

STUDIES ON THE EFFECTS OF PHAGOCYTTIC STIMULATION ON MICROBIAL DISEASE

XI. ACTION OF CHAULMOOGRA DERIVATIVES ON ENDOTHELIAL CELLS OF SKIN VESSELS¹

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The purpose of this series of studies was not to suggest further use of chaulmoogra oil but rather to obtain evidence about some aspects of the behavior of the cellular defense mechanism in leprosy. The problem was approached through the study of the action of chaulmoogra derivatives, which are thought to act on the side of the host rather than directly against the leprosy bacillus. A second purpose of these studies was to demonstrate the possibility of functional stimulation of cells belonging to the reticuloendothelial system (RES).

It is a characteristic of the malign or lepromatous form of leprosy that, although the cells of the RES take up the bacilli, they cannot metabolize or destroy them and thus prevent the development of progressive lesions. Various studies have shown that the bacilli are found in cells which are able to take in particulate matter and vital dyes given intravenously. In view of the large numbers of bacilli that are to be found in the cytoplasm of the lepra cells in active lepromatous lesions, it would seem that these elements of the cellular defense mechanism are paralyzed in the most important function of such cells, i.e., to destroy the bacilli taken in.

Unfortunately, the factors influencing this paralysis of the phagocytic cells are still largely unknown. There is, therefore, a real need of determining these unknown components of the host-parasite relationship. Modern chemotherapeutics are playing an increasingly important role in the treatment of leprosy, but there is an increasing body of opinion that greater therapeutic effectiveness could be attained by simultaneous action on the host with a RES-stimulating agent and on the parasite with an antibacterial drug. Similar studies conducted by us in experimental tuberculosis have been described elsewhere (3, 4, 6, 7).

Chaulmoogra derivatives have, without doubt, a certain therapeutic effect on leprosy, but the mechanism of action of these derivatives is not understood. Some authors have claimed bacteristatic properties for them.

¹ This investigation was supported by grants from the Ministry of Health of the Province of Quebec (Federal-Provincial Health Research Grants) and from "Les Fondations Rhéaume."

According to others, they act on the side of the host. Following the administration of the oils or derivatives the number of leucocytes in the blood increases, supposedly fortifying the natural defense of the organism. Wagner-Jauregg (10), who studied the problem with particular attention and synthesized a long series of chaulmoogra derivatives, did not exclude the possibility of increasing the efficacy of the defense mechanism by the use of chaulmoogra derivatives. Burschkies (2) proved that there is no relation between activity *in vitro* and efficacy *in vivo*.

From an examination of the published investigations of the mechanism of action of chaulmoogra oil and its derivatives, we may conclude that:

1. There has been no agreement about the mechanism of action of chaulmoogra preparations.
2. If the derivatives can in fact act on the parasite, they do not attain an effective concentration in the organism.
3. It is not proved that the active substance is selectively concentrated within the parasite during therapy.
4. Despite the synthesis of chaulmoogra derivatives that are very effective *in vitro*, they did not achieve the expected therapeutic results.
5. If, in fact, the chaulmoogra preparations act upon the host, stimulating the defense mechanism, the mechanism of that action has not been satisfactorily explained.

WORKING HYPOTHESIS

The mesenchymal cells invaded by the leprosy bacilli, in the lepromatous form of the disease, are paralyzed in their most important function, the destruction of the ingested parasites. Jancso (5) showed experimentally that histamine is a physiological activator of the phagocytic activity of the RES. He demonstrated that the endothelial cells of the vessels of the skin undergo a functional metamorphosis under the influence of histamine, and that these cells become active phagocytes. Gözszy and Kátó (3, 6) showed that the same cells could be stimulated to phagocytic activity by the administration of certain derivatives of bicyclo-(0, 3, 5)-decapentane. In both cases this activity was inhibited by antihistamine. Biozzi and associates (1) showed that certain stimuli or chemical or physical actions which may liberate histamine, may also induce phagocytic activity in the endothelial cells of the peripheral vessels.

The working hypothesis of this study is that chaulmoogra derivatives may have histamine-liberating properties, and in this way stimulate the activity of cells belonging to the RES.

METHODS

Experiments were performed as described by Jancso (5). White mice of both sexes, of the CF3 strain, each weighing 20-22 gm., were used. The previous evening, the abdomen of each animal was depilated by means of a barium sulfide paste. The next morning, with animals that were free from lesions in the depilated area, a phagocytic reaction was induced by means of a histamine solution and solutions of chaulmoogra derivatives, which were gently applied to the skin by means of a small cotton swab for a period of two minutes. For the solutions, histamine bichlorhydrate in a 0.5 per cent concentration was dissolved in 70 per cent ethyl alcohol. The various chaulmoogra derivatives used were dissolved in linseed oil. The skin of the animals

servicing as controls was rubbed with the solvent alone. For each substance and each concentration, 4 mice were used in parallel.

Immediately after the application of the solutions to the skin, 0.5 cc. of india ink was injected intravenously in the tail vein of each animal. The ink suspension contained 10 per cent of Pelikan india ink in 0.9 per cent sodium chloride, with 1 per cent gelatine, sterilized at 60°C. After 2 hours, and again after 24 hours, the skin of the animals was examined. A grayish-black spot appeared on the skin if the substances used induced local phagocytic activity. The degrees of the darkening of the spots, when that occurred, were recorded as from 1+ to 4+, as in Table 1. Histological sections were made of the skin of histamine- and chaulmoogra-treated animals showing the phagocytic spots.

RESULTS

At the sites where histamine or active chaulmoogra derivatives were applied, india ink particles were ingested locally by the endothelial cells of skin capillaries. The sections from animals treated with either substance showed the same phenomenon: an intensive engulfment of carbon particles by the endothelial cells of small vessels. This phagocytic activity was brought about very rapidly, and could already be seen 15 minutes after the injection of the india ink. The results observed with the various substances used in the experiment are given in Table 1.

TABLE 1.—*Phagocytic activity of endothelial cells of small skin vessels induced by application of histamine and chaulmoogra preparations.*

Substance tested	Concentration (%)	No. of mice	Phagocytosis	
			2 hrs.	24 hrs.
Histamine bichlorhydrate	0.5	4	4+	4+
Chaulmoogra oil	20.0	4	4+	4+
Chaulmoogra oil	1.0	4	2+	4+
Na chaulmoograte	1.0	4	—	—
Mg chaulmoograte	1.0	4	—	—
Ba chaulmoograte	1.0	4	—	—
Chaulmoogric acid	1.0	4	1+	2+
Chaulmoogryl alcohol	1.0	4	2+	3+
Chaulmoogryl ethyl ester	1.0	4	3+	4+
Chaulmoogryl benzyl ester	1.0	4	4+	4+
Ch-alcohol-zinnamic ester	1.0	4	3+	4+
Chaulphosphat	1.0	4	3+	4+
Control	—	4	—	—

Figure 1 is of a photomicrograph of a histological section of the skin to which chaulmoogra oil was applied. An intense phagocytic activity

of the endothelial cells had been induced by that substance, and india ink particles are to be seen inside those cells. A detailed histological study of the endothelial phagocytosis provoked by histamine has been published by Törö (9).

Effect of an antihistamine.—In a series of experiments under the same conditions, 2 mgm. of a synthetic antihistaminic, mepyramine maleate, was injected subcutaneously in 0.3 cc. of an aqueous solution to each animal 15 minutes before the topical application of the chaulmoogra derivative. In every case the antihistamine inhibited the induced phagocytic activity of the endothelial cells. The inhibiting effect of the antihistamine on the endothelial phagocytosis induced by histamine has been described elsewhere (3, 5, 6).

DISCUSSION

Jancsa (5) concluded from his experiments that histamine is the physiological activator of phagocytosis. He demonstrated that histamine induced phagocytic activity of the endothelial cells of skin capillaries. This observation was confirmed by Törö (9), Matoltsy (8), Biozzi *et al.* (1), and by us (3).

The experiments in this study show that chaulmoogra oil, if applied on the skin, induces the same phenomenon as is induced by histamine—an intense phagocytic activity of the capillary endothelium. The injected india ink particles are ingested by these cells in the area of the skin where the chaulmoogra oil or an active derivative was applied. This endothelial phagocytosis occurred very rapidly, and could be seen as soon as 15 minutes after the application. Normally, these cells have no phagocytic activity, but they may acquire this capacity under the influence of a physiological stimulus of the kind used in this study. Noteworthy, too, is the fact that the induced phagocytic activity could be inhibited by previous administration of a synthetic antihistaminic substance.

This similarity of action of both histamine and the chaulmoogra derivatives on the endothelial cells of skin vessels, and the fact that for both cases the induced phagocytosis could be inhibited by an antihistaminic, permits us to suppose that this action on the part of the chaulmoogra derivatives is brought about as a consequence of the liberation of the histamine. However, this hypothesis remains to be proved by direct quantitative methods.

It is interesting to compare the *in vivo* therapeutic action of chaulmoogra derivatives and their action on the capillary endothelium. Chaulmoogra oil, chaulmoogra acid, chaulmoogra-alcohol-zinnamicester, all induced phagocytic activity of endothelial cells. These same substances are known to be therapeutically active ones. The therapeutically inactive salts that were used, the sodium, magnesium and barium chaulmoogrates, failed to induce the endothelial phagocytosis.

Further observations concerning this problem will be published.

SUMMARY AND CONCLUSIONS

Chaulmoogra oil and certain chaulmoogra derivatives have been found to induce the same intense phagocytic activity of the endothelial cells of the skin capillaries as is induced by histamine. The phenomenon could be inhibited in either case by previous administration of a synthetic antihistaminic substance. From these facts it is supposed that chaulmoogra preparations induce the phenomenon by liberating latent histamine. It is therefore concluded that:

1. By the administration of active chaulmoogra derivatives, a large number of inactive endothelial cells may be stimulated to help the cellular defense mechanism, thus increasing the capacity of the reticuloendothelial system.

2. The experiments suggest that histamine activation is involved in this mechanism, but this needs further direct experimental evidence.

3. If histamine activation is the mechanism of the action of chaulmoogra derivatives, a cycle of defense in which histamine plays an important role may be supposed. Since the phagocytic cells that participate in the pathology of leprosy are paralyzed in their function of destroying the parasite, the active chaulmoogra derivatives could stimulate this section of the defense mechanism, which interferes with bacteriolytic activity.

RESUMEN Y CONCLUSIONES

El aceite de chaulmugra y ciertos derivados chaulmúgricos, según se ha demostrado, inducen la misma intensa actividad fagocítica de las células endoteliales de los capilares cutáneos que induce la histamina. En uno u otro caso, podría inhibirse el fenómeno con la previa administración de una sustancia antihistamínica sintética. Partiendo de esos datos, se supone que las preparaciones chaulmúgricos inducen el fenómeno liberando histamina latente. Dedúcese, por lo tanto, que:

1. Con la administración de derivados chaulmúgricos activos, cabe excitar a un gran número de células endoteliales inactivas a que ayuden el mecanismo de defensa celular, acrecentando así la capacidad del sistema reticuloendotelial.

2. Los experimentos sugieren que la activación de la histamina interviene en este mecanismo, pero esto requiere más pruebas experimentales directas.

3. Si la activación de la histamina es el mecanismo de la acción de los derivados chaulmúgricos, cabe presuponer un ciclo de defensa en el que la histamina desempeña un papel importante. Como las células fagocíticas que participan en la patología de la lepra se ven paralizadas en su función de destruir el parásito, los derivados chaulmúgricos activos podrían excitar este segmento del mecanismo de defensa, que obstaculiza la actividad bacteriolítica.

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DESCRIPTION OF PLATES**PLATE (14)**

FIG. 1. Histological section of the skin of a mouse to which a 20 per cent solution of chaulmoogra oil had been applied before india ink was injected intravenously, showing intensive phagocytic activity of endothelial cells of the small vessels.