Pharmacology, Biochemistry and Behavior 96 (2010) 148–151

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Reduction of aggression during benzodiazepine withdrawal: Effects of flumazenil

L. Saxon [a], S. Borg [a], A.J. Hiltunen [b,*]

[a] Department of Clinical Neuroscience, Section of Dependency Research, Karolinska Institute, Stockholm, Sweden
[b] Department of Psychology, Karlstad University, Universitetsgatan 2, S-651 88 Karlstad, Sweden

ARTICLE INFO

Article history:
Received 24 April 2009
Received in revised form 14 April 2010
Accepted 28 April 2010
Available online 6 May 2010

Keywords:
Aggression
Benzodiazepines
Withdrawal
Flumazenil
Humans

ABSTRACT

Benzodiazepine withdrawal has been associated with hostile and aggressive behavior. The benzodiazepine antagonist flumazenil has reduced, increased or not affected hostility and aggression in animal and human studies. In the present study we analyzed data collected in a placebo-controlled study of the effects of the benzodiazepine antagonist flumazenil in patients previously treated for benzodiazepine dependency, and healthy controls. The aim was to analyze the effects of flumazenil on hostility and aggression. Ten patients and 10 controls received, on two separate occasions, cumulative doses of flumazenil (0.05, 0.1, 0.25, 0.5 and 1 mg at 15 min intervals) or placebo. Withdrawal symptoms were rated after each injection. Patients had been free from benzodiazepines for 47 (4–266) weeks on the first occasion. A three-way interaction (group × treatment × dose) was found, and was explained by: 1) patients rating aggression and hostility higher than controls at all times during placebo, while 2) during the flumazenil provocation i) the initial significant difference between patients and controls was no longer significant above the 0.5 mg dose, and ii) patients rated aggression and hostility significantly lower above the 0.5 mg dose compared to base-line. The results suggest that self-rated aggression and hostility in patients treated for benzodiazepine dependency was reduced by the partial benzodiazepine agonist flumazenil.

1. Introduction

In the clinical setting of treatment of benzodiazepine withdrawal there are some who terminate treatment themselves, or are discharged, as a consequence of intolerable withdrawal related feelings of irritability and aggression. It would therefore be beneficial for the patients, as well as for people close to them and for society if this subgroup of patients could stay in treatment for a controlled withdrawal from medication to maintain a stable life situation. Therefore it is important to investigate the possibility of a pharmacological intervention to reduce abstinence symptoms in this group of patients.

Benzodiazepines were initially known for their "taming" properties (Randall, 1960). It has been shown, in animals, that moderate to high doses of benzodiazepines reduce aggression while low doses may have an intensifying effect in some situations (Miczek et al., 1994). Benzodiazepines are also known to produce paradoxical rage reactions in some individuals of both animals and humans (DiMascio, 1973). Furthermore, benzodiazepines have dependency producing properties (Hollister et al., 1961; Lader, 1991).

Benzodiazepine withdrawal has, among many other symptoms (Ashton, 1991), been associated with irritable, hostile and aggressive behaviors in both animals (Herman et al., 1976; Krsiak et al., 1998; Nath et al., 2000; Votava et al., 2001) and humans (Hallstrom and Lader, 1981; Petursson and Lader, 1981; Lader and Petursson, 1983; Owen and Tyrer, 1983; Ashton, 1984; Fontaine et al., 1984; Lader, 1984). The incidence of such symptoms ranges, in patient populations, from 19 to 75%. Withdrawal symptoms may cause problems with treatment compliance as well as in the social and professional life for some individuals.

A growing evidence suggest that benzodiazepines might increase an aggressive behavior in humans (Wallace and Taylor, 2009), and several possible explanations to this increase has been suggested. The behavioral and emotional control can be lost by GABAergic neurons which synapse with other neurons that control behaviors when activated. Alternatively, anxiolytic effects of benzodiazepines can diminish fear of negative consequences, or lead to change in the self-conscious emotions of shame, guilt or embarrassment. For a more detailed discussion of benzodiazepines and aggression see Wallace and Taylor, (2009). The benzodiazepine antagonist flumazenil, also suggested to have partially agonistic effects at doses higher than 30 mg (File and Pellow, 1986), has been reported to reduce (Allikmets and Rago, 1983; Ushijima et al., 1984; Vasar et al., 1984; Ostrovskaia and Molodavkin, 1985; Uhlirova et al., 2004), increase (Rodgers and Waters, 1984; Beck and Cooper, 1986) or not/inconclusively affect (Polc et al., 1981; Sulcova and Krisiak, 1984; Skolnick et al., 1985; Mos and Olivier, 1986; Mos et al., 1987; Sakaue et al., 2001; Fachinelli et al., 2003; Gourley et al., 2005) hostility and aggression in animal studies. Studies in benzodiazepine-naive humans have shown more self-reported "antagonism" in subjects given flumazenil compared to placebo (Darragh et al., 1983; Higgitt et al., 1986). Flumazenil has also,

* Corresponding author. Tel: +46 54 700 22 02, +46 76 845 80 35 (mobile).
E-mail address: arto.hiltunen@kau.se (A.J. Hiltunen).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.04.023

in animals, been shown to decrease the aggression-heightening effects of the $GABA_A$ receptor modulator alcohol (Miczek and Krsiak, 1979; Weerts et al., 1993; De Almeida et al., 2004). One study has investigated the effects of flumazenil on aggressive responding in humans (Tcheremissine et al., 2005). Doses of flumazenil (2 and 3 mg) did not produce statistically significant changes in aggressive responding. There was, however, some individual variation across subjects and it is speculated if this might be related to the previous history of benzodiazepine abuse in the two subjects responding to the provocation.

It has been suggested that consumption of benzodiazepines causes a receptor shift so that agonists become less effective and inverse agonists become more effective (Nutt, 1990). Bidirectional effects of flumazenil are discussed by File and Hitchcott (1990) To the receptor shift theory File and Hitchcott (1990) have added that individual levels of anxiety influence the effect of flumazenil on benzodiazepine withdrawal. They suggested that flumazenil acts as an agonist when anxiety is high and as an inverse agonist when it is low.

Another possibility would be that flumazenil acts by blocking endogenous inverse agonists that produces the withdrawal related symptoms — as have been a suggested in alcohol withdrawal (Potokar et al., 1997). Still another possible explanation is if withdrawal symptoms continue to be low after the flumazenil provocation. Then it might be that flumazenil revesed a receptor tolerance that have followed as a consequence of the previous benzodiazepine consumption (Savic et al., 1991).

In the present study, we analyzed data collected in a placebo-controlled study of the effects of the benzodiazepine antagonist flumazenil in patients previously treated for benzodiazepine dependency, and healthy controls. The aim was to analyze the effects of flumazenil on self-rated hostility and aggression.

2. Method

2.1. Participants

Ten patients and 10 controls participating in a study of the effects of the benzodiazepine antagonist flumazenil on withdrawal symptoms after treatment for benzodiazepine dependency. The patient group consisted of 5 males and 5 females with a history of benzodiazepine dependence according to DSM-III-R. They had been treated for this by tapering and should have been free from benzodiazepines for at least three weeks, but still complain about characteristic withdrawal symptoms. Their characteristics have been described previously (Saxon et al., 1997). The patients ranged from 28 to 51 (mean 43) years in age and had been benzodiazepine free for 47 (4–266) weeks before the first provocation. All subjects were medically examined prior to provocations and subjects were to be excluded from the study if: 1) using drugs of any kind, 2) were alcohol dependent, or 3) had any signs of previous illness or central nervous system damage. Control subjects consisted of physically and psychiatrically healthy hospital staff (5 males and 5 females) with ages ranging from 34 to 48 years (mean age 42 years). All subjects were screened for benzodiazepines and salicylates in their urine and for indicators of high alcohol consumption in blood.

2.2. Assessments

For subjective ratings, a unipolar 90-item visual analogue self-rating scale previously validated for symptoms commonly associated with benzodiazepine withdrawal and with the use of flumazenil was used; see Saxon et al. (1997). This rating scale is divided into three subscales, consisting of aggregates of Positive- and Negative Psychological Items, as well as Somatic Items. For the present study, six of the items on the Negative Psychological Aggregate were used: “Irritation”, “Temper outbursts you cannot control”, “Having urges to break or smash things”, “Wants to shout or throw things”, “That you easily get annoyed or irritated” and “Having urges to beat, injure, or harm someone”. Each item consisted of a 100 mm scale ranging from “not at all” to “very much”. The subjects were instructed to define the subjective meaning of the extremes, rated to what extent they experienced each symptom at that precise moment — not to indicate changes, and that they should perform the ratings quickly. The subjects were accustomed to the instrument by a practice rating before the experiment began. Self-ratings commenced 7 min after each infusion, and were completed in approximately 6 min. For further information on the self-rating instrument, see Saxon et al. (1997).

2.3. Procedure

Subjects received, on two separate occasions, cumulative doses of flumazenil (0.05, 0.1, 0.25, 0.5 and 1 mg at 15 min intervals) or placebo intravenously. Both occasions were initiated by two single-blind placebo injections. Withdrawal symptoms were self-rated on 100 mm visual analogue scales after each injection.

Subjects gave their consent to participate in the study after receiving verbal and written information about the study, which was approved by the local Ethics Committee. Subjects received some financial compensation for participating. A physician examined the subjects before each experiment.

The experimental protocol was approved by an Institutional Review Committee for the use of Human Subjects and are in compliance with the Declaration of Helsinki for human subjects.

2.4. Data analysis

Group differences, differences between group changes over time (i.e. group × time interaction), over treatment conditions (i.e. group × treatment interaction), and treatment effects over time (i.e. treatment × time interaction), as well as three-way interactions (i.e. group × time × treatment interaction) were tested for significance by analysis of variance (ANOVA, a split-plot design; (Kirk, 1968)). Post-hoc analysis for paired comparisons was carried out using Tukey's HSD Test (Kirk, 1968). The level of significance was set at 5% and sequential Bonferroni was used to control for possible alpha inflation (Rice, 1989).

3. Results

A significant [$F(6,108) = 2.44$, $P = 0.03$] three-way interaction was found between group, treatment and dose (Fig. 1). In post-hoc tests

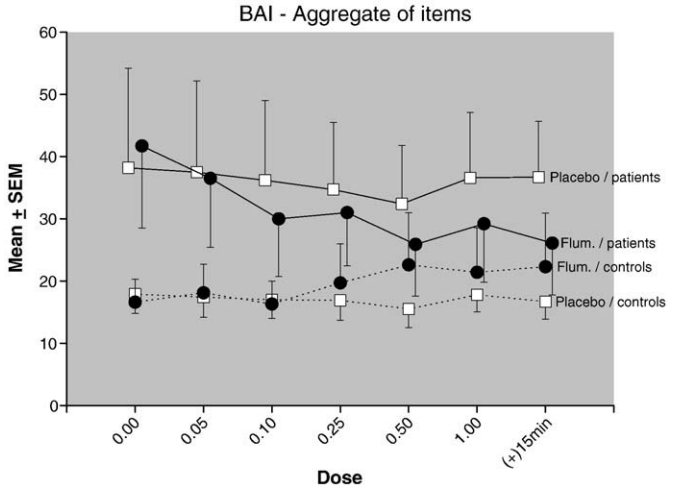

Fig. 1. Sum (±SEM) of aggregate of the six self-rated aggression and hostility items. The 100 mm visual analogue scale ranged from “not at all” to “very much”.

(Tukey's HSD) this was explained by: 1) patients rating aggression and hostility significantly ($P<0.0001$) higher than controls at all times during placebo, while 2) during the flumazenil provocation i) the initial significant ($P<0.01$) difference between patients and controls was no longer significant above the 0.5 mg dose, ii) patients, during flumazenil, did not differ significantly from patients placebo above the 0.5 mg dose, and iii) patients rated aggression and hostility significantly ($P<0.05$) lower above the 0.5 mg dose compared to base-line. Additionally, the possibility of how aggression relates to anxiety was tested. The results showed that Pearson product moment correlation between aggression and anxiety was significant both for the patients ($r=0.80$, $P<0.01$) and for the controls ($r=0.89$, $P<0.01$).

4. Discussion

Benzodiazepine withdrawal has, among many other symptoms, been associated with hostile and aggressive behavior, whereas the benzodiazepine antagonist flumazenil has had variable effects on hostility and aggression in animal studies.

In this analysis of data collected in a study of effects of the benzodiazepine antagonist flumazenil on benzodiazepine withdrawal, we found that patients treated for benzodiazepine dependency experienced stronger feelings of aggression and hostility than controls. This self-rated hostility and aggressiveness was reduced by intravenous administration of flumazenil among patients, whereas controls tended to respond with the opposite effect. Thus, flumazenil seems to have acted as an agonist in the patient group and, possibly, as a weak inverse agonist in the control group. This inverse agonist effect in humans have been described elsewhere (Schöpf et al., 1984), however this inverse agonist effect was not found by Coupland et al. (1997). Our results indicate that previously reported aggression reducing effects of flumazenil in animals may also be shown in humans. The effects do, however, not support the suggestion that benzodiazepine consumption causes a receptor shift that makes inverse agonists act more effectively (Nutt, 1990). Another possibility would be that flumazenil acts by blocking endogenous inverse agonists that produces the withdrawal related symptoms — as have been a suggested in alcohol withdrawal (Potokar et al., 1997). It is not possible to tell, from this study, if this was the case. It could, however, be studied in an experiment with a longer follow-up period — if symptoms return when flumazenil concentrations are decreasing this theory is supported. A third possible explanation is if withdrawal symptoms continue to be low after the flumazenil provocation. Then it might be that flumazenil revesed a receptor tolerance that have followed as a consequence of the previous benzodiazepine consumption (Savic et al., 1991).

Limitations of these results are the limited number of subjects studied and the post-hoc nature of the analyses performed. The results are, however, interesting and further studies should be performed to confirm these results. We observed that the patient with the highest base-line anxiety showed the largest response to flumazenil. It would therefore be interesting to test the hypothesis of File and Hitchcott (1990), that the effects of flumazenil depend on individual levels of anxiety, by comparing effects in larger groups of patients with low and high base-line anxiety.

Flumazenil has also been reported to reduce symptoms of alcohol withdrawal in humans (Nutt et al, 1993; Potokar et al., 1997), and when given in combination with the GABA analogue gabapentin to subjects with alcohol withdrawal, a more favorable effect on subsequent drinking in patients with high alcohol withdrawal symptoms (Anton et al., 2009). Further, in the group with high withdrawal symptoms, there was a significant difference between flumazenil/gabapentin and placebo in reduction of withdrawal symptoms. It would therefore also be of interest to evaluate the influence of anxiety on alcohol induced self-reported aggression during flumazenil provocations, related to a past history of aggressive behavior for both groups.

Acknowledgements

We wish to thank Prof. Paul Hjemdahl for constructive comments of the manuscript as well as Christina Cavalli, Bo Dovborg and Kirsi Laitinen who assisted during the experiments. This study was in part funded by ROCHE – Produkter AB, Sweden.

References

Allikmets LH, Rago LK. The action of benzodiazepine antagonist Ro 15-1788 on the effects of GABA-ergic drugs. Naunyn Schmiedebergs Arch Pharmacol 1983;324:235–7.
Anton RF, Myrick H, Baros AM, Latham PK, Randall PK, Wright TM, et al. Efficacy of a combination of flumazenil and gabapentin in the treatment of alcohol dependence: relationship to alcohol withdrawal symptoms. J Clin Psychopharmacol 2009;29:334–42.
Ashton H. Benzodiazepine withdrawal: an unfinished story. Br Med J (Clin Res Ed) 1984;288:1135–40.
Ashton H. Protracted withdrawal syndromes from benzodiazepines. J Subst Abuse Treat 1991;8:19–28.
Beck CH, Cooper SJ. beta-Carboline FG 7142-reduced aggression in male rats: reversed by the benzodiazepine receptor antagonist, Ro15-1788. Pharmacol Biochem Behav 1986;24:1645–9.
Coupland NJ, Lillywhite A, Bell CE, Potokar JP, Nutt DJ. A pilot controlled study of the effects of flumazenil in posttraumatic stress disorder. Biol Psychiatry 1997;41:988–90.
Darragh A, Lambe R, O'Boyle C, Kenny M, Brick I. Absence of central effects in man of the benzodiazepine antagonist Ro 15-1788. Psychopharmacology 1983;80:192–5.
De Almeida RM, Rowlett JK, Cook JM, Yin W, Miczek KA. GABA(A)/alpha(1) receptor agonists and antagonists: effects on species-typical and heightened aggressive behavior after alcohol self-administration in mice. Psychopharmacology 2004;172:255–63.
DiMascio A. The effect of benzodiazepines on aggression: reduced or increased? Psychopharmacologia 1973;30:95-102.
Fachinelli C, Ison M, Rodriguez Echandia EL. Effects of diazepam and flumazenil on food competition behavior in high- and low-aggression pigeons. Pharmacol Biochem Behav 2003;74:765–70.
File SE, Hitchcott PK. A theory of benzodiazepine dependence that can explain whether flumazenil will enhance or reverse the phenomena. Psychopharmacology (Berl) 1990;101:525–32.
File SE, Pellow S. Intrinsic actions of the benzodiazepine receptor antagonist Ro 15-1788. Psychopharmacology 1986;88:1-11.
Fontaine R, Chouinard G, Annable L. Bromazepam and diazepam in generalized anxiety: a placebo-controlled study of efficacy and withdrawal. Psychopharmacol Bull 1984;20:126–7.
Gourley SL, DeBold JF, Yin W, Cook J, Miczek KA. Benzodiazepines and heightened aggressive behavior in rats: reduction by $GABA_A/\alpha_1$ receptor antagonists. Psychopharmacology 2005;178:232–40.
Hallstrom C, Lader M. Benzodiazepine withdrawal phenomena. Int Pharmacopsychiatry 1981;16:235–44.
Herman ZS, Drybanski A, Trzeciak HI. Increased aggression in rats after withdrawal of long term used oxazepam. Experientia 1976;32:1305–6.
Higgitt A, Lader M, Fonagy P. The effects of the benzodiazepine antagonist Ro 15-1788 on psychophysiological performance and subjective measures in normal subjects. Psychopharmacology 1986;89:395–403.
Hollister LE, Motzenbecker FP, Degan RO. Withdrawal reactions from chlordiazepoxid. Psychopharmacologia 1961;2:63–8.
Kirk RE. Experimental design: procedures for the behavioral sciences. Monterey, California: Brooks/Cole Publishing Co.; 1968.
Krsiak M, Podhorna J, Miczek KA. Aggressive and social behavior after alprazolam withdrawal: experimental therapy with Ro 19-8022. Neurosci Biobehav Rev 1998;23:155–61.
Lader M. Benzodiazepine dependence. Prog Neuropsychopharmacol Biol Psychiatry 1984;8:85–95.
Lader M. History of benzodiazepine dependence. J Subst Abuse Treat 1991;8:53–9.
Lader M, Petursson H. Long-term effects of benzodiazepines. Neuropharmacology 1983;22:527–33.
Miczek KA, Krsiak M. Drug effects on agonistic behavior. In: Thompson T, Dews PB, editors. Advances in behavioural pharmacology, Vol. 2. New York: Academic Press, Inc; 1979. p. 87-162.
Miczek KA, Weerts E, Haney M, Tidey J. Neurobiological mechanisms controlling aggression: preclinical developments for pharmacotherapeutic interventions. Neurosci Biobehav Rev 1994;18:97-110.
Mos J, Olivier B. RO 15-1788 does not influence postpartum aggression in lactating female rats. Psychopharmacology 1986;90:278–80.
Mos J, Olivier B, Van der Poel AM. Modulatory actions of benzodiazepine receptor ligands on agonistic behaviour. Physiol Behav 1987;41:265–78.
Nath C, Saxena RC, Gupta MB. Effect of dopamine agonists and antagonists on the lorazepam withdrawal syndrome in rats. Clin Exp Pharmacol Physiol 2000;27:167–71.

Nutt DJ. Pharmacological mechanisms of benzodiazepine withdrawal. J Psychiatr Res 1990;24:105–10.
Nutt D, Glue P, Wilson S, Groves S, Coupland N, Bailey J. Flumazenil in alcohol withdrawal. Alcohol Alcohol (Suppl) 1993;2:337–41.
Ostrovskaia RU, Molodavkin GM. Antagonism of RO 15-1788 with benzodiazepines in the effect on motivated aggression and the action of analgesics. Biull Eksp Biol Med 1985;99:448–50.
Owen RT, Tyrer P. Benzodiazepine dependence: a review of the evidence. Drugs 1983;25:385–98.
Petursson H, Lader MH. Withdrawal from long-term benzodiazepine treatment. Br Med J (Clin Res Ed) 1981;283:643–5.
Polc P, Laurent JP, Scherschlicht R, Haefely W. Electrophysiological studies on the specific benzodiazepine antagonist Ro 15-1788. Naunyn Schmiedebergs Arch Pharmacol 1981;316:317–25.
Potokar J, Coupland N, Glue P, Groves S, Malizia A, Bailey J, et al. Flumazenil in alcohol withdrawal: a double-blind placebo-controlled study. Alcohol Alcohol 1997;32: 605–11.
Randall LO. Pharmacology of methaminodiazepoxide. Dis Nerv Syst 1960;21:7-10 (Suppl).
Rice WR. Analyzing tables of statistical tests. Evolution 1989;43:223–5.
Rodgers RJ, Waters AJ. Effects of the benzodiazepine antagonist Ro 15-1788 on social and agonistic behaviour in male albino mice. Physiol Behav 1984;33:401–9.
Sakaue M, Ago Y, Murakami C, Sowa C, Sakamoto Y, Koyama Y, et al. Involvement of benzodiazepine binding sites in an antiaggressive effect by 5-HT(1A) receptor activation in isolated mice. Eur J Pharmacol 2001;432:163–6.
Savic I, Widén L, Stone-Elander S. Feasibility of reversing benzodiazepine tolerance with flumazenil. Lancet 1991;337:133–7.
Saxon L, Hjemdahl P, Hiltunen A, Borg S. Effects of flumazenil in the treatment of benzodiazepine withdrawal — a double-blind pilot study. Psychopharmacology 1997;131:153–60.
Schöpf J, Laurian S, Le PK, Gaillard JM. Intrinsic activity of the benzodiazepine antagonist Ro 15-1788 in man: an electrophysiological investigation. Pharmacopsychiatry 1984;17:79–83.
Skolnick P, Reed GF, Paul SM. Benzodiazepine-receptor mediated inhibition of isolation-induced aggression in mice. Pharmacol Biochem Behav 1985;23:17–20.
Sulcova A, Krisiak M. The benzodiazepine-receptor antagonist Ro 15-1788 antagonizes effects of diazepam on aggressive and timide behaviour in mice. Act Nerv Super (Praha) 1984;26:255–6.
Tcheremissine OV, Lane SD, Lieving LM, Rhoades HM, Nouvion S, Cherek DR. Individual differences in aggressive responding to intravenous flumazenil administration in adult male parolees. J Psychopharmacology 2005;19:640–6.
Uhlirova L, Sustkova-Fiserova M, Krsiak M. Behavioral effects of flumazenil in the social conflict test in mice. Psychopharmacology 2004;171:259–69.
Ushijima I, Katsuragi T, Furukawa T. Involvement of adenosine receptor activities in aggressive responses produced by clonidine in mice. Psychopharmacology 1984;83:335–9.
Vasar EE, Maimets MO, Riago LK, Nurk AM, Allikmets LKh. Effect of an imidazobenzodiazepine (RO 15-1788) on aggressive behavior in mice. Biull Eksp Biol Med 1984;98:441–3.
Votava M, Krsiak M, Podhorna J, Miczek KA. Alprazolam withdrawal and tolerance measured in the social conflict test in mice. Psychopharmacology 2001;157: 123–30.
Wallace PS, Taylor SP. Reduction of appeasement-related affect as a concomitant of diazepam-induced aggression: evidence for a link between aggression and the expression of self-conscious emotions. Aggress Behav 2009;35:203–12.
Weerts EM, Tornatzky W, Miczek KA. Prevention of the pro-aggressive effects of alcohol in rats and squirrel monkeys by benzodiazepine receptor antagonists. Psychopharmacology 1993;111:144–52.