ELSEVIER

Journal of Immunological Methods 309 (2006) 1–10

JIM
Journal of Immunological Methods

www.elsevier.com/locate/jim

Research paper

Development of murine monoclonal antibodies to methamphetamine and methamphetamine analogues

Yannic Danger [a], Caroline Gadjou [b], Anne Devys [a], Hervé Galons [b], Dominique Blanchard [c], Gilles Folléa [b,*]

[a] *Etablissement Français du Sang, Laboratoire de Biotechnologie, 34 bd J Monnet, BP 91115, 44011 Nantes cedex 01, France*
[b] *Université René Descartes, Laboratoire de chimie organique 2, Paris, France*
[c] *DB-Biorun S.A.R.L., 1 bis Bd des Américains, 44300 Nantes, France*

Received 27 September 2004; received in revised form 14 September 2005; accepted 3 October 2005
Available online 20 December 2005

Abstract

Methamphetamine and ecstasy are addictive drugs that cause major health problems in young people. Here we report on the development of high-affinity monoclonal antibodies to methamphetamine and its analogues, which may constitute powerful tools for antibody-based therapy. Six haptens, methamphetamine and ecstasy analogues, were synthesized, linked to a carrier protein and injected into mice. Several specific monoclonal antibodies were subsequently obtained following fusion of splenocytes from the immunized animals, with Sp2/O cells. Antibody specificity was fully investigated by competition ELISA, using a series of analogues, to identify specific amphetamine and/or ecstasy-specific antibodies. Antibody affinity was estimated to be in the range of 10^8 M^{-1} with an enantiomeric hapten. Finally, two characteristic hybridoma clones (DAS-M243-6H5 and DAS-M278-4B12), secreting specific and potent mAbs were isolated. The development of drug-specific antibodies as in this study may provide promising therapeutic insight into how to neutralize methamphetamine in vivo during acute intoxication.

Keywords: mAbs; Methamphetamine; Ecstasy; Stereospecificity; Affinity

1. Introduction

Methamphetamine is a drug that was initially synthesized to treat central nervous system (CNS) disorders such as schizophrenia (Sueur et al., 1999). This psychostimulant has now become a major drug of abuse worldwide (Karch et al., 1999). Methamphetamine and its analogues contribute to a growing health problem, especially in the United States (the incidence of methamphetamine-related episodes increased by 30%, from approximately 10,400 in 1999 to 13,500 in 2000) (Hanson, 2002). In high doses, methamphetamine causes hyperactivity, agitation, hyperthermia, hypertension, cardiotoxicity and psychotic disorders such as

Abbreviations: CNS, central nervous system; mAb, monoclonal antibody; THF, tetrahydrofuranne; Boc, Di-*tert*-butyl dicarbonate; TFA, trifluoroacetic acid; Pd/C, Palladium on carbon; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; SMCC, 4-(*N*-maleimidomethyl)cyclohexanecarboxylic acid *N*-hydroxysuccinimide ester; TT, tetanus toxoid; KLH, keyhole limpet hemocyanin; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; OD, optical density; TBS, tris-buffered saline; IgG, immunoglobulin G; RIA, radioimmunoassay; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide; NHS, *N*-hydroxysuccinimide.

* Corresponding author. Etablissement Français du Sang-Pays de Loire, Site de Nantes 34 boulevard Jean Monnet, 44011 Nantes cedex 01, France. Tel.: +33 2 40 12 33 00.
E-mail address: gilles.follea@efs.sante.fr (G. Folléa).

0022-1759/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jim.2005.10.017

paranoia (Buffum and Shulgin, 2001). Even though death is rarely observed following methamphetamine intake, long-term toxicity has been reported in several studies, principally in chronic abusers (Christophersen, 2000; Buchert et al., 2001; Green et al., 2003) where its toxicity is related to interactions with serotonin and/or dopamine transporters.

To date, there are no specific therapies enabling in vivo neutralization of the toxic molecules in the case of hyperacute intoxication. Antibody-based therapy may however be a method of choice to eliminate the drugs, since specific and potent antibodies should be able to capture the drugs in the blood and prevent their diffusion into target organs. Such a strategy has already been used for decades for venom neutralization (Chippaux and Goyffon, 1998) and is also effective in the case of digitalin intoxication (Megarbane et al., 2002). Antibody-based therapy has also been considered for the neutralization of CNS-toxic drugs such as phencyclidines (PCP) (Hardin et al., 1998) as well as for cocaine abuse (Carrera et al., 1995; Deng et al., 2002). Modulation of uptake of cocaine to the brain from the blood stream using specific mAbs has already been demonstrated in animal models (Kosten et al., 2002; Carrera et al., 2000).

Although monoclonal antibodies to methamphetamine have already been described for diagnostic or research purposes, reports on potent antibodies for clinical use have only been published recently (Byrnes-Blake et al., 2001). Methamphetamine is a small molecule (molecular weight of 150 g/mol) that is itself non-immunogenic. Thus, in order to stimulate the immune system and initiate antibody production, the corresponding haptens have to be linked to protein carriers. Two synthesis methods for hapten preparation have been tested in the literature: N-modification of methamphetamine (Inayama et al., 1977; Nam et al., 1993) and ring modification of amphetamine analogues (Terazawa et al., 1991; Byrnes-Blake et al., 2001).

To prepare effective antibodies to fight drug addiction, it is essential to take into account the fact that drug users do not use a pure drug but a mixture containing several co-synthesized products. In the case of methamphetamine addiction, the co-products can be even more toxic than methamphetamine itself (Felgate et al., 1998; Burgess et al., 2000; Kraemer and Maurer, 2002). For this reason, mAbs should be generated with the goal of targeting all toxic products. Another problem is the difficulty in producing mAbs that can distinguish toxic molecules from non-toxic analogues such as endogenous neurotransmitters. The mAbs necessary for application in antibody-based therapy also have to be of high affinity.

This paper reports the generation and characterization of efficient anti-methamphetamine antibodies, using different chemically modified haptens. Our strategy took into account the influence of stereochemistry on toxicity (Spitzer et al., 2001) to induce stereospecific antibodies. This approach therefore enabled us to carefully select two highly specific and potent antibodies directed against methamphetamine and its metabolites in accordance with their biochemical characteristics. These mAbs display therapeutic potential during acute intoxication.

2. Materials and methods

2.1. Drugs and reagents

[^{3}H]-Methamphetamine ((+)-methamphetamine HCl; 9.7 Ci/mmol) was obtained from NEN (Paris, France). 4-Methoxymethamphetamine, 4-methoxyamphetamine, Ephedrine, Ecstasy, 4-methylthioamphetamine, Nethylamphetamine, Nor-ephedrine, 3-hydroxytyramine, Epinephrine, 3-hydroxy-4-methoxyphenetylalamine,Methyl pseudo ephedrine and Amphetamine were either purchased from Sigma Aldrich or were synthesized by ourselves. All other reagents came from Sigma unless otherwise stated.

2.2. Hapten synthesis

The structures of the synthesized haptens are presented in Fig. 1(a). Haptens were synthesized as described by Pouletty et al. (2002), according to the following protocol depicted in Fig. 1(b). Briefly, *RS*- or *S*-methamphetamine was alkylated by benzyl 2-bromoacetate in tetrahydrofuranne (THF) or by 6-bromohexanoic acid benzyl ester following removal of the benzyl group by hydrogenation using palladium on carbon (Pd/C) as a catalyst, which led to the formation of haptens Met1, Met1′ and Met2 with good yields.

Haptens Met4 and Met5 were obtained from (*RS* or *R*)-4-benzyloxyphenylalanine which was reduced by lithium aluminum hydride ($AlLiH_4$) into the 4-benzyloxyphenylalaninols (**1**). Amine groups were protected by acylation with Di-*tert*-butyl dicarbonate (Boc) to obtain the 4-benzyloxy-NBoc-phenylalaninols (**2**). The alcohol function was transformed to tosylate (**3**) with a good yield using *p*-toluenesulfonyl chloride (TsCl). We found that $AlLiH_4$ simultaneously reduced the tosylate and Boc group to the methyl group to generate (*S* or *RS*)-4-benzyloxymethamphetamine (**4**).

Amine groups were protected by acylation with Boc and benzyl-groups were removed by catalytic hydrogenation generating phenols (**5**). The benzyl ester com-