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ASSAY OF HISTIDINE DECARBOXYLASE INHIBITORS IN HUMAN PLASMA*

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Abstract—A technique has been devised for assay of plasma levels of histidine decarboxylase inhibitors in man. It is based upon determination of the power of plasma to inhibit the activity of a preparation of histidine decarboxylase *in vitro* after administration of a drug. This method, which should be generally applicable to assay of all histidine decarboxylase inhibitors, was used to study plasma levels of brocresine and D-2 hydrazino-3-4 (5) -imidazole propionic acid (MK – 785) after oral administration of various single doses. Levels obtained with MK-785 were much higher than with equivalent doses of brocresine. Both the rate at which peak plasma levels were attained and the rate of disappearance from plasma were more rapid with brocresine than with MK-785.

THE BIOSYNTHESIS of histamine in mammalian tissues is accomplished by decarboxylation of the precursor amino acid, histidine; this process is catalyzed by a specific enzyme, histidine decarboxylase. The administration of histidine decarboxylase inhibitors to rats is associated with depletion of rapidly turning over pools of histamine from tissue.[1] Thus, histidine decarboxylase inhibitors have proved useful as tools for exploring possible physiologic and pathologic functions of histamine in the rat.[2] Recently, studies have been initiated on the effects of administration of histidine decarboxylase inhibitors to humans. In man, it is hoped that these agents may prove useful not only as research tools but also as therapeutic agents in some of the many diseases in which histamine is thought to play a pathogenetic role.[3]

This paper describes a method for assay of plasma levels of histidine decarboxylase inhibitors. The method is based upon estimation of the power of plasma to inhibit histidine decarboxylase activity after administration of a drug. This approach to the assay of drug levels was chosen because some histidine decarboxylase inhibitors are so potent that concentrations sufficient to produce significant inhibition *in vitro* would probably defy detection by ordinary chemical means. Because it is based upon histidine decarboxylase inhibition, this method should be generally applicable to assay of plasma levels of any histidine decarboxylase inhibitor. This method was applied to studying plasma levels of D-2 hydrazino-3-4 (5) -imidazole propionic acid and brocresine after oral administration of single doses to normal subjects.

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MATERIALS AND METHODS

The drugs used in these studies and their sources were: D-2 hydrazino-3-4(5)-imidazole propionic acid (MK-785), supplied by Merck Institute for Therapeutic Research; and 4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate (NSD-1055, CL-54, 998, brocresine) supplied by Lederle Laboratories.

Histidine decarboxylase was purified partially from whole fetal rats by a modification[4] of the method of Håkanson.[5] The enzyme preparation was stored at — 20° until used for assay. Assay of enzyme activity was performed by a previously described method.[4] This method is based upon incubation of the enzyme with ^{14}C-histidine labeled in the carboxyl-carbon in the presence of excess cofactor and trapping in hydroxide of Hyamine the $^{14}CO_2$ evolved for assay in a Packard Tri-Carb scintillation spectrometer. In these experiments incubations were done in final volumes of 2·0 ml; 0·8 ml of the water usually added to incubation mixtures[4] was replaced with plasma. Blanks consisted of complete incubation mixtures to which brocresine was added to a final concentration of 0·1 mM.

Plasma from some subjects who had received no drug was found to produce small degrees of histidine decarboxylase inhibition; this inhibition was generally small (0–10 per cent) but in some cases was greater. In these experiments, this inhibition was equated to zero by designating the control (pretreatment) value for any subject as 0 per cent inhibition.

The subjects of these studies were twenty-seven normal volunteers, ages 20–46, of which fourteen were white females; two negro females; and eleven white males. Venous blood was aspirated into 10-ml heparinized Vacutainer tubes (3200 KA) before and at various times after ingestion of drug. Plasma was separated by centrifugation and stored at — 20° until assay.

RESULTS

In preliminary experiments, MK-785 and brocresine were dissolved in pooled human plasma; serial dilutions were made by addition of plasma. Inhibition of histidine decarboxylase activity by drugs dissolved in plasma *in vitro* is illustrated in Figs. 1 and 2.

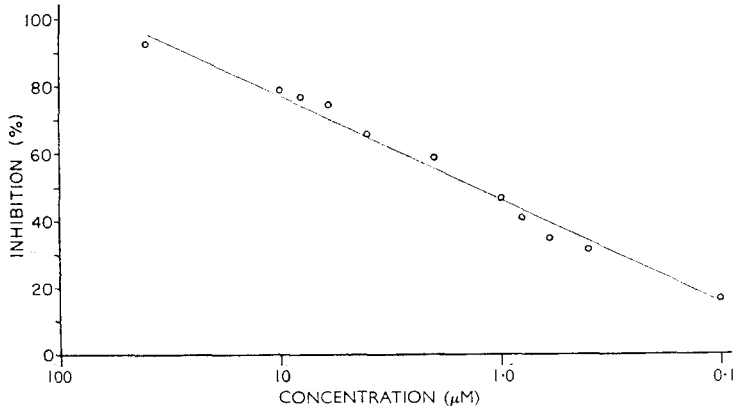

FIG. 1. Inhibition of histidine decarboxylase activity by MK-785 dissolved in plasma *in vitro*. The concentration designated on the abscissa is the concentration of MK-785 in plasma, of which 0·8 ml was added to the incubation mixture to a final volume of 2·0 ml.

For MK-785, histidine decarboxylase inhibition was a linear logarithmic function of drug concentration in plasma over a range of 0·1 to 100 μM. For brocresine, the range of concentration that could be studied reliably was smaller, ranging from 0·1 to 1·0 μM. These curves were used to standardize subsequent studies on plasma levels of histidine decarboxylase inhibitors *in vivo*.

The results of assay of histidine decarboxylase inhibitors in plasma *in vivo* are expressed in Figs. 3 through 6 as concentrations of either brocresine or MK-786. However, it must be emphasized that the results do not necessarily represent true concentration of the drugs in plasma. For example, it is possible that there may be some metabolic or chemical derivatives of the parent drugs which also inhibit histidine decarboxylase activity and which would influence the results of this procedure (see discussion).

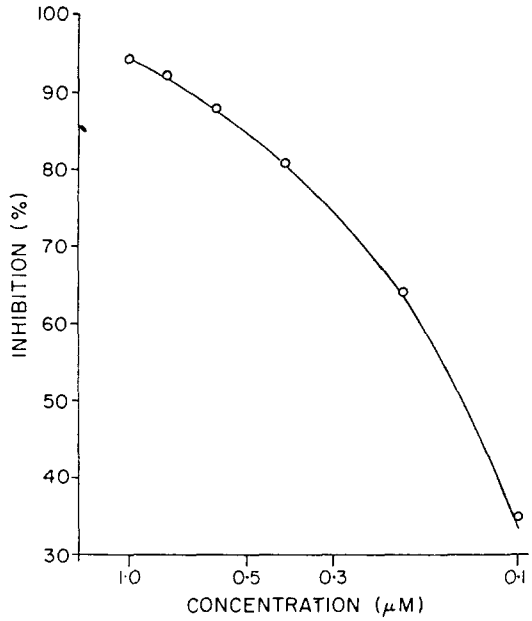

FIG. 2. Inhibition of histidine decarboxylase activity by brocresine dissolved in plasma *in vitro*. The concentration designated on the abscissa is the concentration of brocresine in plasma, of which 0·8 ml was added to the incubation mixture to a final volume of 2·0 ml.

Figures 3 and 4 illustrate plasma levels of MK-785 and brocresine resulting from oral administration of single doses. These figures illustrate that the plasma levels obtained with oral administration of these drugs vary substantially between individuals This variation was more striking with MK-785 than with brocresine.

Figures 5 and 6 illustrate plasma levels of MK-785 and brocresine after oral adminstration of various doses of these compounds. Again the differences between individual are noted. These studies revealed some major differences between the two compounds. Peak plasma levels of MK-785 were much higher than those obtained with equal doses of brocresine. For example, after an oral dose of 50 mg of MK-785, plasma levels reached a peak of 0·5 to 0·6 μM, while double that dose of brocresine produced

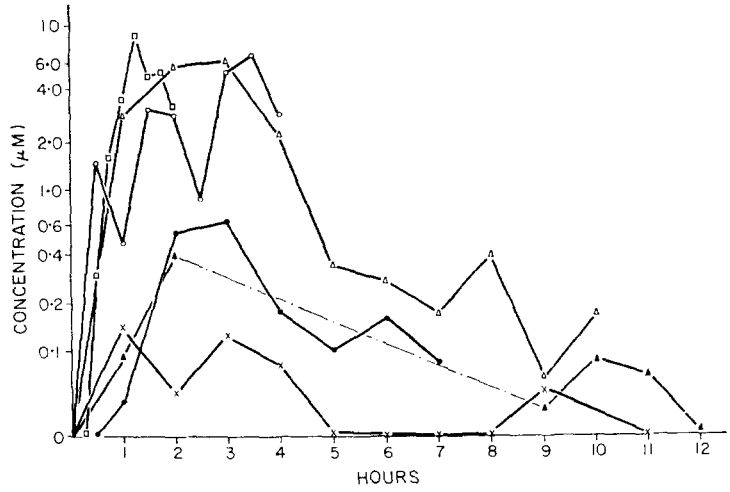

FIG. 3. Concentrations of MK-785 in plasma of six individuals at various times after oral administration of a single dose of 200 mg at zero time.

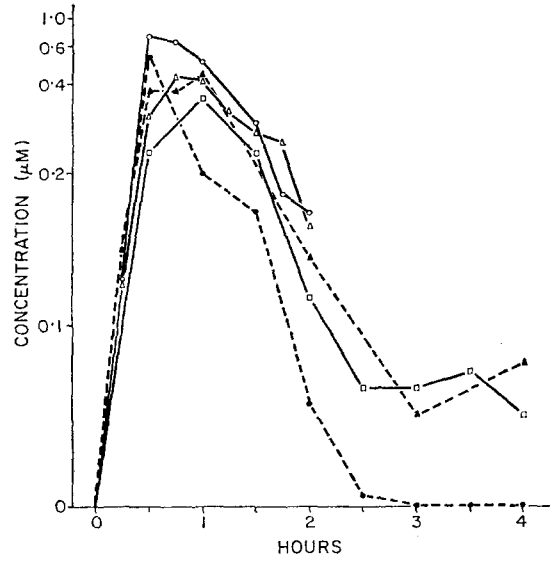

FIG. 4. Concentrations of brocresine in plasma of five individuals at various times after oral administration of a single dose of 400 mg at zero time.

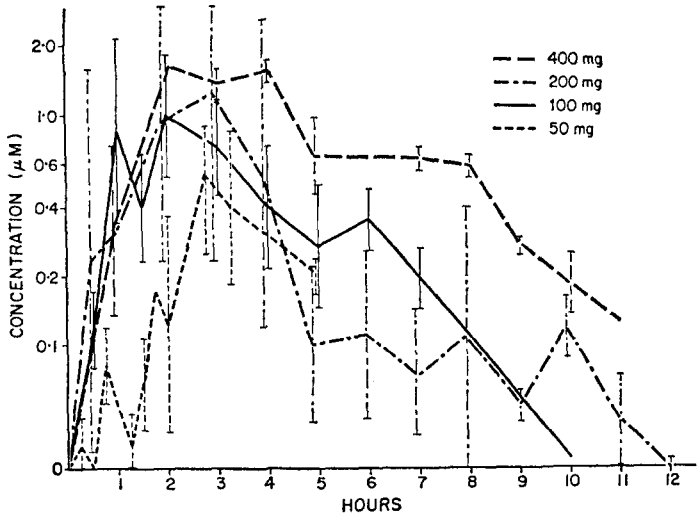

FIG. 5. Plasma levels of MK-785 at various times after oral administration of various single doses. The data are plotted as mean concentrations in all subjects studied. At those points at which results are available from three or more individuals, there are vertical brackets indicating mean deviations (sum of deviations from the mean divided by number of observations).

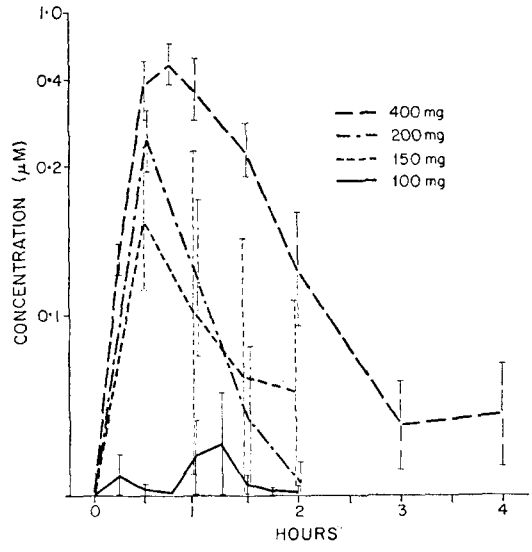

FIG. 6. Plasma levels of brocresine at various times after oral administration of various single doses. The data are plotted as in Fig. 5.

plasma levels that were barely detectable. Peak levels in plasma were reached much earlier with brocresine (30–45 min) than with MK-785 (2–4 hr). Also, it is apparent that brocresine disappeared from plasma much more rapidly than did MK-785.

DISCUSSION

The results of these studies indicate the feasibility of assaying levels of histidine decarboxylase inhibitors in plasma by determining the enzyme-inhibiting potency of plasma after drug administration. We are not aware of other methods having been reported for assay of these agents in plasma. The reasons that this approach was taken to assay of histidine decarboxylase inhibitors and the advantage of general applicability of this type of assay were commented on in the introduction to this paper. One disadvantage of this type of assay is that it is not clear whether it is the drug itself that is being assayed or some chemical or metabolic derivative that also inhibits histidine decarboxylase activity. An example of the sort of chemical derivative that might be involved is the oxime formed by reaction of brocresine with the cofactor for histidine decarboxylase, pyridoxal phosphate; though less potent than brocresine, it still is an effective histidine decarboxylase inhibitor.[6] Brocresine is somewhat less effective an inhibitor of histidine decarboxylase activity in the presence of plasma than in the absence of plasma; this indicates either that brocresine may bind to plasma protein or that it may react with some other normal constituent of plasma to form a product that is less active as an inhibitor of histidine decarboxylase activity.

The availability of this method to assay plasma levels of histidine decarboxylase inhibitors should prove to be a useful adjunct to clinical trials of these drugs. Before this technique became available, studies were done on the effects of brocresine in normal subjects[7] and in patients with various diseases.[7–9] The dose used in these studies was either 200 or 400 mg every 8 hr. The present studies indicate that, although the doses were probably sufficiently large, the frequency of administration was probably too small to maintain effective plasma concentrations of the drug. It seems likely that more effective inhibition of histidine decarboxylase activity might be achieved by administering brocresine at 4-hr intervals[10, 11] or by using a drug that persists longer in plasma such as MK-785. The necessity for maintaining high plasma concentrations of inhibitors in order to produce sustained inhibition of histidine decarboxylase activity *in vivo* is suggested by recent papers indicating very rapid turnover of the enzyme protein; e.g. in gastric mucosa of the rat, the half-life of the enzyme has been estimated at 100[12] to 126[13] min.

It has been noted previously that the efficacy of a histidine decarboxylase inhibitor *in vivo* does not necessarily correlate well with its potency *in vitro*. For example, although brocresine is much more potent than MK-785 *in vitro*, the two drugs are approximately equally effective in rats in decreasing histamine levels in tissues and in urine.[1] Perhaps such discrepancies are related, at least in part, to the rates at which the drugs are cleared from plasma.

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