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(54) Titre : COMPOSITION PHARMACEUTIQUE A BASE DE FLAVOPEREIRINE ET SON UTILISATION CONTRE LE VIH

(54) Title: FLAVOPEREIRINE-BASED PHARMACEUTICAL COMPOSITION AND USE THEREOF FOR TREATING HIV

(57) Abrégé/Abstract:
A composition comprising flavopereirine, or a salt or other pharmacologically acceptable derivative thereof, as the only active principle. The composition is a solid containing around 250-500 mg of the active principle, which may be administered orally in daily doses of around 1-3g. Said composition may be used for treating humans affected by HIV-1 and people with AIDS.

ABSTRACT OF THE DISCLOSURE

A composition comprising flavopereirine, or a salt or other pharmacologically acceptable derivative thereof, as the only active principle. The composition is a solid containing around 250-500 mg of the active principle, which may be administered orally in daily doses of around 1-3g. Said composition may be used for treating humans affected by HIV-1 and people with AIDS.

FLAVOPEREIRINE-BASED PHARMACEUTICAL COMPOSITION AND USE THEREOF FOR TREATING HIV

This invention relates to the antiviral usage of flavopereirine. More specifically, it relates to a pharmaceutical preparation whose sole active ingredient is flavopereirine, and to the use of this preparation for the treatment of viral infections in humans - in particular, infections such as those provoked by the Human Immunodeficiency Virus (HIV).

Flavopereirine is an alkaloid of the beta-carboline class. It is also traditionally referred to as "H or PB 100 composition," and shows UV emission fluorescence at 250-254 and 306 nm.

Flavopereirine may be obtained from the peel (bark) of the Pao Pereira Geissospermum vellosii-Baillon Apocynaceae [see H. Rapoport et al. (J. Am. Chem. Soc. 80, 1601-1608 (1958)); and M. Beljanski and 1, Bugiel: request for first Certificate of Addition # 79 05853 (published March 10, 1980; Publication No. 2,450,607) to French Patent 78 07155, and the document EP-A-0 059 817].

It is known that flavopereirine, administered intracutaneously at a dosage of 200-600 μg or at a dosage of 2.5 - 500 mg/day, preferably 30 mg/day, prevents the appearance and development of viral papules in the case of viruses of the Shope fibrome type and of vaccine.

It is also known that flavopereirine appears to act in vivo against the influenza (RNA) virus, and that it may moreover inhibit the multiplication of the tobacco mosaic virus (TMV) after brief contact with this virus.

Document EP-A-0 059 817 reveals, moreover, that flavopereirine is active against the influenza virus; however, the half-life of a quaternary beta-carboline of this type is too short for efficient use in humans in a galenic form other than

French Patent 88 15845 describes a system for improving immune defense in humans (against RNA viruses - AIDS in particular and DNA viruses). According to this document, the inhibition of the multiplication of the viruses in question is possible only by a combination of four different substances, of which flavopereirine is only one. The pharmaceutical preparation revealed in the document must include at least one representative of each of these four categories of active substances. The flavopereirine included in this combination is administered at a dosage of 0.25 g/day, preferably orally.

It has now unexpectedly been discovered that flavopereirine may on its own represent an effective active agent in the struggle against HIV viruses in mammals, including humans. More specifically, it has been found that flavopereirine is an active agent which on its own, whether in vitro or in vivo, exerts a selective inhibitive action on viral HIV infection, particularly in patients infected by HIV-1.

In one aspect, the invention provides a pharmaceutical composition for the treatment of human immunodeficiency virus (HIV) comprising: flavopereirine, a pharmaceutically acceptable salt thereof, or a derivative thereof, as the sole active ingredient, and a pharmaceutically-acceptable carrier.

In another aspect, the invention provides a pharmaceutical composition as described above wherein the sole active ingredient is present in an amount of about 250 to 500 mg per unit dose.

In a further aspect, the invention provides a pharmaceutical composition as described above, formulated as a tablet or capsule.

The composition is ideally in solid form containing approximately 500 mg of flavopereirine, or one of its salts or other acceptable pharmaceutical derivatives, per dose.

In another aspect, the invention provides use of such a pharmaceutical composition for the treatment of HIV or for the manufacture of a medicament for the treatment of HIV.

These and other aspects will be clarified in this description. The trials and experiments discussed here are given only as illustrations, and do not restrict the scope of the invention in any way.

Flavopereirine is devoid of toxic or side effects in mammals, including humans. The LD50 in Sprague-Dawley EOPS rats is 10.45 g/kg (safety limits: 9.63 - 11.35) orally, and 2.45 g/kg

(safety limits: 2.35 - 2.55) intraperitoneally. The animal dies within 30 to 60 minutes of oral or intraperitoneal administration by respiratory arrest. No change in mortality rates was noticed during the subsequent 14 days.

A sub-chronic oral administration in male and female Sprague-Dawley rats (OPA) showed the absence of toxicity in doses equal to 1/20 of the LD_{50} (viz. 530 $mg.kg^{-1}.d^{-1}$), or of 1/5 of the LD50 (viz. 2120 $mg.kg^{-1}.d^{-1}$). At these doses, no modifications were noted in either body weight or feeding. Neither was any alteration in the globular blood count noted, and hepatic and renal functions remained normal. No lesion of the liver, kidneys, duodenum, myocardia, spleen, thyroid and parathyroid glands, testicles or ovaries was visible by microscope.

Flavopereirine penetrates the hemato-encephalic barrier, as shown by the fact that in male CDI mice which received 10 mg of this preparation orally, the encephalic flavopereirine content was approximately 7 μg (i.e. a concentration of 14 μg/g, for 20 g mice with brains weighing 0.5g).

For use in this invention, the flavopereirine is obtained by extraction from the bark of Pao Pereira Geissospermum vellosii-Baillon. The bark is first ground to obtain a powder. Extraction of the flavopereirine from this powder can be carried out for example by hydrolysis in 1N HCl at 100° C, followed by neutralization by KOH, extraction by ethanol and reduction by distillation. The residue of the distillation is subsequently retaken by chloroform, the excess salt is eliminated by precipitation in cold ethanol, and the residue, which contains mainly flavopereirine, is concentrated.

For the purposes of this invention, the flavopereirine may be utilized either as it is produced, or as a salt or other pharmacologically acceptable derivative.

The following pharmacological tests, relating to the above-mentioned therapeutical indication, were performed. The tests are complemented by the attached diagrams, in which:

- Fig. 1 is a graphic representation of the comparative counts of cells/ml as a function of the number, of hours following infection, the addition of flavopereirine (labeled "H") having been made 12 hours after infection;

- Fig. 2 represents the comparative titration of infectious units (in IU/ml) as a function of the number of hours following infection, without addition of compound H and with such addition at rates of 30 and 60 μg/ml.

- Fig. 3 represents in diagrammatic form the effect of product H (flavopereirine) on the production of interleukin-6 by human monocytes from healthy donors.

- Fig. 4 represents in diagrammatic form the effect of product H on the spontaneous production of interleukin-6 by human monocytes taken from HIV-positive patients.

The antiviral effect of flavopereirine against HIV

The destruction of HIV in the in vitro culture cells without effect on normal or healthy cells was demonstrated by use of the H-9 colony of T4 lymphocytes obtained from Dr. R. Gallo via the Paul Ehrlich Institute (Frankfurt, Germany) and propagated by the Institut für Medizinische Mikrobiologic und Hygiene (University of Bern, Switzerland).

The HIV was obtained as HTLV-III from Dr. Gallo and propagated in the above-mentioned H-9 cells. The surface material produced by the infected H-9 cells culture was stored at -80°C. This material had a concentration of 10^6 IU/ml at the point of use. The H-9 cells were cultivated in an RPMI 1640 medium containing 15% fetal calf serum, 0.002 μm glutamine and 100 IU of penicillin/ml in Falcon vials (25cm^2). 8 to 20 ml. of the medium was used per vial. The vials were left to incubate at 37°C in an upright position. The culture was begun at a concentration of 2×10^5 H-9 cells per ml, and divided when the number of cells reached 1×10^6 Cells/ml. In order to test the

flavopereirine in cultures of both infected and non-infected H-9 cells, microtitration plates were used. In these cases, the cultures were begun at a concentration of 6 x 10^5 cells/ml. After 23 hours incubation, 0.1 mi RPMI 1640 medium containing 10^2 IU of HIV was added to each well containing 0.1 ml of this cellular suspension. This corresponds to an infection multiplication of 1.6 x 10^4, i.e., one infectious unit per 500 cells. 12 hours after infection, 0.1 ml of RPMI 1640 medium, with or without flavopereirine, was added to the infected and non-infected wells. The flavopereirine was used at a concentration of 30 μg/ml and 60 μg/ml. The microtitration plates were covered with an Amersham plate cover, and incubated at 37°C. Cellular counts were determined in a Neubauer chamber. The total quantity of HIV antigen was determined using an HIV antigen kit provided by Abbott Laboratories. For the purposes of titration, a volume of 10 μl (taken from each of the well of the microtitration plate) was diluted in 1 ml of RPMI 1640 medium. Based on this stock, 8 dilutions were made in respective series of 1 to 3 and 1 to 5. A volume of 0.1 ml of each dilution was removed and added to 0.2 mi of a pre-incubated culture of H-9 cells containing 5 x 10^5 cells/ml. After incubation at 37°C, the presence of HIV antigens was tested using an HIV antigen kit provided by Abbott Laboratories.

The results, which are reproduced in graphic form in the attached diagram 1, show that flavopereirine does not affect the multiplication of non-infected cells. By contrast, the quantity of infected cells is around 40% lower when the flavopereirine is present.

What was even more striking (as shown in the presentation of results in diagram 3) was that, although there was an increase in viral particles of untreated infected cells over time, it was not possible, within the limitations of this particular test, to detect the presence of infectious units above

3000 in the series of infected cells treated with flavopereirine (30 μg or 60 μg). This shows that the inhibition of infection exceeds 99% at the very least.

An evaluation of the antiviral effect of flavopereirine was also undertaken by studying the cytopathogenic effect of the HIV virus on MT4 cells, given that a formation of syncytia was observed 4-6 days after infection by HIV-1, followed by the death of the cells.

The flavopereirine was used in the form of an alcoholic solution (40 mg in 100 μl alcohol). Dilutions were made in RPMI medium at 10% of FCS, 1% of PSN and 1% of glutamine. The MT4 cells were left to pre-incubate for two hours at 37°C with a successive dilution of flavopereirine containing 3 x 10^5 cells for 10 μl of flavopereirine solution. The solution was obtained by adding 100 μl of a 10^{-4} dilution of HIV-1 virus, producing a syncytia formation in 4-6 days. After one hour of incubation at 37°C, the infected MT4 cells were washed three times with RPMI solution before being placed in culture (3 x 10^5 cells/ml in microplates with 24 wells) with the presence of different dilutions of flavopereirine. The syncytia count was taken each day in duplicate. The results are summarized in tables I, II and III below.

Table I

H	d3		d4		d6		d7	
400 µg/ml	Tox	Tox						
100	Tox	Tox						
50	(+)	(+)	(+)	(+)	-	-	-	-
10	+	+	+	(+)	+	++	++	++
1	+	(+)	+	+	++	++	++/T	++/T
100 ng/ml	+	+	+	+	++	++	++/T	++/T
HIV-1 only	+	+	+	+	++	++	++	++/T
MT4	-	-	-	-	-	-	-	-

Table II

H	d3		d4		d5		d6		d7		d10
100µg/ml	Tox	Tox									
50.	-	-	-	-	-	-	-	-	-	-	
10	-	-	+	(+)	+	+	+	++	++	++	
1	-	-	+	+	+	++	++	++	++	++/T	
100 ng/ml	-	(+)	+	+	+	+	++	++	++	++	
HIV-1 only	-	-	(+)	+	+	++	++	++	++/T	++/T	

Table III

H	d3		d4		d5		d6		d7	
60 µg/ml	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	(+)	-	++	(+)
10	(+)	-	(+)	(+)	+	(+)	++	++	++	++
1	(+)	(+)	(+)	(+)	(+)	(+)	++	+	++	++
100ng/ml	-	(+)	(+)	(+)	+	(+)	+	++	++	++
HIV-1 only	(+)	(+)	(+)	(+)	+	+	++	++	++	++
MT4	-	-	-	-	-	-	-	-	-	-

Table I shows cellular toxicity for 100 and 400 μg/ml flavopereirine. At 50 μg/ml syncytia had not formed after 7 days culture. From 10 μg/ml to 100 ng/ml, syncytia was observed, as it was in the HIV-1 control,

Table II confirms the protection obtained by flavopereirine at 50 μg/ml, and Table III reconfirms these results: no syncytia were formed at 60 μg/ml after 7 days culture, while a few were observed after 6 days at the dosage 30 μg/ml.

Viral infection test (determination of P24 antigen)

1 nanogram of primary isolates of HIV-1, BRE1 (from an asymptomatic patient) and TIG2 (from an AIDS patient) were inoculated with 10^6 peripheral blood mononuclear cells (PBMC) stimulated with PHA taken from five randomly-chosen HIV-negative donors. After 2 hours of incubation, the cells were rinsed twice and cultivated in 1 ml of RPMI 1640 containing 20 IU of IL-2 per ml (Boehringer Mannheim, Germany), 2 μg of Polybrene (hexadimethrine bromide) per ml (Sigma, St. Louis, MO, USA) and 10^{-7} IU of goat antiserum acting against human alpha interferon (Janssen, Beerse, Belgium) per ml. Half the culture was changed after 72 hours, and thereafter every 48 hours until the 30th day. The surface material of the culture was tested by an immuno-enzymatic (ELISA) assay for the production of antigen P24 (Abbott, Chicago, IL, USA) and the optical density (OD) of the resulting color was converted into P24 concentration from the slope of a standard nomogram, as described by W. Lu and J.-M. Andrieu, Journal of Virology, 66(1): 334-340 (1992).

Testing the efficacy of flavopereirine on the infections capability of HIV-1

1) First experiment: Prior to incubation of the virus in PBMC stimulated by PHA (blast cells), extracellular viral stocks were

pretreated in triplicate with 30 or 60 μg flavopereirine per ml for two hours.

2) Second experiment: Blast cells were pretreated in triplicate with 30 or 60 μg flavopereirine per ml for two hours, and were then rinsed twice before exposure to the viral inoculum.

Cytotoxicity of flavopereirine in PBMC at rest and in blast cells

Prewashed fresh and blastic PBMC taken from five randomly-selected, healthy, HIV-negative donors were treated three times with 30 or 60 μg flavopereirine per ml of alcoholic solution for two hours, After washing twice, the cells were placed in culture in cellular culture medium until day 15. The viability of the cells of each group was examined by exclusion coloring with trypan blue and by quantimetric analysis. Cultures of HIV-negative PBMC without flavopereirine (compound H) were used as controls.

Inhibition of productive HIV-1 infection by compound H

1) A pretreatment of HIV-1 by compound H (at a rate of 30 or 60 μg/ml) completely prevented the infection of target PBMC by primary HIV-1 isolates taken from both symptomatic and asymptomatic patients (see, Table V below).

2) Only pretreatment of target PBMC with 60 μg/ml, however, led to complete inhibition of productive viral infections (see Table VI).

Cytotoxicity of compound H on human PBMC at rest and on blast cells stimulated by PHA

1) The viability of PBMC at rest reduced significantly ($p<0.05$) in the group of cells treated with 60 μg/ml, but this was not the case in the group treated with 30 μg of compound H per ml. (see Table VI).

2) Viability of the blastic cells appeared to be independent of

exposure to compound H (see Table VI).

Inhibition of productive infection by HIV-1 through use of compound H.

A pretreatment of HIV-1 with compound H (in doses of 10, 30, 60, 100, 200 μg/ml) inhibited infection of target PBMC by the virus in a manner dependent on the dose (see Table VII below). Doses equal to or higher than 60 μg of compound H per ml appeared to represent the required concentration for complete inhibition of productive viral infections.

In the primary human PBMC culture system, the efficacy of compound H on the inhibition of wild HIV-1 remained unchanged when the medicine was placed for incubation in a culture medium containing 50% human serum before the inhibition experiment (see Table VIII).

Cytotoxicity of compound H in human PBMC stimulated by PHA

The viability of blast PBMC diminished significantly ($p<0.001$) in the group of cells treated with 200 μg/ml, but this was not the case in the groups treated with 100, 60, 30 and 10 μg/ml compound H respectively (see Table IX).

The replication of HIV-1 was totally inhibited by 60-100 μg of compound H per ml. These concentrations are 2 to 4 times weaker than the cytotoxic concentrations. This effect did not appear to be influenced by the constituents of the human serum.

Effects of product H on the production of primary (IL-1β and TNF-α) and secondary (IL-6) cytokines by normal monocytes taken from HIV-positive patients.

The adhesive monocytes used were taken from the blood of two types of donors:

- Normal donors - voluntary blood donors who attended the blood bank of the Pitié-Salpêtrière Hospital, Paris, France;

- HIV-positive donors from medical consultations. These individuals are in the early stages of HIV infection and, at the moment of taking the blood, were not undergoing any treatment. The risk factors involved were intravenous drug abuse for six of the donors, sexual transmission for three and blood transfusion (in Zaire) for one patient. No correlation was found between the risk factors and the results of the experiments.

In the case of the HIV-positive donors, a number of anomalies were found; these related mainly to the polynuclear and lymphocytic lines;

- Hypersegmented or hyposegmented (Pelger type) polynuclears in four cases out of 10.

- Hyperbasophilic lymphocytes were also found in four cases out of 10 (not the same cases as the above).

No correlation was found between these anomalies and the results obtained in the experiments.

In immunophenotypical analysis, no expression of the differentiation antigens (CD34, CD33, CD13 and CD11b) examined was found deficient.

I. Normal donors

The ten donors in these experiments showed a considerable coherence of results;

- No spontaneous production of either primary cytokines (IL-Iβ and TNF-α) or of interleukin-6, a secondary cytokine;

- The stimulation of monocytes over a period of 48 hours with interferon-- (1000 U/ml) did not cause any production of primary cytokines, except in donor #10 in IL-1β, or of secondary cytokine, except for donors #1 and 5.

- As expected, the stimulations obtained with LPS (lipopolysaccharide) and the combination interferon-γ + LPS they varied in amplitude from donor to donor; they were always

significant, with a synergic effect in the case of double stimulation.

Effect of product H

Product H was not used in these experiments at a rate of more than 20 μg/ml water, since above this rate, net toxicity could have been produced. Four donors were previously tested with 30, 50, 100 μg/ml with virtually total cytotoxicity: above 40% at 30 μg/ml, and 100 μg/ml above that rate (analysis of the culture surface material was not prepared).

The doses of 5, 10 and 20 μg/ml were chosen from the second series of experiments onwards, after the results from the first two donors had shown that doses of less than 5 μg/ml proved inactive.

Direct activity of product H:

- On the production of primary cytokines:

  * Increase in the production of TNF-α (donor 7), in one dose only; this effect was also detected in the response to IFN-γ.

  * Increase in the production of IL-1β (donors #3, 5 and 6) with a dose effect, though this was marginal. Donor #10, however, responded very well to doses of 10 and 20 μg/ml.

- On the production of secondary cytokine (interleukin-6):

  * No modification in response was observed.

Indirect Activity of product H:

- On the production of primary cytokines:

  * Production of TNF-α: There was a sharp reduction in production, but solely in the case of a heavy dosage of the product (20 g/ml) ($p<0.05$).

  * Production of IL-1β: there was no significant modification of the responses to LPS or to IFN-γ + LPS ($p<0.05$).

- On the production of secondary cytokine (interleukin-6):

  * There was a sharp reduction in the production of

IL-6; this inhibition was never total, and, contrary to the case of TNF-α, it was dose-dependent ($P<0.05$).

These different results are organized in diagrams 3 and 4, in which the measured doses are shown in μg/ml of cytokines, and the standard deviations are not precise, since these are always 10% less than the average.

II, HIV-positive donors

The response of the monocytes taken from the various HIV-positive donors was tested in terms of spontaneous response. The quantities of cells received were low (the donors were not affected by cytapheresy), and the experiments were therefore limited by the number of cells available.

Direct activity of product H:

- On the production of primary cytokines.

No modification on the base production was observed, whether for TNF-α or for IL-1β.

- On the production of secondary cytokine.

8 out of 10 donors showed a significant spontaneous response in interleukin-6.

Indirect activity of product H:

- Effect on the production of primary cytokines.

The effects obtained were the same as those for healthy donors, namely an almost total reduction in the production of TNF-α (only at a dosage of 20 μg/ml) and an absence of any impact on the, production of interleukin-1 at the three dosages employed ($p<0.05$).

- Effect on the production of interleukin-6.

Interleukin-4 was used at the same time as product H, since it has been shown prior to these experiments that this cytokine blocks the spontaneous production of interleukin-6 in certain HIV-positive patients.

In five cases, interleukin-9 was used for comparison,

since this, too, blocks the production of IL-6 in normal monocytes stimulated by LPS.

The results obtained were as follows:

* Interleukin-4 inhibited the production of IL-6, but this inhibition was never total ($p<0.05$).

* Interleukin-9 partially inhibited (up to a maximum of 50%) spontaneous production; it never has a compound effect to that of interleukin-4 (indeed, in two out of five cases, it caused the neutralization of these effects; these results are not shown in the tables).

* Product H showed an inhibitory effect with a very clear dosage effect (@<0.05). However, it never led to the total inhibition of the spontaneous production of IL-6. On the contrary, in the presence of interleukin-4, an amplification effect was almost always obtained (except in case #4), with total disappearance of production at 3 μg/ml of product H (and often even from 1 μg/ml) ($p<0.05$).

In conclusion:

- Product H proved toxic at dosages higher than 20 μg/ml in vitro on the cells used within the framework of these experiments, viz. human monocytes taken both from healthy and from asymptomatic HIV-positive donors;

- Product H proved able to modulate the production of cytokines: this was true directly for primary cytokines, though this effect was weak; indirectly, this modification was marked in the case of the production of TNF-α and IL-6, though not IL-1. Product H also inhibited the spontaneous production of IL-6 shown in some HIV-positive patients. This inhibitory effect, which in these experiments was never total, was amplified in the presence of IL-4;

- The normalization of the IL-6 and TNF-α responses in the HIV positive subject, except for the inhibition of the production of IL-1, was highly significant, since product H did

not modify the potential immune relations between monocytes and lymphocytes, or the majority of the inflammatory reactions necessary for survival, such as the stimulation of the stock cells of the bone marrow and the establishment of a defense reaction on the general level.

Clinical tolerance and efficacy of flavopereirine

The clinical study was carried out on 24 HIV-positive patients with total T4 lymphocyte counts at absolute values ranging from 0.2 - 0.4 x 10^9/l. All the patients were informed about the active ingredient being used and about the other anti-retrovirus medicines in use at the time of the study. The selection of patients was made on the basis of the absolute T4 lymphocyte count: men and women above 18 years of age, having a Karnofsky index equal to or higher than 90%, showing presence of anti-HIV I antibodies in two successive tests (ELISA method), from groups CDC 11, CDC III, CDC IV C2, CDC IV E in the CDC 87 classification; with hemoglobin higher than 100-120 g/l, neutrophile polynuclears higher than 1.5 x 10^9/l, platelets numbering more than 80 x 10^9/l, T4 lymphocytes numbering no more than 0.2 x 10^9/l and no less than 0.4 x 10^9/l, and the absence of anti-retroviral therapy, particularly by AZT.

20 patients were accepted on the basis of the above criteria. Before inclusion in the study, a pretherapeutic study was made for each patient: postclinical and therapeutic history, clinical examination including determination of fever, anorexia and nausea, headaches, pruritus, cough and expectoration, diarrhea, adenopathies, buccal mycoses, seborrheic dermatitis and Kaposi's lesions. A biological study was also undertaken; this included red corpuscles, platelets, lymphocytic sub-groups (CD2, CD4, VD8, CD19, CD4/8), determination of antigen P24 and microglobulin beta-2, of DHL (dehydrogenated lactate), plasmatic ferritin, ALAT (alanine-amino-

transferase), ASAT (aspartate-aminotransferase) and plasmatic creatinine.

The flavopereirine (compound H) was administered in the form of 600 mg capsules, at a daily does of 1-3 g, preferably at least around 1 g, which is generally active for one day, The average length of treatment was 43 ±11 weeks. Side effects were few, occurring only in the first three months. Neither blood nor renal toxicity was observed; nor was there any significant modification in ALAT or ASAT. No degradation in the CDC classification and no infections were noticed; the Karnofsky's index remained around the 100% level in all cases- Physical and professional activity on the part of the patients remained completely normal.

Immune response to the treatment was expressed mainly in a significant increase in CD4+ cells (p.<0.05), as well as in CD19+ cells (p<0.05). The negative decline in CD4+ was reversed in 18 out of 19 patients (p<0,05).

All the in vitro and in vivo results clearly indicate that the flavopereirine compound exerts a significant inhibitory effect on the viral infectional capacity of HIV, both in vitro in human cells and in vivo in HIV-1 infected patients.

In 10 of the patients treated over a year, the following significant variations were further noted:

- Increase in red cells at 9 months;
- Increase in hemoglobin at 9 months;
- Increase in the total lymphocyte mass at 9 and 12 months;
- Increase in CD2 at 9 months;
- Increase in CD4 at 12 months;
- Increase in CD8 at 9 months;
- Increase in CD19 at 6-9 and 12 months;
- Increase in microglobulin beta-2 at 3-6 and 12 months.

In practice, we recommend ideally oral administration in solid form, such as tablets or capsules, for example. A

unitary dosage of around 250-500 mg of active ingredient is particularly appropriate.

The recommended dosage, in the light of the above results and the indications of toxicity, is around 1-3 g, which are generally active for one day (g/d) and preferably at least around 1 g/d, most profitably taken at successive intervals over the course of the day.

The dosages and/or galenic forms retained may, however, vary according to the state of the patient and the stage of viral attack being treated. Their adaptation to the specific case concerned in each particular treatment may be easily achieved by the professional on the basis of his relevant experience and, if necessary, with the assistance of routine preliminary tests. In this respect, it is particularly recommended that close attention be paid to the data providcd by a pharmacokinetic study of the patient made in order to establish the half-life, of the active ingredient being administered, and, if necessary, to adapt the form of pharmaceutical preparation for administration accordingly. The latter may, for example, take the form of time-release galenic preparations.

Apart from the active ingredient or a salt or other derivative thereof, the doses for administration include at least one pharmaceutical support or vector, as well as excipients, carriers and standard perfumes and/or colorants.

Table IV

Pretreatment of viral inoculum with compound H (experiments in triplicate)

Viral stock (1 ng/ml)	HIV pretreated with H for 2 hours	Post-infection production of HIV P24 (pg/ml)				
		d3	d5	d14	d21	d30
HIV-$1_{Asym.}$	Control	250±25	>1500			
(Stock$_{Brat}$)	+30 µg/ml	-	-	-	-	-
	+60 µg/ml	-	-	-	-	-
HIV-1_{AIDS}	Control	575±129	>1500			
(Stock$_{Tigr}$)	+ 30 µg/ml	-	-	-	-	-
	+ 60 µg/ml	-	-	-	-	-

Reunified peripheral blood mononuclear cells (PBMC) taken from five randomly-chosen, healthy, HIV-negative donors.

Table V

Pretreatment of target cells with compound H (experiments in triplicate)

Viral stock (1 ng/ml)	PBMC* pretreated with H for 2 hours	Post-infection production of HIV P24 (pg/ml)				
		d3	d5	d14	d21	d30
HIV-$1_{Asym.}$	Control	250±25	>1500			
(Stock$_{Brat}$)	+30 µg/ml	113±7	>1500			
	+60 µg/ml	-	-	-	-	-
HIV-1_{AIDS}	Control	575±129	>1500			
(Stock$_{Tigr}$)	+ 30 µg/ml	515±103	>1500			
	+ 60 µg/ml	-	-	-	-	-

* Reunified PBMC taken from five randomly-chosen, healthy, HIV-negative donors.

Table VI

Cytotoxicity of compound H in human PBMC at rest and in PBMC stimulated by PHA (blastic) (experiments repeated 5 times)

Target cells	Cells treated with H (2 hrs.)	Viability (%) of PBMC after exposure to compound H				
		d3	d7	d11	d13	d15
PBMC*	Control	97±2	95±3	98±2	91±8	85±9
	+30 µg/ml	95±4	93±2	88±7	82±10	81±11
	+60 µg/ml	56±6	23±4	17±4	12±5	25±8
Blast.**	Control	86±5	84±6	76±4	75±5	68±7
	+30 g/ml	88±3	85±7	71±3	76±9	69±8
	+60 g/ml	79±4	74±5	70±4	71±8	63±7

* Reunified PBMC taken from five randomly-chosen, healthy, HIV-negative donors.

** Blastic cells stimulated with PHA taken from five randomly-chosen, healthy, HIV-negative donors.

Table VII

Pretreatment of viral inoculum with compound H in the absence of human AB group serum (experiment repeated 5 times)

HIV pretreated with H for 2 hrs.	Production of P24 in HIV (pg/ml)			
	d4	d10	d14	d21
Control	510±235	>1500		
+200 µg/ml	-	-	-	-
+100 µg/ml	-	-	-	-
+60 µg/ml	-	-	-	-
+30 µg/ml	173±102	>1500		
+10 µg/ml	388±124	>1500		

Reunified peripheral blood mononuclear cells (PBMC) taken from five randomly-chosen, healthy, HIV-negative donors.

Table VIII

Pretreatment of viral inoculum with compound H in the presence of human AB serum (experiment repeated 3 times)

HIV pretreated with H for 2 hrs.	With/w.out (-/+) 50% of AB serum	Production of P24 in HIV (pg/ml)			
		d4	d10	d14	d21
Control	-	510±235	>1500		
+200 µg/ml	+	-	-	-	-
+100 µg/ml	+	-	-	-	-
+60 µg/ml	+	-	-	-	-
+30 µg/ml	+	275±98	>1500		
+10 µg/ml	+	384±83	>1500		

Reunified peripheral blood mononuclear cells (PBMC) taken from five randomly-chosen, healthy, HIV-negative donors.

Table IX

Cytotoxicity of compound H on human PBMC stimulated with PHA (blasts) in the presence or absence of human AB group serum (experiment repeated 5 times)

Cells treated with H (2 hrs.)	With/w.out (-/+) 50% of AB serum	Cytotoxicity of compound H after exposure to PBMC*			
		d4	d10	d14	d21
Control	-	85±1.4**	86±1.3	80±2.7	77±3.1
+200 µg/ml	-	45±6.3	33±7.8	24±7.6	15±5.4
+100 µg/ml	-	84±1.6	79±3.1	73±5.3	74±4.7
+60 µg/ml	-	86±1.5	82±2.4	82±2.6	80±3.3
+30 µg/ml	-	81±2.8	83±2.6	80±3.9	78±4.5
+10 µg/ml	-	87±1.2	86±1.4	84±1.5	81±2.3
+200 µg/ml	+	37±6.8	24±6.6	17±7.9	11±5.6
+100 µg/ml	+	83±2.3	80±4.5	77±5.6	73±4.4
+60 µg/ml	+	85±1.2	84±1.7	82±2.1	79±3.5
+30 µg/ml	+	88±1.3	84±2.5	82±3.7	81±4.6
+10 µg/ml	+	87±1.1	88±1.4	84±3.2	82±3.1

* Blastic cells stimulated with PHA taken from five randomly-chosen, healthy, HIV-negative donors.
** Percentage (average ± standard deviation) viable cells

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A pharmaceutical composition for the treatment of human immunodeficiency virus (HIV), comprising:

(a) flavopereirine, or a pharmaceutically-acceptable salt thereof; and

(b) a pharmaceutically-acceptable carrier.

2. A pharmaceutical composition according to claim 1, wherein component (a) is present in an amount of about 250 to 500 mg per unit dose.

3. A pharmaceutical composition according to claim 1 or 2, formulated as a tablet or capsule.

4. A pharmaceutical composition according to claim 1, 2 or 3, formulated in a time-release galenic form.

5. A pharmaceutical composition according to any one of claims 1 to 4, wherein the composition is formulated to deliver 1 to 3 grams per day of component (a).

6. A pharmaceutical composition according to any one of claims 1 to 5, wherein component (a) is obtained by extraction from the bark of Pao Pereira Geissospermum vellosii-Baillon.

7. A pharmaceutical composition according to any one of claims 1 to 6, in solid form.

8. Use of flavopereirine, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of HIV.

9. A use according to claim 8, wherein the medicament is formulated in solid form containing 250 to 500 mg flavopereirine, or a pharmaceutically-acceptable salt thereof.

10. A use according to claim 8 or 9, wherein the medicament is formulated to deliver 1 to 3 grams of flavopereirine, or a pharmaceutically-acceptable salt thereof.

11. A use according to claim 8, 9 or 10, wherein the medicament is in the form of a tablet or capsule.

12. A use according to any one of claims 8 to 11, wherein the medicament is formulated in a time-release galenic form.

13. A use according to any one of claims 8 to 12, wherein component (a) is obtained by extraction of the bark of Pao Pereira Geissospermum vellossii-Baillon.

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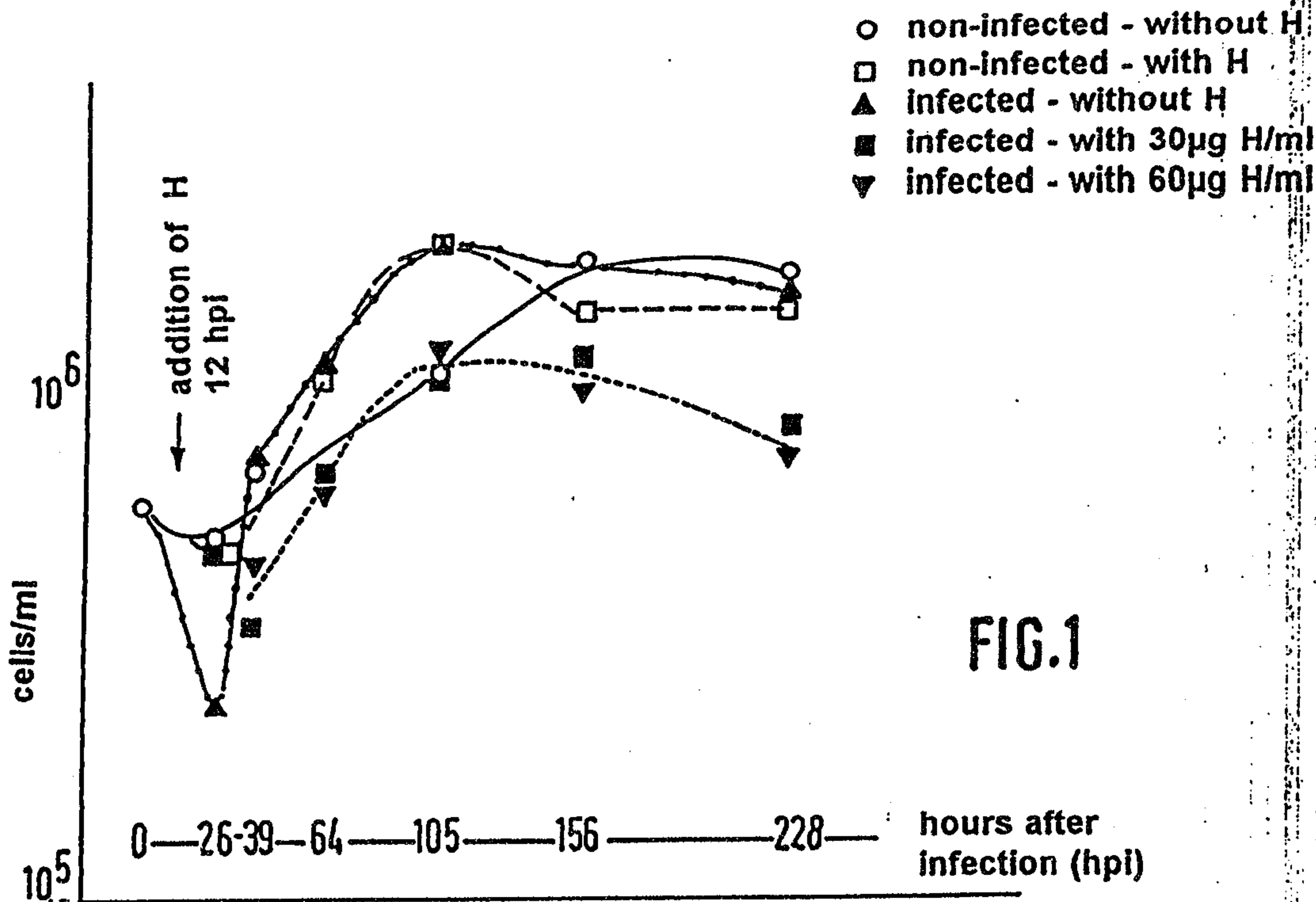

FIG.1

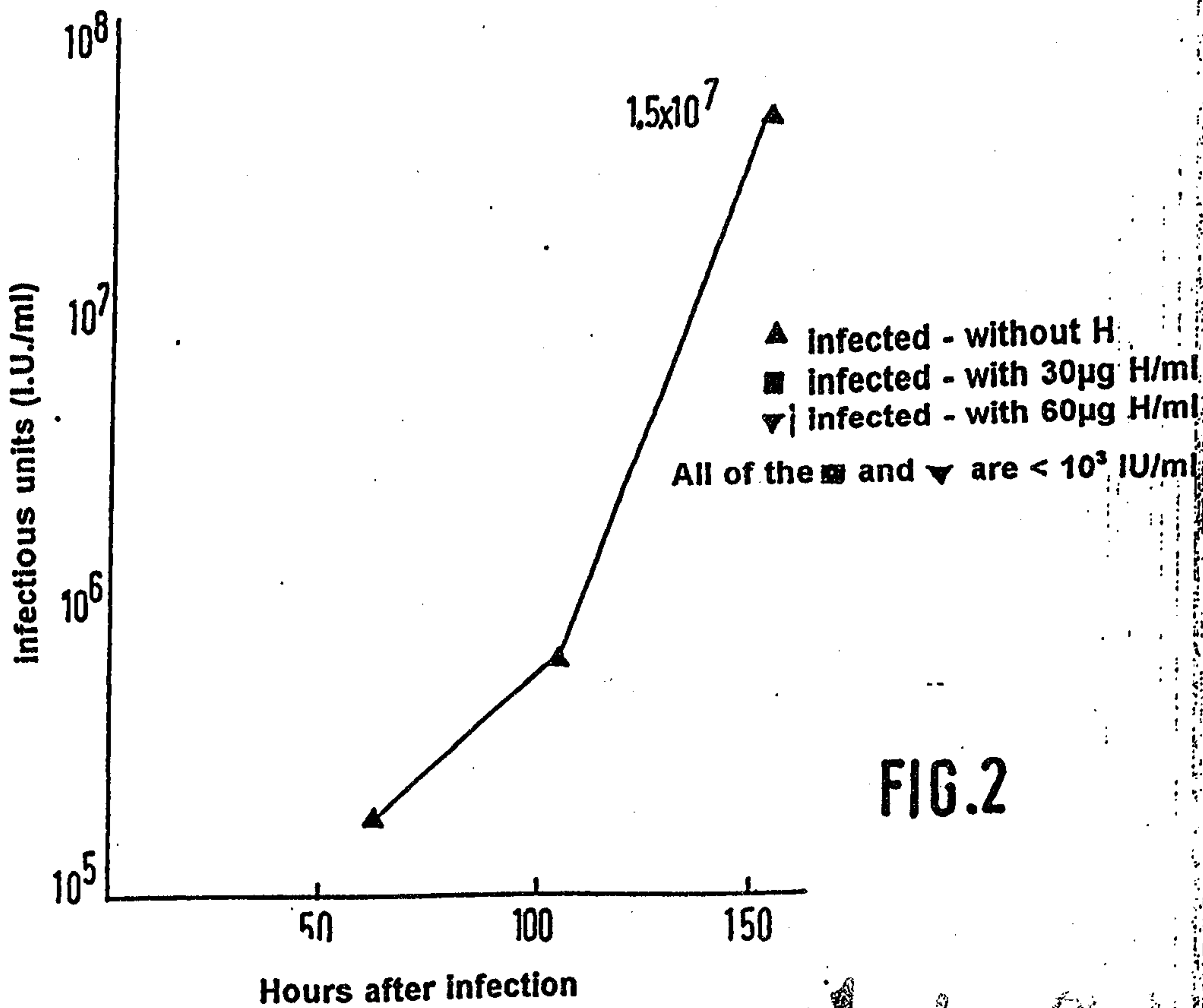

FIG.2

Effect of Product H on the Production of Interleukin-6 by Human Monocytes Obtained from Healthy Donors

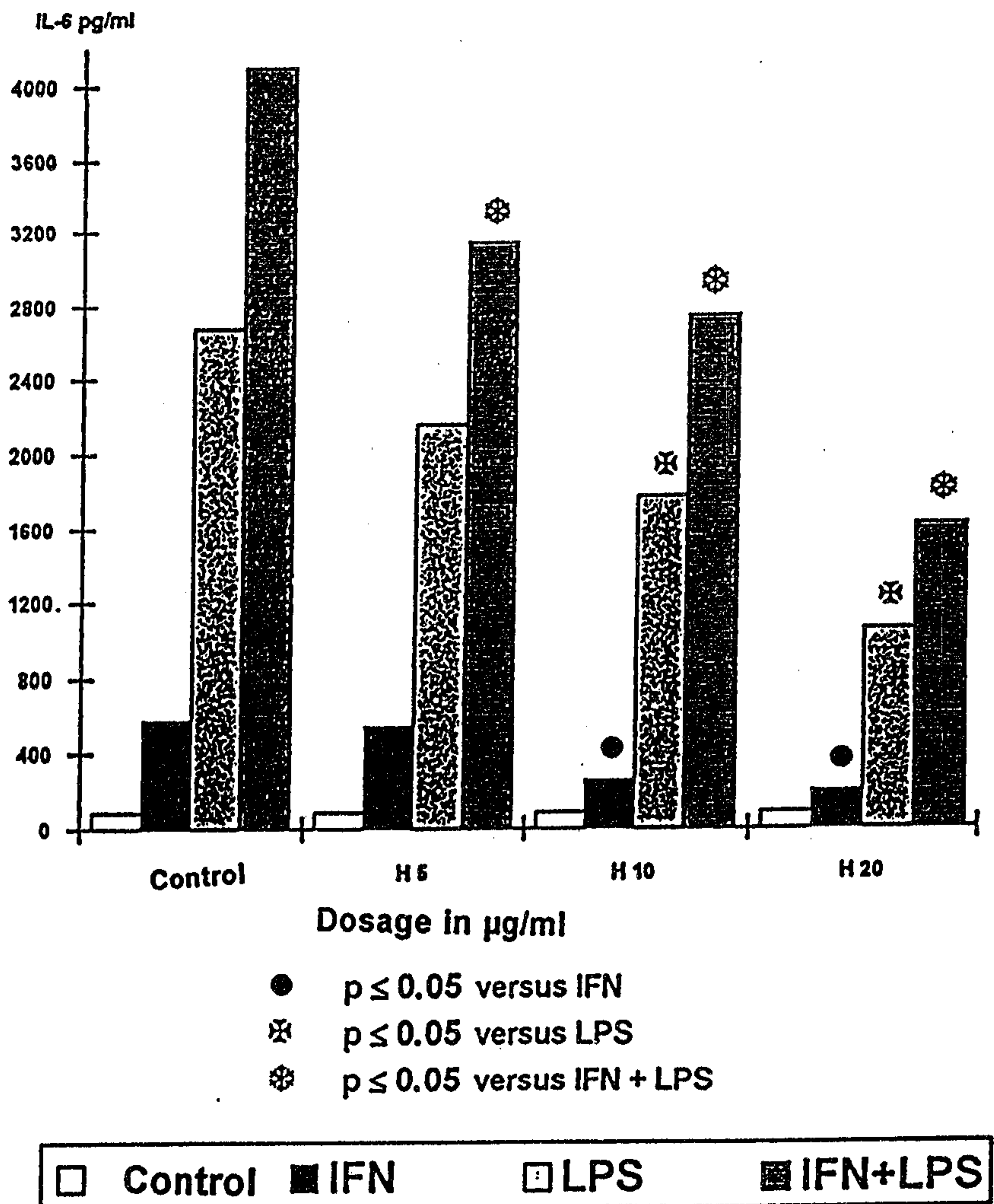

FIG.3

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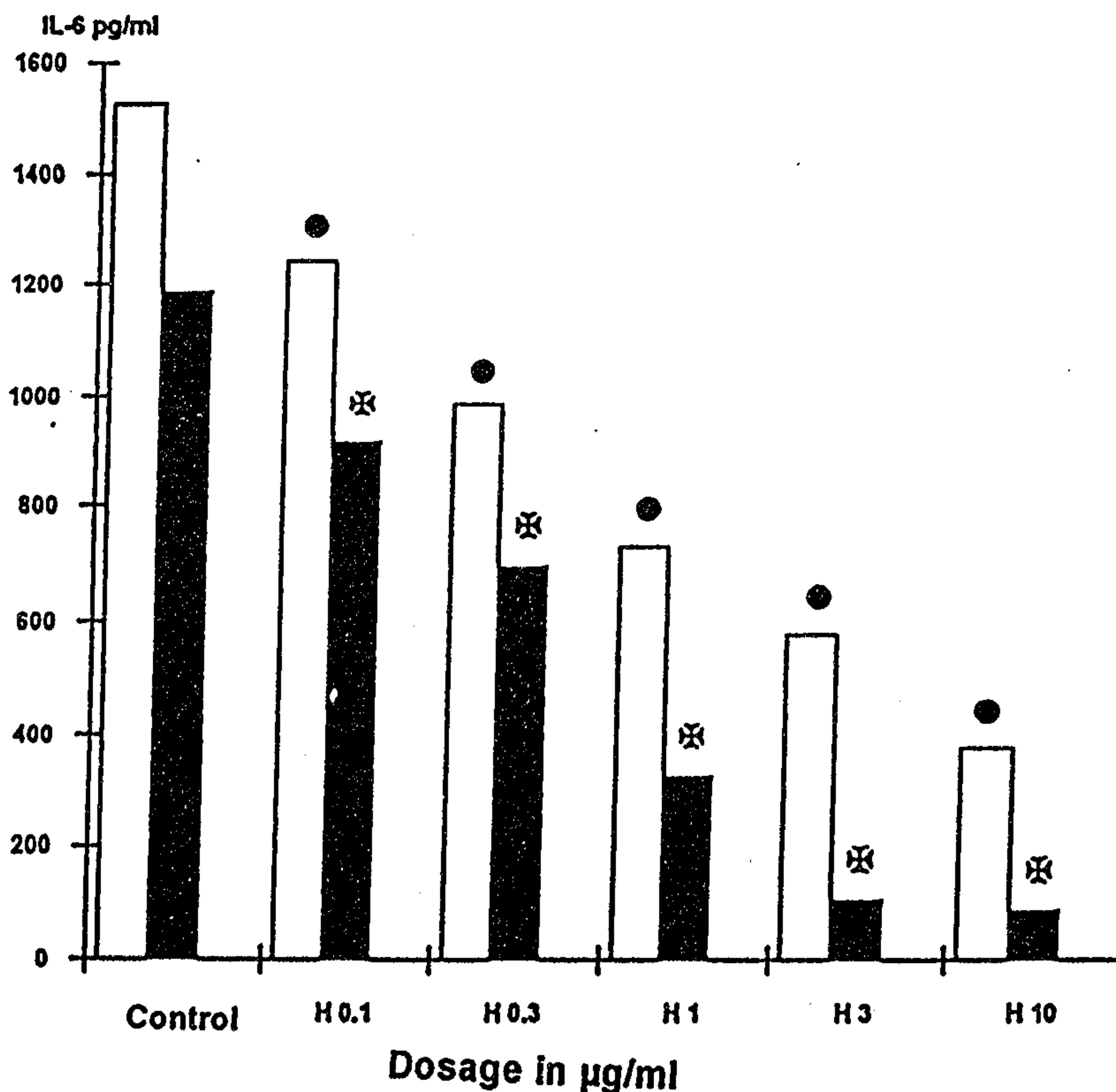
Effect of Product H on the Production of Interleukin-6 by Human Monocytes Obtained from Sero-Positive Donors



● $p \leq 0.05$ versus Control

✠ $p \leq 0.05$ versus Interleukin-4

□ Control

■ Interleukin-4

FIG. 4