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Perspective

Lysozyme – An Underexploited Molecule with High Therapeutic Potential but of Little Interest to the Pharmaceutical Industry

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Abstract

The discovery of lysozyme has accomplished its hundredth anniversary. Lysozyme is mainly known for its bactericidal, bacteriostatic, and antiviral properties, but has also antifungal, anti-inflammatory, immunostimulant, and anti-cancer functions. Lysozyme-based medical applications could be developed not only for infectious but also for a variety of chronic diseases, including cancer, hypertension, and diseases related to insulin resistance with the advantage of having no adverse effects. Also, lysozyme has been associated with the prevention of infections and other diseases and has anti-inflammatory properties. Therefore, it could be also considered as a functional food and for clinical nutrition. Despite its affirmed and promising medical applications, the need for developing new treatments against antibiotic-resistant strains, and affordable treatments especially for diseases with high social, societal, and economic impact, today, surprisingly, lysozyme-based approved drugs and treatments for humans are still almost not existing. The development or re-profiling of lysozyme-based drugs appears to be unattractive for the industry. One probable reason for that is, that already well-known sources, such as egg-white lysozyme, are unlikely to be patentable due to a lack of innovation. In the current system, the development of unpatentable drugs can only be attractive with a sufficiently long market exclusivity granted. Today, drug development depends exclusively on the economic interests of the industry. However, for more sustainable drug development and full exploitation of available resources, a more public interest-driven system may be needed. Also, for the targeted use of lysozyme as a functional food and for clinical nutrition public resources for research and clinical trials are necessary.

Keywords: Clinical nutrition; Disease prevention; Drug approval; Exclusivity; Forgotten food; Functional food; Health Policy; Lysozyme; Patentability; Public health; Tailored incentives; Therapeutic potential; Underexploited drug.

Introduction

A hundred years ago, in 1922, Alexander Fleming discovered lysozyme. Lysozyme (muramidase) is a small alkaline polypeptide whose name is given by its well-known capacity to hydrolyze the mucopolysaccharides of the cell walls of many pathogenic, in particular Gram-positive bacteria. Lysozyme breaks the β-1,4 glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the cell wall of bacteria, with the cell wall rupture causing the death of the bacteria. Lysozyme is present in

several mucosal secretions and tissues of vertebrates, invertebrates, and plants acting as an endogenous antibiotic, protecting the organism against bacteria, viruses, and fungi, but not only. It also has immunomodulating functions and is involved in both pro- and anti-inflammatory processes. High serum lysozyme levels are associated with infections, inflammatory diseases, cancer, and also renal insufficiency. In fact, lysozyme can be used as a biomarker for several diseases and their progression [1-7]. These may be the same diseases where exogenous lysozyme may have therapeutical value, but this raises then the question of whether exogenous lysozyme could provide additional benefit to the already altered endogenous levels. On the other hand, this same condition also suggests the possibility of well-tolerated therapeutical strategies. Another concern is that although the utility of lysozyme as a potent topical anti-biological agent has been recognized, it is still widely believed that it cannot be an effective systemic drug due to its size which may be too large to travel between cells [8]. This conviction could be one of the reasons for the substantial limitation of the development of lysozyme-based medications. However, first of all, this would not preclude the development of drugs with topical effects. Lysozyme could be used in lotions, eye drops, suppositories, and aerosols, for example. It also may be encapsulated with drug release and topical effects in the intestine. Secondly, increased serum lysozyme concentrations after oral lysozyme administration have been demonstrated [9-11] which seems to be in contradiction to the above-mentioned conviction. Intestinal absorption of lysozyme without decomposition into amino acids was suggested [12], and systemic effects of exogenous lysozyme have been demonstrated [13]. However, hen egg-white lysozyme may be partially resistant to digestion in the duodenum [14,15], and when orally administered, a previous fasting state may be necessary to significantly increase serum levels [9]. Lysozyme is even able to penetrate the blood-brain barrier when encapsulated in positively charged liposomes [16]. Also, though involving a greater risk of anaphylaxis, in addition to oral, parenteral treatments could be considered [13]. In the following sections, we want to illustrate where exogenous lysozyme has already approved benefits for the treatment of human diseases and where it could have benefits. The great advantage of lysozyme treatments is, that, except for possible allergic reactions, it appears to have no toxic side effects [13,17-20]. In addition to disease treatment lysozyme may also be considered for disease prevention and in that case, without necessarily competing with endogenous lysozyme.

The most efficient lysozyme source appears to be human lysozyme which can be also expressed in transgenic plants or animals, for example in rice *calli* [21] or the mammary gland of transgenic goats [22]. Nevertheless, the most commonly used lysozyme source is chicken egg-white, which contains not only large amounts of lysozyme but is also very economical to produce. As an alternative to egg white, also donkey milk contains high lysozyme levels. In terms of lysozyme contents, one hen egg corresponds to about one glass of donkey milk, containing both about 100mg of lysozyme [23-25].

Applications in the Food and Feed Industry

Currently, the use of lysozyme as a drug for the treatment of diseases is very limited both in specific therapeutic applications, as well as in frequency. The main use of lysozyme concerns food conservation, such as cheese, wine, and beer [26], where it is used as an additive (E 1105), and it is also used for livestock production [27, 28] in alternative to growth-promoting antibiotics, especially for antibiotic-free production systems. In the animal production sector, lysozyme products are gaining market given the increasing regulatory restrictions concerning on-farm antibiotic use as a response to increasing antibiotic resistance of pathogens. Lysozyme may be also used as a sweetener in the food industry [29], and it is proposed as a molecule in activated packaging to enhance safety and extend food shelf life. Lysozyme incorporated in cold plasma-activated biodegradable polylactic acid packages of fruit juices can inhibit the growth of *Listeria monocytogenes*, with increasing impact at increasing storage temperatures [30]. In this context, lysozyme is also raising interest for its applications in nanomedicine and biocatalysis capitalizing on its adsorption properties on silica matrices [31]. Finally, lysozyme is commercialized as a food supplement with an immune-stimulating effect.

Current Medical Applications

As for approved medical applications, a Japanese pharmaceutical company has produced and distributed a lysozyme medication for over 50 years, reaching Costa Rica, the Dominican Republic, El Salvador, Hong Kong, Japan, Malaysia, Singapore, and Taiwan, where it was employed against bacterial and viral infections, inflammation as well as for wound repair. In particular, it was used in cases of chronic sinusitis, difficulty in expectoration associated with respiratory diseases, bronchitis, asthma, and periodontitis, and the usual daily dosage of lysozyme chloride ranged between 60 and 270mg [32]. The drug is considered safe but was voluntarily recalled in 2016 after the Pharmaceutical Affairs and Food Sanitation Council of Japan had declared a “decrease of the medical usefulness of lysozyme in the present medical environment and that its usefulness could not be confirmed at this point in time.” In the same year, two articles were published [32, 33], both testing the efficacy of lysozyme as an add-on to standard treatment in patients with chronic obstructive pulmonary disease (COPD) in clinical trials. The first study [33] was a small-cohort, but cross-over study where it was demonstrated that the co-administration of lysozyme with regular treatment following COPD guidelines had beneficial additive effects on spirometry,

airway function, and impulse oscillometry system-assessed measures of respiratory resistance and reactance. The second clinical trial was far larger, but instead of a cross-over design, where each patient received both, the lysozyme and the control treatments, a parallel design was applied, where the patients were assigned to either the lysozyme or the control treatment. In that second study, there was no statistical difference between the two groups, and it was concluded that adding lysozyme to standard therapy was not able to prevent COPD exacerbation. It might be the latter study that was considered by the Pharmaceutical Affairs and Food Sanitation Council of Japan for its decision. However, the authors of this second trial highlighted some limitations of the study suggesting, for example, some possible expert-related variations in the judgments of the COPD classification. They also observed that lysozyme might reduce the exacerbation rate and the median time to first exacerbation in patients with airway-dominant phenotype. They reported as well, that, even though not statistically significant, the levels of improvement in forced expiratory volume in 1 second and COPD assessment test scores were always greater in the lysozyme group than in the placebo group. As for the experimental design, in that context, it should be noted that in the case of treatments that are not too different in terms of the expected clinical outcome, a cross-over study design is supposed to be the appropriate choice as it is more sensitive to detect differences compared to a parallel design [34].

Currently, lysozyme medications seem to be only approved in France, the Republic of Latvia, and Italy. The approval of these drugs implies the demonstration of the clinical evidence for efficacy [35-37]. In France and the Republic of Latvia, it is proposed for the treatment of mild inflammation of the throat in patients without fever. A lysozyme medication has been produced in Italy since the mid-forties and is approved as a co-adjuvant in the case of Herpes Zoster and Herpes simplex infections. Daily oral doses of over 1g are suggested for that application, and a prescription is necessary.

Possible Future Medical Applications

Lysozyme functions are reported and possible therapeutical fields of applications are suggested in recently published reviews [24, 38-40], and these fields of applications are far beyond the currently approved therapeutical indications. Besides the better-known antibacterial and antiviral functions, these reviews discuss also antifungal, immunomodulatory, anti-cancer, and neuroprotective functions, as well as lysozyme effects on hypertension. Even if only possible topical effects of lysozyme were considered, supplemental lysozyme could have value in the treatment of various diseases. Some specific functions and possible therapeutical fields are listed here below:

(1) Anti-inflammatory agents

Lysozyme decreases the oxidative burst and chemotaxis in neutrophils [41], its peptides obtained by simulated physiological gastrointestinal digestion have strong antioxidant activity [42], lysozyme induces transcriptional modulation of the TNF-α/IL-1β pathway genes in U937 monocytes and has, therefore, anti-inflammatory activity. These effects can be achieved with the supplementation of relatively low concentrations of hen egg-white lysozyme for a short time of exposure [43]. In a porcine model of dextran sodium sulfate-induced colitis, hen egg-white lysozyme significantly reduced the local expression of pro-inflammatory cytokines TNF-α, IL-6, IFN-γ, IL-8, and IL-17 while increasing the expression of the anti-inflammatory mediators IL-4 and TGF-β [44]. Lysozyme also attenuated the inflammation symptoms and restored the epithelial barrier function suggesting that hen egg-white lysozyme could be a promising alternative for the treatment of infammatory bowel disease [44]. Similar observations of alleviated inflammation in colitis-induced mice were made when administered a fungal lysozyme [45]. In atherosclerotic rats, lysozyme stimulated gastric mucus production and prevented mucosal tissue damage [46]. In 2007, recombinant human lysozyme was patented in China as an inhalant for the treatment of bronchial asthma (CN1583167A) [47].

Lysozyme could also protect against the renal damage that occurs in diabetes. It binds to advanced glycation end products (AGEs) associated with generating reactive oxygen species, inflammatory cell activation, and growth factor/cytokine production. Lysozyme reduces their serum concentration and their deposition in the kidneys of early-stage diabetic rats [48] preventing the development of glomerular and renal hypertrophy as well as the overexpression of AGE receptors [49]. In cancer treatment, lysozyme reduced pain and ameliorated the global features of inflammatory and necrotic processes [13, 50] (see also (3) below). When fed to premature infants with mixed pathology, a more rapid sanation of the infectious inflammatory foci and an increase in body weight were observed [51] suggesting lysozyme administration for clinical nutrition. When chronic crural ulcerations refractory to previous treatment were subjected to local treatment with a solution of egg-white lysozyme in normal saline, ulcerations were quickly cleared of pus, granulation tissue developed, the inflammatory reaction around the ulcers decreased and pains were no longer felt [52].

(2) Antibacterial and antiviral agents

Given the challenge of antibiotic resistance in human infectious diseases, lysozyme is currently regaining worldwide interest and has been reconsidered, especially during the COVID pandemic [38]. When lysozyme was administered as a simple feed ingredient to piglets that were previously orally challenged with *E.coli*, ileal, cecal, and fecal *E. coli* counts decreased without

altering lactobacillus counts. Also, white blood cells, epinephrine, and cortisol concentrations were reduced [53]. In 2004, the intratracheal administration of lysozyme was patented in the US suggesting its treatment for a variety of pulmonary diseases and infections (US6776989B2) [54]. A similar patent was published in 2008, specifying human lysozyme as the lysozyme source (US20080031868A1) [55]. It was demonstrated in a clinical trial that lysozyme has synergistic effects with antibiotics [56]. Children with acute pneumonia had a more rapid elimination of fever, toxic and cardiorespiratory syndromes, cough, and physical signs of the disease when additional lysozyme was administered. In children with pyelonephritis, the treatment with additional lysozyme resulted in complete remission in 81% of the cases against 56.4% in the patients treated with antibiotics alone.

The supposedly limited antimicrobial spectrum of lysozyme may be a reason why systemic antibacterial lysozyme drugs were not developed. However, it has been shown that when hen egg-white lysozyme was hydrolyzed using digestion with pepsin followed by trypsin, the antimicrobial activity was broadened to include also Gram-negative bacteria [57]. Not only the acidic environment of artificial gastric juice can hydrolyze lysozyme in vitro to form active micropeptides, but also the ability to inhibit nucleic acid replication appears to be greatly improved compared with that of intact lysozyme suggesting an enhancement of bacteriostatic and antiviral effects [58]. Also, an innovative pharmaceutical form of administration was developed to increase lysozyme effects and broaden its antimicrobial spectrum for the treatment of enteric infections [59].

Lysozyme inhibits virus entry by binding with cell receptors or viruses, binding nucleic acid, and inhibiting virus-induced cell fusion [39]. Human clinical trials with lysozyme have shown antiviral effects against herpes, measles, and hepatitis [38]. Lysozyme extracted from a marine bacterium appears to have a strong inhibitory effect on the rabies virus [60], and both, human and hen egg-white lysozyme appear to have antiviral effects against HIV [61, 62]. In summary, lysozyme could be an effective agent against infectious diseases. In addition to, and compared to conventional treatments, lysozyme has two great advantages, that should also be considered, first, to also exerting anti-inflammatory functions and second, to not having adverse effects.

(3) Anti-cancer agents

The anti-tumor properties of lysozyme are known since the late fifties and have been confirmed by many in vivo studies on animal tumors, in vitro studies with human carcinoma and sarcoma cells, as well as studies on the application of lysozyme in human neoplastic diseases. Most of this research was performed from the sixties through the eighties. The effects have been verified through tumor cell mixing, local peritumoral and intra-tumoral treatments, and systemic treatments through subcutaneous, intramuscular, intraperitoneal injections, and oral administrations [13]. The anti-tumor mechanism is associated with both, direct activation of immune effectors and indirect potentiation of host immunity, with treatments at an early stage having a higher antitumor effectiveness. Lysozyme may affect both primary tumor growth and metastasis development. In mice with Lewis lung or MCa mammary carcinoma, lysozyme had antimetastatic activity [63-65]. In any case, in animal models, the survival time of treated animals increased by 20-30% including those which did not respond with a reduction of neoplastic growth [13]. Also, when combined with chemotherapeutic drugs, the efficacy of the chemotherapy in increasing the postsurgical survival time of mice carrying solid metastasizing neoplasms, improved significantly [13].

Even though lysozyme was patented in Japan in 1978 for cancer patients, it has still no established place in the treatment of tumors and regained only recently research interest when other lysozyme sources than egg-white lysozyme, and egg-white lysozyme with a physically altered structure were evaluated. Both, recombinant human lysozyme and self-assembled nanostructured hen egg-white lysozyme appear to have strong in vitro anti-proliferative activity against gastric and breast cancer cells, respectively [18, 66]. It was also demonstrated that marine lysozyme from a marine bacterium can inhibit tumor growth of subcutaneous xenograft of S180 sarcoma and hepatoma 22 in mice [19], and this source of lysozyme appeared to have a greater anti-tumor potential compared to egg-white lysozyme.

The same advantages of lysozyme as already mentioned above, apply also to the treatment of cancer. Besides the fact, that there are no toxic side effects, lysozyme has also anti-inflammatory properties that may be beneficial in cancer therapy [13]. Lysozyme administration may alleviate for example oral mucositis during radio- or chemotherapy reducing the density of ulcerations and pain intensity when consuming food [50]. In addition to that, a third rationale for lysozyme administration during cancer is the immunomodulatory function of lysozyme that may help prevent cancer patients from opportunistic infections (see also (4 below). The potentiation of the depressed immunological status of advanced uterine cervical cancer patients [67] and patients with head and neck cancer [68] has been demonstrated. Taking this information into account, lysozyme may therefore also be considered for clinical nutrition accompanying cancer therapy.

(4) Disease prevention

Lysozyme has immune-stimulating properties. In pigs, hen egg-white lysozyme reduced *Campylobacter* shedding, and alleviated the response to oral challenges of *E. coli* [69-73]. First of all, lysozyme peptides obtained by simulated physiological

gastrointestinal digestion have strong antioxidant activity [74]. In piglets, serum superoxide dismutase, glutathione peroxidase, and total antioxidant capacity were increased promoting detoxification [75]. Hen egg-white lysozyme has been shown to protect against oxidative stress-induced tissue damage, by suppressing the generation of reactive oxygen species and decreasing the expression of oxidative stress genes [46]. Lysozyme administration also appears to affect gastrointestinal morphology and alter the intestinal microbial population. In piglets, lysozyme administration increased villus heights and crypt depths, indicating improved intestinal health [69, 71]. Lysozyme also altered the gastrointestinal microbial population in pigs [70] and broiler chickens [76], decreasing the abundance of bacteria associated with disease and increasing bacteria associated with health.

Lysozyme administration may help prevent infectious diseases and could be particularly beneficial to people who are immunocompromised. A weakened immune system can either result from a medical condition or the receipt of immunosuppressive medications or treatments and may also include elderly people and preterm babies. Aging is associated with declines in adaptive and innate immunity [77]. As for babies, at the time of term birth, the immune system has not fully matured, and immaturity of the immune system is even more pronounced when born preterm [78]. When 200ml of donkey milk were daily administered to a group of healthy, 72 to 97 years old people, for a month, increasing levels of the serum cytokines IL-8 and IL-6 were detected [79]. During the Covid-19 emergency, the protective effects of lysozyme on human corneal epithelial cells to SARS-CoV-2 infection were investigated [80]. The preventive treatment with lysozyme markedly decreased the production of pro-inflammatory molecules, such as cytokine TNF-α, induced by the viral spike proteins at infection. As a consequence, the viral entry into the human corneal epithelial cells which is enhanced by cytokine TNF-α was reduced. In another study, preventive lysozyme administration reduced the incidence of post-transfusion hepatitis and icteric hepatitis in patients with orthopaedic diseases [81].

Lysozyme could also help prevent cancer. When normal mice were administered two lysozyme injections (1mg/day x 2 consecutive days) before inoculation of tumor cells into the peritoneal cavity, there was a decrease in tumor growth and host survival improved with increases in life span of 32, 24, and 15% in sarcoma 180, Ehrlich's carcinoma, and Dalton's lymphoma animals, respectively [82]. Furthermore, as discussed below (5), lysozyme administration could also help protect against hypertension, insulin resistance, and neurodegenerative diseases.

(5) Other yet less-studied functions of lysozyme with possible benefit in the prevention or treatment of hypertension, insulin resistance, and Alzheimer's

The inhibition of angiotensin I-converting enzyme (ACE) has proven to be an effective strategy in the prevention and treatment of hypertension and related diseases. Hen egg-white lysozyme subjected to simulated gastrointestinal digestion appeared to have a strong ACE inhibitory activity in vitro [42], and it was suggested that these peptides could be beneficial ingredients to be used in functional foods. Similar observations were made with lysozyme obtained from turtle egg white [83].

Obesity and insulin resistance are associated with circulating soluble dipeptidyl peptidase 4 (DPP-4) [84] and a possible causative link between soluble DPP-4 and insulin resistance is suggested [85]. Incretins, e.g., glucagon-like peptide (GLP)-1, enhance glucose-stimulated insulin release from pancreatic β cells, and DPP-4 inhibitors are developed to stabilize incretins and thereby boost post-prandial insulin and lower blood sugar [86]. In diabetic mice, DPP-4 inhibitors lowered glucose by inhibiting DPP-4 in the gut [86], and DPP-4 inhibitors are now in widespread use in type 2 diabetes. In silico hydrolysation of egg proteins with pepsin and trypsin generate peptides that had in vitro inhibitory activity of both ACE and DPP-4 useful for controlling both hypertension and hyperglycemia, respectively [87]. The Zucker, diabetic fatty rat model of type 2 diabetes mellitus was used to evaluate the in vivo bioactivity of an egg lysozyme hydrolysate [88]. The hydrolysate and positive control, Vildagliptin, were administered by oral gavage and were evaluated over a 6h period. The hydrolysate resulted in 25% inhibition of plasma DPP-4 after 90min and the time course of the response was similar to that of Vildagliptin. This response appeared to be sufficient to increase active (GLP)-1 and reduce hyperglycemia during an oral glucose tolerance test in Zucker rats [89]. For this discovery, the patent WO2009128713 [88] was filed as an invention for the treatment or prophylaxis of insulin resistance, i.e., diabetes, diabetes type 2 or non-insulin diabetes mellitus, or glucose intolerance.

Studies on diabetes-related Alzheimer's disease rat models have demonstrated that GLP-1 also positively affects learning and memory [90, 91]. It has been further demonstrated that DPP-4 inhibitors not only increase peripheral insulin sensitivity but also improve cognition and brain mitochondrial function of insulin-resistant rats [92] and in Alzheimer's disease mice models [93]. Lysozyme may therefore have also potential as a therapeutic target for Alzheimer's disease. It appears to be neuroprotective for increasing Aβ toxicity [94]. In the cerebrospinal fluid of

Alzheimer's disease patients, the level of lysozyme appears to be significantly increased and lysozyme co-localized with $A\beta_{1-42}$ in plaques. In a Drosophila model of $A\beta_{1-42}$ toxicity, lysozyme co-expression reduced $A\beta_{1-42}$ levels, extended survival, and improved the activity of the flies [94, 95] showing also that lysozyme binds with $A\beta_{1-42}$ inhibiting its aggregation. The authors suggest that the existence of lysozyme in mature plaques may be a residual effect of unsuccessful inhibition of oligomer formation, perhaps due to insufficient lysozyme levels or the lack of persistence of sufficient levels to achieve this function.

Factors Hindering the Development of Lysozyme-based Medicines

Despite the already affirmed bactericidal, bacteriostatic and antiviral properties, and the public need for the development of new treatments against antibiotic-resistant strains, approved lysozyme-based treatments for humans are almost not existing. Also, lysozyme appears to have an immense therapeutic potential that still needs to be fully explored and unleashed, even though already one century has passed since its discovery. Even though lysozyme has recently aroused a discrete research interest with promising results, lysozyme-based medicines may not be developed. Part of the problem could be associated with allergies to lysozyme. Both egg-white and donkey milk lysozymes are considered allergens identified by the WHO/IUIS Allergen Nomenclature Sub-Committee as Gal d 4 and Equ a 6, respectively. In that sense, lysozyme in donkey milk may have a great advantage over egg-white lysosome as eggs contain up to 24 different other antigenic protein fractions in addition to lysozyme, which could also be present in the egg-white lysozyme extracts, and may significantly contribute to the frequency of allergic reactions [96].

However, the main reason for not developing lysozyme-based medicines may overall rely on the fact that to be marketable, drugs need to be approved to guarantee both safety and effectiveness. These drug approval procedures are very expensive. Drugs won't be developed without the prospect for potential payoffs. Among the key counterweights to drug development costs are drug pricing, patents, and marketing exclusivity. Patents protect intellectual property rights and marketing exclusivity prevents competition for a designated period of time. Unlike a patent, which is generally acquired early in development, and runs considerably longer, marketing exclusivity is granted only upon approval of a drug and puts into place a period of time during which no other applications can be accepted and/or approved for the same active ingredient.

Often approved drugs are protected by more than one patent [97] suggesting that companies may seek additional market protection. Also, even though for patents the evidence for the therapeutic utility needs to be provided from laboratory or animal experiments, clinical trials are not yet required. The very cost-intensive clinical trials become mandatory further on for drug approval, necessitating the investment of a pharmaceutical company. For these reasons, a successfully patented drug may not necessarily become marketable. The human use of lysozyme in cancer patents, in fact, was patented in Japan in 1978 [13]. Also, the treatments of respiratory disorders by intratracheal administration of lysozyme (US6776989B2), and of recombinant human lysozyme in preparation of medicine for bronchial asthma (CN1583167A), were patented in the years 2004 [54] and 2007 [47], respectively. Nevertheless, none of these drugs appear to have been approved and except for the last patent, which expires in the year 2024, these patents have already expired.

A major problem with patents is that drugs cannot be patented when the idea is insufficiently new or inventive, even when that drug has not yet been proven safe and effective in clinical trials [98]. It is not uncommon for scientific publications to disclose a drug in a manner that later prevents it from being patented. This occurs even in many universities with large medical-research programs [98]. A cursory disclosure containing already little information about a drug is often enough to prevent it from being patented later. There are also research institutes, such as the Mario Negri Institute of Pharmacological Research, in Milan, Italy, that deliberately decide to not patent any of their research. On the other hand, universities, research institutes, and researchers need to publish their results. The application for a lysozyme-based drug patent (US20080031868A1) [55] was withdrawn without disclosing the reason to the public. This patent was for the prevention and treatment of pneumonia, tracheitis, faucitis, or amygdalitis, caused by virus, bacterium, drug-resistant bacteria or chlorine, as well as for the treatment of tracheitis, pneumonia, or abscess of the lung, caused by virus or severe acute respiratory syndrome, with human lysozyme medicine in the form of an aerosol. The application for another drug patent (WO2009128713) [88] for the treatment of diabetes with egg hydrolysates, with DPP-4 inhibitory activity, was rejected because of insufficient innovation. In that sense, all the research published over the years on lysozyme sources, such as egg-white or human lysozyme hinders further patenting of these already-known compounds. Unless the findings have been already patented at the time of publication, this translates into an immense waste of time, knowledge, capital, and funds, as well as of possible precious therapeutic resources that are easily available and have no toxic side effects.

In those countries where lysozyme has gained drug approval, lysozyme could be repurposed for other therapies. The maximum exploitation of drugs with already known safety, tolerability, and pharmacological parameters should be a sound strategy of greatest public interest as it addresses both aspects, the environmental and economic sustainability of healthcare. Re-profiling of drugs for the treatment of a different patient population or different

diseases than for which they were initially developed, is suggested as a valid alternative to traditional drug development [99]. This procedure allows the pharmaceutical industry to save a lot of money, as information about drug metabolism, bioavailability, pharmacokinetics, and toxicity has been already established. In addition, obtaining "Second Medical Use" patents for known medicaments is possible, but as discussed before, only if there is sufficient innovation. In this context, repurposing over 880 past medicines with likely pharmaceutical benefits, that are no longer available since drug approval became more rigid, were suggested as potential starting points for future developments of drugs [100]. However, the same authors noted only a weak response from the private sector to such opportunities. They concluded that there is a need for additional incentives provided by regulatory agencies to incentivize the repurposing of medicines, particularly for indications of high priority for public health. Pharmaceutical companies that conduct additional clinical trials on a previously approved drug can receive three years of market exclusivity in the United States for the necessary clinical investigation [101]. However, this may not be a sufficiently attractive incentive for pharmaceutical companies. In Europe, the current situation is even worse as the regulation does not provide any additional exclusivity or incentivization at all for repurposed drugs.

Importance of Tailored Incentives and Regulations Provided by Regulatory Agencies for the Re-profiling and Efficient Exploitation of Lysozyme

States have a legal obligation to make essential medicines available to those who need them at an affordable cost. Neither the development of non-patentable drugs nor the re-profiling of drugs may be the first choice of the pharmaceutical industry which would usually turn their efforts and investments towards the development and marketing of drugs with the highest prospect for profit but should be a priority of the governments if these drugs significantly contributed to contain private and public expenses for healthcare and to improve public health. This seems to be the case with lysozyme. Its possible therapeutic fields are not limited to urgently needed alternative treatments for infections but may cover also a wider spectrum of chronic diseases, all of them with high social, societal, and economic impact. In that sense, the governments may intervene with appropriate incentivization and regulation for research, drug development, approval, and commercialization but not only. Lysozyme is also a food supplement and as such it may be applied for disease prevention and clinical nutrition. It is already commercialized especially in the feed industry, and also, even if still less propagated, as a food supplement to boost the immune system. It is readily available for everyone at a low cost, without any restriction. Through the commercialization of lysozyme as a food supplement, all the expensive approval procedures and necessary clinical trials are avoided, and from an economic point of view, this may be a more profitable and at the same time, risk-free strategy, compared to the development of lysozyme-based medicines that could be considered insufficient to offset the concomitant financial risks. However, clinical trials would be also indispensable for any targeted use as a food supplement, especially when it comes to clinical nutrition, and therefore, also in this context, incentives for research to specify the conditions for beneficial use with proper posology, are needed.

In summary, any intervention provided by regulatory agencies will need to be very well studied. To overcome the problem of the patentability of drugs, a grant of prolonged exclusivity such as it is, for example, for orphan drugs, has been suggested [98]. This grant alone may not be sufficiently attractive for the industry. The use of priority review vouchers to speed up the application process of a future potentially more profitable drug may represent a further powerful incentive. However, in the longer term, for sustainable drug development and commercialization, a more public interest-driven system may be needed where drug research and development become also a public enterprise, replacing the current drive of profit maximization via patents [102].

Author Contributions

IS had the idea, developed the framework, and wrote the draft, corrections, and final submitted version. VMM contributed with literature research, reading, and commenting on the draft and versions. All authors contributed to the article and approved the submitted version.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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