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Regulation of alloreactivity in the popliteal lymph node assay by the new immunosuppressants: malononitrilamides

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Abstract Malononitrilamides (MNAs) represent a new class of low molecular weight immunosuppressants and have been shown to prevent and reverse ongoing acute allograft rejection and effectively prolong xenograft survival in rodents. MNAs were also found to be potent inhibitors of B and T cell-mediated autoimmune processes and mediate their effects by binding specifically to dihydro-orotate dehydrogenase (DHODH), inhibiting de novo pyrimidine biosynthesis, thereby blocking T and B cell proliferation and strongly suppressing the IgM and IgG antibody production. Here we evaluated the effects of the MNAs (HMR 1279 and HMR 1715) on the in vivo lymphoproliferation that occurs after challenge with allogeneic cells in a local graft-versus-host reaction in Lewis × Brown Norway F_1 hybrid rats by measuring the enlargement of the popliteal lymph nodes (PLN) draining the site of allogeneic cell injection. Oral administration of the MNAs dose-dependently prevented the localized lymphoproliferative response in the PLN assay and suppressed the lymph node hyperplasia. The MNAs even acted therapeutically when they were given during an ongoing alloreactivity as late as days 4 or 5 after challenge. Consistent with the mode of action, a complete reversal of the immunosuppression on the lymphoproliferation in vivo was attempted in this protocol by the addition of exogenous uridine during days 0–5. These data suggest the HMR 1279 and HMR 1715 mediate their antiproliferative and immunosuppressive effects in the PLN assay in vivo by decreasing the activity of DHODH in the lymph node cells and thereby inhibiting pyrimidine biosynthesis.

Key words Immunosuppression · Alloreactivity · Malononitrilamides · Popliteal lymph node assay · Local graft-versus-host reaction

Introduction

Malononitrilamides (MNAs) represent a new generation of low molecular weight immunosuppressive agents and belong to the derivatives of leflunomide's primary metabolite A 771776. The MNAs (HMR 1279 and HMR 1715) are structurally unrelated and distinct in their mode of action to any other of the currently used immunosuppressants (Fig. 1). They have been shown to prevent and reverse established acute allograft rejection in various rodent models [5, 9] and effectively prolong xenograft survival [4, 7]. These derivatives of leflunomide's active metabolite have also been used effectively as immunosuppressive agents against certain types of B cell-mediated processes in rodent models of autoimmune diseases [6, 8, 10]. They mediate their effects by binding specifically to dihydro-orotate dehydrogenase (DHODH) and inhibiting de novo pyrimidine biosynthesis [11]. Thereby they block B and T cell proliferation and strongly suppress the IgM and IgG antibody pro-

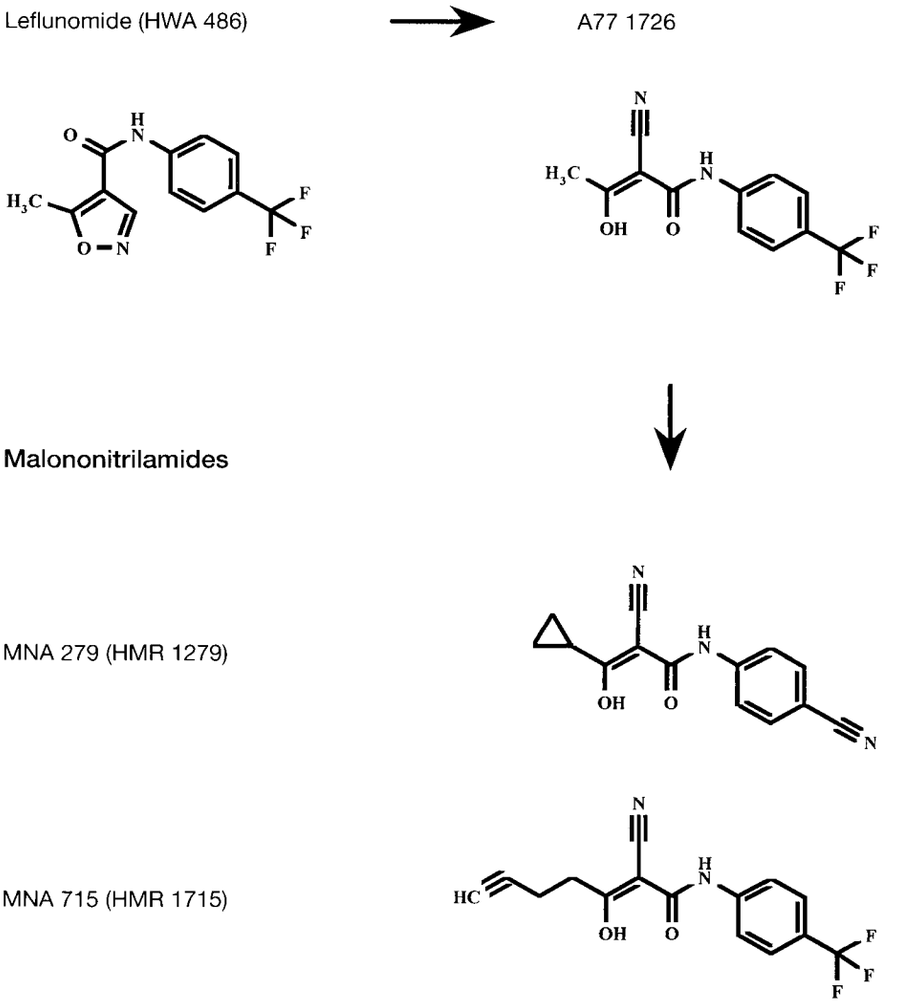

Fig. 1 Chemical structure of the malononitrilamides (MNAs) HMR 1279 and HMR 1715

duction [2, 3]. Prompted by their immunopharmacological abilities we evaluated the effects of HMR 1279 [2-cyano-N-(4-cyano-phenyl)-3-cyclopropyl-3-oxo-propan-amide] and HMR 1715 {2-cyano-3-hydroxy-N-[4-(trifluoromethyl)-phenyl]-6-heptynamide} on the in vivo lymphoproliferation that occurs after challenge in Lewis × Brown Norway F_1 hybrid rats by measuring the enlargement of the popliteal lymph nodes (PLN) draining the site of allogeneic cell injection.

Materials and methods

Animals

Lewis rats and Lewis × Brown Norway F_1 hybrid rats were all obtained from Charles River Wiga (Sulzfeld, Germany). All animals were fed a standard rat diet and drinking water ad libitum.

PLN assay

To test the alloreactivity in vivo, a localized T cell-dependent lymphoproliferative response to allogeneic cells in Lewis ↘ Brown Norway rats was examined. For this local graft-versus-host (GvH) reaction the PLN assay was used. This test is based on the enlargement of the draining lymph node as a result of injecting immunocompetent cells (1×10^8 parenteral Lewis spleen cells) into the hind foot pad of Lewis ↙ Brown Norway F_1 recipients. The PLN assay is usually measured at day 6 after challenge as a gain in lymph node weights and was performed as described by Ford et al. [1].

Substances

Both MNA 279 HMR 1279) and MNA 715 (HMR 1715) were supplied in pure form as a white powder (Hoechst Marion Roussel, Wiesbaden, Germany) and were prepared daily fresh as a homogenous suspension in 1% carboxymethyl cellulose (1% CMC) at varying concentrations. All the animals were given the test drugs each morning by gastric gavage and were treated with the drugs at the concentrations and schedules given in the text. Control animals received the vehicle solution (1% CMC) only.

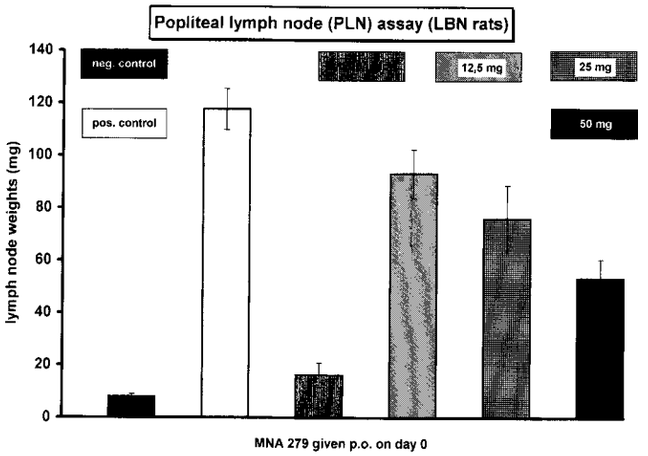

Fig. 2 Prevention of the localized lymphoproliferative response and popliteal lymph node (PLN) swelling by MNA 279. Animals were treated with 12.5, 25, or 50 mg/kg of the drug by oral gavage *(p.o.)* on day 0 together with the challenge and showed a dose-dependent inhibition of the lymph node hyperplasia. A therapy control group was treated with cyclophosphamide Norway *(cyclophosph.)* (50 mg/kg, i.v.). (*neg.* negative, *pos* positive, *LBN* Lewis × Brown Norway)

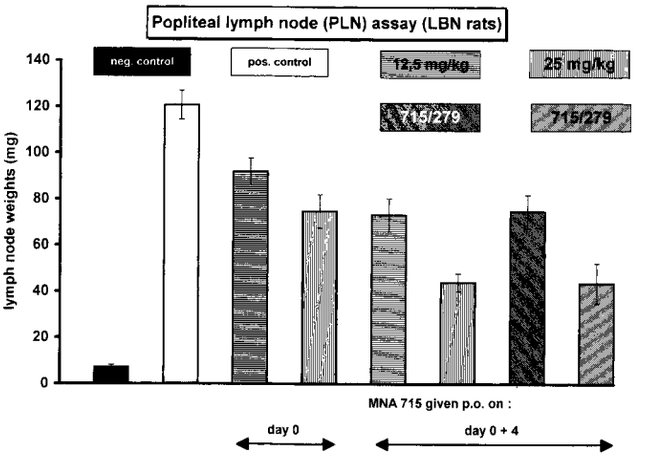

Fig. 3 Two oral administrations of MNA 715 (12.5 or 25 mg/kg) on days 0 and 4 relative to the challenge with spleen cells had a better inhibitory effect on PLN enlargement. Cross-over treatment with both MNAs was equally as effective as monotherapy with one MNA alone

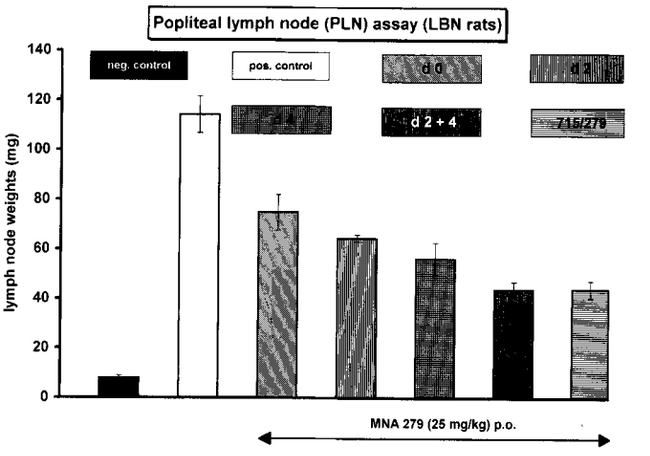

Fig. 4 Therapeutic effects of MNA 279 on the local graft-versus host reaction in the PLN assay. Even a single administration of 25 mg/kg either on day *(d)* 2, day 4, or days 2 and 5 resulted in a significant inhibition of this lymphoproliferative response. Later application seems to have better therapeutic activity in treating lymph node hyperplasia

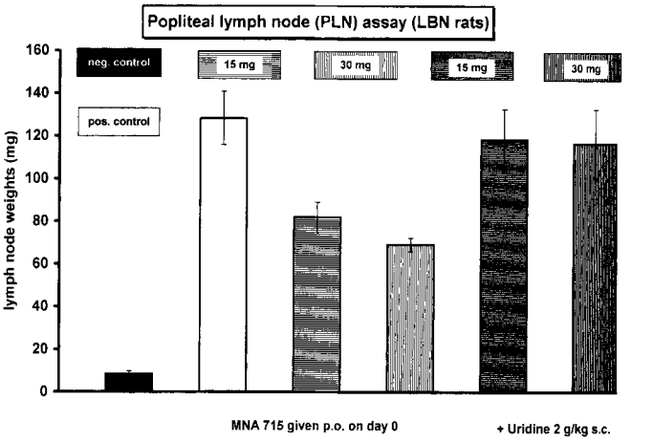

Fig. 5 Antiproliferative and immunosuppressive effects of MNA 715 (15 or 30 mg/kg) were completely antagonized when 2 g/kg of exogenous uridine were given in addition s.c. on days 0 to 5

Results and discussion

Prevention of alloreactivity in a local GvH reaction (PLN assay)

In this system there is an enlargement of the draining lymph node at the site of allogeneic cell injection relative to the node draining the syngeneic cell injection site. A single administration of either one of the two MNAs (12.5, 25, or 50 mg/kg), given by oral gavage on day 0 together with the challenge, dose-dependently prevented the localized lymphoproliferative response and suppressed the lymph node hyperplasia (Fig. 2). The development of in vivo alloreactivity and the PLN swelling was always inhibited, independently to the time when the nodes were harvested and the lymphoproliferation was measured. Two oral administrations of 25 mg/kg of the MNAs on days 0 and 4 relative to the challenge with spleen cells had a better and more

pronounced inhibitory effect on the development of the in vivo alloreactivity and showed a higher suppression of the PLN enlargement (Fig. 3). Cross-over treatment with both MNAs on days 0 and 4 was equally as effective as the monotherapy with one MNA alone. The lymph node hyperplasia in response to a localized injection of allogeneic cells was significantly suppressed. HMR 1279 and HMR 1715 abrogated ensuing local GvH reaction and inhibited PLN swelling in a dose-dependent manner as was measured by lowered weights.

Therapy of localized lymphoproliferative response in the PLN assay

Even a single administration of 25 mg/kg of the MNAs, when given orally during an ongoing alloreactivity as late as on day 4 or 5 after challenge with the alloantigen resulted in a clear inhibition of this lymphoproliferative response. Therapeutic treatment (later application) seems to have better efficacy in treating lymph node hyperplasia than earlier application. Both HMR 1279 and HMR 1715 acted therapeutically and only 1 day after drug application the PLN enlargement was already suppressed (Fig. 4). These results presented here clearly indicate that the new immunosuppressants, the low molecular weight MNAs, can even inhibit ongoing aberrant immune responses.

The suggested molecular mechanism by which MNAs inhibit alloreactivity in vivo

Here we demonstrate that HMR 1279 and HMR 1715 mediate their antiproliferative and immunosuppressive activity in vivo on the alloreactivity in a local GvH reaction in the PLN assay by decreasing the DHODH activity and inhibiting the de novo pyrimidine biosynthesis of the infiltrating lymphocytes. The prevention of PLN enlargement by the MNAs (15 or 30 mg/kg) was antagonized by uridine supplementation. Consistent with the mode of action, a complete reversal of the immunosuppression was attempted in this protocol by the addition of exogenous uridine during days 0–5 at 2 g/kg twice a day given subcutanously, thereby antagonizing the antiproliferative effects of the MNAs (Fig. 5). These data suggest that HMR 1279 and HMR 1715 mediate their antiproliferative and immunosuppressive effects in the PLN assay in vivo by decreasing the activity of DHODH in the lymph node cells and thereby inhibiting the pyrimidine biosynthesis.

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References

1. Ford WL, Burr W, Simonsen G (1970) A lymph node weight assay for the graft-versus-host activity of rat lymphoid cells. Transplantation 10: 258–266
2. Kurrle R, Bartlett RR, Ruuth E, Lauffer L, Schorlemmer HU (1996) Malononitrilamides inhibit T- and B-cell responsiveness. Transplant Proc 28: 3053–3056
3. Kurrle R, Ruuth E, Bartlett RR, Lauffer L, Schorlemmer HU (1997) Malononitrilamides inhibit T- and B-cell responsiveness in different species. Transplant Proc 29: 1302–1303
4. Lin Y, Segers C, Waer M (1996) Efficacy of the malononitrilamide X 920715 as compared with leflunomide in cardiac allo- and xenotransplantation in rats. Transplant Proc 28: 3036
5. Morris RE, Huang X, Cao W, Zheng B, Shorthouse RA (1995) Leflunomide (HWA 486) and its analog suppress T- and B-cell proliferation in vitro, acute rejection, ongoing rejection, and anti-donor antibody synthesis in mouse, rat, and cynomolgus monkey transplant recipients as well as arterial intimal thickening after balloon catheter injury. Transplant Proc 27: 445–447
6. Schorlemmer HU, Bartlett RR (1997) Malononitrilamides (MNA 279 and MNA 715) have therapeutic activity in acute and chronic relapsing experimental allergic encephalomyelitis (EAE). Inflamm Res 46: S163–S164
7. Schorlemmer HU, Kurrle R (1996) Control of mouse-to-rat skin xenograft rejection by malononitrilamides. Transplant Proc 28: 3037–3030
8. Schorlemmer HU, Brendel S, Bartlett RR (1996) Malononitrilamides prevent the development of murine systemic lupus erythematosus-like diseases in BDF1 hybrid mice and MRL/lpr autoimmune mice. Transplant Proc 28: 3040–3042
9. Schorlemmer HU, Schwab W, Ruuth E, Kurrle R (1996) Acute skin graft rejection can be prevented and treated in rat models by malononitrilamides. Transplant Proc 28: 3048–3050
10. Schorlemmer HU, Bartlett RR, Kurrle R (1997) Analogues of leflunomide's primary metabolite, the malononitrilamides, prevent the development of graft-versus-host disease. Transplant Proc 29: 1298–1301
11. Williamson RA, Yea CM, Robson PA, Curnock AP, Gadher S, Hambleton AB, Woodward K, Bruneau JM, Hambleton P, Moss D, Thomson TA, Spinella-Jaegle S, Morand P, Courtin D, Sautes C, Westwood R, Hercend T, Kuo EA, Ruuth E (1995) Dihydroorotate dehydrogenase is a high affinity binding protein for A 771726 and mediator of a range of biological effects of the immunomodulatory compound. J Biol Chem 270: 22467–22472