CHAULMOOGRA OIL AND UNSATURATED FATTY ACIDS IN LEPROSY

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Summary

Chaulmoogra oil and cooking oils were fed to mice per oral for a while before peritoneal macrophages were harvested from them and challenged in vitro with suspensions of M. leprae. Smears were made at intervals from the incubating cell-bacillary cultures, stained with acid fast stains and examined for phagocytic activity. Cytocentrifuge slides were also made from the incubating cultures, stained with acid fast stains and the phagocytic activity compared to that of the smears.

There was increased phagocytic activity by the oil-fed mice. DNA synthesis in the spleen and peritoneal macrophages was also increased. It is suggested that extra stimulation of the reticulo endothelial system by unsaturated fatty acids could be harnessed in augmenting chemotherapy in leprosy management.

Introduction

For centuries chaulmoogra (Hydnocarpus) oil has been considered the basis of all specific therapy in leprosy until it was largely discarded when sulphones were introduced (Cochrane and Davey[1]). In 1916 it was shown (Rogers, 1916a[2]; Rogers, 1916b[3]) that the active principle of chaulmoogra and hydnocarpus acid was the lower melting-point fatty acid. The beneficial effect of palm oil in the management of leprosy in Nigeria was also reported by Lengauer[4].

High molecular weight unsaturated fatty acid has been found to stimulate the reticulo-endothelial system, conferring thereby an enhanced phagocytic and bacteriolytic capability to histiocytes: this stimulation has been exploited in the treatment of experimental murine leprosy[5]. There have been two recent reports on the antileprosy activity of hydnocarpus and chaulmoogra acid, using the footpad technique[6,7]. Lately Hughes and his collaborators[8] reported the suppression of experimental allergic encephalomyelitis in the guinea-pig by oral linoleic acid therapy. These reports prompted this study for determining the effect on macrophages and reticulo-endothelial system of the mice, of cooking oils-palm oil, corn oil, sun flower oil as well as chaulmoogra oil-each containing varying quantities of unsaturated fatty acids. (Table 1).

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Materials and Methods

Some 150 outbred mice, 5-6 weeks old, each weighing 18-20 grams were used in this investigation. The mice were divided into groups of thirty mice each. Four types of oil were used for feeding each of the four groups; the

TABLE I

Percentage of saturation and unsaturation of some oils found in Nigeria and used in this investigation

	Saturated Mainly Palmitic	Mono-unsaturated Mainly Oleic	Poly-unsaturated Mainly Linoleic	Total Unsaturation
Palm oil	47.8	42.5	9.6	52.1
Corn oil	8.7 — 16.2	23.5 — 49.6	34.3 — 60.8	83.8 — 91.3
Sunflower oil	9.2 — 12.0	22.5 — 57.8	37.9 — 58.2	88.0 — 91.8

(Data supplied by Dr. M. O. Otuedoh of NIFOR, NIGERIA)

fifth group was the control mice, not fed with any oil. In each of the four oil-fed groups each mouse was fed orally, using a plastic canule, with 0.2 ml chaulmoogra oil, palm oil, corn oil or sunflower oil; this was done three times a week for four weeks. Eight mice in the chaulmoogra group died shortly after the first feeding, and five more on the second occasion, all through shock. These deaths limited the feeding of the chaulmoogra group to only two days. All the mice were left for four weeks before harvesting. At the expiry of four weeks all the mice were healthy and free from infection; there were no further deaths. Two mice at a time from each group were killed by rapid cervical dislocation, the fur damped with alcohol and the peritoneum opened up with sterile scissors and forceps.

Two ml of heparinized saline (15 units of heparin in one ml saline) was injected into the peritoneal cavity. After circulating the injected fluid by prodding, the peritoneal exudates were aspirated with a sterile pasteur pipette in each case, and in within five minutes of the introduction of heparinized saline. The exudates were collected in siliconised glass tubes held at about 0°C (crushed ice). The cells for each mouse were separately investigated, and not pooled together. The killing continued until all the mice had been sacrificed.

Counting of the cells was done by adding 0.05 ml of the cells suspension to 0.45 ml of the counting fluid (Crystal violet in 4% acetic acid) and then counting in a haemocytometer chamber. The remaining cell suspension was spun down @ 1100 rpm for 10 minutes, the top pipetted off and the remainder re-suspended in 1 ml RPMI - 1640 medium, containing 10% new born calf serum. Cell concentration in all cases was between 1-2 x 10^6 cells/ml.

Mycobacterial Suspension

The *M. leprae* suspension used in this observation was processed from the biopsy of a histoid lesion from a DDS resistant patient whose B.I. was 4 and M.I. 3%. After trimming the tissue free of epidermis and subcutaneous fat, it was minced with scissors and homogenized in a manual glass grinder. The homogenate was centrifuged for 5 minutes at 200 x g, the supernatant pipetted off and centrifuged again for one hour @ 2000 x g. The sediment which contained the bacilli was then suspended in 2 ml of 1% human serum albumin saline, exactly according to method of Samuel et al[9]. The acid-fast bacilli (AFB) were counted by the method of Hart and Rees[10]. The suspension was diluted with cold RPMI medium to give a concentration of 1 x 10^6 AFB/ml.

Infection and Phagocytic Indices

0.5 ml of the cell-suspension prepared above was pipetted into two castor wells (i.e. four wells for the two animals in each group), and 0.5 ml of the AFB suspension added to each

well. The trays of wells were then incubated at 37°C. Using sterile pasteur pipettes, drops of the incubating cell-bacillary mixtures were introduced onto clean slides and smears made at 30 minutes, 1½ hours, 2½ hours and 3½ hours. The slides were heat-fixed (lukewarm), stained with Ziehl-Neelsen stains and counter-stained with toluidin blue, before determining phagocytic indices (number of bacilli per cell).

Cytocentrifuge Slide Preparations

At 3½ hours some material from the respective incubating cell-bacillary mixtures were pipetted out, diluted 1 : 2 and 2 : 5 with saline, introduced into centrifuge tubes with coverslips of 12 mm diameter and spun @ 550 for 10 minutes. The slides so made were heat-fixed (lukewarm), stained as described above and phagocytic indices as determined here were compared to those of the slide smears.

Spleen cell preparation

Whole spleen organs which were also taken out after harvesting peritoneal exudates were put into sterile watch glasses. Using bent needles fixed on syringes the spleen cells were expressed out of their sheaths into saline, and broken up with pasteur pipette. The cell-count was done as was described for peritoneal macrophages. Since the numbers were about 100 times higher than peritoneal cells they had to be diluted 1 in 100 with RPMI medium.

DNA Synthesis

The peritoneal and spleen cells for each animal were separtely spun down and resuspended in 1 ml RPMI medium containing 10% new born calf serum. 200μ quantities of each suspension was pipetted into each of six wells, 25μ quantities of tritiated thymidine added and incubated at 37°C. for 16–18 hours in 5% CO_2 incubator. These were harvested, dried and then counted in a liquid scintillation counter. All these determinations were repeated five times.

Histology

Chunks of spleen and liver organs of both the experimental and control mice were also fixed in formol saline, stained with haematoxylin and cosin (H and E) and Deticulin stains and examined.

Results

Table 2 shows the mean phagocytic indices. There was three to eight fold enhancement of phagocytic activity.

The DNA synthesis investigation shows a statistically significant difference between the oil-fed mice and the control. The phagocytic indices and DNA synthesis results of the chaulmoogra oil group which was fed for only two days before harvestation four weeks later show that chaulmoogra oil is a strong RES stimulator.

TABLE 2

Mean Phagocytic Indices of Oil-fed and Control mice

Oils	Phagocytic index after 30 minutes	After 1½ hrs	After 2½ hrs	After 3½ hrs	Cytocentrifuge after 3½ hrs
Chaulmoogra-fed mice	3.16	4.0	3.6	3.0	2.8
Palm oil-fed mice	3.42	4.75	3.2	2.96	3.5
Corn oil-fed mice	4.60	5.22	5.28	3.64	3.94
Sunflower-fed mice	5.84	6.66	5.04	4.0	4.1
Control mice	0.92	0.81	0.72	0.80	0.75

TABLE 3

Thymidine incorporation (DNA synthesis) by peritoneal and spleen macrophages
Values are expressed as mean ± standard deviation and standard errors of counts per minute

Oils	Mean Count of	Peritoneal Macrophages	Standard Error	Spleen Macrophages	Standard Error
Chaulmoogra oil	6	1241 ± 533	106.68	819 ± 62	12.51
Palm oil	6	799 ± 114	22.88	688 ± 87	17.46
Corn oil	6	1298 ± 137	27.36	1065 ± 44	8.89
Sunflower oil	6	1101 ± 89	17.97	854 ± 86	17.22
Control	6	238 ± 77	15.36	314 ± 50	9.9

Histologically liver showed occasional granulomatous foci having histiocyte and mononuclear cells in H and E. In reticulin (or silver) staining fine argyrophilic fibres were seen lining the luminal surfaces of central lobular veins, as well as among and in between the liver cells.

Spleen

In H and E the white and red pulp showed a large number of giant cells, varying in size and shape; some resembled foreign body giant cells. A good number of histiocytes was found in the white pulp. **Reticulin staining**: Moderate quantities of argyrophilic fibres were seen running along the trabeculae.

Discussion

In this study cooking oils and chaulmoogra oil have been fed to the mice for four weeks and two days respectively before harvestation. The enhanced phagocytic indices shown by the peritoneal macrophages suggest that the unsaturated fatty acids from those oils stimulated the peritoneal macrophages. This finding is comparable to the observation by Schroit and Gallily[11] of more than two-fold increase in the phagocytic ingestion rates of macrophages which were incubated with unsaturated fatty acids. The enhanced DNA synthesis in the peritoneal and spleen macrophages should be seen as a direct result of the stimulation by the unsaturated fatty acids of the oils employed.

The histology of the liver and spleen of the oil-fed mice showing moderate formation of argyrophilic fiber-type histiocytes confirmed the report of Sasaki[5] who employed a high grade unsaturated fatty acid as the inducing agent. He utilized the enhanced phagocytic and bacteriolytic potentialities of this type of histiocytes in treating experimental murine leprosy, and in decreasing the bacterial load of the skin of a few clinical cases (Lepromatous leprosy patients), fed with the same purified unsaturated fatty acid (Sasaki et al[12]). It is conceivable that in the presence of a chronic infection like leprosy more adequate domestic use of these cooking oils (including usage for Salads) can be judiciously made and profitably employed in augmenting the chemotherapeutic effects of drugs.

Chaulmoogra oil and the cooking oils used in this investigation seem, through their unsaturated fatty acid components, to have a stimulatory effect on the immune system, enhancing phagocytosis and possibly intracellular killing through the formation of special argyrophilic fiber-type histiocytes as demonstrated by Sasaki and his collaborators. (Sasaki[5], Sasaki et al[12]). The effectiveness of chaulmoogra oil in leprosy management therefore is not likely a direct mycobactericidal action, but indirectly by the enhanced stimulation of the RES. This may well explain its lamentable limitations in the

pre-sulphone era when its therapeutic potential was overestimated. Consequently, the only logical therapeutic value of chaulmoogra oil today should be as an adjuvant, as it is now used in suspending DDS for intramuscular injections.

Less than a decade ago Waters and his co-workers[13] detected small numbers of viable bacilli in long-term dapsone-treated LL patients which Smelt and his co-workers[14] believe are the likely cause of relapse of the disease when chemotherapy is interrupted. At the same time Convit and his collaborators[15] showed that by injecting BCG with large amounts of killed *M. leprae* into lepromatous skin, the epitheloid cell granuloma induced by BCG will also eliminate killed *M. leprae*, suggesting that the lepromatous macrophages when appropriately activated are capable of digesting *M. leprae*.

It is suggested that the elimination of these persisting bacilli could well be effected by the additional stimulation of macrophages and RES by unsaturated fatty acids, thereby utilizing a little more fully, the hitherto untapped immunological potentials of unsaturated fatty acids.

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