Vol. 82, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 14, 1978 Pages 840-846

ANTIMETABOLIC ACTIVITIES OF 2-FLUORO-L-HISTIDINE

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Received April 14, 1978

Summary: 2-Fluoro-L-Histidine inhibits protein synthesis in various cell cultures, as measured by ^{3}H-leucine incorporation. This histidine analog also inhibits the cytopathogenicity of a number of RNA and DNA viruses in primary and continuous cell cultures; it blocks the transformation of normal mouse (MO) cells by murine sarcoma virus, and partially suppresses the release of murine leukemia virus by a continuously infected mouse cell line (JLSV5). In human skin fibroblasts, it reduces the interferon-inducing capacity of poly(I)·poly(C). Inhibition of cell protein synthesis may be the common cause of the various effects. 4-Fluoro-L-histidine is essentially inert in all of the test systems examined.

Fluorine-substituted aromatic and heteroaromatic compounds often display interesting and useful biological properties. The physiological activity of ring-fluorinated analogs is not surprising in view of the dimensions of the fluorine atom, the covalent and van der Waals radii of which are not much larger than those of the hydrogen atom; however, replacement of hydrogen by fluorine creates changes in electron-density distribution which may significantly alter enzyme-substrate or receptor-substrate interactions.

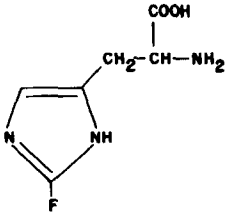
A large number of fluorinated amino acids (1) and nucleosides (2) have been synthesized, typical examples of which are p-fluorophenylalanine (FPhe) and 5-fluoro-2'-deoxyuridine (FUdR). In bacterial systems, FPhe is incorporated at random into proteins at the expense of phenylalanine, without discrimination among different sites in the polypeptide chain (3). FPhe has also been shown to inhibit both interferon production (4) and interferon action (5). FUdR functionally resembles uridine, being phosphorylated in the cell to its 5'-monophosphate (FdUMP). FdUMP competitively blocks thymidylate

Abbreviations: FPhe, p-fluorophenylalanine; 2-FHis, 2-fluoro-L-histidine; 4-FHis, 4-fluoro-L-histidine; PFU, plaque-forming units; VSV, vesicular stomatitis virus; HSV-1, herpes simplex virus (type 1); Coxs. B4, Coxsackie (type B4); MSV, murine (Moloney) sarcoma virus; PRK, primary rabbit kidney; VERO, African green monkey kidney cell line; MO, a 3T3 mouse cell line; HSF, human skin fibroblast; JLSV5, a Balb/c mouse cell line carrying murine (Rauscher) leukemia virus.

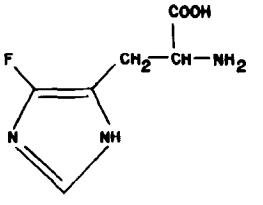
0006-291X/78/0823-0840$01.00/0

synthetase, the enzyme that converts dUMP to dTMP. FUdR itself inhibits the multiplication of various DNA viruses, at least in cell cultures (6).

We had previously shown that substitution of a fluorine atom for the amino group at C-5 of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR) results in an analog with broad-spectrum antiviral properties (7). This antiviral activity appeared to parallel an inhibition of host cell RNA and DNA synthesis (7). We now report studies with two other fluoroimidazoles, 2-FHis and 4-FHis. While 4-FHis showed only minimal activity, 2-FHis was found to inhibit cytopathogenicity of a variety of RNA and DNA viruses; this antiviral activity correlates quite well with an inhibition of host cell protein synthesis, and may be a consequence of such inhibition.



2-fluorohistidine



4-fluorohistidine

MATERIALS AND METHODS:

Compounds. 4-FHis was prepared according to our published method (8). A modification of the published procedure (9,10) was used for the synthesis of 2-FHis. α-N-Trifluoroacetyl-L-histidine methyl ester was coupled with an aryldiazonium salt at C-2, and the product was hydrogenolyzed to the 2-amino-histidine derivative. The latter material was diazotized and irradiated in fluoroboric acid solution. The protected fluorohistidine was isolated by extraction and, finally, the protecting groups were removed by alkaline hydrolysis. Before use, the fluorohistidines were dissolved in phosphate-buffered saline (0.01 M in 0.14 M NaCl, pH 7.2) at 1 mg/ml, and the solutions were stored at 4° throughout the course of the experiment. DL-FPA was purchased from Serva Feinbiochemica, Heidelberg, Germany. L-Leucine [^{3}H-4] (30 Ci/mmole) was obtained from the Centre d'Etudes Nucleaires de Saclay (Gif-sur-Yvette, France).

Cell cultures (PRK, HeLa, VERO), viruses (VSV, HSV-1, vaccinia, Coxs. B4, polio I, measles) and the methodology for measuring inhibition of virus-induced cytopathogenicity have been described previously (7), as has the microtechnique for measuring inhibition of infectious center formation in MO cells inoculated with murine (Moloney) sarcoma virus (11).

Inhibition of Cellular Protein Synthesis. To measure the effects on cellular protein synthesis, confluent cell cultures (in 60mm Falcon plastic petri dishes), which had or had not been infected with virus, were exposed to 100 μg/ml of the compounds in Eagle's minimal essential medium (12) supplemented with 3% calf serum. The cultures were incubated for 24 h or 72 h at 37°, were washed (3x) with Eagle's medium, and were then incubated for 30 min at 37° with [^{3}H-4]leucine (1 μCi/ml medium/petri dish). The cells were further processed for determination of acid-insoluble radioactivity (7).

TABLE 1

Effect of 2-Fluorohistidine and 4-Fluorohistidine on Virus-induced Cytopathogenicity in Various Cell Cultures

Virus	Cell Culture	Minimal Inhibitory Concentration (µg/ml)	
		2-Fluorohistidine	4-Fluorohistidine
VSV	PRK	30	100
HSV-1	PRK	30	>100
Vaccinia	PRK	30	>100
VSV	HeLa	30	>100
Coxs. B4	HeLa	30	>100
Polio I	HeLa	30	>100
Measles	VERO	10	>100
Coxs. B4	VERO	30	>100

The minimal inhibitory concentration is defined as the lowest concentration of compound inhibiting cytopathic effect by 50%. The cell cultures were inoculated with 100 cell culture-infecting dose-50 of virus for 1 h at 37° and, immediately thereafter, were exposed to the compounds. Viral cytopathic effect was recorded as soon as it reached 100% in the control cell cultures: 2 days with VSV in PRK or HeLa; 2 days with Coxs. B4 or polio I in HeLa; 3 days with HSV-1 or vaccinia in PRK and with Coxs. B4 in VERO; and 7 days with measles in VERO cells.

Oncornavirus shedding by the transformed cell line JLSV5 was monitored by determination of virion-associated reverse transcriptase activity (13). JLSV5 cell cultures were also employed for measuring VSV multiplication (11). In HSF cell cultures, interferon production was measured according to a recently described superinduction scheme (14). In vivo antiviral activity was assessed in mice inoculated intranasally with either HSV-1 (12 day-old mice) or VSV (20 day-old mice). These experimental herpes and rabies model infections have been fully documented in earlier reports (15,16).

RESULTS AND DISCUSSION:

2-FHis, when added to PRK, HeLa, or VERO cell cultures immediately after these cells had been infected with either VSV, HSV, vaccinia, polio, Coxsackie or measles virus, completely arrested the development of viral cytopathogenicity at a concentration of 100 µg/ml; 4-FHis showed no effect (Table 1). No microscopically visible alteration of cell morphology was witnessed at this concentration unless the cells were incubated with 2-FHis for 3 days or longer, after which period shrinking or flaking occurred. Quite similar results were obtained in MO cells which had been inoculated with MSV and, immediately thereafter, treated with either 2-FHis or 4-FHis. At 100 µg/ml, 2-FHis completely suppressed virus-induced focus formation (Fig. 1); at the same concentration, 4-FHis showed no inhibitory effect.

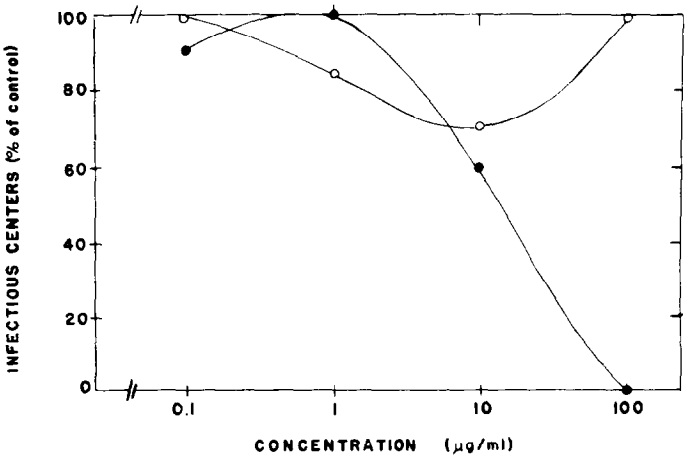
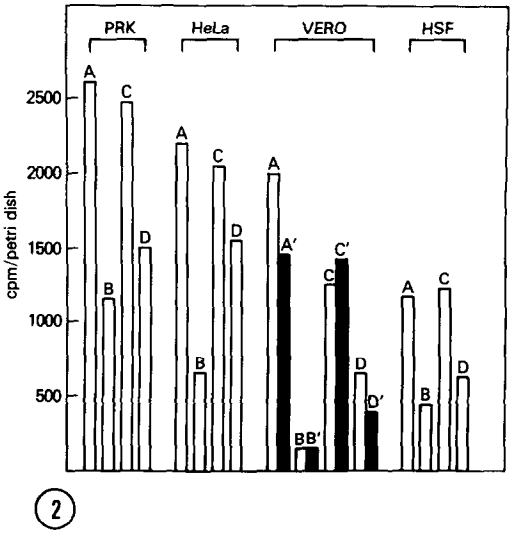


Figure 1. Effect of 2-fluorohistidine (•) and 4-fluorohistidine (o) on the development of infectious centers in MO cells infected with MSV.

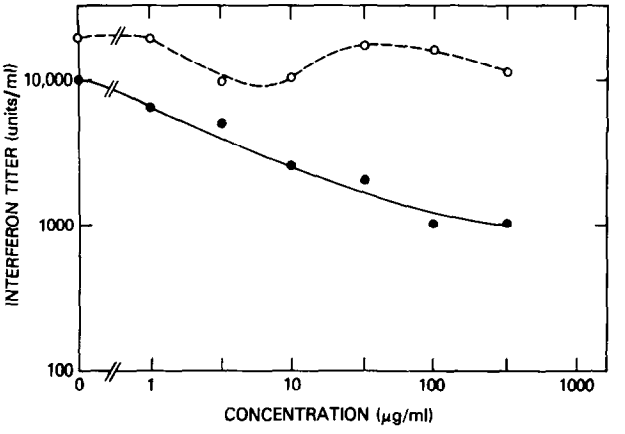
In rationalizing the antiviral activity of 2-FHis, one might expect the analog to be incorporated into the growing polypeptide chain and/or to inhibit protein synthesis, in analogy with FPhe. 2-FHis did, indeed, inhibit protein synthesis, as monitored by [^{3}H-4]leucine incorporation in either PRK, HeLa, VERO, or HSF cell cultures (Fig. 2). The effect was more pronounced after 72 h than after 24 h contact of 2-FHis with the cells and, in all types examined, 2-FHis proved distinctly superior to FPhe in inhibiting protein synthesis. Under the same conditions, 4-FHis exhibited little, if any inhibitory activity (Fig. 2). Virus-infected cells did not differ markedly from uninfected cells with regard to the extent of protein synthesis inhibition by the fluorinated amino acids (Fig. 2). In all experiments shown, the analogs were used at 100 µg/ml; at 10 µg/ml, no inhibitory effects on protein synthesis could be observed.

When exposed to JLSV5 cells, a transformed cell line harboring Rauscher leukemia virus, 2-FHis (at 100 µg/ml) effected a 20% suppression in the release of reverse transcriptase-containing virus particles. This inhibitory effect on oncornavirus shedding by JLSV5 cells cannot be interpreted as specific, since 2-FHis was also found slightly effective (ca. 7%) in curtailing the replication of an exogeneous virus (VSV) in these cells. 2-FHis, at 10 µg/ml, or 4-FHis, at either concentration, failed to affect either oncornavirus production or VSV multiplication in JLSV5 cells.

Similarly to earlier results reported for FPhe (4), 2-FHis counteracted the interferon-inducing activity of poly(I)·poly(C) in human skin fibroblasts



(2)



(3)

Figure 2. Inhibition of protein synthesis in various cell cultures, as monitored by [^{3}H-4]leucine incorporation: A, control; B, 2-FHis; C, 4-FHis; D, FPhe; A'-D', inoculated with measles virus. Cell cultures were incubated with 100 μg/ml of the compound for 72 h, at which time [^{3}H-4]leucine was added. Assays were performed after 30 min at 37°; values shown are averages for 2 to 6 petri dishes.

Figure 3. Effect of 2-fluorohistidine on interferon production in HSF cells induced with poly(I)·poly(C) and superinduced with cycloheximide and actinomycin D. Before induction with poly(I)·poly(C) (100 μg/ml), the cells were exposed for 24 h to varying concentrations of 2-FHis, dissolved in Eagle's minimal essential medium containing histidine (——) or free of histidine (---).

(Fig. 3), but only after the cell cultures had been depleted in a histidine-free medium. 4-FHis did not affect interferon production, even when applied in a histidine-free medium.

The *in vivo* antiviral potentials of 2-FHis were explored in mice, infected intranasally with either HSV-1 or VSV. These experimental model infections have been employed previously (15,16) to assess the prophylactic and/or therapeutic efficacy of a number of potentially useful antivirals. Despite its inhibitory effects in cell culture (Table 1), intraperitoneally injected 2-FHis failed to protect mice against a lethal HSV-1 or VSV challenge. When mice were given a total amount of 1 mg of 2-FHis spread over 5 daily doses of 0.2 mg, the first of which was administered immediately after virus inoculation, they died at the same rate as untreated mice. At a higher level (5 mg/mouse), 2-FHis itself proved 100% lethal for both 12 day- and 20 day-old mice, whether administered as a single dose or as repeated doses of 1 mg each.

Two major features emerge from the findings reported herein: (1) 2-FHis

shows distinct antimetabolic and antiviral properties, while 4-FHis fails to show such effects. This sharp distinction between the isomeric analogs has been observed previously in studies with E. coli (7); while no explanation, based on structural or electronic properties, can yet be offered for the difference, the problem is under active investigation. (2) At concentrations which correspond quite well to those required for antiviral activity, 2-FHis inhibits cellular protein synthesis; the antiviral activity does not appear to be particularly specific, and may be a direct consequence of the inhibition of host cell protein synthesis. A similar relationship had been established (7) with 5-fluoroimidazole-4-carboxamide ribonucleoside, the antiviral activity of which appears to result from an inhibition of host cell DNA and RNA synthesis. Exploratory studies on the mechanism of inhibition of protein biosynthesis indicate that 2-FHis is incorporated into newly synthesized proteins at the expense of histidine (18), and that the resulting protein "analogs" may be nonfunctional. The analog also appears to suppress histidine biosynthesis in E. coli (work in progress) and either, or both, pathways must be considered.

ACKNOWLEDGMENTS:

This investigation was supported, in part, by grants from the Belgian F. G. W. O. (Fonds voor Geneeskundig Wetenschappelijk Onderzoek) and the Katholieke Universiteit Leuven (Fonds Derde Cyclus). The technical assistance of Anita Van Lierde, Miette Stuyck, Frieda De Meyer, Chris Dillen, Francine Cornette, and Rene Conings is gratefully acknowledged.

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