

Successful Reduction of SARS-CoV-2 Viral Load by Photodynamic Therapy (PDT) Verified by QPCR – A Novel Approach in Treating Patients in Early Infection Stages

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Abstract

Background: The Covid-19 pandemic is affecting Global Health and the world's economy dramatically since early 2020. After a temporary slowdown in summer 2020 the infection numbers and death rates have been increasing again in recent months leading to various restrictions of social and economic life in many countries. Latest developments of new vaccinations seem to be promising; however large-scale production and worldwide distribution logistics take time while questions such as length of immune protection, long-term side-effects etc. are remaining unclear at this point. Furthermore, vaccination is a preventive approach and not a therapy for acutely infected patients. Thus, there are still therapies needed to help people with Covid-19 Infections. The objective of this study was to evaluate if Photodynamic Therapy (PDT) with Riboflavin and a specially designed light treatment kit would be able to fill this gap and to help people in early stages of infection. This may lead to a relief of the hospitals and intensive care stations.

Methods: This study was made up of two groups with 20 patients each with the experiment (verum) group receiving Photodynamic Therapy and daily testing and a control group receiving conventional care plus testing. All patients in both groups had positive Covid-19 test results at the beginning of the study being in an early infection stage with mild symptoms like fever, dry cough, headache, hard breathing, fatigue etc. QPCR tests with CT-viral load were performed on day 1, 2, 3, 4, 5 and 7 in the experiment group and on day 1, 3, 5 and 7 in the control group.

Results: All 20 patients in the experiment group showed significant improvement in clinical symptoms and viral load assessment within the 5 days of PDT treatment. 14 out of 20 patients had a negative QPCR test after 5 days of treatment with PDT while the other 6 patients also showed significantly reduced viral load. 20 patients in the control group with conventional care were tested 3 times within 5 days and no significant improvement could be seen clinically or in viral load assessment.

Conclusion: In this primary study the potential of Photodynamic Therapy (PDT) against SARS-CoV-2 could be shown in early infections stages. PDT proved to be successful in improving clinical symptoms, lowering viral loads and in preventing hospitalisation and intensive care treatments. This treatment is easy to perform, cost effective and can be done at home. It can be used for prevention after contact with infected people or in case of positive testing but also in early cases with mild to moderate clinical symptoms.

Background

The principle of Photodynamic Therapy (PDT)

Photodynamic Therapy (PDT) is one of the most interesting and promising approaches for treatment of various cancers and infectious diseases [1, 2]. PDT has a long history and is already approved for several superficial cancers on the skin or on superficial layers in the esophagus, bronchial system, stomach, bile duct,

colon and bladder. In contrast to chemotherapy or radiation PDT-treatments normally do not go along with severe side effects. The principle is the stimulation of a light sensitive drug (photosensitizer) which is applied either on the skin as a cream, applied orally or injected into the blood circulation. In cancer treatment the photosensitizer is integrated into tumor tissue by endocytosis and is subsequently irradiated with light of specific wavelength that

matches the absorption spectrum of the photosensitizer. This light activation process induces various chemical processes such as the development of radical oxygen species that ultimately lead to the destruction of tumor tissue or microbes and viruses.

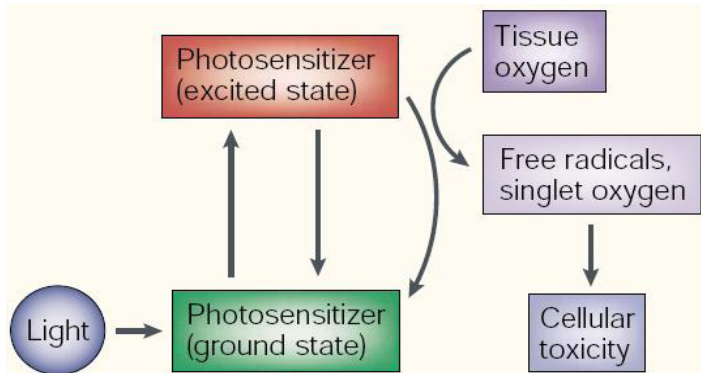


Figure 1: The principle of Photodynamic Therapy

Most of the photosensitizers used in therapeutic applications are derived from natural substances, especially from porphyrin like Haem or Chlorophyll and thus are called Haematoporphyrines or Chlorines [3]. Additionally, there are other natural substances like Curcumin, Hypericin or Riboflavin available which are effective photosensitizers and can be stimulated according to their light absorption spectrum as well [4].

Anti-viral Photodynamic Therapy:

It is known for a long time that PDT cannot only target and destroy tumor cells but also bacteria, viruses and any other types of microbes. Antimicrobial Photodynamic Therapy (also called aPDT) is a treatment option based on the combination of a photosensitizer that is selectively localized in the target tissue and the application of light of appropriate wavelength to activate the photosensitizer, resulting in photodamage and cell death. Exposure of the photosensitizer to light of a specific wavelength will lead to light absorption by the photosensitizer, thereby lifting it from a short-lived (nanoseconds) state to an excited singlet electronic state. The singlet-state photosensitizer can then undergo an electronic transition to a much longer-lived (microseconds) triplet state. The longer lifetime allows the triplet photosensitizer to react with ambient (ground state) oxygen by one of two different photochemical pathways, called Type 1 and Type 2 photochemical pathway. Type 1 involves an electron transfer to produce superoxide radicals and then hydroxyl radicals, while Type 2 involves energy transfer to produce excited state singlet oxygen. Superoxide, hydroxyl radicals and excited state singlet oxygen are highly reactive oxygen species (ROS) that can damage nearly all types of biomolecules (proteins, lipids and nucleic acids) and kill cells by inducing irreparable oxidative damages [5]. These processes are called photodamage. Typical examples for those damages are the inhibition of protein synthesis and molecular alterations of DNA strands through DNA-protein cross-linking or strand breaks. These processes alter the transcription of the genetic material during its replication (mutagenic effect) and finally lead to microbial death [6]. The killing of microbial cells via PDT is rapid and only takes a few seconds (while the action of antibiotics or other medications can take hours or days).

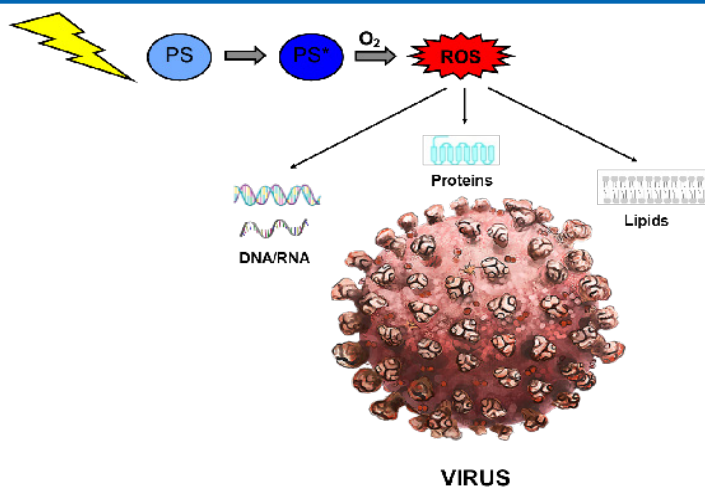


Figure 2: The mechanism of anti-viral Photodynamic Therapy:

Light stimulation boosts the photosensitizer into the excited state (PS*). The reaction with oxygen is leading to reactive oxygen species (ROS) which are able to damage the virus proteins, lipids and nucleic acids.

Riboflavin: A natural photosensitizer with a long history

Riboflavin, also known as Vitamin B2, is a vitamin found in food and used normally as a dietary supplement. It is used supplementary to treat Vitamin B2 deficiency and for prevention of migraine. It can be applied orally or by injection. It is normally very well tolerated, even it's safe in pregnancy [7]. Riboflavin is required by the body for cellular respiration. It was discovered in 1920, isolated in 1933 and first made in 1935. It is on the World Health Organisation's list of essential medicines. Riboflavin is available as generic medication and over the counter.

In humans there is no evidence for Riboflavin toxicity produced by excessive intakes, because it has lower water solubility than other B-vitamins and absorption becomes less efficient as doses increase [8]. Even when 400 mg of Riboflavin per day was given orally to subjects in one study for 3 months to investigate the efficacy in the prevention of migraine headache, no short term side effects were reported [9]. Although toxic doses can be administered by injection, any excess at nutritional relevant doses is excreted in stool and urine imparting a bright yellow color when taken in large quantities. Vitamin B2, along with other B-complex vitamins, is important for metabolism, where it is a co-factor of enzymes involved in the metabolism and catabolism of proteins, fats, carbohydrates, and purines. In addition, Vitamin B2 is required for the generation of energy. Vitamin B2 is also involved in the metabolism of other B-vitamins, in the production of hormones in the adrenal cortex, and in the body's antioxidant defense system, where it is a co-factor of the glutathione reductase enzyme. Thus, Vitamin B2 is of vital importance during all life stages. It is required for heart, brain and cognitive function, bones, joints and muscles, eyes, skin, immune system, liver, and more [10].

Dosage form, formulation, route of administration and pharmacokinetics

Riboflavin can be administered orally or intravenously. Oral administration of Riboflavin can be regarded as absolutely safe

because the extent of absorption in the small intestine is limited. Absorption occurs at a specialized segment of the mucosa; drug absorption is limited by duration of drug's contact with this area. Studies have shown that not more than 25 to 30 mg Riboflavin can be absorbed from a single dose [8]. This makes the oral application very safe. Oral absorption is increased when Riboflavin is taken with a meal. So even with very high doses of Riboflavin no side effects can be expected because the excess will be excreted by stool. The amount of absorption can be calculated from urine excretion which contributes to 50 % of the overall removal of Riboflavin from the plasma. After a single oral dose, biologic half-life is about 66 to 84 minutes in healthy people. Riboflavin is widely marketed as a food supplement and available doses in the formulations vary from 100 – 400 mg. Pure Riboflavin should be replaced by Riboflavin-5-phosphate which has much better water solubility and is the active form of Riboflavin. We recommend and used a safe dose of 100 mg Riboflavin-5-phosphate in this study for both systemic and local effects.

Data and literature supporting the proposed use for Covid 19

The potential role of Riboflavin in the photoinactivation of microorganisms and viruses has been known for over half a century [6, 11]. As it is a naturally occurring compound in the human body, this facilitates the use of Riboflavin for medical applications. From the beginning, the application of Riboflavin was used for photodynamic inactivation of viruses which has found most interest. It is still the most intensively investigated field especially in the decontamination of blood products [12-15]. Due to the extensive knowledge of this naturally occurring compound, Riboflavin has been qualified by the US FDA as GRAS (Generally Regarded As Safe). It binds to the nucleic acid bases of virus RNA and upon UV-irradiation, specifically oxidizes the guanine bases in nucleic acids by a single electron transfer reaction. In follow-up reactions, $\frac{1}{2}$ O₂, hydrogen peroxide and hydroxyl radicals are formed. This results in irreversible single strand breaks in nucleic acids with damaging of the pathogens. Riboflavin PDT has been shown to be effective against enveloped as well as a number of non-enveloped viruses – including HIV, West Nile virus, VSV, IAV, porcine parvovirus, pseudorabies virus, human hepatitis A virus (HAV), encephalomyocarditis virus, Sindbis virus, the MERS coronavirus among others [6]. Riboflavin is the active photosensitizer in the MIRASOL Pathogen Reduction Technology System (Terumo BCT, Lakewood, CO, USA), which is used to treat platelet and plasma products [14]. Moreover, it is also in use for pathogen reduction in whole blood [16]. A new study published in the US in April 2020 showed that Covid-19 virus in plasma products can be eliminated below the limit of detection in a short time with Riboflavin and UV light [17, 18]. At this point we want to emphasize that other substances like Chlorines, Haematoporphyrines, Porphycenes, Curcumin, Methylene Blue or Chlorophyllin can work as photosensitizers as well; however substances like Haematoporphyrines and Chlorines need to be applied by injection and are very expensive while Curcumin or Methylene Blue could only be applied locally without any

systemic effects because of their low resorption in the intestine [6]. Therefore we decided for Riboflavin as it is cheap, non-toxic and well absorbed. Another big advantage is that it works with blue and UVA light. Both wavelengths and especially UVA have already an inhibitory effect on viruses and microorganisms and fulfill all safety requirements [18].

Safety requirements for in vivo use and rationale for UVA/blue light usage

The above mentioned studies on virus elimination in blood products have all been performed in vitro [12-15]. The applied light for Riboflavin stimulation in the in vitro studies was produced in UV lamps delivering a broad spectrum of UV light including UVC, UVB and UVA light. Range of UVC-light is from 100 – 280 nm, UVB from 280 – 315 and UVA from 315 – 400 nm. The Mirasol system for cleaning blood products from viruses is CE approved but uses UV light with wavelengths between 280 and 400 nm (UVA and UVB together). Other studies showed effective virus inactivation by high energy UVB/UVC light which is also used in sterilisation boxes (i.e. for mobile phones) for home-use nowadays. However such an approach with UVB/UVC light cannot be used in humans as such shorter wavelengths in the UVC and UVB spectrum with high energy are known to be potentially dangerous for cancer development or damaging healthy cells. A possible explanation for the usage of such wavelengths is that all studies have been done in vitro; thus it was not necessary to worry about potential side effects in humans.

Due to those safety issues we developed a new and safe protocol based on photodynamic mechanism with Riboflavin as photosensitizer in combination with UVA/blue light stimulation. By using a photodynamic process lower light energy with higher wavelengths is necessary to trigger virus inactivation. This approach is considered to be safe for in vivo applications as no side-effects should be expected.

According to the absorption spectrum of Riboflavin-5-phosphate maximum absorption and stimulation can be found at 375 and 447 nm (fig.4). In a new study from Rezaie et al from August 2020 all safety issues of narrow banded UVA light including cell, animal and histological studies were investigated carefully showing no relevant risk for clinical application in humans [18].

Additionally, blue light is absorbed by the erythrocytes especially through thin mucous membranes in mouth and throat without pigmentation. Red blood cells carry oxygen and also nitric oxide (NO) in a complex. Irradiation with blue light of the erythrocytes releases free nitric oxide leading to quick vasodilatation of the blood vessels [19]. This effect can be regarded as an additional benefit for thrombus and embolism protection [20]. The benefit of NO for Covid-19 treatment is currently under investigation in different studies. Thus, the light system in our presented study was equipped with multiple light diodes with a combination of 375 nm narrow banded UVA and 447 nm blue wavelengths for maximum safety and therapeutic efficacy.

Photodynamic Process by interaction of Riboflavin with light:

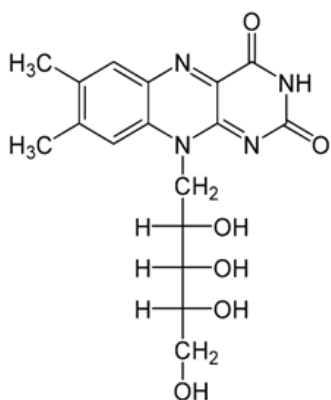
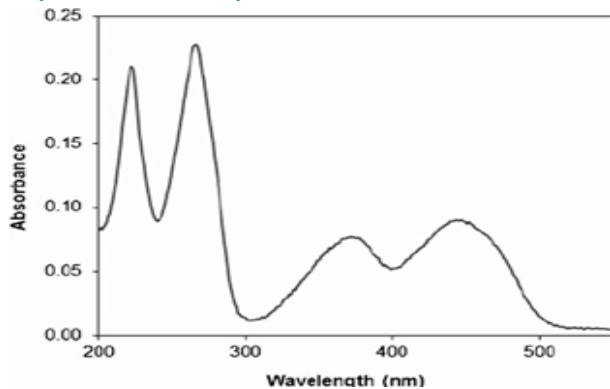


Figure 3: Absorption spectrum and chemical structure of Riboflavin in the literature

Figure 3 shows that Riboflavin absorbs light in the UVC-, UVB-, UVA- and blue range. For treatment in humans we have to neglect the UVB and UVC peaks with 220 and 280 nm because of risk for tissue damage or cancer development.

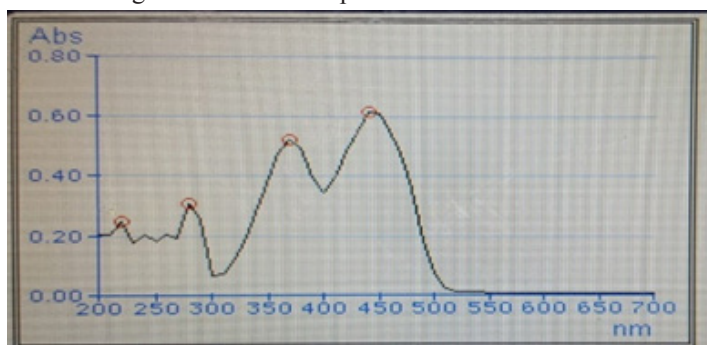


Figure 4: Physiological absorption spectrum of Riboflavin-5-phosphate used in this study

Fig.4 shows a different spectrum of Riboflavin-5-phosphate from Ultrabotanica (USA) with higher peaks in UVA and blue range. This formulation has been used in our presented study.

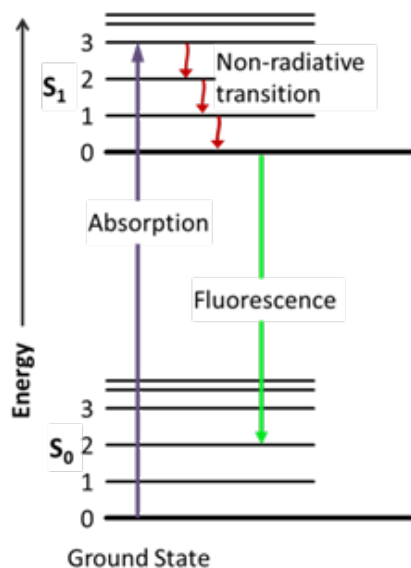


Figure 5: Fluorescence effect after activation

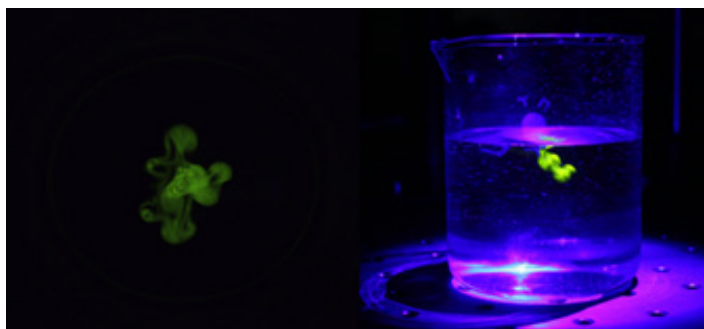


Figure 6: Fluorescence of Riboflavin with blue light

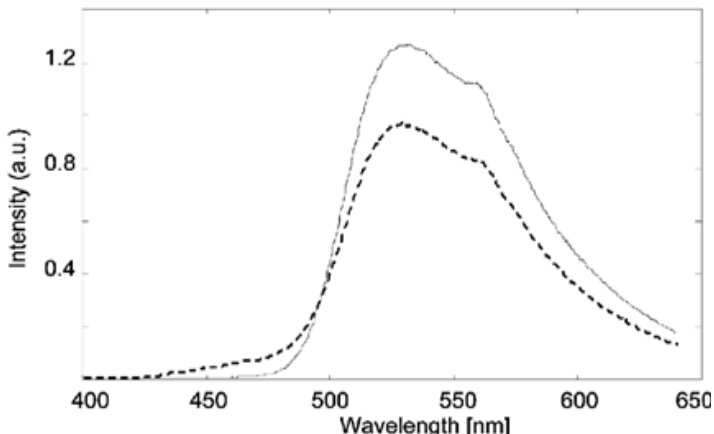


Figure 7: Fluorescence spectrum of Riboflavin after activation with UVA/blue light

After activation of the photosensitizer with light according to its absorption spectrum a different wavelength of light (color) is emitted as fluorescence. This fluorescence effect can be used in diagnostics.

Fig 8 and 9 are showing the fluorescence effect of Riboflavin after activation with UV and blue light.

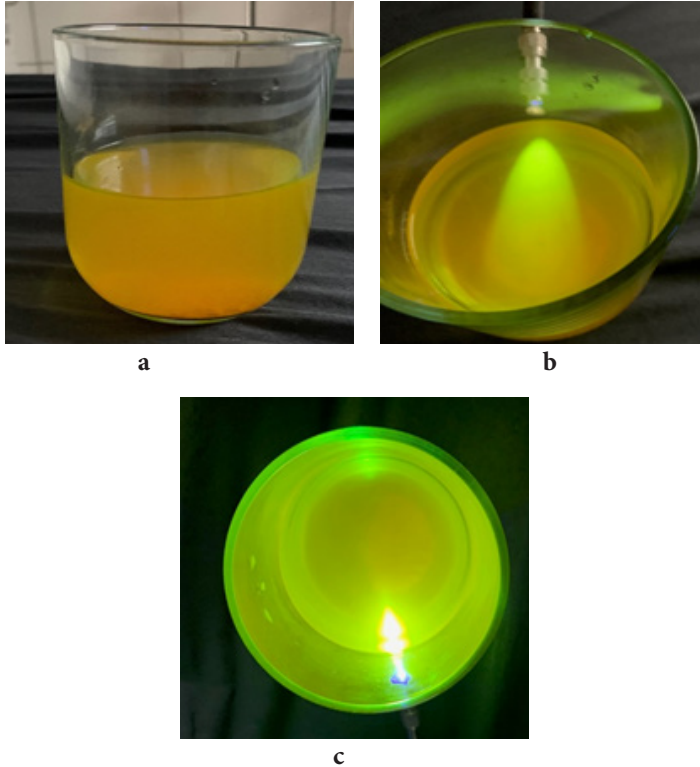


Figure 8a: Yellow Riboflavin solution in water

Figure 8b: Stimulation with UVA 375 nm with green fluorescence 532 nm

Figure 8c: Stimulation with Blue 447 nm with green fluorescence 532 nm

Figure 8 a,b,c show the fluorescence effect of Riboflavin by irradiation with UVA (375 nm) and blue (447 nm) light which is leading to a fluorescence response with green light (532 nm). The same effect can be shown on a human tongue (Figure 9).



Figure 9: UV 375 nm on the tongue and after spraying a Riboflavin-5-phosphate solution on the tongue

Materials and methods

Riboflavin-5-phosphate (100mg capsules) was provided by UltraBotanica (Oklahoma, USA). All analysis certificates and product specifications were provided by UltraBotanica [21, 22].

The anti-viral equipment was developed and provided by W Medical Systems GmbH (Germany) and consists of a small aluminium suitcase with

- 60 capsules Riboflavin-5-phosphate 100 mg
- 1 Light treatment device (“Spectra Watch” with 4 red laser diodes and 2 blue, 2 green and 2 yellow LEDs) for systemic treatment of blood via the wrist arteries
- 1 spray bottle with mouth and nose applicator
- 1 nose treatment applicator with 1 blue LED (447 nm) and 1 UVA LED (375 nm)
- 1 mouth treatment applicator with 14 blue LEDs (447 nm) and 14 UVA LEDs (375 nm)



Figure 10: Antiviral Equipment Box



Figure 11: Riboflavin-5-phosphate



Figure 12: Spray bottle



Figure 13a: Laser/LED watch front side

Figure 13b: Laser/LED watch back side

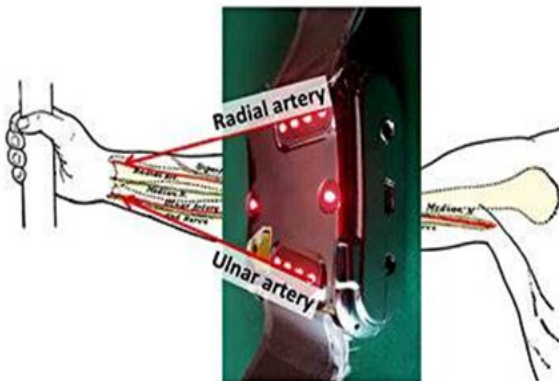


Figure 14: Spectra Laser Watch and systemic irradiation of wrist arteries

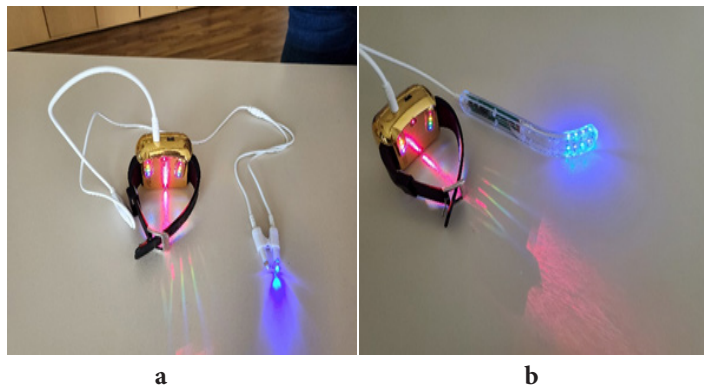


Figure 15a: Laser light watch with connected nose applicator and below

Figure 15b: Laser light watch with connected mouth applicator

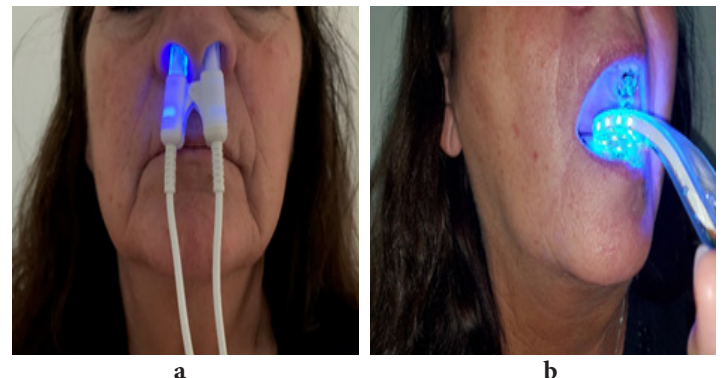


Figure 16a: 20 minutes treatment with nose applicator

Figure 16b: 20 minutes treatment with mouth applicator

Light Treatment kit and rational for use:

As we know that the infection route in Covid 19 patients starts in the upper respiratory tract the light treatment kit used in this study is targeting the nose, mouth and throat area. Local treatment of the respiratory tract is done by special applicators for nose and mouth/throat: The nose adapter contains 1 blue LED diode (447nm) and 1 UVA LED diode (375 nm) while the mouth/throat applicator contains 14 blue LED diodes (447 nm) and 14 UVA LED (375 nm). The diodes in this applicator are built in a way that irradiation goes up, down, lateral and in front. Due to the curved end it is possible to irradiate deeply into the throat.

The Spectra Watch for systemic treatment is equipped with 4 red laser diodes (658 nm), 2 green LED diodes (532 nm), 2 blue LED diodes (447 nm) and 2 yellow LED diodes (589 nm). The device is fixed on the wrist so that the light can penetrate into the arteries (Figure 14) for systemic effects of the different wavelengths and stimulation of the circulating Riboflavin.

Litscher et al. could demonstrate an improved microcirculation after usage of the laser watch with improved oxygen supply [22]. Other effects like improved immune stimulation, anticoagulating and anti-inflammatory effects, elevated nitric oxide and improved oxygen uptake by erythrocytes can be derived from data of intravenous laser therapy [23, 24]. However we decided for a non-invasive approach as we were looking for an effective home-use treatment tool.

Most important in this context is the systemic stimulation of the circulating Riboflavin by the blue diodes (447 nm). We know today that Covid-19 infections also affect the inner organs like liver, kidney, heart and brain, sometimes without or low respiratory symptoms. Hagman et al. could show that the risk of critical disease and mortality correlates with the amount of virus RNA detected in the blood stream [25]. So Photodynamic Therapy of the blood via the wrist arteries might have a preventive effect on disease progression to critical state.

Treatment protocol:

1. Take 1 capsule Riboflavin-5-phosphate (100mg) with a meal for systemic application
2. Open a second capsule (100mg) and dissolve it in a glass of

- 200 ml water (for local application in nose, mouth and throat)
3. After 1 hour fix the light treatment device ("Spectra Watch") on the wrist and switch on to 60 minutes (for additional systemic effects)
 4. After 15 minutes fill up the spray bottle with the dissolved Riboflavin solution and spray 3 times into both nostrils
 5. Flush the mouth three times with the rest of solution of the glass with gargling (and drinking is recommended)
 6. After 15 minutes, attach nose and mouth applicator to the laser watch and treat each nostril for 10 minutes with blue and UVA light (switch sides after 10 minutes) and inside the mouth and throat for 20 minutes

Study design

Definition of Infection Stages for our study rationale:

Stage 1: In the first step after infection the Covid-19 virus is bound and detected in cells and tissue of the upper respiratory tract. In this time the patient has already a positive PCR test but is mostly still asymptomatic.

Stage 2: In this stage (normally after 5-7 days) typical symptoms are arising with sore throat, fever, coughing and loss of smell and taste. The infection is still in the upper respiratory tract but may also start to infect the bronchial system and lung.

Stage 3: In case of progressed disease with lung infection patients usually need to receive oxygen supply; however without artificial respiration or intubation.

Stage 4: This stage is referring to patients in hyperinflammation phase with acute respiratory distress syndrome (ARDS) and other organ failures. Patients are usually intubated and receive artificial respiration.

The treatment presented in this study is recommended in early cases of stage 1 and 2 patients where the treatment can be done by the patient at home or by supervision of a local physician for prevention of hospitalisation, intubation, artificial respiration and late disease complications.

The focus of the presented treatment protocol is on treating the infected areas like nose, mouth and throat. Additionally we achieve a systemic effect by applying the light into the wrist arteries with the "laser watch". PDT could also be applied in stage 3 or 4 patients with a different protocol by applying Riboflavin and light directly in the intrabronchial system by special fiberoptic technology. This is under investigation as well and should be discussed further in another publication.

Patients selection and study protocol

Inclusion criteria: Men and women, 10-90 years, stage 1 or 2 according to definition above. Exclusion criteria: Stage 3 or 4 patients, pregnancy, children below 10 years

For this study 20 stage-2 patients (according to the definition above) were chosen as experiment (verum) group. All of them had PCR tests and Covid-19 related symptoms like fever, sore throat, coughing etc.; none of them had to be supplied with oxygen at that time. 13 women and 7 men with age between 18 and 80 years were enrolled into the study.

On day 1 an extensive clinical evaluation took place including QPCR test and medical questionnaire. Afterwards the first PDT treatment was initiated. On the following 4 days QPCR tests were done every day before the PDT treatment including CT (cycle threshold) value for viral load evaluation. The patients received a total of 5 PDT treatments within 5 days. The last tests were done on the 5th and 7th day.

20 additional patients under care of the same clinic served as a control group for this study (12 women and 8 men, with age between 22 and 77 years). They were supplied with conventional treatment modalities according to the recommended standards for Covid-19 management; none of those patients received PDT additionally.

This group was evaluated by QPCR and medical questionnaire, too.

QPCR and CT values were done by Omnid Hospital laboratory in Teheran, Iran.

Results

Clinical symptoms of patients from the experiment group before PDT treatment are summarized in table 1. 18 out of 20 (90%) patients in the experiment group had fever before PDT treatment. 17 out of 20 (85%) had a dry cough and additionally 9 out of 20 (45%) suffered from headache. 19 out of 20 (95%) reported chest pain/pressure. All 20 (100%) patients suffered from tiredness. 11 out of 20 (55%) had sleep disorder and 18 out of 20 (90%) had lost of taste or smell.

Table 2 shows the post treatment symptoms of the patients from the experiment group (after 5 treatment days): No (0 out of 20) patient had fever anymore. Only 6 (30%) reported a dry cough and 5 (25%) still had headaches. The pain conditions got better in 14 (70%) patients, while only 5 (25%) still reporting chest pain/pressure. Tiredness improved and later tiredness went down in 8 (40%) patients, with 12 people still feeling fatigue. 4 (20%) patients achieved a better sleep. The loss of taste or smell remained in all 18 (90%) affected persons.

Table 1: Symptoms of 20 patients in the experiment (verum) group before therapy

Experiment group			SYMPTOMS						
Gender	Case number	FEVER	DRY COUGH	HEADACHE	HARD BREATHING, OR CHEST PAIN OR CHEST PRESSURE	FATIGUE OR TIREDNESS	SLEEP DISORDER	LOSS OF TASTE OR SMELL	How many days before you felt bad
F	1	■	■		■	■	■	■	5
M	2	■	■			■		■	7
F	3	■	■		■	■		■	5
F	4	■	■		■	■	■	■	3
F	5	■	■		■	■	■	■	3
F	6	■		■	■	■	■	■	4
M	7	■	■	■	■	■	■	■	7
F	8	■	■		■	■		■	7
M	9	■	■		■	■			7
M	10	■	■		■	■		■	8
M	11	■	■		■	■		■	3
F	12	■	■	■	■	■	■	■	2
M	13				■	■		■	4
F	14	■			■	■			3
F	15	■		■	■	■	■	■	4
F	16	■	■	■	■	■	■	■	5
F	17		■	■	■	■	■	■	2
F	18	■	■	■	■	■	■	■	4
M	19	■	■	■	■	■		■	5
F	20	■	■	■	■	■	■	■	3

Table2: Symptoms of the experiment (verum) group after 5-day PDT

Experiment group		SYMPTOMS						
Gender	Case number	FEVER	DRY COUGH	HEADACHE	HARD BREATHING OR CHEST PAIN OR CHEST PRESSURE	FATIGUE OR TIREDNESS	SLEEP DISORDER	LOSS OF TASTE OR SMELL
F	1		■		■	■	■	■
M	2					■		■
F	3							■
F	4						■	■
F	5		■				■	■
F	6			■				■
M	7		■	■				■
F	8					■		■
M	9					■		
M	10				■	■		■
M	11		■		■	■		■
F	12		■	■		■	■	■
M	13					■		■
F	14							
F	15			■				■
F	16			■	■			■
F	17				■	■	■	■
F	18					■	■	■
M	19					■		■
F	20		■			■	■	■

QPCR Cycle Threshold measurements:

What does Ct mean: in a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct (Cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (ie exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (ie the lower the Ct level the greater the amount of target nucleic acid in the sample). Real time assays undergo 40 cycles of amplification. Interpretation of the Ct is as follow

Ct < 29 are strong positive reactions indicative of abundant target nucleic acid in the sample
 Ct of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid
 Ct > 38 = A marker of probable very low levels of virus in the specimen, near the levels to undetectable
 Negative: Indicate that the pathogen may present at a level below the lower limit of this assay

Table3.: Test results with CT values in the experiment (verum) group

Experiment group			QPCR CYCLE THRESHOLD VALUE							
AGE	Gender	Case number	PRE DAY0	AFTER DAY1	AFTER DAY2	AFTER DAY3	AFTER DAY4	AFTER DAY5	DAY6 NO TREATMENT	DAY7
49	F	1	+	37.2	38.9	Negative	Negative	Negative		Negative
52	M	2	+	35.2	34.5	36.8	37.8	Negative		Negative
24	F	3	+	28.3	30.3	35.7	35.1	Negative		Negative
18	F	4	+	35.1	39.3	Negative	Negative	Negative		Negative
37	F	5	+	28.3	30.5	32.2	35.2	Negative		Negative
27	F	6	+	29.8	30.2	31.2	33.4	Negative		Negative
40	M	7	+	29.7	29.2	35.4	37.2	Negative		Negative
57	F	8	+	29.2	29.2	34.3	37.8	Negative		Negative
50	M	9	+	22.7	27.3	25.6	30.1	33.7		35.1
31	M	10	+	27.1	30.2	32.2	36.7	Negative		Negative
53	M	11	+	23.9	24.6	27.7	34.4	35.1		34.1
80	F	12	+	34.6	Negative	Negative	38.8	39.1		Negative
39	M	13	+	34.1	35.7	38.8	39.1	Negative		Negative
25	F	14	+	24.1	26.6	29.7	35.7	Negative		Negative
50	F	15	+	26.7	28.8	33.4	37.2	Negative		Negative
67	F	16	+	35.5	34.3	36.7	38.1	Negative		Negative
39	F	17	+	35.4	37.1	38.9	39.2	Negative		Negative
45	F	18	+	31.2	32.9	35.4	35.5	37.2		38.1
39	M	19	+	21.4	25.2	23.4	26.9	32.2		34.2
37	F	20	+	23.6	27.9	25.4	26.2	29.7		30.3

The result of the QPCR (cycle threshold) measurements are summarized in table 3 for the experiment group. All patients in this group had a positive pre-treatment test. Between day 1 and 3

the average cycle threshold value increased by 3.99 between day 3 and 5 by 4.98. On day 5, 14 out of 20 patients had cycle threshold value below detection limits.

Table 4: Symptoms of the control group first day

control group			SYMPTOMS						
Gender	Case number	FEVER	DRY COUGH	HEADACHE	HARD BREATHING, CHEST PAIN OR CHEST PRESSURE OR PAIN IN CHEST	FATIGUE OR TIREDNESS	SLEEP DISORDER	LOSS OF TASTE OR SMELL	How many days before you feel bad
F	1		■	■	■	■		■	6
F	2	■	■	■	■	■	■	■	3
F	3	■	■		■	■		■	4
F	4	■	■		■		■	■	5
F	5	■	■	■	■		■	■	3
F	6	■	■		■	■		■	3
M	7		■		■	■	■		5
F	8		■	■	■	■	■	■	3
F	9	■	■	■	■	■			7
M	10	■		■	■			■	9
M	11	■	■	■	■	■	■	■	3
M	12	■	■		■	■	■	■	3
F	13	■	■	■	■	■	■	■	4
F	14	■	■		■	■	■	■	7
F	15	■		■	■	■	■	■	2
F	16	■	■	■	■	■	■	■	5
M	17	■	■	■	■	■	■	■	3
M	18	■	■		■	■		■	6
M	19		■	■	■	■		■	5
M	20		■	■	■	■		■	3

Clinical symptoms of patients from the control group on day 1 are summarized in table 4: 15 out of 20 (75%) patients in the control group had fever on day 1. 18 out of 20 (90%) had a dry cough and additionally 13 out of 20 (45%) had headache. All patients

reported chest pain/pressure. 17 out of 20 (85%) patients suffered from tiredness. 12 out of 20 (60%) had sleep disorder and 18 out of 20 (90%) had lost their taste or smell.

Table 5: Symptoms of the control group after 5 days without PDT

control group			SYMPTOMS					
Gender	Case number	FEVER	DRY COUGH	HEADACHE	HARD BREATHING OR CHEST PAIN OR CHEST PRESSURE OR PAIN IN CHEST	FATIGUE OR TIREDNESS	SLEEP DISORDER	LOSS OF TASTE OR SMELL
F	1		■	■	■	■		■
F	2							
F	3					■		■
F	4		■		■		■	■
F	5	■	■	■	■		■	■
F	6	■	■		■	■		■
M	7		■		■	■	■	
F	8							
F	9	■	■	■		■		
M	10	■		■				■
M	11		■	■	■	■	■	■
M	12		■			■	■	■
F	13	■	■	■	■	■	■	■
F	14	■	■		■	■	■	■
F	15	■		■	■	■	■	■
F	16	■	■	■	■	■	■	■
M	17	■	■	■	■	■	■	■
M	18	■	■		■	■		■
M	19		■	■	■	■		■
M	20		■	■	■	■		■

Table 5 shows the clinical symptoms of the patients from the control group after 5 days of conventional care: 2 of the patients had to be hospitalized within the 5 days and left the control group. 10 out of 18 (56 %) patients still had fever after 5 days. 15 out of 18 patients (83%) were still suffering from dry cough and 11 out

of 18 (61%) from headaches. The pain conditions got better in 4 (22%) patients, with 14 (78%) still reporting chest pain/pressure. Tiredness was still affecting 15 out of 18 (83%) patients. 10 out of 18 patients still had sleep disorder (56 %). The loss of taste or smell remained in all affected persons.

Table 6: Test results with CT values of the control group without PDT

AGE	Gender	Case number	QPCR CYCLE THRESHOLD VALUE								
			PRE DAY0	AFTER DAY1	AFTER DAY2	AFTER DAY3	AFTER DAY4	AFTER DAY5	DAY6	DAY7 AFTER PDT	
55	F	1	+	21.3		22.1		26.2		28.4	
45	F	2	+	18.4	ICU						
57	F	3	+	27.8		28.4		31.9		33.2	
55	F	4	+	30.5		31.5		32.6		34.1	
77	F	5	+	21.8		21.1		21.7		30.1	
73	F	6	+	24.6		24.6		23.2		23.3	
75	M	7	+	24.1		25.6		25.1		-	
45	F	8	+	15.4	ICU						
23	F	9	+	21.6		20.3		20.8		-	
34	M	10	+	26.1		25.5		26.2		-	
55	M	11	+	22.7		22.7		21.3		-	
54	M	12	+	21.6		18.7		24.8		27.3	
22	F	13	+	29.4		30.4		32.1		35.2	
35	F	14	+	31.5		32.0		31.1		-	
45	F	15	+	33.2		34.1		37.0		-	
33	F	16	+	26.7		26.6		28.1		-	
45	M	17	+	23.1		22.1		21.9		-	
46	M	18	+	30.4		28.8		28.2		-	
51	M	19	+	30.9		30.6		30.6		-	
27	M	20	+	31.3		30.2		31.2		-	

Table 6 shows the results of the QPCR (cycle threshold) measurements of the control group after 5 days of conventional care. All patients in this group had a positive pre-treatment test. 2 of the patients had to be hospitalized within the 5 days and left the control group. The other 18 patients showed no significant improvements: Between day 1 and 3 the average cycle threshold value increased by 0.37, between day 3 and 5 by 0.48. All patients still had a positive QPCR test result after 5 days.

A post verum treatment after 5 days testing was offered to all patients of the control group with 7 people accepting the PDT treatment. Improvement could be shown in all 7 patients.

Discussion

The efficacy of Photodynamic Therapy (PDT) against several viruses including SARS-CoV-2 could be shown in several in-vitro experiments over the last couple of years [6, 12-18]. A newly designed PDT treatment kit had been developed in early 2020 for in vivo application of Photodynamic Therapy against viral infections. Several patients with influenza and infections of the upper respiratory tract were treated in the author's clinic with immediate clinical improvements in summer and fall 2020. Our presented study is the first practical approach in using PDT against SARS-CoV-2. The results indicate high effectiveness in improving clinical symptoms and reducing viral load in patients suffering from early stages of Covid-19.

While no significant improvements with conventional care could be observed in the control group, basically all 20 patients from the experiment group had a significant reduction of clinical symptoms like fever, dry cough, chest pain etc. Furthermore, viral load assessment by QPCR showed very encouraging results with 70 % tested negatively after 5 days and the other 30 % showing a significant reduction of viral load to low or moderate levels. PDT also proved to be successful in preventing hospitalisation and intensive care treatments.

Additionally, we emphasize that PDT led to no side-effects at all; this is in accordance with all the in-vitro and safety data for Riboflavin-based Photodynamic Therapy. Another advantage of PDT is the simple and cheap application which can be done even at home by the patient in early disease stages. This would not only relieve the hospitals with intensive care units but also prevents excessive expenses for health insurances and damage of global economy (due to economic and social lockdowns). Conventional medication is showing very limited success in prevention of severe Covid-19 infections. Latest developments of new vaccinations seem to be promising; however, vaccination is a preventive approach and not a therapy for acutely infected patients. Furthermore, there are many questions still to be answered regarding vaccinations (especially new mRNA vaccines); i.e. length of immune protection and side effects.

However, our therapeutic approach is not be considered to be in competition with vaccinations. The combination of both preventive vaccinations and therapeutic interventions like Photodynamic Therapy (PDT) for infected people is necessary for successful management of this global pandemic. Additionally there is potential that Photodynamic Therapy might also lead to a natural vaccination effect. Further investigations to check if IgG or CD8+T cells will be developed by our immune system after PDT are already under progress. Because of the encouraging presented data additional studies with bigger amount of patients will be initiated shortly.

Conclusion

The presented study shows first evidence for a successful treatment of Covid patients in early stages of disease by Photodynamic Therapy. The applied protocol with a combination of oral Riboflavin and UVA and blue light proved to be effective in reducing viral load of patients and in improving clinical symptoms significantly.

This therapeutic intervention is suitable for early stages of infection in the incubation phase after positive testing but also when first clinical symptoms like fever, sore throat or breathing problems arise. Consequently, it is expected to accelerate recovery and prevent patients from getting into advanced stages.

The procedure can also be used in a preventive manner after contact with infected people or after meetings with many people. If used widely this Photodynamic Therapy approach can assist in lowering Covid-19 cases in addition to vaccinations and other treatments. Since not all patients of the world can be vaccinated quickly and the long term success is still unclear it is an efficient treatment tool that everybody can use at home.

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