Alternative therapies in antibiotic-resistant infection

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Abstract

Case report: A 24-year-old woman suffering from post-influenza otitis media infection was initially treated with several series of a steroid (Elocon) and a combination of steroids and antibiotics (Atecortin, Dicortineff) without significant medical benefit. The isolated bacterial strains were identified as *Staphylococcus homis* and *Staphylococcus epidermidis*. Specific phage therapy applied sequentially over a period of three weeks resulted only in a partial reduction in inflammation and limited improvement in overall health condition. Oral application of lactoferrin (LF; 50-mg daily oral doses for seven days with two-week intervals) led to a complete clearance of both bacterial strains and full recovery of the patient. The recovery was associated with increased myelopoiesis and a sustained elevation of serum endogenous LF. In conclusion, specific bacteriophage therapy combined with the administration of lactoferrin proved to be effective in the treatment of antibiotic-resistant external ear infection.

Key words: infection, inflammation, antibiotic resistance, bacteriophages, lactoferrin.

Introduction

The renaissance of interest in phagotherapy in recent years is associated with the aggravating problem of resistance of bacterial strains to antibiotics [1]. Phage therapy is highly effective in curing suppurative infections otherwise resistant to antibiotic therapy and provides long-term specific resistance (for review, see [2]). Recently we also demonstrated that lactoferrin (LF), an iron-binding protein contained in the neutrophils and secretory fluids of mammals, provides adjunct therapy benefits in various experimental designs in mice [3-5]. Our earlier investigations on human volunteers [6] revealed that LF, given orally, regulates several immune parameters in healthy subjects, including a rise in the percentage of circulating neutrophil precursors. LF has also been shown to exhibit direct and indirect antibacterial actions [7]. The aim of this report was to test the effectiveness of combined bacteriophage and LF therapy in a patient with external ear infection.

Case

The investigation was conducted on a 24-year-old woman suffering from post-influenza otitis media infection. The patient was initially treated topically with a steroid (Elocon) and a combination of steroids and antibiotics (Atecortin, Dicortineff). None of the applied drugs proved effective. The patient then reported (April 12, 2000) to the Laboratory of Bacteriophages for the isolation and identification of the pathogen(s) involved in the infection. The isolated bacterial strains were identified as *Staphylococcus homis* (left ear) and *Staphylococcus epidermidis* (right ear). The patient was subjected to two series (beginning May 12 and June 16, 2000) of oral phage therapy with a phage specific to *Staphylococcus aureus* (676/T) from the collection of the Institute of Immunology [8]. After treatment, the two original bacterial strains were still identified at the sites of inflammation. Therefore, the patient was additionally treated (July 2000) with bacteriophages specific to *Staphylococcus* (A3/R and 676/T) for three weeks, both orally and topically in the form of droplets. Although some improvement in health condition was registered by endoscopic examination, the patient complained of persisting pain and discomfort. Eventually the patient was subjected to oral treatment with bovine lactoferrin in capsule

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Table 1. **The studied clinical parameters during therapy in the patient**

Parameters	Phage therapy		LF treatment	
	before treatment	after 4 months of treatment	one week after treatment	two months after treatment
leukocytosis (mm^3)	4600	5000	4800	5100
phagocytic index (%)	72	71	57	73
serum LF (ng/ml)	168	238	930	753
neutrophil precursors (bands) (%)	3	4	7	10
neutrophils (%)	54	54	52	48
basophils (%)	2	1	1	2
eosinophils (%)	3	1	2	1
monocytes (%)	0	0	0	0
lymphocytes (%)	38	40	38	39

form (50 mg daily for seven days, three repeats with two-week intervals). The lactoferrin was a gift from Pharma Review, Houston, USA. The undesired symptoms alleviated in the course of the LF treatment.

Results

Tab. 1 illustrates the alterations in the magnitudes of the studied parameters during the first series of phage therapy and during treatment with LF. The results show that the levels of neutrophils and their precursors as well as the level of serum lactoferrin and the neutrophils' ability to phagocytize *Staphylococcus aureus* did not significantly change after three weeks of phage therapy. However, following LF treatment the values of the studied parameters began to increase (*Tab. 1*). Some of the changes were even more profound two months after treatment and included a very strong increase in the level of neutrophil precursors, to 10% of the total cell count, accompanied by a decrease in mature neutrophils and still a significant elevation in serum LF concentration (an over threefold increase).

Discussion

Resistance to antimicrobial agents continues to be a major cause of increased morbidity, mortality, and healthcare costs. The foremost contributing factor to the problem of antibiotic-resistant infections is the widespread use of certain antibiotics. Here we demonstrate the utility of specific bacteriophage and lactoferrin therapy to combat such antibiotic-resistant infections. This study shows a direct interdependence between the effectiveness of antibacterial therapy and changes in the level of endogenous serum LF as well as those of the most efficient phagocytes: circulating neutrophils and their precursors. Although it is difficult to ascertain the exact mechanism of action of LF, in part because other therapeutic agents were used in this protocol, the hitherto accumulated knowledge on LF suggests LF-induced upregulation of proinflammatory mediators. Among these mediators is IL-8, a cytokine responsible for the degranulation of neutrophils [9]. According to the hypothesis of Rich et al. [9], endogenous LF, released from neutrophils, generates a "demand" signal for an increased recruitment of neutrophils from the bone marrow, generated by LF-inducible proinflammatory cytokines including G-CSF (granulocyte-colony stimulating factor). Such a hypothesis may explain the significant increase in lactoferrin serum level and neutrophil precursors in the course of LF treatment (Tab. 1) and the decreased percentage of mature neutrophils. Thus LF accelerates neutrophil turnover. A similar phenomenon was observed in human volunteers treated with LF [6] as well as in an animal model using mice [5]. It is, however, more difficult to explain why the ability of neutrophils to phagocytize bacteria transiently decreased during the treatment with LF. A similar phenomenon was observed in our larger study of patients subjected to phage therapy [10], which could be simply connected with patient recovery and a decreased necessity to phagocytize bacteria. The decreased ability to phagocytize bacteria may also be compensated by the increased recruitment of new neutrophils. Nevertheless, by the end of the treatment the ability of neutrophils to phagocytize bacteria had returned to the levels observed at the beginning of the monitoring (73% vs 72%). The causes of LF therapeutic action in the local infection may be more complex and involve the previously described direct and indirect antibacterial properties of lactoferrin [4-7]. We are of the opinion that the direct antibacterial action of LF is accomplished by the distinctly elevated endogenous (human) LF (*Tab. 1*), as determined by the immunoenzymatic method using antibodies specific to human LF. It is also possible that LF, given orally, may reach distant sites of infection, as was demonstrated in the case of mice with *Staphylococcus aureus* urinary infection [11].

In conclusion, phage therapy, combined with oral lactoferrin treatment, appeared to be effective in curing external ear infection otherwise resistant to antibiotic therapy. The therapeutic effect correlated with a stimulation of myelopoiesis and a rise in serum LF level, parameters crucial in the defense against bacterial infections. However, in the described case, a self recovery should also be taken into account.

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