Long-term administration of galactoside-specific mistletoe lectin in an animal model: no protection against \(N\)-butyl-\(N\)-(4-hydroxybutyl)-nitrosamine-induced urinary bladder carcinogenesis in rats and no induction of a relevant local cellular immune response

Received: 16 June 1999 / Accepted: 3 August 1999

Abstract  Aqueous extracts from leaves of the European mistletoe (\(Viscum album\) L.) are postulated to exert an anticancer efficacy by cytotoxic and/or immunological mechanisms of action. Although popular as an unconventional therapy modality, no controlled randomized clinical trials are available, reliably documenting a clinically beneficial antineoplastic potential of the various commercial mistletoe preparations. Since previous investigations have focused on the purified galactoside-specific lectin (\(Viscum album\) L. agglutinin, VAA) as major biological response modifier in the low-dose range, the objective of the present experimental study was to examine its effect on \(N\)-butyl-\(N\)-(4-hydroxybutyl)-nitrosamine (BBN)-induced carcinogenesis in the urinary bladder of rats, a suitable animal model for human disease. The carcinogen was fed by gavage in three fractionated low doses (150 mg/kg body weight each) to obtain low-grade and low-stage transitional cell carcinomas. From the onset of the experiment VAA was injected subcutaneously twice a week (1 ng/kg body weight) continuously for either 6 or 15 months. Following an experimental period of 6 months the incidence of bladder carcinomas was 10.2% in rats given exclusively BBN and 6.7% in those additionally treated with VAA. After an experimental time of 15 months 25.8% of the rats fed BBN only and 19.7% of the animals additionally receiving VAA had developed urothelial carcinomas. The differences of the tumor incidences did not reach the level of statistical significance, neither after an experimental duration of 6 (\(P = 0.88\)) nor of 15 months (\(P = 0.71\)). A difference was found in the size of the transitional cell carcinomas. They proved to be significantly larger (\(P = 0.02\)) in the rats additionally treated with VAA for 15 months (mean maximum diameter: 3.31 mm) than in those without lectin treatment (mean maximum diameter: 1.88 mm). Quantitative immunocytochemistry analyzing a panel of immune cells yielded no evidence for the ability of the lectin to provoke a substantial, biologically relevant local cellular immune response in the wall of tumor-free and tumor-bearing bladders. From the current experiment it is obvious that galactoside-specific mistletoe lectin failed to protect against, inhibit, delay or reduce development of chemically induced urothelial carcinomas of the urinary bladder even after long-term administration in the clinically recommended schedule. It seems highly unlikely that adjuvant treatment with mistletoe extracts or VAA might favorably influence bladder cancer in patients by immunological effector mechanisms.

Key words  Urinary bladder cancer · \(N\)-butyl-\(N\)-(4-hydroxybutyl)-nitrosamine · Mistletoe · Lectin · Agglutinin · Cellular immune response · Alternative cancer therapies

Abbreviations  VAA galactoside-specific mistletoe lectin · \(MNU\) \(N\)-methyl-\(N\)-nitrosourea · \(BBN\) \(N\)-butyl-\(N\)-(4-hydroxybutyl)-nitrosamine

Introduction

Worldwide, unconventional, unorthodox or alternative therapies to fight cancer become increasingly popular and lead to enormous costs, although there is a lack of acceptable scientific evidence that any of the treatments are actually effective (McGinnis 1991; Drings et al. 1995; Haustein et al. 1998; Klebingat 1998). In central Europe, (Germany, Austria and Switzerland) particularly aqueous extracts from leaves of the European mistletoe
(Viscum album L.) have been used in adjuvant cancer therapy for decades, on the basis of anthroposophic medicine of mythical and philosophical origin introduced by Steiner around 1920 (for review and comments see Stramann 1993; Burkhard 1998). However, the postulated clinical antitumor activity of mistletoe is so far unproven, the possible underlying pharmacological mechanisms of action are unknown, except for a general toxicity of lectins and viscosities, and – apart from numerous anecdotal case reports – no controlled randomized clinical trials are available documenting any antineoplastic potential for the various plant preparations (Hauser 1989, 1993a; b; Kiene 1989; Kleijnen and Knipschild 1994; Oopen 1994; Burkhard 1995; Drings et al. 1995; Marx 1995; Gabius et al. 1996; Gabius 1997; Kaegi 1998; Sauer 1998).

It is believed that cytotoxic or immunological effects or a combination of both may be involved in mistletoe’s putative anticancer activity. During the last 10 years most interest has been focused on the galactoside-specific lectin or agglutinin (VAA, formerly ML1) – the main component of most mistletoe extracts – which has repeatedly been demonstrated to trigger a cell-mediated and humoral immune response by stimulating and activating peripheral blood mononuclear cells as a result of protein-carbohydrate interactions (for review of the literature see Gabius and Gabius 1994, 1998a, b). It is thus tempting to assume that the mistletoe lectin, applied at its immunomodulatory dose of 1 ng/kg body weight, possibly plays a role in limiting tumor growth by humoral and/or cellular effector mechanisms. Two previous long-term animal experiments found no evidence at all for the ability of immunomodulatory galactoside-specific mistletoe lectin to inhibit tumor development induced by N-methyl-N-nitrosourea (MNU) in the urinary bladder of rats or to provoke a substantial local cellular immune reaction following continuous biweekly administration for 15 months in clinically recommended doses (Kunze et al. 1997, 1998).

We have now started an additive histopathological and immunocytochemical approach evaluating the effect of VAA on carcinogenesis in the urinary bladder of rats induced by N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN). This potent urothelial carcinogen is well-known to produce a histopathological spectrum of bladder cancer identical to that observed in the human bladder, thus providing a suitable animal model for human disease (Kunze and Schauer 1977; Kunze 1979, 1994; Kunze and Chowaniec 1990). Unlike MNU, which exerts its carcinogenic effect directly on the urothelium following intravesical instillation and spontaneous decay, orally administered BBN needs to be metabolized enzymatically in the liver to become a urothelial carcinoen, the ultimate carcinogenic metabolite coming in contact with the transitional cell epithelium via the bloodstream (Druckrey et al. 1967).

Bladder cancer appears to be particularly suited to the study of the immunomodulating and possibly tumor-inhibiting effects of VAA, since nonspecific immuno-

therapy with bacillus Calmette-Guérin (BCG) is known to stimulate the natural immune system of the bladder remarkably both at a humoral and at a cellular level. This treatment has been consistently shown to reduce recurrences of low-grade and low-stage transitional-cell carcinomas and to control the formation of urothelial carcinoma in situ in humans (for review of the literature see Catalona and Ratliff 1990; Lamm 1994; Herr et al. 1995). On the basis of this clinical experience, we decided to choose an experimental model using a low dose of BBN that results in the development of mainly exophytic noninvasive papillary transitional-cell carcinomas. If VAA given at its immunomodulatory dose were capable of favorably influencing urothelial carcinogenesis by activating the immune defense system, this effect must become evident especially in low-grade and low-stage rather than in high-grade and high-stage cancers.

Materials and methods

Animals

Pathogen-free female Wistar rats with an initial weight of 190–210 g (Animal Breeding Farm Harlan-Winkelmann, Borchen, Germany) were used. Groups of three rats were housed in plastic cages and maintained under standardized conditions in an air-conditioned room at a temperature of 18–21°C and a relative humidity of 45%–55% in a professional animal facility on a 12-h light/dark cycle. The animals had free access to tap water and were fed a standard ground commercial diet (Altromin 1324; Altromin Company, Lage, Germany). The experimental protocol was approved by the “Animal Care Committee” of the Faculty of Medicine of the University of Göttingen.

Carcinogenesis

N-Butyl-N-(4-hydroxybutyl)-nitrosamine (BBN), prepared by Dr. Zelesny, Deutsches Krebsforschungszentrum Heidelberg, Germany, was dissolved in 1,2-propanediol as a vehicle and administered by gavage in three fractionated doses of 150 mg/kg body weight each (total dose per animal: 450 mg/kg body weight) at intervals of 24 h from the beginning of the experiment. For each gavage feeding, the rats received 1 ml freshly prepared carcinogen-containing solution. BBN was given in fractionated low doses rather than in a single high dose to prevent toxic effects causing death of the experimental animals.

Purification of galactoside-specific mistletoe lectin

Commercially available dried mistletoe leaves were extracted with phosphate-buffered saline. After centrifugation of the extract, the lectin was purified by affinity chromatography on lactosylated Sepharose 4B (obtained after the ligand had been coupled to divinyl-sulfone-activated resin) and gel filtration as described in detail previously (Gabius 1990). Purity was assessed by gel-electrophoretic analysis and N-terminal sequence analysis. Protein determinations with the Bradford reagent in microtiter plates were routinely performed with bovine serum albumin as a standard, and controls were also carried out to exclude the possibility of contamination by endotoxin (Gabius 1990; Gabius H.-J. et al. 1992).

Administration of galactoside-specific mistletoe lectin

Galactoside-specific mistletoe lectin (VAA) was dissolved in 20 mM sterile phosphate-buffered saline (pH 7.4) containing carbohydrate-