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BL-20803, a New, Low-Molecular-Weight Interferon Inducer

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BL-20803 induced interferon in mice when administered via oral or parenteral routes. During multiple dosing with the drug at 48-h intervals, animals exhibited a hyporesponsive state to the third treatment. Inhibition of vaccinia virus infection was correlated with interferon induction.

Most chemical agents thus far described which stimulate interferon production in animals are compounds of relatively high molecular weight (6). Interesting and possibly important exceptions are certain fluorenones, a class of low-molecular-weight compounds which elicit high levels of circulating interferon in mice (5). We report on another low-molecular-weight interferon inducer, BL-20803 (Fig. 1), which is 4(3-dimethylaminopropylamino)-1,3-dimethyl-1H-pyrazolo [3,4-b] quinoline dihydrochloride (7).

After oral or parenteral administration of BL-20803 to 15-g female CD-1 mice (Charles River Breeding Laboratories), there appeared in mouse serum within 6 h a virus-inhibitory activity which protected mouse L cells against infection by vesicular stomatitis virus (VSV) or mouse picornavirus GD7 (Fig. 2). Activity broadly peaked from the 16th to the 24th h, declined rapidly thereafter, and was not detectable by 40 h after dosing. That this antiviral factor is interferon was confirmed by the following criteria: (i) it was species-specific and failed to protect either human or hamster cells from viral challenge; (ii) it did not sediment at 100,000 $\times$ *g*; (iii) it was stable to prolonged incubation at pH 2 at 4 C; and (iv) it was readily degraded by the proteolytic enzyme trypsin (8).

The magnitude of the circulating interferon response in mice was dose-dependent. At 400 mg/kg orally or 300 mg/kg intraperitoneally, the compound elicited from 1,000 to 10,000 units of interferon per ml of mouse serum as measured by a plaque reduction assay. Less than 5,000 units was usually obtained at lower dose levels, and no activity was detectable below 200 mg/kg by the oral or 100 mg/kg by the intraperitoneal route of administration of the drug. The capacity of BL-20803 to suppress vaccinia virus-induced tail lesions was closely related to its ability to elicit interferon (Table 1). The drug showed antiviral activity only to 200 mg/kg, the minimal dose necessary to elicit a significant circulating interferon response.

Mice rapidly became hyporesponsive to repeated stimulation by interferon inducers (2). The development of the hyporesponsive state to BL-20803 was compared with the response to diethylaminoethyl-fluorenone (Tilorone; 5) and polyinosinic-polycytidylic acid (poly I:C; 4). In our hands, the doses chosen were nontoxic and elicited consistently high circulating interferon production which peaked at or near the times when the animals were sacrificed for assay (see legend of Fig. 3). Whereas animals became hyporesponsive to restimulation after a single encounter with Tilorone or poly I:C, this refractory state was not reached until sometime during the response to the second treatment with BL-20803 and became overt after the third dose (Fig. 3). In fact, concentrations of interferon in the serum stimulated by the second dose of the compound appeared to be almost twice as high as that elicited by the first dose. The same delay in expression of the hyporesponsive state was also observed when BL-20803 was followed by either Tilorone or poly I:C (not shown in Fig. 3).

The acute toxic LD_{50} of BL-20803 in mice is 250 mg/kg intraperitoneally and 1,600 mg/kg orally. Therapeutic ratios are therefore calculated as 2.5 and 8, respectively. Structural relatives have been synthesized in an effort to increase activity and improve therapeutic ratios. Detailed studies on their properties will be reported in a subsequent paper.

We gratefully acknowledge our debt to Robert Gardier, who

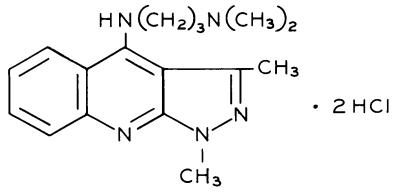

FIG. 1. *Structure of BL-20803.*

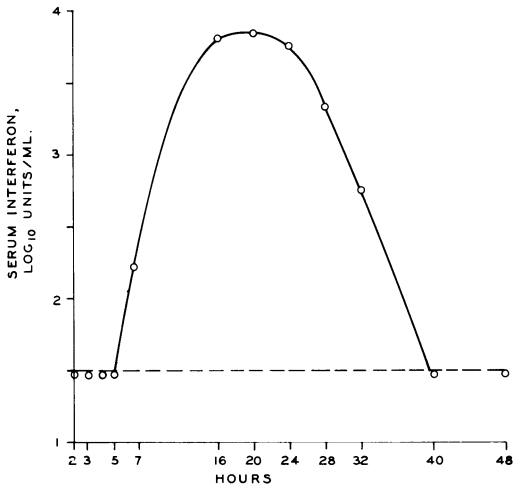

FIG. 2. *Time course of appearance of circulating interferon after oral administration of BL-20803 to mice at 400 mg/kg. (Virtually identical kinetics are observed at 300 mg/kg given intraperitoneally.) The lower limit of sensitivity of the assay as denoted by the broken line is 1.5 $\log_{10}$ units/ml due to nonspecific interference from mouse serum.*

TABLE 1. *Relationship between interferon-eliciting activity and supression of vaccinia virus infection by BL-20803*[a]

Dose (mg/kg)	Interferon (units/ml)	Percent reduction of tail lesions
400	12,600	89
200	3,802	84
100	<32	16
50	<32	0

[a] Mice at each dose level were given BL-20803 orally. Twenty hours later, three mice from each group were bled out and serum was assayed for interferon by the plaque reduction procedure (1) employing VSV infection of a clonal derivative of the mouse L cell. The remaining mice, including an undosed group of six, were infected via the tail vein with a sufficient input of vaccinia virus IHD strain to produce 20 to 30 tail lesions per mouse (3). Lesions were enumerated 7 days later. Reduction in the lesion score of greater than 50% is considered significant.

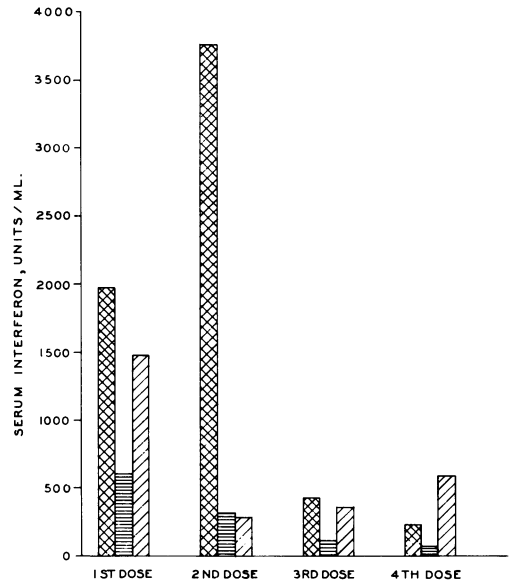

FIG. 3. *Effect of repeated dosing of mice with inducers on interferon production. Each compound was given at 48-h intervals, and three mice were sacrificed for interferon assay 24 h after receiving either BL-20803 or Tilorone or 6 h after receiving poly I:C. The bars in each set represent (left to right): BL-20802, 400 mg/kg orally; Tilorone, 400 mg/kg orally; and poly I:C, 15 mg/kg intraperitoneally.*

observed that unusual basophilic granules induced by BL-20803 in peripheral rat lymphocytes resembled those caused by diethylaminoethyl-fluorenone (M. Rohovsky et al., Toxicol. Appl. Pharmacol. **17**:556, 1970). The observation stimulated the studies reported in this paper.

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