Effects of Microwave Radiation on Anti-infective Factors in Human Milk

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ABSTRACT. In intensive care nurseries it has become common practice to use microwave thawing of frozen human milk for more rapid accessibility. Twenty-two freshly frozen human milk samples were tested for lysozyme activity, total IgA, and specific secretory IgA to *Escherichia coli* serotypes 01, 04, and 06. The samples were heated by microwave for 30 seconds at a low- or high-power setting and then reanalyzed. One-mL aliquots of 10 additional human milk samples were microwaved at low (20°C to 25°C), medium (60°C to 70°C), and high (≥98°C) setting before the addition to each of 1 mL of diluted *E coli* suspension. *E coli* growth was determined after 3½ hours of incubation at 37°C. Microwaving at high temperatures (72°C to 98°C) caused a marked decrease in activity of all the tested antiinfective factors. *E coli* growth at ≥98°C was 18 times that of control human milk. Microwaving at low temperatures (20°C to 53°C) had no significant effect on total IgA, specific IgA to *E coli* serotypes 01 and 04, but did significantly decrease lysozyme and specific IgA to *E coli* serotype 06. Even at 20°C to 25°C, *E coli* growth was five times that of control human milk. Microwaving appears to be contraindicated at high temperatures, and questions regarding its safety exist even at low temperatures. *Pediatrics* 1992;89:667–669; *microwave radiation, human milk, lysozyme, IgA, host defenses.*

Human milk is used by many intensive care nurseries in the nutritional support of high-risk premature infants because these babies have both a recognized susceptibility to infection and a relatively immature digestive system. A variety of host-defense factors have been demonstrated in fresh human breast milk.[1] Two of these important protective factors are *lysozyme*, which catalyzes the hydrolysis of β-1,4-glycosidic bonds in bacterial cell walls, and *specific secretory IgA* against a wide array of enteric and respiratory pathogens; this IgA has the ability to attach itself to the mucosal epithelium of the intestine and prevent the attachment, and possibly invasion, of specific infectious agents.

To provide a continuous human milk supply for an intensive care nursery, fresh human milk must be frozen for proper storage. Such freezing has been shown to have little impact on IgA[2] or on the bacterial-growth-inhibiting activity of human milk.[3]

Recently, with the proliferation of microwave ovens, some nurseries have used this type of radiation either to thaw frozen human milk or to warm freshly expressed, refrigerated human milk. Because of the paucity of data concerning the use of microwaves and human milk, the present study was designed to evaluate the effects of microwave treatment on several of the anti-infective factors in human milk.

METHODS

Milk Samples

Breast milk samples were obtained from 22 term and preterm lactating mothers after the first week of postnatal life, using a sterile breast pump or manual expression (using proper collection techniques[4,5]) and sterile glass jars. Sterility was checked on each specimen by culturing on trypticase soy with 5% sheep blood agar plates. Breast milk samples containing any of the Enterobacteriaceae were excluded from the study. Samples were frozen immediately at −18°C and used within 2 to 7 days.

Milk Processing

Each frozen sample was allowed to thaw at room temperature. Two-milliliter aliquots from each sample served as controls. These samples were not microwaved. After thawing, they were assayed for lysozyme activity, total IgA, and specific IgA to *Escherichia coli* serotypes 01, 04, and 06. In addition, two 2-mL aliquots from each sample were placed into individual glass test tubes. These were placed in a plastic stand in the center of a Litton microwave (model 1010) and exposed to radiation for 30 seconds at either a low or high power setting. Temperatures were measured immediately after radiation (samples were stirred with the thermometer). Mean temperature was 33.5°C at the low setting, with a range of 20° to 53°C. Mean temperature at the high setting was 90.5°C, with a range of 72° to 98°C. Samples were then placed on ice at 4°C and centrifuged at 7000 × *g* at 4°C for 30 minutes. Fat and sediment were discarded, and the supernatant was used for assay of lysozyme, total IgA, and specific IgA to *E coli* serotypes. All 22 samples were stirred before aliquoting.

Lysozyme

Lysozyme activity was measured from the rate of lysis of a suspension of lyophilized *Micrococcus lysodircticus* cells, slightly modified by Litwack.[6] Egg white lysozyme (Sigma) was used as the standard.

Estimation of Immunoglobulin

Total IgA was estimated by the "precision" single radial immunodiffusion technique. For the quantitation of IgA the Sigma antibody was based on the use of *11S* (not 7S or secretory component) human IgA. Specificity was tested by enzyme-linked immunosorbent assay against human IgG, human IgM, and human IgA, and only the IgA showed reactivity. Sensitivity was tested by enzyme-linked immunosorbent assay in which 200 ng/mL of human IgA was used to coat a well (we estimate that 50 ng/mL would be the maximum to stick in a 250-μL aliquot). Then serum was diluted

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from 1:100 to 1:32 000. Dilution plotted against optical density was linear. Thus, the test appears to be sensitive.

Specific IgA to *E coli* serotypes 01, 04, and 06 were assayed by enzyme-linked immunosorbent assay as specified by Liebhaber et al.[2] Each sample (positive control) on each microtiter plate was arbitrarily assigned a value of 100, and all readings were adjusted accordingly by using the following formula:

$$\text{adjusted test value} = \frac{100}{\text{positive control value}} \times \text{test reading.}$$

Bacterial Growth

Processing of 10 additional milk samples was similar to that described above except that a Panasonic model NE-7650A was used at low, medium, and high setting. Temperatures were measured and exposure was continued until the temperature reached 22° to 25°C (low), 60° to 70°C (medium), or ≥98°C (high).

We used the model of Hernandez et al[3] to assess the ability of human milk to suppress bacterial growth. *E coli* was grown in trypticase soy broth for 18 to 24 hours and spectrophotometrically standardized to 0.250 absorbance units at 600 nm, approximating 9×10^7 colony-forming units per milliliter.[6-10] The actual number of colony-forming units per milliliter was determined by plating a 10-μL aliquot of 10^{-5} serial diluted *E coli* suspension in sterile phosphate-buffered saline on blood agar plates. One milliliter of diluted *E coli* suspension (9×10^7 colony-forming units per milliliter) was added to 1 mL of sample (microwaved human milk; for the control, untreated human milk was used). Using a 10-μL calibrated loop, duplicate aliquots from each mixture were plated on 5% sheep blood agar plates at 0 hour. After 3½ hours of incubation at 37°C, the tubes containing the mixtures were immediately placed in an ice bath (to retard bacterial growth) and duplicate aliquots of 10 μL were again plated from each mixture. After the plates were incubated at 37°C for 24 hours, the number of colonies formed was determined.

Statistical analysis of all data was determined by the Student's *t* test (samples in each group compared with control).[11] Additionally, the Fisher's protected least significant difference test was performed on all our data.[11]

Of the 22 milk samples analyzed for total IgA and lysozyme, adequate documentation on whether the milk came from mothers of preterm or term babies was available for 18 babies (9 preterm, 9 term) for lysozyme and 16 babies (8 preterm, 8 term) for total IgA. Control IgA (milligrams per deciliter) was 90.63 ± 39.61 (mean ± SEM) for preterm and 65.56 ± 13.65 for term. Control lysozyme (milligrams per deciliter) was 21.22 ± 5.37 for preterm and 16.27 ± 2.67 for term. Unpaired *t* test of control term milk vs control preterm milk failed to show a significant difference for either lysozyme or total IgA.

RESULTS

Lysozyme activity was significantly lower than control values following either low or high power microwave treatment (see Table 1). Total IgA and IgA directed against *E coli* O antigen group 01 and 04 were not significantly different at low microwave treatment but were adversely affected at high microwave treatment. IgA against *E coli* O antigen 06 was significantly lower than control at either microwave setting. Inhibition of bacterial growth (Table 2) was adversely affected, allowing 5 times, 10 times, and 18 times greater *E coli* growth at 3½ hours after exposure to low, medium, and high microwave treatment, respectively.

TABLE 2. *Escherichia coli* Growth at 3½ Hours*

	No.	Colony Count
Control	10	$8.4 \pm 2.7 \times 10^7$
Low microwave	10	$43.9 \pm 11.4 \times 10^7$†
Medium microwave	10	$90.1 \pm 24.1 \times 10^7$‡
High microwave	10	$152 \pm 43 \times 10^7$‡

* Results are mean ± SE. All significant differences were also confirmed by the Fisher's protected least significant difference test.
† $P = .005$ compared with control.
‡ $P = .001$ compared with control.

DISCUSSION

Microwave ovens have become a commonplace tool in both the home and workplace. These devices provide convenience, speed, and economy in food preparation, especially in large institutional facilities such as hospitals. Thus, it would seem reasonable to thaw frozen human milk using this rapid method, since Goldblum et al[12] showed that rapid, high-temperature treatment did not destroy immunologic qualities of human milk. However, our data indicate that microwave radiation is not a suitable heat treatment modality, as there is significant loss of immunologic properties.

In the study of Goldblum et al[12] there was no change in concentration of lactoferrin, secretory IgA, and sIgA antibody activity following rapid heat treatment to 72°C for 15 seconds. Lysozyme activity actually increased. At 87°C IgA was either rapidly denatured or antigenically altered so that it could not be detected immunologically. The sIgA antibodies were affected similarly.

Jason et al[13] obtained breast milk samples twice from one donor and microwaved 10-mL aliquots for 0 to 35 seconds on a high setting or 0 to 25 seconds on a medium setting to temperatures between 25°C and 67°C. IgA levels were stable until a temperature

TABLE 1. Impact of Microwaving on Anti-infective Factors in Human Milk*

	No.	Control	Low Microwave	High Microwave
Lysozyme activity, μg/mL	22	23.7 ± 4.0	19.2 ± 3.4 $P < .005$	0.9 ± .72 $P < .0005$
Total IgA, mg/dL	22	73.3 ± 16.1	48.9 ± 15.8 NS†	1.55 ± 1.54 $P < .0005$
Ag-specific Ab to *Escherichia coli* serotype				
01	22	100%	91 ± 9.2‡	24.9 ± 10.0‡
04	22	100%	90.3 ± 6.5‡	12.3 ± 3.7‡
06	22	100%	79.8 ± 5.7‡ $P < .005$	17.1 ± 3.6‡ $P < .0005$

* Results are means ± SE. All significant differences were also confirmed by the Fisher's protected least significant difference test.
† Not significant.
‡ Percent of control.

greater than 56°C was obtained, at which point they declined by 27%. At baseline cell viability was 83.5%; after 25 seconds on the high setting (approximately 58°C), cell viability was 0%. Cells were stable up to 39°C.

Sigman et al[14] analyzed breast milk samples from 20 lactating mothers. Frozen samples were treated as follows: (1) placed in the refrigerator (10°C) overnight for 16 to 18 hours; (2) defrosted under running water (44° to 49°C) until the endpoint temperature registered 37°C (98.6°F); (3) placed in a water bath (62.5°C ± 2°C) for 30 minutes—modified "holder" pasteurization; or (4) placed in a microwave oven for 50 seconds at 700 W. For IgA there were significant *losses* for both holder pasteurization and the microwave treatments (%losses = 16.2 ± 21.3 and 30.5 ± 32.6, respectively). There was no significant change with the refrigerator or running water methods. Total colony counts and total coliform counts were much less with holder pasteurization and microwaving than the other methods. In their study, it appeared that IgA destruction was dependent on the internal temperature with little loss occurring until the temperature reached approximately 60°C, beyond which there appeared to be accelerated loss.

Our findings were similar to those of Jason et al[13] and Sigman et al.[14] At temperatures of 20° to 53°C there was no adverse effect on total IgA; at temperatures above 58°C, we found no IgA or sIgA activity against *E coli*, possibly signifying denaturation of antibody.

The adverse effects on anti-infective factors are difficult to explain on the basis of hyperthermia alone since the temperatures used were not excessive, according to the study of Ford et al.[15] There is some controversy whether there are nonthermal effects of microwave radiation.[16] Even if it were possible to determine the precise mode of the biologic effects of microwaves, it would be difficult to regulate the dose of radiation, since location in the oven, proximity to heated surfaces, and the variation in load all affect the dose delivered. Furthermore, there is difficulty in regulating absorption in seemingly homogenous liquids such as milk, since absorption and reflection of microwaves can modify the output of the microwave generators.[17] Initial experiments to thaw frozen human samples often resulted in variable thawing. This made it difficult to achieve a liquid state without excessive heating.

SUMMARY

Microwaving at high temperatures (72° to 98°C) caused a marked decrease in activity of all the tested anti-infective factors of human milk. Those results correlated well with the subsequent studies of *E coli* growth—after microwaving human milk to ≥98°C, bacterial growth was 18 times that of control human milk. Microwaving at low temperatures (20° to 53°C) had no significant effect on total IgA or specific IgA to *E coli* 01 and 04, but it did significantly decrease lysozyme and specific IgA to *E coli* serotype 06. Even when the temperature was further controlled to 20° to 25°C, *E coli* growth was 5 times that of control human milk.

This preliminary study suggests that microwaving human milk could be detrimental. Further studies are needed to determine whether and how microwaving could safely be done.

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