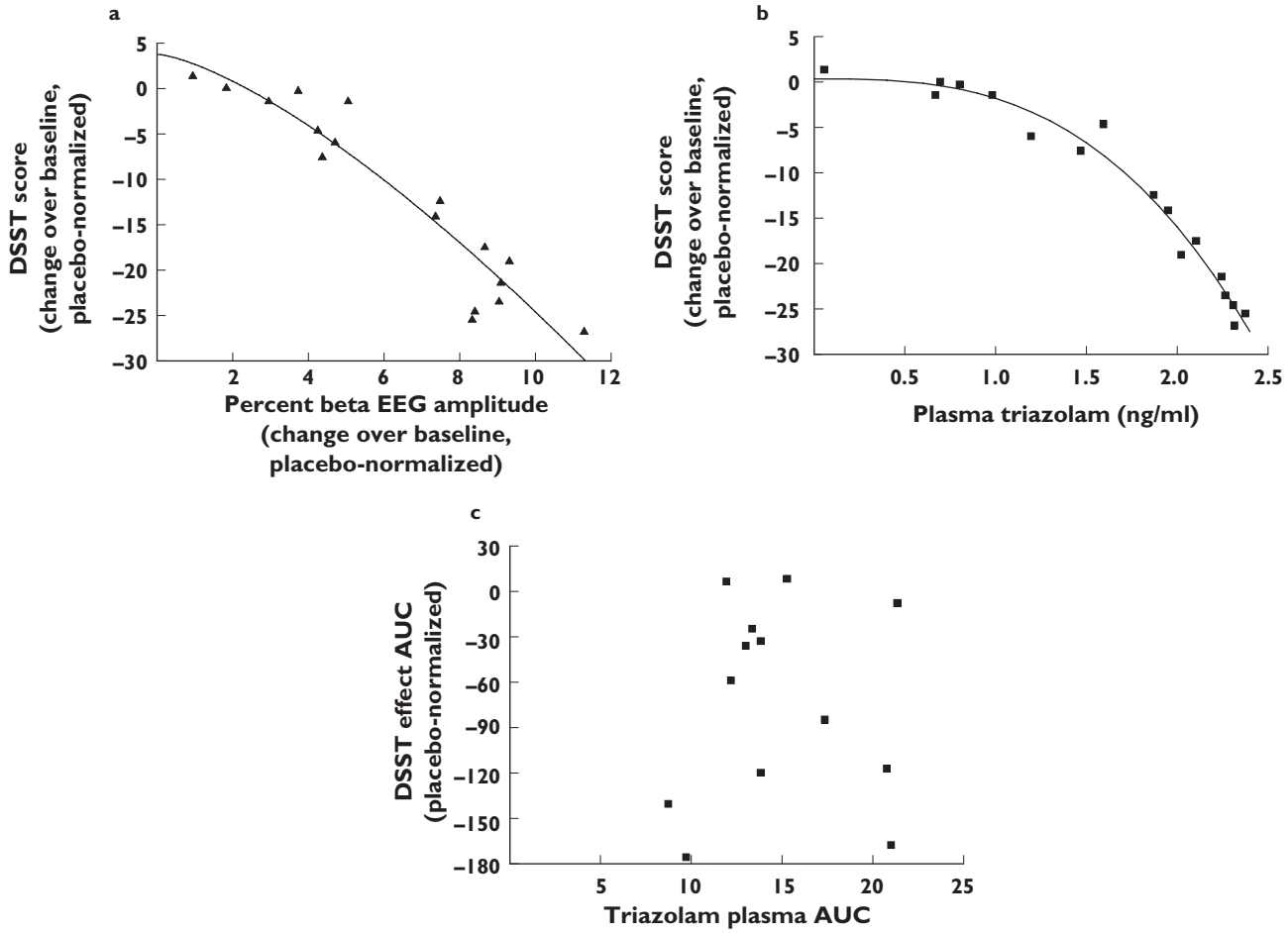

Figure 2

(a) Correlation between DSST and EEG measures using mean values of EEG change and DSST change at corresponding times (r = –0.94, P < 0.001) based on an exponential equation ($y = -1.13x^{1.4} + 3.78$). Relative asymptotic standard errors (in per cent) for parameter estimates in the fitted function were: 1.13 (±103%); 1.4(±29%); 3.78 (±95%); (b) The relation of mean plasma triazolam concentrations to mean DSST changes over baseline (placebo-normalized) at the corresponding time (r = –0.99). Solid line represents an exponential equation [$E = -2.14C^{2.93} + 0.33$] fitted to data points using nonlinear regression. Relative asymptotic standard errors (in percent) for parameter estimates in the fitted function were: 2.14 (±33%); 2.93 (13%); 0.33 (±288%); (c) Relation between the plasma triazolam AUC and the placebo-normalized AUC_{effect} for DSST change score among 13 subjects (r = 0.04, NS). Units for AUC_{effect} (y-axis) are: DSST change score × h. Units for plasma AUC (x-axis) are: ng ml^{-1} × h

DSST scores and an increase in beta amplitude on the EEG (Figure 1). The changes returned to baseline levels by 4–8 h after dosing. Mean (±SD) AUC_{effect} values for triazolam and placebo for DSST score were: –57 ± 58 (95% CI: –93 to –21) *vs.* 16 ± 34 (95% CI: –6 to 37; $P < 0.001$). For the EEG, values were: 32 ± 35 (95% CI: 10–54) *vs.* –14 ± 24 (95% CI: –29 to 1; $P < 0.005$). CV values among 13 subjects were: 89% for placebo-normalized DSST AUC_{effect}, and 95% for placebo-normalized EEG AUC_{effect}.

Mean values of EEG change and DSST change for 13 subjects at corresponding times were strongly correlated ($r = -0.94$, $P < 0.001$), based on an exponential function of the form: $y = Bx^A + K$, as described previously (Figure 2). However, values of AUC_{effect} for each individual were not significantly correlated ($r = -0.418$, NS).

Mean DSST changes and mean plasma triazolam concentrations at corresponding times were highly correlated ($r = -0.99$, $P < 0.001$) based on an exponential function (Figure 2). Mean EEG beta amplitude changes and mean plasma concentrations were similarly correlated ($r = 0.94$, $P < 0.001$) through an exponential function. For both DSST and EEG, no evidence of clockwise or counterclockwise hysteresis was found.

Plasma triazolam AUC for the 13 subjects was not significantly correlated with AUC_{effect} for the DSST ($r = 0.04$, NS) (Figure 2) or the EEG ($r = 0.21$, NS).

Discussion

The benefits and disadvantages of various subjective and objective measurement techniques used to delineate the time-course and intensity of benzodiazepine agonist compounds are described previously [5–10]. The DSST is a classic psychomotor performance test that is well-established as a procedurally straightforward, inexpensive, and sensitive index of benzodiazepine agonist effects. However the DSST is also influenced by practice and adaptation as may occur both within and between testing sessions. The EEG is a fully objective, quantitative, and practice-insensitive measure of central benzodiazepine action, but requires specialized instrumentation and may be sensitive to artefact [5, 11]. Also, the mechanism of EEG changes associated with benzodiazepine against treatments is still not established. In the present study we compared these two approaches using the benzodiazepine derivative triazolam as a representative full-agonist ligand.

DSST decrements and increments in EEG beta amplitude both clearly distinguished triazolam from placebo, either at individual time points, or based on integrated 8-h effect areas. Both measures had a time-course that matched plasma triazolam concentrations. Based on mean values at corresponding time points, there was a highly significant correlation between plasma level and DSST or EEG change. Further, DSST and EEG changes themselves were highly intercorrelated. However, plasma AUC values appeared unrelated to placebo-normalized AUC_{effect} for both DSST and EEG, and AUC_{effect} for DSST and AUC were poorly correlated with each other. Finally, the between-subject coefficient of variation for plasma AUC (standard deviation 28% of the mean) was much less than the coefficient of variation for placebo-normalized values of AUC_{effect} for DSST (89%) or EEG (95%).

The results of this study indicate that between-subject variability in triazolam kinetics is exceeded by variability in response. Variance in response for DSST and EEG is similar, suggesting that factors other than intrinsic variability should be considered in distinguishing these two pharmacodynamic methods. Based on values aggregated across subjects at individual times, plasma concentrations, DSST changes, and EEG changes had a similar time course and were highly intercorrelated. However net kinetic and dynamic exposure measures between subjects were poorly correlated, indicating high variability in individual sensitivity to benzodiazepine agonist effects.

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