



THERAPEUTIC POTENTIAL OF BIOCONVERTED CONJUGATED LINOLEIC ACID IN DRUG INDUCED IMMUNOSUPPRESSED AND INFECTIVE ORGANISM INDUCED *PLASMODIUM BERGHEI*.

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ABSTRACT

Conjugated linoleic acid (CLA) is a fatty acid with many health effects. On the other hand Probiotics have been shown to convert linoleic acid (LA) into conjugated linoleic acid. Conjugated linoleic acid (CLA) can ameliorate the immune responses as well as acts as anticancerous. Hence the Present study was conducted to therapeutic potential of Bioconverted conjugated linoleic acid in Drug induced immunosuppressed and Infective organism induced *Plasmodium berghei*.

Keywords: CLA- Conjugated linoleic acid, Drug induced immunosuppressed, *Plasmodium berghei*.

INTRODUCTION

Conjugated linoleic acid (CLA) is a generic name for a mixture of isomers of linoleic acid with conjugated double bonds. The double bonds can be in several possible positions either as *cis* or *trans* isomers. Double bonds of CLA are mainly found at positions 9 and 11, or 10 and 12³ CLA has potential health or nutritional effects, including anticarcinogenic activity, antiatherogenic activity^{9,12}, the ability to reduce the catabolic effects of immune stimulation², The *cis*-9, *trans*-11 CLA isomer also is the predominant isomer found in the diet. On the other hand probiotics (*Lactobacillus*) have been reported many health benefits. The Health impact with the consumption of microflora consisting of probiotics is reported in humans and in animals^{6,10,11}.

On the other hand CLA can be formed from linoleic acid by the ruminal bacteria (*Butyrivibrio fibrisolvens*) and other probiotics *Lactobacillus*.^{1,6,7}

Hence the Present study was conducted to therapeutic potential of Bioconverted conjugated linoleic acid in drug induced immunosuppressed and Infective organism induced *Plasmodium berghei*.

MATERIALS AND METHODS

The *Lactobacillus acidophilus* (MTCC 447) convert linoleic acid into Bioconverted Conjugated linoleic acid (B.CLA) formed was used to study its impact on immune response *i.e.* humoral immune response, cell-mediated immunity and macrophage function in Drug induced immunosuppressed host and Infective organism induced immunosuppression (*Plasmodium berghei*) in Swiss albino mice.

Various Parameters and Techniques Employed to Evaluate the Immune Status *i.e.* Humoral immune response, cell-mediated immunity and Macrophage function

Enzyme Linked Immunosorbant Assay (ELISA)

Anti BSA antibodies were measured by ELISA as described by Hudson and Hay 1989⁴.

Development of anti SRBC antibody by Direct Haemagglutination (HA) Test (Hudson and Hay, 1989⁴. (Humoral immunity)

Delayed type of Hypersensitivity (DTH) response by Hudson and Hay 1989⁴. (Cell-mediated immunity)

Nitroblue Tetrazolium Chloride (NBT) reduction test Hudson and Hay, 1989⁴. Bactericidal activity by Raghuramulu et al.,1983¹³. Inducible Nitric Oxide (iNO'S) test Stuehr and Marletta, 1987¹⁴. (Macrophage function)

Animals: Six to eight weeks old Swiss Albino mice of either sex, weighing 20-25 g obtained from Animal House, Central Research

Institute (CRI), Kasauli, H. P., were employed in the present study. The animals were housed in University Animal House under pathogen free conditions on standard laboratory diet (Kisan Feeds Ltd, Mumbai, India) and water. The animals were acclimatized for two weeks before starting the experiments. The experimental protocol was approved by the University Animal Ethic Committee and care of the animals was done as per the ICMR guidelines recommended by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 107/1999/CPCSEA).

Selection of dose of conjugated linoleic acid

Swiss albino mice were given doses of Conjugated linoleic acid @ 5mg per kg bodyweight. The seven doses of Conjugated linoleic acid were injected intraperitoneally (i.p.)

Induction of immunosuppression

Animal were made immunosuppressed by giving i.p. injection of hydrocortisone (HC) (Glaxo SmithKline, Pune, India) @ 20mg/kg weight/dose/mouse single dose of 0.2 ml HC/dose/mouse i.p.

Induction of infection (*Plasmodium berghei* infection)

The animals were infected with *Plasmodium berghei* by giving 1 × 10⁶ infected RBC's in sodium citrate-buffer @ 200 µl /mouse by i.p. route. 1

1) Therapeutic potential of Bioconverted conjugated linoleic acid (B.CLA) was checked in Drug induced immune-suppressed host to assess the immune response

For this animals were divided in to following four groups:

Groups	Treatment
Control group I	Untreated + normal diet.
Test group II (CLA)	B.CLA
Test group III (CLA+HYD)	B.CLA+ Hydrocortisone (HC)
Test group IV (HYD)	Hydrocortisone (HC)

The mice were made immunosuppressed by Hydrocortisone (HC) @ 20mg/kg body weight in single dose of 0.2 ml HC/dose/mouse i.p. On the fifth day of HC dose the mice were given to B.CLA treated.

Follow up of experiment

Blood was collected by puncturing of retro-orbital plexus in mice. Animals were bled to check immune response and later sacrificed to collected the spleen. Splenocytes were collected as various immunological parameters were used to study cell mediated immune responses and macrophage functions.

2) Impact of B.CLA on the outcome of *Plasmodium berghei* in Swiss Albino mice

Animals were infected with *P. berghei* and then were divided into following three groups. Each group consisted of 10 mice (n = 10).

Groups	Treatment
Control group I	<i>P. berghei</i> + normal diet
Test group II	<i>P. berghei</i> + B.CLA

Test groups II animals were treated with B. CLA from the day of infection

From the day 1 of induction of infection blood smears from tail vein were prepared daily and stained with Giemsa stain (Hi-media Pvt. Ltd., Mumbai, India) and checked for rate of infection under light microscope (100X). Percent parasitemia was calculated and mortality rate was recorded.

RESULTS

Effect of Bioconverted conjugated linoleic acid (BCLA) on immune response in immunosuppressed mice

The effect of CLA in immunosuppressed mice was evaluated in animals divided in different groups *i.e.* untreated Control group, hydrocortisone treated control group (HYD), BCLA treated group and HYD and BCLA treated group (HYBCLA).

Effect of Bioconverted conjugated linoleic acid (BCLA) on humoral immune response

The results of humoral immune response are shown in Table 1 In direct Haemagglutination the anti SRBC's antibody titers of immunosuppressed HYD group (1:8) was 2 times lower than control animals, whereas treatment with BCLA resulted in a increase in antibody titers of immunosuppressed animals (HYBCLA). The HYBCLA animals showed the increase in anti-SRBC antibody titer to 1: 64, which was 4 times higher than only hydrocortisone treated group.

The results of ELISA were similar to those of direct Haemagglutination *i.e.* the anti BSA antibody titers in HC treated Test groups (1:8) was found to 4 times lower than control group. However, the treatment of BCLA increased the titer (1:128) in immune suppressed host which was 16 times higher than that of the only hydrocortisone treated group.

Effect of Bioconverted Conjugated linoleic acid (BCLA) on Cell mediated immunity

The results of DTH response was determined at 24hr 48 hr and 72 hr by measuring the foot pad thickness in all the treated and untreated groups and are shown in Table 2 it was observed that after 24 hrs all the groups showed the rise in footpad thickness ($p < 0.05$) as compared to control group.

Table 1: Effect of BCLA on humoral immune response in immunosuppressed mice

Titer	Group of animals			
	Control	BCLA treated	Hydrocortisone (HYD)	Hydrocortisone BCLA (HYBCLA)
Anti-SRBC	1:32	1:256	1:8 (4 ↓)	1:128 (16 ↑)
Anti-BSA	1:16	1:128	1:8 (2 ↓)	1:64 (4 ↑)

(↑) = time increase as compared to hydrocortisone treated group; (↓) = time decrease as compared to control group.

Table 2: Effect of BCLA on cell mediated immune response in mice

Time (In hours)	Groups of animals and foot pad thickness (mm)			
	Control group	BCLA treated	Hydrocortisone (HYD)	Hydrocortisone BCLA (HYBCLA)
0	1.71±0.534	1.69±0.243 ^a	1.69±0.307 ^a	1.69±0.004 ^{ab}
24	1.73±0.001	2.21±0.004 ^a	1.69±0.025 ^a (0.9 ↓)	2.04±0.259 ^{ab} (1.2 ↑)
48	2.47±0.345	3.01±0.056 ^a	1.76±0.700 ^a (0.7 ↓)	2.35±0.034 ^{ab} (1.3 ↑)
72	1.71±0.204	1.70±0.076 ^a	1.68±0.026 ^a (0.9 ↓)	1.69±0.038 ^{ab} (1.0 ↑)

Data are mean ± SEM ; a= $p < 0.05$ vs. Control group

b= $p < 0.05$ vs. Hydrocortisone (HYD) treated group; (↑) = Time increase as compared to hydrocortisone treated group; (↓) = Time decrease as compared to control group.

The animals treated with HYD only showed the (↓) in footpad thickness even at 24, 48 and 72 hr as compared to untreated control group. The treatment of immunosuppressed animals with BCLA (HYBCLA) showed the rise in footpad thickness and there was 1.2, 1.3 and 1.0 times increase in footpad thickness at 24, 48 and 72 hr as compared to hydrocortisone treated group.

Effect of bioconverted conjugated linoleic acid (BCLA) on Macrophage function:

The results showed significantly higher ($p < 0.05$) NBT activity in HYBCLA treated animals as compared to only hydrocortisone treated group (HYD). It was 25.37%±2.324, 54.25%± 2.003, 27.99%±1.204 and 9.1%±4.085 in Control, BCLA treated, HYBCLA and only Hydrocortisone treated respectively Table 3 The treatment of immunosuppressed animals with BCLA resulted in 3 times increase in activity as compared to only hydrocortisone treated

animals which, showed 2.7 times decrease in activity as compared to control group.

The iNO's activity also increased in immunosuppressed animals when they were treated with CLA. The statistical analysis revealed a significant ($P < 0.05$) higher iNO's activity in Immunosuppressed animals treated with CLA (HYBCLA). The Control group showed 20.1%±3.475, activity while hydrocortisone treated group showed 3.12%±3.405 whereas when the animals were treated with CLA activity rose to 32.02%±3.845. The treatment of immunosuppressed animals with BCLA resulted in 4.9 times increase in activity as compared to only hydrocortisone treated animals which, showed 4.6 times decrease in activity as compared to control group. Like other activities bactericidal activity was also found to be 3.12%±3.405 in only hydrocortisone treated group, which increased to 32.0%±3.845 when immunosuppressed host treated with BCLA

as well. The treatment of immunosuppressed animals with BCLA resulted in 10 times increase in activity as compared to only

hydrocortisone treated animals which, showed 6.4 times decrease in activity as compared to control group.

Table 3: Effect of BCLA on macrophage functions in immunosuppressed mice

Test	Groups of Animals			
	Control Group	BCLA treated	Hydrocortisone (HYD)	Hydrocortisone BCLA
NBT Reduction	25.37±2.324	54.25±2.003 ^a	9.1±4.085 ^a (2.7 ↓)	27.99±1.204 ^{a,b} (3 ↑)
Bactericidal Activity	19.8±1.439	48.96±2.634 ^a	4.3±2.102 ^a (4.6 ↓)	21.25±4.702 ^{a,b} (4.9 ↑)
iNO's activity	20.1±3.475	49.9±2.535 ^a	3.12±3.405 ^a (6.4 ↓)	32.0±3.845 ^{a,b} (10 ↑)

Data are mean ± SEM ; a=p<0.05 vs. Control group

b=p<0.05 vs. Hydrocortisone (HYD) treated group

(↑) = time increase as compared to hydrocortisone treated group

(↓)= time decrease as compared to control group

Effect of bioconverted conjugated linoleic acid on the outcome of *Plasmodium berghei* in mice

The results of effect of Bioconverted conjugated linoleic acid on the outcome of *P. berghei* infection results showed that administration of Bioconverted conjugated linoleic acid and affected the outcome of infection in mice. The animals treated with BCLA showed the expression of parasitemia on day 3 while the control group showed the expression of infection day 2nd day only. It was observed that animals treated with BCLA enhanced the survival time by 14 days as compared to control group, which survived for 11 days only.

DISCUSSION

The health effects of probiotics are well known but since live probiotics cannot be given in immunocompromised/ immunosuppressive/old patients. CLA seem to be an alternate for the same. Since probiotics in live form may show some side effects in normal or immunocompromised host. On the other hand CLA can be formed from linoleic acid by the ruminal bacteria and other probiotics *Lactobacillus*¹.

Hence the present study was further extended to see the effects of CLA in drug immunosuppressed host. Our results revealed that CLA induced immunosuppressed mice could restore the immune response towards normalcy of drug immunosuppressed mice. The CLA can be employed as safe immunomodulatory even in immunosuppressed host.

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