4-METHYLUMBELLIFERONE, AN HYALURONAN SYNTHASE INHIBITOR, PREVENTS THE DEVELOPMENT OF ONCOLOGICAL, INFLAMMATORY, DEGENERATIVE AND AUTOIMMUNE DISORDERS

Review

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Received September 24, 2024

Revised November 24, 2024

Accepted December 08, 2024

Abstract. In present review we consider numerous experiments on tissue cultures, animal models of diseases and the first clinical studies providing the prospects of creating new drugs based on 4-MU. We believe that along with many receptors and transcription factors, the main pharmacological target of 4-MU is the hyaluronan synthase, which produces the main component of the extracellular matrix, glycosaminoglycan, hyaluronic acid (HA). The pharmacological effects of 4-MU in oncological, autoimmune, degenerative and hypercompensated regenerative processes (fibrosis, scarring) are associated with inhibition of HA synthesis. Clinical drugs based on 4-MU will be the first in the class for the treatment of a wide range of diseases.

Keywords: *4-methylumbelliferone, odeston, hymecromone, hyaluronan synthase inhibition, hyaluronan synthase*

DOI: 10.31857/S03209725250101e7

"The most fruitful basis of the discovery of a new drug is to start with an old one."
Sir James Black, Nobel Prize Laureate 1988

INTRODUCTION

In the post-genomic era, the development of new therapeutic drugs relies on a detailed knowledge of signaling pathways and the key effectors involved in them, or pharmacological targets: enzymes, receptors, and transcription factors. At the same time, physiologically active substances still play an important role in the identification and validation of pharmacological targets. To date, a large amount of experimental data has been accumulated to validate the therapeutic effects of 4-methylumbelliferone (4-MU), a natural coumarin-type compound, in animal models of cancer, autoimmune, degenerative and hyperproliferative diseases. This review is devoted to the validation of hyaluronan synthase as the main pharmacological target of 4-MU, which is a necessary step in the development of new in class of drugs - inhibitors of hyaluronic acid (HA) synthesis.

THE ROLE OF EXTRACELLULAR MATRIX IN NORM AND PATHOLOGY

The extracellular matrix (ECM), which forms the basis of connective tissue, is a highly organized extracellular structure that provides mechanical integrity and intercellular interaction.

The ECM consists of polymeric carbohydrates - glycosaminoglycans (GAGs) and various proteins (mainly fibrillar) and proteoglycans (PGs). The VSM is both a barrier and depot for peptide hormones and cytokines. It also directly generates chemical and mechanical signals necessary to maintain tissue homeostasis. Pathological processes in a number of systemic diseases lead to VSM reorganization, changes in its structure, which ultimately contributes to changes in tissue architecture and promotes the development of such diseases as fibrosis, osteoarthritis, and cancer [1, 2].

HA, a polymer composed of D-glucuronic acid and D-N-acetylglucosamine residues linked alternately by β-1,4- and β-1,3-glycosidic bonds; it is the major mass component of the extracellular

matrix. HA homeostasis is maintained by synthetic activity of hyaluronan synthases (HAS) and degradation by hyaluronidases and chemical degradation mainly by reactive oxygen species. Three isoforms of the hyaluronan synthase enzyme are known: HAS1, active in embryogenesis; HAS2, the major isoform both in embryogenesis and in most tissues in the postnatal period, synthesizing high molecular weight forms (HMFs) of 1000-6000 kDa; HAS3, synthesizing low molecular weight forms of HA (LMFs), less than 250 kDa. High molecular weight HAs are generally associated with anti-inflammatory, anti-angiogenic, and anti-cancer properties. In contrast, low molecular weight fractions of HA exhibit pro-inflammatory and pro-angiogenic effects and promote cell adhesion. Although these properties of HA are generally recognized, the mechanisms underlying them are not fully understood and remain a subject of research [3].

In addition, there are different types of hyaluronidases in the human body, cleaving HA. The most extensively characterized are HYAL-1 and HYAL-2. HYAL-2 cleaves HA into fragments of approximately 50 monomer length (≈20 kDa), whereas HYAL-1 cleaves HA into tetrasaccharide fragments (≈1600 Da), which subsequently undergo further degradation in lysosomes [4]. Pathologic processes such as disruption of GC metabolic pathways, cancer, tissue damage and inflammation can alter this balance by increasing the concentration of NMFs of GC. There is strong evidence for the involvement of HA in the pathological processes of chronic inflammation inherent in diseases such as type 2 diabetes, liver cirrhosis, asthma, as well as in cancer progression and metastasis. Thus, HA increases the adhesion and motility of metastasizing melanoma cells [5], increases the motility of pancreatic [6] and prostate [7] cancer cells, impedes drug delivery to tumors [8-10], promotes the development of drug resistance [11], enhances cell division [12], and acts as a factor in the regulation of immunity [13]. Increased GC expression in tumor stroma is a negative prognostic sign [14-18].

The level of HA in the blood is a marker of liver fibrosis. GC synthesis in fibrotic liver is carried out by fibroblasts originating from activated stellate cells. Normally, stellate cells do not express the main enzyme producing HA in adult tissues, hyaluronan synthase type 2 (HAS2), and do not synthesize HA, but liver injury leads to the production of TGF-β, triggering the transdifferentiation of stellate cells into myofibroblasts and dramatically increasing the expression of *HAS2* in them [19]. Accumulation of HA in parenchyma causes activation of Notch1 signaling pathway in stellate cells, leading to their activation, increased intercellular matrix synthesis and fibrosis development [20]. Thus, HAS2 and HAS3 are important pharmacological targets in the

therapy of diseases associated with pathological activation of HA synthesis, in particular, liver fibrosis.

Molecular mechanisms of HA synthesis by mammalian hyaluronan synthases are becoming important in connection with the targeted search for specific inhibitors - potential drugs. These mechanisms are most fully considered in the review by DeAngelis and Zimmer [21]. Within a few years after the discovery of the bacterial enzyme from Streptococcus, SpHAS, three vertebrate HAS isoforms (HAS1, -2, -3 isoenzymes) and a viral HAS (CvHAS of Chlorella virus *Paramecium bursaria*, PBCV-1) were discovered. CvHAS shows similarities in the overall architecture of the transmembrane (TM) domains, with two TM helices at the N-terminus and four at the C-terminus, and the cytoplasmic domain with the enzyme active center. All these enzymes belong to the first class of glycosyltransferases, but vertebrate HAS and viral CvHAS add sugars from the non-reducing end (Fig. 1), unlike SpHAS, which has chain growth from the reducing end.

Fig. 1. Schematic of HA synthesis involving hyaluronansynthase. UDP-GlcNAc - uridine-5'-diphosphate-N-acetylglucosamine; GlcA - glucuronic acid

The vertebrate enzymes, CvHAS and SpHAS, have a type 2 glycosyltransferase domain (GT-2) that allows reactions with both monomers: both uridine-5'-diphosphate-glucuronic acid (UDP-GlcA) and uridine-5'-diphosphate-N-acetylglucosamine (UDP-GlcNAc). The three-dimensional structure of the viral enzyme CvHAS was established by electron cryomicroscopy [22]. A ubiquitously used and the only well-characterized inhibitor of HA synthesis is 4-MU, which can significantly reduce the expression of hyaluronan synthases HAS2/HAS3 [1]. In clinical medicine, 4-MU is known under the trade name "Gymecromone" or "Odeston". This drug is approved for use in European and Asian countries and is routinely used as a hepatoprotector and for treatment of spasms and dyskinesia of bile ducts. Thus, in Italy the drug is distributed under the name "Cantabilin" and is authorized by the Italian Medicines Agency (AIC no. 02130002).

MECHANISM OF ACTION OF 4-MU ON HYALURONIC ACID SYNTHESIS

There are no data on competitive inhibition or even direct interaction of 4-MU with the enzyme. 4-MU does not affect the enzymatic activity of the solubilized enzyme [23]. The most common hypothesis postulates that 4-MU is a competitive substrate for uridine-5'-diphosphate-glucuranosyltransferase (UGT), thus depleting the cellular pool of uridine-5'-diphosphate-glucuronic acid (UDP-GlcA) required for GC synthesis [24-26] (Fig. 2).

Fig. 2. Putative mechanism of action of 4-MU on HA synthesis. *a* - The scheme represents the normal pathway of HA synthesis. *b* - The scheme shows the substitution of UDP by the 4-MU pool, as a consequence of which HAS cannot synthesize HA. GlcNAc, N-acetylglucosamine; GlcA, glucuronic acid; 4-MU, 4-methylumbelliferone; UDP, uridine-5'-diphosphate; UGT, uridine-5'-diphosphate-glucuranosyltransferase; UDP-GlcNAc - uridine-5'-diphosphate-N-acetylglucosamine; UDP-GlcA - uridine-5'-diphosphate-glucuronic acid; HAS - hyaluronan synthase; GlcNAc -β(1,4)-GlcA - N-acetyl-glucosamine-β(1,4)-glucuronic acid. Figure borrowed from Nagy et al., 2015 [26]

This hypothesis is also not confirmed experimentally. The fact that 4-MU does not affect the synthesis of other glycosaminoglycans, which include the same monomers as in HA, testifies against it. In addition, coumarins with alkylated hydroxyl 7, which cannot be a substrate for UGT, still have a high inhibitory capacity in *in vitro* studies [27]. 4-MU has been shown to reduce the expression level of *HAS2* mRNA [25, 28, 29] while simultaneously increasing the expression level of hyaluronidase 1 (*Hyal1*) [30], as well as reducing the levels of uridine-5'-diphosphate-glucose phosphorylase and dehydrogenase [31]. At the same time, it is unknown how exactly transcriptional regulation of GC synthesis is realized and to what extent the mechanism is selective for these mRNAs (Fig. 3).

Fig. 3. Schematic representation of the pathways of inhibition of HA synthesis by 4-MU. The action of 4-MU is accomplished in several ways: either by depletion of the HA precursor, UDP-glucuronic acid; inhibition of the expression of the gene encoding HAS2 in the nucleus; or mediated inhibition of hyaluronan synthase activity. 4-MU, 4-methylumbelliferone; HAS2, hyaluronan synthase. Figure

borrowed from a review by Vitale et al. [1]

4-MU, as shown by us and other researchers [32, 33], has multiple targets not directly related to metabolism. It is possible that the decrease in HA accumulation in the medium during *in vitro* studies is a cumulative effect of several parallel processes, including possible substrate depletion, as well as the experimentally demonstrated decrease in *HAS2* expression and increase in *Hyal1* expression [30]. It is also known that *HAS2* expression is regulated by nuclear receptors, in particular, glucocorticoid receptor, and *HAS2* expression is almost completely inhibited by dexamethasone [34]. Studies have revealed changes in the cell cycle and p53 pathway upon exposure to 4-MU [35, 36].

ACTION 4MU ON VARIOUS CANCERS, AUTOIMMUNE AND INFLAMMATORY PROCESSES

4-MU has an effect on tumor progression-related processes such as migration, proliferation, invasion of cancer cells and angiogenesis. The cells of the tumor microenvironment, represented by endothelial cells, immune system cells and fibroblasts, are also affected by 4-MU. All these processes are associated with active changes in the structure and composition of the intercellular matrix, the main component of which is HA. These works justify the development of drugs aimed at altering the properties of the intercellular matrix. This approach holds promise in the treatment of various cancers, and 4-MU represents an already approved drug that could be used in a novel capacity.

In a mouse model of liver fibrosis induced by carbon tetrachloride, we showed that the formation of collagen strands is preceded by the synthesis of HA along the borders of hepatic lobules. 4-MU interfered with the initial formation of HA ties and resulted in a significant reduction of collagen fibrillar formations around the hepatic lobules [30]. It is indicative that in our subsequent work, knockdown of the gene encoding HAS2 using specific siRNA reproduced the effect of 4-MU on a number of signaling pathways and transcription of a number of key genes, leading to suppression of liver fibrosis [37].

The possibility of using 4-MU for the treatment of brain diseases is of particular interest. The VSM of malignant gliomas and glioblastomas is characterized by an increased content of HA, which stimulates adhesive and invasive processes of the tumor [38]. 4-MU is a small molecule capable of crossing the blood-brain barrier and inhibiting the synthesis of the main component of the extracellular matrix, HA, which created prerequisites for the study of this substance for the therapy of gliomas and glioblastomas. Thus, it was shown in mouse models that high doses of 4-MU decreased HA synthesis, simultaneously increasing apoptosis and reducing proliferation and migration of glioblastoma cells [39 - 41]. The effect of 4-MU on glioma cells was to reduce proliferation *in vitro* and *in vivo* by regulating autophagy processes [42]. In addition, in the work of Chistyakov et al. [43] proved the ability of 4-MU to inhibit the inflammatory response of astrocytes. Oral administration of 4-MU led to a significant reduction of HA in the spinal cord and brain of mice, a decrease in synaptic stability and reactivation of neuroplasticity, which resulted in memory improvement [44].

Data on preclinical studies of 4-MU for the treatment of different types of diseases are summarized in Table 1.

Table 1. Preclinical studies of the effect of 4-MU on various diseases

Authority/system	Disease under study	Year	Type of study	Reference
Inflammation	acute respiratory distress syndrome (ARDS)	2013	*in vitro*	[45]
		2015	*in vitro*	[46]
	allergic inflammation	2022	*in vitro*	[47]
	allergic rhinitis	2022	*in vitro/in vivo*	[48]
	inflammation	2022	*in vitro*	[49]
Head and neck	oral squamous cell cancer	2022	*in vitro*	[50]
Biliary tract	bile duct dyskinesia	1984	*in vivo*	[51]
	biliary colic	1995	*in vivo*	[52]
Immune response	Graves' orbitopathy	2020	*in vitro*	[53]
	graft rejection	2021	*in* vitro/in *vivo*	[54]
	Autoimmune response to transplanted Langerhans islets	2020	*in* vitro/in *vivo*	[55]

	acute rejection of a lung allograft	2021	*in* vitro/in *vivo*	[56]
Bone marrow	chronic myeloid leukemia	2013	*in vitro*	[57]
		2016	*in vitro*	[58]
		2017	*in vitro*	[59]
Lungs	pleural mesothelioma	2017	*in* vitro/in *vivo*	[60]
	pulmonary fibrosis and pulmonary hypertension	2017	*in vivo*	[61]
Mammary glands	breast cancer	2019	*in vitro*	[62]
		2022	*in vitro*	[63]
Bladder	bladder cancer	2017	*in* vitro/in *vivo*	[64]
Peripheral nervous system	malignant peripheral nerve sheath tumor	2017	*in* vitro/in *vivo*	[65]
Liver	hepatocellular carcinoma	2012	*in* vitro/in *vivo*	[66]
		2015	*in* vitro/in *vivo*	[67]
		2019	*in* vitro/in *vivo*	[29]
		2021	*in* vitro/in *vivo*	[68]
		2022	*in* vitro/in *vivo*	[69]
	melanoma metastasizing to the liver	2005	*in* vitro/in *vivo*	[70]
	liver fibrosis	2019	*in vivo*	[30]
	steatohepatitis	2021	*in* vitro/in *vivo*	[71]
Pancreas	pancreatic cancer	2006	*in* vitro/in *vivo*	[72]
		2016	*in* vitro/in *vivo*	[73]
		2017	*in* vitro/in *vivo*	[74]
		2018	*in* vitro/in *vivo*	[75, 76]
	pancreatic ductal adenocarcinoma	2019	*in vitro*	[77, 78]
Kidneys	renal cell carcinoma	2013	*in vitro*	[79]
	ischemia-reperfusion injury of the kidneys	2013	*in vivo*	[80]
	metastatic renal cell cancer	2020	*in vitro*	[81]
	diabetic kidney disease	2021	*in vivo*	[82]
	advanced renal cell carcinoma	2022	*in* vitro/in *vivo*	[83]

Prostate	prostate cancer	2010	*in vitro*	[84]
		2015	*in* vitro/in *vivo*	[85]
Connective tissue	fibrosarcoma	2017	*in vitro*	[86]
		2019	*in vitro*	[87]
		2020	*in vitro*	[88]
		2021	*in vitro*	[89]
Large intestine	colorectal carcinoma	2015	*in* vitro/in *vivo*	[90]
Central nervous system	glioblastoma	2021	*in vitro*	[39, 40]
		2022	*in* vitro/in *vivo*	[91]
Endometrium	endometriosis	2016	*in* vitro/in *vivo*	[92]
		2020	*in vitro*	[93]
		2023	*in vivo*	[94]
Ovaries	ovarian cancer	2014	*in vitro*	[95]
		2019	*in* vitro/in *vivo*	[96]
		2020	*in vitro*	[97]

THE PROSPECT OF TREATING VARIOUS DISEASES WITH COMBINATIONS WITH 4-MU. EFFECT ON THE PHYSICAL BARRIER OF THE TUMOR. APPLICATION OF 4-MU AS AN INHIBITOR OF HYALURONIC ACID SYNTHESIS

The GC-rich ECM surrounding the cells constitutes a biological barrier of the tumor microenvironment. This barrier regulates the work of immune effectors [13, 98], inhibits the diffusion of drugs [99], impedes the uptake of DNA-transgenic complexes during gene therapy [100], and plays an important role in acquisition of resistance to anticancer drugs [1, 11, 101].

The possibility of changing the properties of the tumor microenvironment in order to improve the outcome of various antitumor therapies is currently under active investigation. The pathological tumor microenvironment is characterized by hypoxia and high intrathecal fluid pressure, leading to tumor progression and development of resistance to treatment [102]. Increased intrathecal pressure is considered as the most important barrier for effective drug distribution within the tumor. The causes of increased tumor intrathecal pressure are numerous and include the presence

of a well-developed network of blood vessels in the tumor, underdevelopment of lymphatic vessels, alteration of extracellular matrix components, and pressure generated by continuously dividing tumor cells [103, 104]. The increased content of HA in the tissues surrounding the tumor contributes to an increase in the volume of the extracellular matrix and, consequently, to an increase in the pressure inside the tumor [105, 106]. High HA content in the tumor microenvironment creates a physical barrier that limits the access of monoclonal antibodies and immune cells to tumor tissue. This is one of the mechanisms of tumor tissue resistance to immunotherapy [107].

The ability of 4-MU to inhibit GC synthesis creates prerequisites for its use as adjuvant therapy in cancer treatment in combination with primary therapy. It has been shown in various models that the use of 4-MU as adjuvant therapy for various types of cancer increases the effectiveness of treatment, reduces the toxicity of antitumor drugs and helps to overcome the resulting chemoresistance (Table 2).

Table 2. Preclinical studies of the efficacy of treatment of various cancers with drug combinations with 4-MU

Disease under study	Main treatment	Type of study	Year	Reference
Hepatocellular carcinoma.	Immunotherapy: adenovirus encoding IL-12 (AdIL-12)	*in vitro*	2018	[111]
Glioblastoma	temozolomide	*in vitro*	2023	[41]
Malignant pleural mesothelioma	trametinib	*in* vitro/in *vivo*	2017	[60]
Colorectal carcinoma	Cyclophosphamide with immunotherapy (AdIL-12)	*in* vitro/in *vivo*	2015	[90]
Melanoma	vemurafenib	*in vitro*	2021	[109]
Esophageal squamous cell cancer	dichloroacetic acid	*in* vitro/in *vivo*	2019	[108]
Oral squamous cell cancer.	radiotherapy	*in vitro*	2022	[50]
Renal cell carcinoma	sorafenib	*in vitro*	2013	[79]
Progressive renal cell carcinoma	sorafenib	*in* vitro/in *vivo*	2022	[83]
Pancreatic cancer	5-fluorouracil	*in* vitro/in *vivo*	2018	[76]

Pancreatic cancer	gemcitabine	*in* vitro/in *vivo*	2006	[72]
Ovarian cancer	carboplatin	*in* vitro/in *vivo*	2019	[96]
Urothelial carcinoma of the bladder	cisplatin or doxorubicin.	*in vivo*	2019	[110]
Fibrosarcoma	radiotherapy	*in vitro*	2021	[89]
		in vitro	2019	[87]
		in vitro	2017	[86]
Chronic myeloid leukemia.	imatinib	*in vitro*	2017	[59]
Chronic myeloid leukemia.	doxorubicin	*in vitro*	2016	[58]

According to the available data on the study of 4-MU as an additive to the main therapy, 4-MU increases the radiosensitivity of radiation-resistant oral squamous cell carcinoma cells [50] and fibrosarcoma cells [86-89]. Sorafenib in combination with 4-MU more effectively inhibits proliferation, invasion, capillary formation and induces apoptosis of renal cell carcinoma cells and endothelial cells [79, 83]. 4-MU enhances the efficacy of 5-fluorouracil [68] and gemcitabine [72] against pancreatic cancer by inhibiting cell proliferation and leading to a decrease in the size of primary tumors and metastases, as well as increasing the survival of diseased animals. 4-MU increases the sensitivity of glioblastoma cells to temozolomide, enhancing the drug's effect on cell death [41]. 4-MU increases the cytotoxic effect of carboplatin on chemoresistant ovarian cancer cells [96]. Co-administration of dichloroacetate and 4-MU in a model of esophageal squamous cell carcinoma enhanced apoptosis and inhibited tumor growth [108]. Myeloid leukemia cells upon treatment with 4-MU became more sensitive to doxirubicin [58] and increased the rate of senescence [59]. On melanoma cells, the combination of vemurafenib with 4-MU was shown to more effectively reduce cancer cell survival compared to vemurafenib monotherapy [109]. 4-MU increases the chemosensitivity of urothelial bladder carcinoma cells to doxirubicin and cisplatin [110]. 4-MU significantly reduces tumor intratumor pressure and improves tumor perfusion, promoting more efficient expression of adenoviral transgene in IL-12 immunotherapy (AdIL-12) for the treatment of colorectal cancer [90]. In a study in a liver cancer model, 4-MU in combination with AdIL-12 resulted in more pronounced inhibition of tumor growth and increased survival of mice compared to monotherapy [111].

USE OF 4-MU AS A HEPATOPROTECTANT AND CHOLESTATIC TO REDUCE HEPATOTOXICITY OF PRIMARY THERAPY

Immune checkpoint inhibitors, cytokines and antibodies to them, are used as immunomodulators to enhance the body's immunological response in response to tumors and foci of chronic inflammation in rheumatoid, autoimmune, and inflammatory diseases [112, 113]. These drugs have successfully completed clinical trials and are approved for use by the European and American drug agencies [114]. However, up to 17% of patients receiving such immunotherapy suffer from complications associated with liver and bile duct damage [115-119].

Depending on the severity of hepatotoxicity, discontinuation of immune checkpoint inhibitors is recommended for the treatment of hepatotoxicity, corticosteroids are indicated in some cases, and immunosuppression is indicated in more severe cases [120-123]. In case of development of cholestatic form of hepatotoxicity, when corticosteroids are ineffective, ursodeoxycholic acid helps to improve liver parameters [124-127]. It has proven hepatoprotective and choleretic properties and is considered the standard therapy for cholestatic liver diseases with an autoimmune component, such as primary biliary cirrhosis, primary sclerosing cholangitis [128-130]. Currently, no published data on the effect of 4-MU on the risk of hepatotoxicity in response to immunotherapy have been found, but, as in the case of ursodeoxycholic acid, the proven cholestatic and hepatoprotective properties of 4-MU make it a promising candidate for such studies.

The presented data create prerequisites and indicate the necessity to conduct full clinical trials of 4-MU as adjuvant/supplementary antitumor therapy, which contributes to the reduction of GC content, has an effect on ECM and tumor microenvironment, reduces intratumoral pressure, improves tumor perfusion, facilitates drug access and has hepatoprotective and cholestatic effects, thus reducing the risks of hepatotoxicity during immunotherapy.

TOPICAL APPLICATION OF 4-MU AS A SUBSTANCE PREVENTING THE FORMATION OF STRETCH MARKS, SCARS, KELOID SCARS, SUNBURNS AND FOCI OF HYPOPIGMENTATION

Topical application of 4-MU leads to effective inhibition of HA synthesis in the skin [131]. It has been shown that 4-MU prevents keratinocyte activation, reduces epidermal hyperproliferation [132] and the rate of keloid keratinocyte migration, reducing the probability of keloid scar formation [133].

4-MU enhances the processes of melanogenesis, which makes it a promising candidate for the treatment of skin conditions associated with hypopigmentation, as well as for use as a cosmetic agent to create a natural tan [134].

METABOLISM 4. TOXICITY AND SAFETY FOR HUMANS

4-MU, like all coumarins, is poorly soluble in water. It is a nonpolar molecule and therefore easily crosses the lipid barrier in the intestine, is almost completely absorbed during oral administration and excreted with urine and bile [26]. The methyl group at position 4 ensures low toxicity of the drug, preventing its metabolism to coumarin-3,4-epoxide under the influence of cytochrome P450, and weak anticoagulation properties, compared to other coumarins, such as dicoumarin and warfarin [135].

Upon ingestion, 4-MU is metabolized very rapidly and almost completely in the liver and small intestine to 4-methylumbelliferone-beta-D-glucuronide (4-MUG), which has so far limited its use for the treatment of bile ducts only [1, 136-138]. When 4-MUG is administered orally, less than 3% of the initial drug dose reaches systemic levels unchanged. When 4-MU is administered intravenously, its concentration in the blood is 10-30 times higher [26, 139]. The half-life of 4-MU when administered orally is only 28 minutes for humans and 3 minutes for mice [140, 141]. At the same time, the median plasma concentration of 4-MUG is more than 3000 times higher than that of 4-MU [26, 141]. It can be said that when the drug is administered, most of it is in the body in the form of its metabolite 4-MUG. But despite the low bioavailability and short half-life of the drug, 4-MU has an effective inhibitory effect on HA synthesis when taken orally. 4-MUG was found to be as effective as 4-MU in inhibiting HA synthesis, and within the cell it is hydrolyzed back to 4-MU [137]. Therefore, the effect of its metabolite, 4-MUG, must be taken into account to evaluate the pharmacodynamics of the drug. These data create prerequisites for the use of 4-MU for the

treatment of diseases far beyond the biliary tract. Thus, being a small nonpolar molecule, 4-MU is able to cross the blood-brain barrier and effectively inhibit glioma cell division [42].

The typical dosing regimen of 4-MU for an adult is 900-2400 mg/day [26]. No mutagenic or genotoxic effects have been observed [1, 142, 143]. Clinical trials in the USA on patients with chronic hepatitis B and C (NCT00225537), as well as on healthy people and patients with respiratory system diseases (NCT02780752) [144] proved the safety of 4-MU see Table 3).

Table 3: Clinical studies of 4-MU in the therapy of various diseases

Disease under investigation/study objective	Status	Year	Link/Identifier .gov
Interstitial lung diseases (The SOLID Study)	Phase II; recruitment of participants has not started	2024	NCT06325696
Primary sclerosing cholangitis.	Phase II; enrollment underway	2022	NCT05295680
COVID-19	unknown	2022	NCT05386420
Pulmonary hypertension, including interstitial lung disease (The SATURN Study)	phase II; completed	2021	NCT05128929
Healthy participants; study of the effect of 4-MU on HA synthesis	phase I; completed	2016	[144]/ NCT02780752
Stage 2 biliary sludge	no information	2016	[145]
Chronic hepatitis C and hepatitis B virus	unknown	2005	NCT00225537
Bile duct dyskinesia	no information	2005	[146]
Bile duct dyskinesia	no information	2001	[147]
Bile duct dyskinesia	no information	1995	[52]
4-MU bioavailability study	no information	1993	[141]

Symptoms after bile duct surgery	no information	1988	[148]
Bile duct dyskinesia after cholecystectomy	no information	1984	[51]

CONCLUSION

Despite the plethora of experimental work demonstrating the efficacy of 4-MU in various animal models of cancer, immune and degenerative diseases, it must be recognized that reliable molecular mechanisms of action remain hypothetical. Nevertheless, there is evidence that, at least in a model of liver fibrosis, knockdown of the gene encoding HAS2 leads not only to suppression of fibrosis but also to changes in the transcriptome similar to the effects of oral administration of 4-MU [37]. At the same time, it cannot be excluded that some of the mechanisms of action of 4-MU may be independent of inhibition of HA synthesis. Thus, effects of 4-MU have been described [62, 149]. Also, 4-MU may have different antitumor mechanisms depending on the type of cancer. However, taken together, the multiple data on the efficacy of 4-MU that we have reviewed prove the need to move to a detailed study of the pharmacokinetic and pharmacodynamic aspects that determine the treatment regimen (route of administration, doses affecting its bioavailability, time of interval between them, and schedule of administration). The first toxicologic studies in phase I clinical trials have already been conducted [144], which makes it possible to move now to clinical studies of the drug's efficacy (phase IIa). A similar trend is observed in the world. Thus, clinical trials of 4-MU for the treatment of interstitial lung diseases and cholangitis are currently planned (see Table 3).

In clinical trials of 4-MU, doses need to be tailored to the specific pathology, excretion rate of its metabolites and bioavailability. The creation of new forms, such as nanoparticles containing 4-MU, is an important factor that will not only increase bioavailability, but also create prerequisites for patent protection of a new drug.

Finally, if we accept our arguments that the main pharmacological target of 4-MU is hyaluronan synthase, the development of new chemical compounds using 3D models of HAS2/HAS3 and docking of potential ligands using artificial intelligence approaches should inevitably lead to the development of original first-in-class targeting drugs based on hyaluronan synthase inhibitors.

AUTHORS' CONTRIBUTION

Y.V. Kotelevtsev participated in the formulation of the general concept of the review, selection of the main articles and supervised his own experimental work reflected in the review. A. Citrina and N. Halimani provided the main conclusions from their experimental articles and participated in the discussion of the review text and made changes. V.V. Fedorova collected and analyzed the literature and wrote the main text of the review.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS DECLARATION

This article does not describe any research involving humans or animals as subjects.

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