0022-3565/87/2401-0145$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 240, No. 1
Printed in U.S.A.

Ribavirin Inhibits Mast Cell Mediator Release[1]

DIANA L. MARQUARDT, HARRY E. GRUBER and LINDA L. WALKER
Department of Medicine, University of California, San Diego, San Diego, California
Accepted for publication October 9, 1986

ABSTRACT

Ribavirin (1-β-D-ribofuranosyl-1,2-4-triazole-3-carboxamide) is a promising antiviral agent as well as a structural analog of guanosine. Although at different concentrations it has been reported to induce either immunosuppression or immune stimulation, its effects upon immediate hypersensitivity reactions are largely unknown. Because purine metabolism appears to be important in mast cell secretion, the effects of ribavirin on mouse bone marrow-derived mast cell functions were investigated. When ribavirin was added to mast cells at the time of stimulation with A23187 or specific antigen, no effect on the release of β-hexosaminidase, a preformed mediator, was evident. However, mast cells cultured in 1 to 20 μM ribavirin for 1 to 7 days exhibited dose- and time-dependent inhibitions of stimulated β-hexosaminidase and leukotriene C_4 releases without altering mast cell mediator content. This inhibition occurred even when ribavirin had no effect on cell growth. A concomitant decrease in antigen-challenged mast cell intracellular Ca concentration was also observed after ribavirin treatment. Chronic ribavirin exposure *in vitro* inhibits mast cell secretory processes stimulated by both immunoglobulin E- and nonimmunoglobulin E-related signals. Its precise mechanism of action and any potential efficacy as an antiallergic agent remain to be elucidated.

Ribavirin has recently been reported to inhibit HTLV-III viral replication *in vitro* (McCormick *et al.*, 1985) and has proved to be effective in the clinical treatment of lassa fever (McCormick *et al.*, 1986) and respiratory syncytial virus infections (Hall *et al.*, 1983). However, the biochemical mechanism of its efficacy in viral illnesses is poorly understood, although proposed mechanisms have involved guanine nucleotides (Goswami *et al.*, 1979), RNA polymerase (Smith, 1984) or reverse transcriptase (McCormick *et al.*, 1985). In an immunologic sense, ribavirin (5–60 μg/ml) has also been reported to inhibit antigen- and mitogen-mediated lymphocyte proliferation (Jolley and Suchil, 1984), though much lower doses may have some immunostimulatory effects (Powers *et al.*, 1982). Given the cellular immune system alterations induced potentially by ribavirin, the effects of this agent on IgE-mediated immunologic events may be of interest as well. In support of this premise is the fact that purine nucleosides and nucleotides have been shown to be important as modulators of mast cell secretion (Marquardt *et al.*, 1978, 1984a,b), and ribavirin, with its structure similar to guanosine, or ribavirin triphosphate, speculated to be a competitive inhibitor of other nucleotides (Smith, 1984), could alter mast cell purine metabolism.

Mouse bone marrow cultured with a source of interleukin 3 produces a relatively pure culture of mast cells that contain histamine and β-hex and that generate small quantities of prostaglandin D_2 and larger amounts of LTC_4 upon appropriate stimulation (Razin *et al.*, 1982). This "atypical" mast cell population has proved to be useful in studying long-term exposure of various agents that alter mast cell mediator release by a variety of mechanisms (Marquardt *et al.*, 1984a, 1986). Therefore, this mast cell population was utilized in the studies described.

Methods

Chemicals. The following were purchased from the manufacturers indicated: ado, concanavalin A, N-acetyl-β-D-glucosaminide, 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO); RPMI-1640, penicillin/streptomycin, minimal essential medium nonessential amino acids, fetal calf serum, L-glutamine (GIBCO, Grand Island, NY); calcium ionophore A23187 (Calbiochem, La Jolla, CA); quin-2 (Amersham International, Amersham, UK) and [^{3}H]LTC_4 radioimmunoassay kit (New England Nuclear, Boston, MA).

The following were generously donated: deoxycoformycin (Developmental Therapeutics Program, Chemotherapy Division, NCI, Bethesda, MD); mouse hybridoma antiDNP IgE antibody and DNP-conjugated DNP-BSA antigen (F. Liu and D. Katz, La Jolla, CA) and ribavirin (ICN Nucleic Acid Research Institute, Costa Mesa, CA).

Mast cell culture conditions and media. Bone marrow obtained from Balb/C mice femurs was cultured in a 1:1 mixture of Razin media and conditioned media, produced by coculturing splenocytes from C57B1/6J and C3H mice in the presence of concanavalin A (Razin *et al.*, 1981). After weekly passaging and at least 15 days in tissue culture, the resulting cells were >90% pure mast cells and >95% viable as

Received for publication February 14, 1986.
[1] This work was supported by U.S. Public Health Service Grants AI-21504 and AM-35108 and the American Lung Association.

ABBREVIATIONS: IgE, immunoglobulin E; β-hex, β-hexosaminidase; LTC_4, leukotriene C_4; ado, adenosine; quin-2, quin-2 tetraacetoxymethyl ester; DNP, dinitrophenyl; DNP-BSA, DNP-bovine serum albumin.

assessed by Trypan blue exclusion. Viability was assessed further by lactic dehydrogenase releases, which were no different in ribavirin-treated and control cells. Cells exposed to ribavirin in culture were washed 3 times before use in experiments. Parallel cultures of cells grown in media alone were used as controls for pharmacologically manipulated mast cells. Cell growth was assessed by counting cells at particular time points and comparing actual numbers of ribavirin-treated cells to numbers of cells grown in media alone.

Assessment of mast cell mediator release. β-hex was chosen as a representative granule-associated, preformed mast cell mediator because it is easily quantitated and its release nearly identically parallels that of histamine (Schwartz *et al.*, 1979). Mouse bone marrow-derived mast cells were centrifuged at 200 × *g* for 5 min, washed 3 times in Tyrode's buffer lacking divalent cations, sensitized for 30 min at 37°C with antiDNP IgE (1 μg/10^6 cells) and challenged with either DNP-BSA antigen (175 ng/3 × 10^5 cells) or A23187 (1.0 μg/ml/3 × 10^5 cells) in 400 μl of complete Tyrode's buffer for 10 min at 37°C. Reaction mixtures were centrifuged at 200 × *g* for 10 min, and supernatant and pellet β-hex concentrations were assayed by the hydrolysis of *p*-nitrophenyl-β-D-glucosamide as described elsewhere (Schwartz *et al.*, 1979). Spontaneous β-hex release was determined in unchallenged cells. The net percentage of β-hex released is defined as follows:

$$\text{net \% } \beta\text{-hex release} = \frac{\text{supernatant } (\beta\text{-hex})}{\text{supernatant } (\beta\text{-hex}) + \text{pellet } (\beta\text{-hex} - \text{spontaneous } (\beta\text{-hex}) \text{ release}}$$

When exogenous ado was present in reaction mixtures, it was added simultaneously with the secretagog.

Mast cell supernatant LTC_4 concentrations were quantitated as an example of a generated mediator. Cells were prepared and challenged as described above for β-hex release except that the reactions were allowed to proceed for 20 min before centrifugation. Supernatants were stored at −20°C for up to 4 weeks before immunoreactive LTC_4 concentrations were assessed utilizing a [^{3}H]LTC_4 radioimmunoassay kit. This radioimmunoassay identifies LTC_4, some LTD_4 and a small amount of LTE_4 and compares favorably with high performance liquid chromatography assessment of leukotrienes in this relatively pure, serum-free cell system (Razin *et al.*, 1983). Ten microliter aliquots of each supernatant sample were used in the assay, which is accurate between 0.025 and 1.6 ng of LTC_4/100 μl.

Assessment of mast cell intracellular free calcium. Mast cells are suspended in Hank's balanced salt solution containing 1 mM $CaCl_2$ and 0.5 mM Mg^{++} in a volume of 500 μl containing 100 μM quin-2. After 20 min at 37°C the final volume is increased to 5 ml and antiDNP IgE antibody is added for an additional 40 min. After 2 washes, cells are placed in cuvettes and stirred at 37°C to assess quin-2 fluorescence on a spectrofluorometer (White *et al.*, 1984). Basal tracings are established over 2 to 5 min, and 200 ng of DNP-BSA antigen are added to the cuvette during continuous recording, with a stable quin-2 fluorescence identified over the next 2 to 5 min. The addition of Triton to lyse the cells is done to establish a maximal-free Ca^{++} level, and the addition of 10 mM ethylene glycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid to chelate the calcium established a minimum level of less than 5 nM calculated-free Ca^{++}. The intracellular calcium concentration in resting or stimulated cells is calculated using the formula:

$$(Ca^{++}) = \frac{115 \times 10^{-9} \text{ M } (F - F_{min})}{(F_{max} - F)}$$

where F is the quin-2 fluorescence reading obtained and the K_D for quin-2/Ca^{++} is 115 × 10^{-9} M as determined in this system. pH was controlled in the presence of ethylene glycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid by the addition of HCl.

Statistical analysis. Statistical significance was assessed utilizing the paired, two-tailed Student's *t* test. Results are expressed as means ± S.E. unless otherwise specified.

Results

β-hex release from ribavirin-treated mast cells. Mouse bone marrow-derived mast cells challenged with A23187 or DNP-BSA antigen released 8 to 15% of total cell β-hex, a preformed, granule-associated mediator. In the additional presence of 10^{-5} M ado, mediator release increases to 20 to 40% as described previously (Marquardt *et al.*, 1978). Ribavirin (10 μM) added at the time of mast cell stimulation does not affect β-hex release or did a 60-min preincubation with ribavirin. However, mast cells incubated for 3 to 7 days in 10 μM ribavirin, washed and challenged with A23187 with or without exogenous ado exhibited a marked attenuation of β-hex release compared to parallel cells cultured in media alone (fig. 1). Ribavirin exposure did not alter mast cell mediator content (*i.e.*, total cell β-hex concentration) or cell viability, and spontaneous release of β-hex was similar in the two cell groups. The dose-response relationship between ribavirin exposure and preformed mediator release is depicted in figure 2. Although 1 μM ribavirin for 6 days inhibits mediator release significantly, maximal inhibition is evident between 10 and 20 μM. This continues to hold true when 10^{-5} M ado is added to augment mediator release and allow the identification of alterations in ado responsiveness induced by ribavirin.

The time course of ribavirin's inhibitory effects on mast cell mediator release was studied by adding 10 μM ribavirin to cultured cells and challenging the cells after 1 to 6 days of ribavirin treatment. After only 24 hr of exposure, a decrease in mast cell mediator release was observed that plateaued between 3 and 6 days (fig. 3). Spontaneous β-hex release remained the same throughout the time period.

Mast cell growth was inhibited by chronic ribavirin exposure,

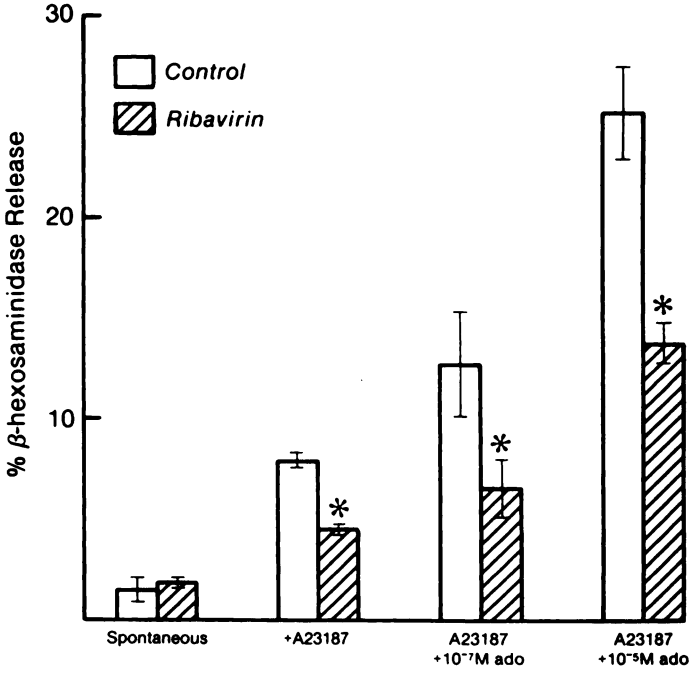

Fig. 1. β-hex release from control and ribavirin-treated mast cells. Mouse bone marrow-derived mast cells cultured for 3 to 7 days in media alone (□) or 10 μM ribavirin (▨) were challenged with the Ca ionophore with or without the additional presence of ado. The percentages of β-hex release from resting and stimulated cells are shown as means ± S.E. of duplicate values from 7 experiments. *Significantly different from control cells ($P < .05$). Similar results were obtained with DNP-BSA antigen stimulation of antiDNP IgE-sensitized mast cells.

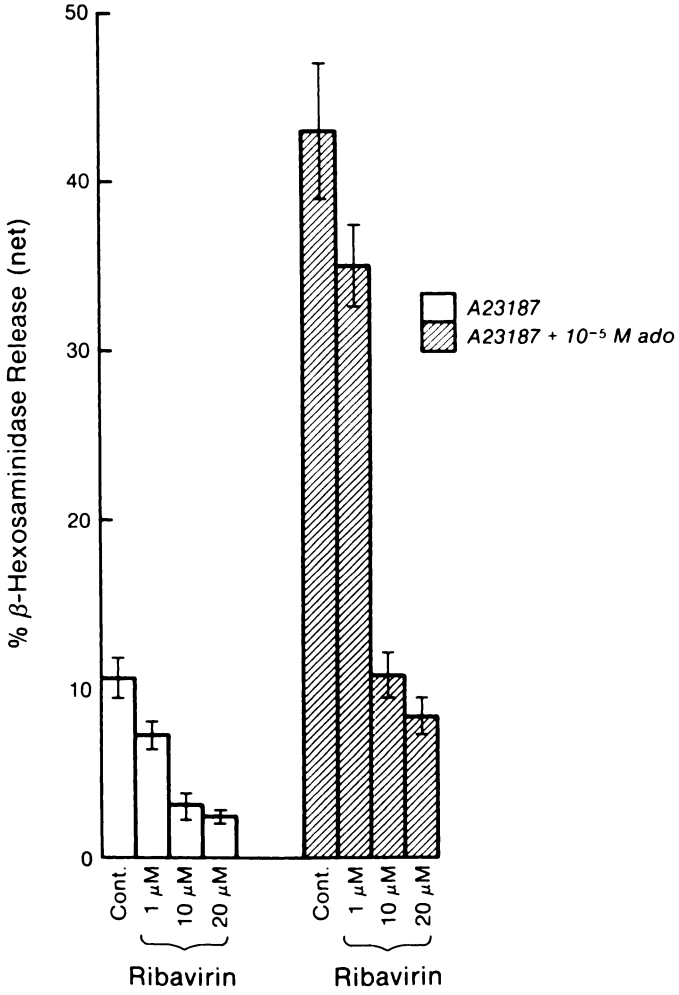

Fig. 2. Dose-response effects of ribavirin on mast cell β-hex release. Mast cells were cultured in media alone (controls) or 1, 10 or 20 μM ribavirin for 6 days, washed and challenged with A23187 (□) or A23187 + 10^{-5} M adenosine (▨), and net β-hex release was quantitated. Ribavirin-treated cells at all concentrations tested released significantly less β-hex when challenged with A23187 with or without 10^{-5} M adenosine ($P < .05$). Mediator content and spontaneous release were no different in control and ribavirin-exposed cells. Depicted are means ± S.E. of duplicate determinations from 3 experiments.

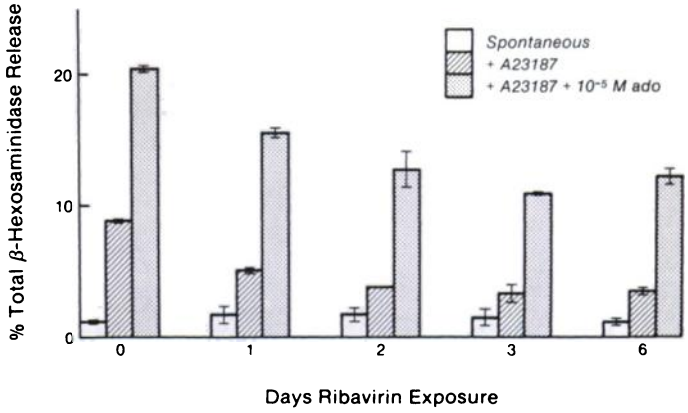

Fig. 3. Time course of ribavirin inhibition of mast cell mediator release. Ribavirin (10 μM) was added to cultured mast cells for the number of days indicated with parallel cultures of cells of the same age and from the same mice used as controls (*i.e.*, 0 days ribavirin exposure). Total β-hex release from cells that were resting (□), A23187-stimulated (▨) and A23187-stimulated in the presence of 10^{-5} M adenosine (□) was measured. All stimulated values in ribavirin-exposed cells (days 1–6) were statistically different from control cells (day 0) ($P < .01$). Spontaneous release of β-hex were not significantly different at any time point ($P > .1$). Shown are means ± S.E. of values from 3 experiments.

TABLE 1

Cell growth *vs.* mediator release with ribavirin exposure

Ribavirin Exposure	Cell Growth	A23187-induced β-hex Release
days	*% of Control*	*% of Control*
1	78 ± 2.5	58 ± 2.3
2	99 ± 0.3	43 ± 2.4
3	75 ± 1.9	38 ± 3.2
6	51 ± 5.2	40 ± 2.1

by approximately 50% after 6 days. However, the inhibition of mast cell mediator release described did not require a concomitant attenuation of cell growth and cannot be explained solely on this basis (table 1).

LTC_4 production in ribavirin-treated cells. The release of immunoreactive LTC_4 from antiDNP IgE-sensitized, DNP-BSA antigen-stimulated mast cells provides a measure of arachidonic acid metabolism and lipoxygenase product generation. LTC_4 is the primary mouse bone marrow mast cell arachidonic acid product, and its generation is at times dissociated from mast cell degranulation. Specifically, exogenous ado potentiates mast cell histamine and β-hex releases without affecting LTC_4 production (Marquardt *et al.*, 1984a). The effect of long-term ribavirin exposure on mast cell LTC_4 release was studied in a way identical to that described for β-hex release except that reactions proceeded for 20 min after antigen challenge and supernatant LTC_4 concentrations were quantitated by a radioimmunoassay that correlates well with high performance liquid chromatography measurements of LTC_4 in these cells (Razin *et al.*, 1983). Resting supernatant LTC_4 levels were nearly identical in ribavirin-grown and control cells. Antigen challenge resulted in a release of nearly 50 ng of $LTC_4/10^6$ control cells, but only 25 ng of $LTC_4/10^6$ ribavirin-treated cells. The additional presence of exogenous ado (10^{-4}–10^{-10} M) did not significantly augment LTC_4 levels in either cell population (fig. 4). Ribavirin added at the time of secretagog or with a 60-min preincubation did not affect stimulated LTC_4 production.

Ca fluxes in ribavirin-treated mast cells. Quin-2 fluorescent dye was used to quantitate alterations in intracellular-free Ca^{++} concentrations in control and ribavirin-exposed mast cells. Mouse bone marrow-derived mast cells exhibit relatively low resting intracellular-free Ca^{++} levels that quadruple 1 to 2 min after antigen stimulation (fig. 5). Ribavirin-treated mast cells demonstrate modestly but statistically significantly greater basal intracellular-free Ca^{++} concentrations than control cells, and the increase in Ca^{++} after antigen challenge is only one-half as large as that seen in control cells.

Discussion

In addition to its actions on lymphocyte proliferation (Jolley and Suchil, 1984) and erythrocyte viability (Shulman, 1984), ribavirin has now been demonstrated to inhibit stimulated mast cell mediator release in an ado-independent fashion (fig. 1). The concentrations of ribavirin used here *in vitro* are close to levels obtained with a standard oral regimen of 15 mg/kg/day (1.7–5.3 μM in plasma) (McCormick *et al.*, 1986) or peak plasma levels of 2.7 to 13.2 μM after inhaled drug (Hall *et al.*, 1983). Although a precise mechanism of action has not been identified,

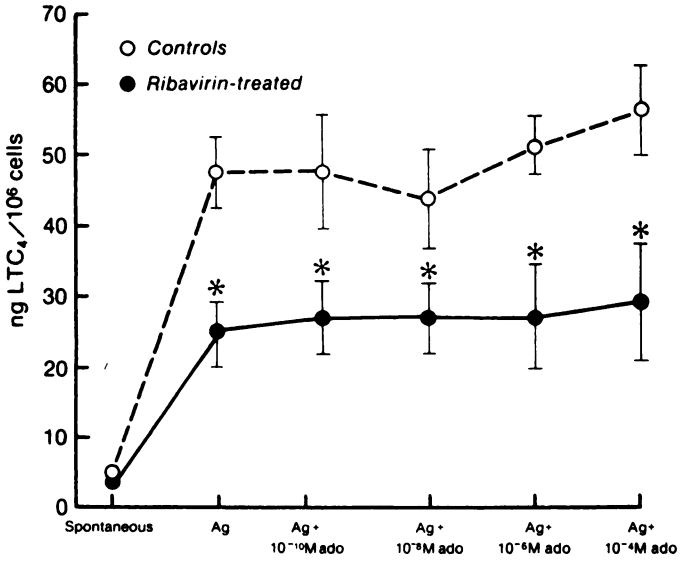

Fig. 4. LTC_4 release from control and ribavirin-treated mast cells. Mast cells cultured from 4 to 6 days in the presence of media alone (O) or media containing 10 μM ribavirin (●) were sensitized with antiDNP IgE and challenged with DNP-BSA antigen. Immunoreactive LTC_4 concentrations in cell supernatants were assessed in the absence and presence of exogenous adenosine. Shown are means ± S.E. of duplicate determinations from 5 experiments. *Statistically different from controls ($P < .05$).

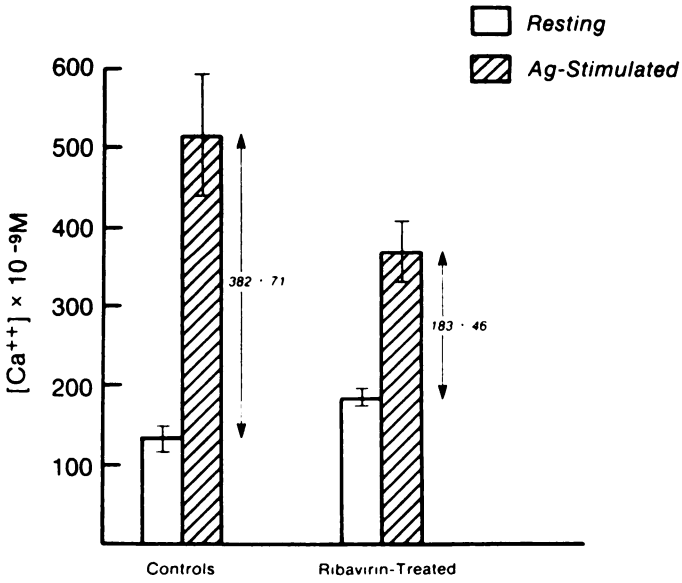

Fig. 5. Ribavirin-induced changes in mast cell calcium fluxes. Intracellular-free Ca concentrations in resting (□) and DNP-BSA antigen-stimulated (▨) control and ribavirin-exposed mast cells were assessed using quin-2. Resting ribavirin-treated cells' intracellular Ca^{++} concentrations were significantly higher and antigen-stimulated Ca^{++} concentrations significantly lower ($P < .05$ in each case) than comparable values in control cells. The differences between resting and stimulated Ca^{++} concentrations in the two cell populations were also statistically significant ($P < .01$). Depicted are means ± S.E. of values from 9 experiments.

it has been established that the inhibition of β-hex release requires chronic (>24 hr) exposure (fig. 3) and is dose-dependent with an optimal concentration of approximately 10 μM ribavirin (fig. 2). The effect does not appear to be due to alterations in IgE receptors, as similar inhibition is evident using both IgE and nonIgE-mediated stimuli. The mediator release inhibition does not depend on a concomitant inhibition of mast cell growth (table 1) or alterations in cell mediator content, spontaneous mediator release ("leak") or cell viability.

The generation of mouse bone marrow-derived mast cell arachidonic acid metabolites as assessed by measuring immunoreactive LTC_4 concentrations in cell supernatants is attenuated by ribavirin to a similar degree as β-hex release (fig. 4). Thus, although ado itself appears to exhibit differential effects on degranulation and mediator generation in mast cells (Marquardt *et al.*, 1984a), ribavirin under these experimental conditions acts on both processes to a similar degree. This provides additional evidence that ribavirin does not exert its inhibitory effects *via* ado receptors.

Mast cell resting and stimulated intracellular-free Ca^{++} concentrations were studied in ribavirin-treated cells because calcium influx has been associated with mast cell mediator release induced by many, but not all, stimuli (Beaven *et al.*, 1984b; White *et al.*, 1984). Coinciding with the generation of the calcium signal is the breakdown of phosphatidylinositol to inositol phosphates, although the precise interaction of these two events remains uncertain (Beaven *et al.*, 1984a). Agents that directly activate protein kinase C may induce mediator release without exhibiting this augmentation of quin-2 fluorescence (Castagna *et al.*, 1982). Because the reduction of intracellular Ca^{++} influx in ribavirin-grown cells is nearly identical to the reduction in mast cell mediator releases (fig. 5), one might predict that the ribavirin effect is a relatively early one in the cascade of biochemical events associated with mast cell secretion. However, whether ribavirin itself directly affects the mast cell calcium signal, phosphatidylinositol metabolism or the molecules that themselves alter calcium fluxes is unknown. Clearly many biochemical processes are at least indirectly affected by ribavirin exposure.

How ribavirin might alter mast cell function is still open to speculation. The hypothesis that ribavirin in some way alters mast cell purine metabolism is interesting in that ribavirin has been reported to be a guanosine analog in other systems (Goswami *et al.*, 1979) and ribavirin could enter purine metabolic pathways in mast cells *via* the enzymatic action of ado kinase as does 5-amino-4-imidazolecarboxamide riboside, a compound of similar structure (Marquardt and Gruber, 1985). The subsequent production of ribavirin triphosphate could result potentially in competition between this nucleotide and ado triphosphate. Although resting ado triphosphate levels in ribavirin-treated cells do not differ substantially from controls (data not shown), competition at an undefined regulatory site is conceivable. The time required for ribavirin to exert its actions could suggest a need for protein synthesis, perhaps of an inhibitory protein of some type. Clearly these modes of action of ribavirin are only speculation and further studies of mast cell purine metabolism and the complex series of biochemical events involved in mast cell mediator release may lead to a better understanding of agents that alter these processes.

One cannot directly compare mast cells derived from mouse bone marrow to human lung mast cells, but the two populations have numerous similarities in terms of mediators and pharmacologic responses (Srendi *et al.*, 1983). In any case, the identification of ribavirin as an apparently nontoxic mast cell inhibitor may have interesting implications as this drug comes into more widespread use as an antiviral agent. The possibility that ribavirin could possess some antiallergic or antiasthmatic efficacy remains to be seen.

Acknowledgments

The authors thank Dr. Steve Wasserman for advice and Ms. Kim Finley for technical assistance.

References

BEAVEN, M. A., MOORE, J. P., SMITH, G. A., HESKETH, T. R. AND METCALFE, J. C.: The calcium signal and phosphotidylinositol breakdown in 2H3 cells. J. Biol. Chem. **259:** 7137–7142, 1984a.

BEAVEN, M.A., ROGERS, J., MOORE, J. P., HESKETH, T. R., SMITH, G. A. AND METCALFE, J. C.: The mechanism of the calcium signal and correlation with histamine release in 2H3 cells. J. Biol. Chem. **259:** 7129–7136, 1984b.

CASTAGNA, M., TAKI, Y., KAIBUCHI, K., SANO, K., KIKKAWA, K. AND NISHIZUKA, Y.: Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J. Biol. Chem. **257:** 7847–7851, 1982.

GOSWAMI, D. B., BOREK, E., SHARMA, O. K., FUJITAKI, J. AND SMITH, R. A.: The broad spectrum antiviral agent ribavirin inhibits capping of RNA. Biochem. Biophys. Res Commun. **89:** 830–836, 1979.

HALL, C. B., MCBRIDE, J. T., WALSH, E. E., BELL, D. M., GALA, C. L., HILDRETH, S., TEN EYCK, L. G. AND HALL, W. J.: Aerosolized ribavirin treatment of infants with respiratory syncytial viral infection: A randomized double-blind study. N. Engl. J. Med. **308:** 1443–1447, 1983.

JOLLEY, W. B. AND SUCHIL, C.: Effects of ribavirin on the immune system (second report). *In* Clinical Applications of Ribavirin, ed. by R. A. Smith, V. Knight and J. A. D. Smith, pp.93–96, Academic Press, New York, 1984.

MARQUARDT, D. L. AND GRUBER, H. E.: Inhibition of mast cell mediator release by 5-amino-4-imidazolecarboxamide riboside (AICAR) (Abstract). Fed. Proc. **44:** 1323, 1985.

MARQUARDT, D. L., GRUBER, H. E. AND WASSERMAN, S. I.: Adenosine release from stimulated mast cells. Proc. Natl. Acad. Sci. U.S.A. **81:** 3978–3982, 1984b.

MARQUARDT, D. L., PARKER, C. W. AND SULLIVAN, T. J.: Potentiation of mast cell mediator release by adenosine. J. Immunol. **120:** 871–878, 1978.

MARQUARDT, D. L., WALKER, L. L. AND WASSERMAN, S. I.: Adenosine receptors of mouse bone marrow-derived mast cells: Functional significance and regulation by aminophylline. J. Immunol. **133:** 932–937, 1984a.

MARQUARDT, D. L., WALKER, L. L. AND WASSERMAN, S. I.: Cromolyn inhibition of mouse bone marrow-derived mast cell mediator release. Am. Rev. Respir. Dis. **133:** 1105–1109, 1986.

MCCORMICK, J. B., KING, I. J., WEBB, P. A., SCRIBNER, C. L., CRAVEN, R. B., JOHNSON, K. M., ELLIOTT, L. H. AND BELMONT-WILLIAMS, R.: Lassa fever. Effective therapy with ribavirin. N. Engl. J. Med. **314:** 20–26, 1986.

MCCORMICK, J. B., MITCHELL, S. W., GETCHELL, J. P. AND HICKS, D. R.: Ribavirin suppresses replication of lymphadenopathy-associated virus in cultures of human adult T lymphocytes. Lancet **2:** 1367–1369, 1985.

POWERS, C. N., PEAVY, D. L. AND KNIGHT, V.: Selective inhibition of functional lymphocyte subpopulations by ribavirin. Antimicrob. Agents Chemother. **22:** 108–114, 1982.

RAZIN, E., CORDON-CARDO, C. AND GOOD, R. A.: Growth of a pure population of mouse mast cells *in vitro* with conditioned medium derived from concanavalin A-stimulated splenocytes. Proc. Natl. Acad. Sci. U.S.A. **78:** 2559–2561, 1981.

RAZIN, E., MENCIA-HUERTA, J. M., LEWIS, R. A., COREY, E. J. AND AUSTEN, K. F.: Generation of leukotriene C_4 from a subclass of mast cells differentiated *in vitro* from mouse bone marrow. Proc. Natl. Acad. Sci. U.S.A. **79:** 4665–4667, 1982.

RAZIN, E., MENCIA-HUERTA, J. M., STEVENS, R. L., LEWIS, R. A., LIU, F.-T., COREY, E. J. AND AUSTEN, K. F.: IgE-mediated release of leukotriene C_4, chondroitin sulfate E proteoglycan, β-hexosaminidase, and histamine from cultured bone marrow-derived mouse mast cells. J. Exp. Med. **157:** 189–201, 1983.

SCHWARTZ, L. B., AUSTEN, K. F. AND WASSERMAN, S. I.: Immunologic release of β-hexosaminidase and β-glucuronidase from purified rat serosal mast cells. J. Immunol. **123:** 1445–1450, 1979.

SHULMAN, N. R.: Assessment of hematologic effects of ribavirin in humans. *In* Clinical Applications of Ribavirin, ed. by R. A. Smith, V. Knight and J. A. D. Smith, pp. 79–92, Academic Press, New York, 1984.

SMITH, R. A.: Background and mechanisms of action of ribavirin. *In* Clinical Applications of Ribavirin, ed. by R. A. Smith, V. Knight and J. A. D. Smith, pp. 1–18, Academic Press, New York, 1984.

SRENDI, B., FRIEDMAN, M. M., BLAND, C. E. AND METCALFE, D. D.: Ultrastructural, biochemical, and functional characteristics of histamine-containing cells cloned from mouse bone marrow: Tentative identification as mucosal mast cells. J. Immunol. **131:** 915–922, 1983.

WHITE, J. R., ISHIZAKA, T., ISHIZAKA, K. AND SHA'AFI, R. I.: Direct demonstration of increased intracellular concentration of free calcium as measured by quin-2 in stimulated rat peritoneal mast cell. Proc. Natl. Acad. Sci. U.S.A. **81:** 3978–3982, 1984.

Send reprint requests to: Diana L. Marquardt, M.D., c/o University of California San Diego Medical Center, H-811-G, 225 Dickinson Street, San Diego, CA 92103.