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Immuno-Multi-Therapy and Prophylaxis Efficacy against COVID-19

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Abstract

In the context of the current COVID-19 pandemic, strategies for finding effective therapies advocate the so-called "therapeutic repositioning" approach, i.e., the use of existing molecules on the pharmaceutical market, whose toxicity and therapeutic efficacy are known.

The immunotherapy proposed in this study consisted to use four well-known components of validated therapeutic drugs, namely: interferon type 1 (IFN α), interferon type 2 (IFN γ), chloroquine (Chloroquine phosphate) and antioxidants (Vitamins A, C, E, trace elements and lycopene). Such non pathogen specific treatment was curative and preventive (i.e., prophylaxis), and expected to enhance the patient's innate response. A selected cohort consisted of 122 patients tested positive by RT-PCR (SARS-CoV-2 infection). Among them, 89 patients were asymptomatic and 43 symptomatic (COVID-19). Due to the compassionate nature of this therapeutic approach, age, and gender were randomized. Four types of treatment were selected using a multi-therapy approach applied for a duration of five days. Among the medical team in

charge of the protocol ten (10) were subjected for prophylactic purposes to a three-day tri-therapy treatment.

A total of 132 participants received a multi-therapy treatment as curative treatment (122 patients) and as prevention (10 health workers). All participants were tested for RT-PCR before treatment, all patients tested positive while the health workers tested negative. After two weeks all participants tested negative by RT-PCR. Clinical follow-up showed a total and rapid recovery at the early stage of tri-immunotherapy while repeated RT-PCR testing for the participants with preventive treatment remained negative.

Conclusion. Such multi-immunotherapy protocols against SARS-CoV-2 efficacy appear substantial for treatment and potentially efficient for health worker prevention. Due to our limited subject and the compassionate context, all multi-immunotherapy protocols would require a control study to evaluate their efficacy.

Keywords

SARS-CoV-2; COVID-19; Interferon; Chloroquine; Antioxidant

Abbreviations

BT: Dual Therapy; IFN: Interferon; NK: Natural-Killer Cell; CTL: Cytotoxic T-Lymphocyte; TH1: Helper T1 Cell; TTA: Triple A Therapy; TTG: Triple G Therapy; NK-ADCC: Natural Killer Cell-Antibody Antigen Dependent Cytotoxicity; RT-PCR: Reverse Transcription-Polymerase Chain Reaction; RNS: Reactive Nitrogen Species; ROS: Reactive Oxygen Species

Introduction

In the current COVID-19 pandemic context, strategies for finding effective therapies are based on the so-called "therapeutic repositioning" approach, i.e., the use of existing molecules on the pharmaceutical market whose toxicity and therapeutic efficacy are demonstrated and validated [Serafin, et al., 2020, James Nurton 2020]. In the present study, the choice of the therapeutic drugs was thus made on such basis of their validated therapeutic effects.

The exogenous Interferon (IFN) allows to reinforce the interferon-Natural-Killer cell (NK) system, i.e., to increase the expression of APOBEC3G/3F (Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide 3G or 3F), Tetherin, INFITMs (INF-Induced Transmembrane proteins) which are the major antiviral effectors of the interferon system, capable of inhibiting viral entry, translation, maturation and dissemination [1-3]. Moreover, IFN over-activates

infected phagocytes (monocytes, macrophages, poly nuclear neutrophils, etc.) to produce toxic oxygen and nitrogen derivatives leading to an oxidative explosion for the destruction of the pathogen [1,4-6]. Interferon type 1 (alpha or beta) acts autocrine and stimulates the infected cell to produce antiviral proteins (i.e. Tetherin (or CD317, cluster of differentiation 317)) responsible for the binding of "viral envelope - cell membrane", when the trapped virion dies by endocytosis.

Chloroquine is an inhibitor of endosome acidification which, by preventing the formation of the phago-lysosome, promotes the cytotoxicity of NK cells. Among other adaptive system immunity pathway Chloroquine blocks the CMH2 (Cytotoxic T-Lymphocyte (CTL)-Major Histocompatibility Complex type 2) pathway, promotes the CTL-CMH1 (Cytotoxic T-Lymphocyte (CTL)-Major Histocompatibility Complex type 1 CMH1 (TDC8 (cytotoxic T8 cell) and TH1 (Helper T1 cell), NK-ADCC (antibody-antigen-dependent cytotoxicity) anti infectious agent response. Moreover, Chloroquine can destabilize the envelope glycoproteins [7,3].

Antioxidants prevent the excessive formation of free radicals, Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), which are the source of oxidative stress that interfere with cell signalling and accelerate the progression of certain types of infection [Alain, et al., 2005] or systemic dysfunction [8].

Altogether, the combination of these molecules tends to strengthen the immune system through its different metabolic pathways [2,8,4,3]. In collaboration with the Institut Pasteur of Paris, an original immunotherapy approach was developed that combines type I interferon, IFN (IFN α , β) and type II interferon (IFN γ) associated with chloroquine phosphate and several antioxidants [7].

Materials and Methods

Population and study sites: The participants enrolled in this study were patients attending the Mwangeji General Reference Hospital (Lualaba province, Kolwezi) and screened for COVID-19 diagnosis by RT-PCR performed by the laboratory of the National Institute of Biomedical Research (INRB, England National Institute of Biomedical Research). RT-PCR for SARS-CoV-2 viral RNA detection was carried out following manufacturer instruction [Boditech Med Inc.'s]. Participant populations were prioritized among asymptomatic travelers and hospitalized symptomatic patients.

Material: Interferons including IFN α and IFN γ (Corbiopharm S.A., Belgium), chloroquine phosphate, Nivaquine (Syncom Formulations Ltd.) and, the antioxidant Hercules [Hercules - A Complete Antioxidant, BIOGENICS SARL] with several antioxidants including: Three vitamin C, A, and E; two trace elements (selenium, zinc); and lycopene [9]. The combination of these components and their dosage has been defined as the therapeutic protocols "BELA

UNILU.20" (Balaka Ekwanga-Lungu Anzwal University of Lubumbashi 2020) including the following posology: A single dose of interferon alpha, 200 IU a day; two doses of chloroquine 100 mg a day; two doses of 1 antioxidant capsule a day following manufacturer recommendation (Hercules Glow Pharma) [10,11].

Methods: Asymptomatic patients were treated for five consecutive days according to three standardized protocols with the above defined doses: 1) Triple A Therapy (TTA) including: interferon alpha, chloroquine, and antioxidant [INF α , CQ, A]; 2) Triple G Therapy (TTG) including: interferon gamma, chloroquine, and antioxidant [INF γ , CQ, A]; 3) Dual therapy (BT) including: interferon alpha and antioxidant [INF α , A]. Symptomatic patients were treated for five consecutive days according to the above-defined protocols: 1/ combined TTA and TTG protocols; 2/ TTA protocol; 3/ TTG protocol; 4/ BT protocol.

Dual Therapy (BT) was the preferred approach for four (4) patients who had failed prior therapy (chloroquine and azithromycin), for five (5) other patients who had a history of heart and kidney disease, and for three (3) patients who had an allergy to chloroquine.

All patients had a control test (RT-PCR diagnosis) at the end of treatment (i.e., day 5). Because the difficulties and cost of transportation between homes and screening site two symptomatic patients had an early test on day 3 of treatment. All patients tested negative.

Participating health workers (i.e., medical staff members of the clinical trial team volunteer) were preventively treated with the TTA protocol (interferon alpha, chloroquine and antioxidant) for three (3) days. All participants were monitored for clinical signs and symptoms until the end of the study on day 20 after treatment.

Ethics: In the absence of treatment for COVID-19 in DRC, a therapeutic repositioning framework was developed, approved and accepted by the Ethics Committee of the participating Hospital Board of Physicians. Each treatment was offered on a voluntary basis to all patients tested positive by RT-PCR and a verbal consent applied to each one or family member responsible. The same ethical procedure was used for volunteer participants (i.e., members of the clinical trial team).

Results

A total of 122 participants, from one to 71 years old, including 33 females and 89 males, were treated with the BELA-UNILU.20 protocols showing in a short-term after treatment a negative RT-PCR virus detection test (Table 1) and drastic improvement of the clinical signs (Table 2). Among them, 79 asymptomatic patients were treated according to three different and predefined treatment protocols: TTA (37); TTG (35); BT (7). The 43 symptomatic patients were treated according to four different approaches: TTG+TTA (6); TTA (15); TTG (17); BT

[5]. The 10 participants, medical staff members treating COVID-19 patients who were treated preventively (TTA protocol) remain negative for SARS-CoV2 RT-PCR to date (Table 1).

Immunotherapy (protocol) 79 Participants	Asymptomatic: RT-PCR Positive/Tested	
	Before Therapy	After Therapy
IFN α , CQ, An (TTA)	37 /37	0/37
IFN γ , CQ, An (TTG)	35/35	0/35
IFN α , An (BT)	7-Jul	0/7
Immunotherapy (protocol) 43 Participants	Symptomatic: RT-PCR test positive / tested)	
	Before Therapy	After Therapy
IFN α , IFN γ , CQ, An (TTA+TTG)	6/6	0/6
IFN α , CQ, An (TTA)	15/15	0/15
IFN γ , CQ, An (TTG)	17/17	0/17
IFN γ , An (BT)	5-May	0/5

Table 1: SARS-CoV2 RT-PCR screening of COVID-19 Asymptomatic and Symptomatic Patients Treated by a five-day Immunotherapy.

While two symptomatic patients tested negative as early as day 3 of treatment, signs and symptoms did not persist among all symptomatic patient after the 5-day treatment including: 44% (19/43) without clinical sign on day 3, and respectively 42% (18/43) on day 4, 12% (5/43) on day 4, and the remaining 2% (1/43) on day 6 (Table 2).

Although the RT-PCR control tests could not be performed at the same time after treatment for all patients, all tests were performed in full for all patients (Table 3) between day 3 and day 27.

RT-PCR Control Test Time	Symptomatic Patient Immunotherapy Protocol	Asymptomatic Patient Immunotherapy protocol	Total
Day 3 to 5	2* BT	0/0	0 / 2
Day 6 to 10	2 BT, 5 TTA, 6 TTG, 1 TTA+TTG	4 BT, 9TTG, 9TTA	0 / 36
Day 11 to 15	1 BT, 5 TTA, 6 TTG, 4 TTA+TTG	3 BT, 10TTG, 12TTA	0 / 41
Day 16 to 20	1 BT, 1 TTA, 2 TTG	6TTG, 10TTA	0 / 20
After day 20	1 BT, 3 TTA, 2 TTG 1 TTA+TTG	9TTG, 7TTA	0 / 23
Total	43/43	79/79	0 / 122

Table 2: Timeline of participants tested negative by RT-PCR.

	Clinical Profile	Day of Treatment					*	RT-PCR Negative	ITT*
		1	2	3	4	5	6		
1	Ageusia, anosmia							5	TTG
2	Back pain							14	TTG
3	Cold							7	TTG
4	Cold							7	TTG
5	Cold							14	TTA
6	Fever, anosmia, ageusia							5	TTG
7	Fever, anosmia, ageusia, muscle aches							7	TTG
8	Fever, muscle aches							13	TTA
9	Fever, dry cough							5	TTA
10	Fever, dry cough							11	TTG
11	Fever, dry cough							13	TTG
12	Fever, dry cough							20	TTA
13	Fever, dry cough							20	TTG
14	Curvature							14	TTA+ TTG
15	Fever, dry cough, aches and pains, anosmia, headache, colds							12	TTA
16	Fever, dry cough, aches and pains, headache, colds							13	TTA
17	Fever, dry cough, muscle aches							10	TTG
18	Fever, dry cough, muscle aches							15	TTA

19	Fever, dry cough, muscle aches							8	TTG
20	Fever, dry cough, headache, cold							8	TTG
21	Fever, dry cough, anosmia, ageusia, muscle aches, dyspnea							10	TTA
22	Fever, dry cough, anosmia, ageusia, anorexia, chest pain, mild breathing difficulties.							9	TTG
23	Fever, wet cough, muscle aches, headache, cold, shortness of breath.							18	TTG
24	Cold, dry cough							6	BT
25	Cold, dry cough							14	BT
26	Cold, dry cough							11	TTA
27	Cold, dry cough							8	TTA
28	Cold, dry cough							12	TTA
29	Dry cough							27	TTA
30	Dry cough							15	TTG
31	Dry cough							21	TTG
32	Dry cough							17	TTA
33	Dry cough							3	TTG
34	Dry cough							3	BT
35	Dry cough							20	BT
36	Dry cough							9	TTA
37	Dry cough							9	TTA
38	Dry cough							27	TTA+ TTG

39	Fever, dry cough, aches and pains, cold, headache							13	TTA+TTG
40	Dry cough, cold, chest pain							10	TTA+TTG
41	Dry cough, cold, dysphagia							13	TTA+TTG
42	Dry cough, chest pain							13	TTA+TTG
43	Dry cough, dyspnea							7	BT

Table 3: Timeline of clinical profile of symptomatic patients at the day of treatment on onward. Legend: Gray cell indicates the timeline of the observation of clinical symptoms listed in the Clinical Profile; *= day after treatment; ** = Immunotherapy protocol.

Overall, this immunotherapy did not induce any intolerance or side effects, except in three individuals to chloroquine with transient signs (2 days) including: transient puffiness of the face (1); mild pruritus (2). In these patients the signs immediately regressed without sequelae after discontinuation of chloroquine treatment.

Discussion

Asymptomatic and symptomatic patients (RT-PCR positive before treatment) tested PCR negative after 5 days of treatment with the tri- bi-immunotherapy protocols implemented. All ten medical team preventively treated with TTA protocol at the beginning of the study (i.e., more than 6 months ago) repeatedly tested negative by RT-PCR until now.

It is remarkable about this is first and foremost the disappearance of clinical signs of any kind in a very short period of time mostly at the inception of the treatment. This is in favor of the efficacy of the treatment given that the patients have been seen and treated within 2 or 3 days after onset and 24 hours after testing positive by RT-PCR. At the end of treatment (D6), no persistence of any of the clinical signs was observed [12-15].

Although, the BELA UNILU.20 immunotherapy treatment protocols against COVID-19 appear to be effective, more hindsight is necessary (e.g., clinics, immune response). Nevertheless, given the potential efficacy and safety of the BELA UNILU.20 protocols and within the context of limited resources, it will be appropriate to integrate these protocols into the COVID-19 treatment exercise in DRC. Therefore, these encouraging results led us to an enlarged the cohort, such work is in progress and integrate other parameters in the management for a precise clinical and therapeutic follow-up.

Conclusion

These clinical trials have established, at this stage, the efficacy and safety of BELA-UNILU.20 treatment protocols against COVID-19. This treatment deserves to be integrated into the treatment of COVID-19 in the Democratic Republic of Congo and elsewhere in the world.

However, the encouraging results already obtained to date, and in a context of limited resources, need to be further developed. Research should therefore continue through larger cohorts and by considering other parameters in the management for a precise clinical and therapeutic follow-up, as well as for a better evaluation of the immune response.

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