Effect of human milk on a model of giardiasis in mice

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Giardia intestinalis, the cause of human giardiasis, is the most commonly diagnosed flagellate in the intestinal tract of human, especially in the children. Some observations suggest that interactions of G. intestinalis with non-immune intestinal factors may influence the course of infection. The killer effect of unsaturated free fatty acids and other lipolytic products that are present in non-immune, normal human milk was questionable. In this study, Giardia muris which is a good experimental model for giardiasis was used to investigate the lethal effect of normal human milk in vivo. As the study group 10 BALB/c mice infected with G. muris were taken and fed only with human milk for 7 days period, and as the control group other 10 BALB/c mice infected with G. muris were taken and fed normally. At the end of the time period faecal samples were taken from all mice and no cyst or trophozoite forms of G. muris were seen in the study group. Also, all mice were killed and duodenal biopsies were performed. Although no evidence of G. muris was found in the biopsies of the study group, cyst forms were found in the biopsies of the control group. As a result, in this study in vivo protective effect of human milk on G. muris infection is demonstrated. [Turk J Med Res 1997; 15(1):12-14]

Key Words: Giardiasis, Human milk

Giardia intestinalis, a flagellate was first defined in 1681 by Leeuwenhoek who had shown it in his own faeces. It is one of the intestinal protozoan which frequently attacks children living in temperate climate. When originality of reservoir, body dimensions and different structural specialities as stated with the studies performed are taken into consideration, Giardia types are defined in groups of Giardia agilis, Giardia muris and Giardia intestinalis. However the exact discrimination of the different types is still impossible (1).

Symptoms of giardiasis start with a severe diarrhea that proceed with malabsorption in children. Although the infection may disappear in a few days in some days in some patients, the symptoms may stay indistinct for years (2).

Non-immune intestinal factors were shown to affect the progress Giardia infections with the studies performed. Food mixture, digestion enzymes and their products that change continuously especially in the transverse colon affect the colonisation ability of the parasite.

Bile stimulating lipase (BSL) substance, a bile salt stimulator which is not present in cow and goat milk is found excesssively in human milk and this substance is shown to have killer effect on G. intestinalis trophozoites (3,4). At the same time the killer affect unsaturated free fatty acids and other lipolytic products that are present in human milk on the G. intestinalis trophozoites is shown in vitro (5).

In our study G. muris which is an intestinal parasite that is seen the most frequently in mice is used as an experimental model to investigate the lethal effect of human milk.

Received: Feb. 15, 1997 **Accepted:** March 4, 1997

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MATERIALS AND METHODS

As the study group 20 BALB/c mice infected by G. muris were taken. 10 of these mice were fed with human milk of mothers who did not have a history of giardiasis or any another parasite for seven days. Other on BALB/c mice were fed with normal diet for seven days as the control group. Milk samples were drawn from mothers were brought to the laboratory within 2 hours and were given to the mice. This procedure was carried on twice in a day for seven days and consumption of another food was prevented. Fresh samples of faeces of all mice were obtained on the seventh day and were prepared for microscopic study. These samples were studied with x40 magnification and were examined for G. muris cysts and trophozoites.

Both groups of mice were killed on the seventh day and duodenums were removed. The duodenum samples were prepared after the parts were suspended with a 0.5% NaCl solution in a sterile petri dish. G. muris trophozoites were examined in the fresh samples at x40 mag-

nification. Duodenum part of a mouse infected with G. muris was suspended with 3ml 0.5% sterile NaCI solution in a sterile petri dish and the suspension was transferred to two separate sterile test tubes in equal amounts. 0.5 cc human milk was added to one of the tubes. Both tubes were stored in a 37°C steriliser. Fresh samples from both tubes were examined for G. muris trophozoites at the end of every 15 minutes at x40 magnification by the microscope.

RESULTS

G. muris cysts were detected in the fresh samples prepared from faeces specimens obtained before study from groups of mice. At the seventh day G. muris cysts or trophozoites were not present in faeces of the group fed with human milk while there were G. muris cysts present in the faeces of the control group which were fed with normal diet.

Also no G. muris cyst or trophozoite forms were seen in the fresh duodenum sample of the group fed with human milk but G. muris trophozoites were present in the duodenum samples of the control group.

To study the lethal effect of human milk on G. muris trophozoites in vitro, duodenum sample of a mouse infected with G. muris suspended with 3ml 0.5% NaCI and was transferred to two sterile test equal amounts. 0.5 cc human milk was added to one of the test tubes and both were led to stay in 37°C steriliser. Fresh samples obtained from each tube were studied every 15 minutes and G. muris trophozoites from both tubes lost their motility at the end of first hour.

DISCUSSION

G. intestinalis, an agent of giardiasis, is seen every region of tropical and subtropical climate zone of the world. Climate is seems not to have much effect on distribution of the disease but poor personal hygiene conditions, deficient water supplies or less usage of water, crowded living conditions and poor social economic conditions are factors for high contamination. The reason why this parasite which is seen frequently in children and is not present in babies fed with human milk is related to bile salt stimulator lipase (BSL), a pancreatic carboxyle ester hydroxylase like substance (6). Besides this, in the study of Reiner and friends, BSL and other lipolytic products were shown to change the living conditions of the parasite and have toxic effects an G. intestinalis (7). Gillin and friends related the lethal effect of the human milk on intestinal parasitic protozoal to lipase in a different structure that is not present in milk of other mammalian (8). Hernell and friends proved this different lipase to be bile salt stimulating lipase and showed existence of it in human milk (9).

In another study, Marrow and friends, in Mexico, came across 17% symptomatic giardiasis with ELISA test in 197 babies , eldest 18 months. They had the speculation of the protective effect of human milk since none of the patients were feeding with mother's milk (10). In another study of Crouch and friends showed that infant formula fortified with arachidonic, linoleic and palmitic acid had protective effects like human milk (11).

Rohra and friends showed the same lethal effect is formed with unsaturated fatty acids in their in vitro study on G. intestinalis trophozoites (12). In a similar way Andrew and friends observed the recovering of G. muris infection in mice fed with human milk for five days (13). Kaplan, in his study on G. muris showed the mouse's own phagocytic activity acted together with IgG, s-IgA in human milk had protective effect against giardiasis (14).

In the in vivo part of our study in the group fed with human milk for seven days, G. muris infection was observed to be cured. For this reason, the protective effect of human milk on G. muris infection in vivo is speculated. In the in vitro part of our study, the trophozoites of both experiment and control group lost their motion ability and died at the end of the first hour, this maybe due to unavailable conditions. Since we don't have media which allows long time cultivation of Giardia spp., an absolute proof could not be obtained on the in vitro effect of human milk.

Farelerde oluşturulan giardiasis modeline insan sütünün etkisi

Bir kamçılı protozoa olan Giardia intestinalis'e insan bağırsağında, özellikle de çocuklarda çok sık rastlanmaktadır. Yapılan çalışmalar G. intestinalis enfeksiyonlarının seyrini immun olmayan intestinal faktörlerin etkileyebildiğini göstermiştir. Aynı zamanda, anne sütünde bulunan doymamış serbest yağ asitlerinin ve diğer lipolitik ürünlerin Giardia üzerine olan öldürücü etkilerinin varlığı henüz tam olarak incelenememiştir. Bu çalışmamızda, giardiasis için en iyi laboratuar modeli olan ve farelerin paraziti Giardia muris üzerine normal insan sütünün öldürücü etkisi in vivo olarak araştırılmıştır. Çalışmamızda, G. muris ile enfekte 20 adet BALB/c cinsi fare alınmış ve bunların 10 tanesi 7 gün süreyle sadece insan sütüyle beslenirken, kalan 10 tanesi de kontrol grubu olarak normal diyetleriyle beslenmiştir. 7 gün sonunda her iki grubun önce dışkı örnekleri incelenmiş ve kontrol grubunda G. muris kistlerine rastlanırken, insan sütü ile beslenen farelerin dışkı örneklerinde G. muris kist veya trofozoitine rastlanmamıştır. Daha sonra tüm farelerin duodenumları çıkarılarak buradan alınan taze örnekler incelenmiş ve yine sadece kontrol grubu farelerde G. muris trofozoitleri saptanmıştır. İnsan sütü ile beslenen farelerde ise G. muris kist veya trofozoitlerine rastlanmamıştır. Sonuç olarak, normal anne sütünün Giardia üzerine öldürücü etkisinin varlığı in vivo olarak kanıtlanmıştır. [T Klin Araştırma 1997; 15(1):12-14]

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