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Feature Paper

Photodynamic therapy in oncology

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One of the greatest problems of the medical world is cancer, the second most important cause of death on Earth. During the last decade an increase of malignant tumors was seen in Romania, cancer being, after the cardiovascular diseases, the most frequent cause of mortality. The powerful impact of cancer on human life is obvious not only in the death rate but in the number of new cases and living diseased. Therefore, early diagnosis and treatment of cancer has become one of the priorities of the medical world. Current ways of treating cancer (surgery, radiotherapy, chemotherapy) are sometimes only palliatives, that's why research for new methods to cure cancer is a continuous struggle around the world. Such research have lead to new ways of dealing with this disease based on the induced photochemical effect of light radiation on tumors, known as photochemotherapy or photodynamic therapy. Photodynamic therapy is a method of local treatment of the tumor by administrating a photosensitizing substance which is selectively absorbed by the tumor cells and which, during the irradiation with optical radiations, ends in destroying the tumoral tissue. Although in experimental phase, the obtained results of phototherapy in the latest period are remarkable and have lead to growing interest to this method. The application of photodynamic therapy in current practice raises some problems: the *type of potosensitizer* (the way of administration, how to prepare it, therapeutic concentration), the *radiation source* (type, irradiation parameters: wavelength, energy, exposure time, pulse duration, pulse frequency, etc*), methods of determining the biologic response*, etc. Until now, there are numerous studies on each of these matters. A synthesis of these studies is the subject of this article.

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1. Introduction

Cancer is the second cause of death of people around the world. Cancer therapy is a current practice in the latest decades and hard efforts are made to find new harmless and effective solutions. Nowadays, chemotherapy, renewed surgical technique to remove tumors, radiotherapy and, newly, photodynamic therapy are aimed at prolonging and giving a better standard of life to patients diagnosed with neoplastic disease.

Surgery is the oldest (and once the only) way of treating cancer. In the case of micro metastasis, surgery is obsolete and the association of other techniques, as radiotherapy is mandatory. Like surgery, radiotherapy has its limitations. When dealing with large tumors, there are some risk that the central cells are not affected, which leads to recurrences. That's why chemotherapy has its indications. All these methods are sometimes only palliatives, so research is continuing everywhere to understand the mechanisms of cancer evolution and find new ways of effectively deal with it.

Such researches have lead in the last years to photochemotherapy or photodynamic therapy (PDT).

2. Photodynamic therapy

Photodynamic therapy is a method of local treatment of cancer based on administration of a photosensitizing substance selectively absorbed by the tumor cells which leads, after the exposure to optical radiation, to tumor destruction.

The photodynamic action of nonionizing radiation was discovered in 1898 by the student Oscar Raab who demonstrated the effect of light and some colorants on paramecia [1]. He noticed that when paramecia was treated with acridine orange dye and exposed to sunlight a lethal effect has appeared. The paramecia treated with acridine orange dye but kept in dark and the ones exposed to sunlight without acridine orange had survived. Herman von Tappeiner, in Munich, has continued the experiments, and has introduced together with Joldbauer in 1904 the term of *photodynamic reaction* containing light, the photosensitizing substance and oxygen [2].

Since 1904 many researchers have developed the photodynamic therapy demonstrating its value in the treatment of malignant tumors and have reported many studies and even elaborated some protocols accepted by the medical authorities (Table 1).

Table 1. Current status of approved photodynamic therapy.

Year	Photosensitizer	Disease
1993	Porfimer sodium	Early bladder tumors
1996	Porfimer sodium	Obstructive tumor of esophagus
1997	Porfimer sodium	Advance lung and esophageal tumors
1997	Porfimer sodium	Advance lung and esophageal tumors
1997	Porfimer sodium	Early lung cancer
1997	Porfimer sodium	Early esophageal, lung, gastric, cervical cancer
1998	Porfimer sodium	Endobronhial cancer
2000	Verteporfin	Age-related macular degeneration

Although in experimental phase, the obtained results of phototherapy in the latest period are remarkable and have lead to growing interest to this method.

PDT is optimal in endoscopic applications: treatments of carcinomas in GIT, bronchial carcinomas, some carcinomas of bladder and urinal paths etc. Penetration of light with wavelengths within the range of 630 - 700 nm, which are utilized in PDT for excitation of most of the preparations, seems to be the limiting factor as their penetration is up to 6 mm only. Therefore PDT is suitable for treatment of early stages of solid carcinomas and/or paliative treatment.

The application of photodynamic therapy in current practice raises some problems:

- choosing "right" photosensitizing substance
- how to prepare and administer this substance
- how much time should pass from the moment of administration of the substance till the exposure to the light radiation
- choosing the type of the radiation source and setting the parameters of the light radiation (wavelength, time exposure, pulse duration, pulse frequency, etc)
- methods of evaluating the biologic response
- how long is the organism still sensitized to radiation, etc.

Until now there were a lot of studies aimed at analyzing these factors. The most important results are summarized in the following pages.

2.1. The mechanisms of photodynamic therapy

Photodynamic therapy is based on photooxidation of living matter invalving three basic constituents: the photosensitizer (S), the radiation (with a wavelength appropriate to the maximum absorption of the substance in use), and oxygen. Oxygen species, in different toxic forms, represent the most important agents of the cellular death processes during the photodynamic therapy.

Active oxygen species can be generated through a type II reaction mechanism (Fig. 1) between the photosensitizer molecule, (being in an active triplet state - $^3S^*$) and the biologic environment (B). The sequence of the reactions is:

$$^3S^* + {}^3O_2 \Rightarrow {}^1S + {}^1O_2{}^*$$
$$^1O_2{}^* + B \Rightarrow B_{ox}$$

where: $^3O_2$ is the triplet excited state of oxygen, $^1O_2$ is the singlet oxygen molecule, and B_{ox} is the target substrate oxidated.

Molecular oxygen, as a singlet, ($^1O_2$) acts rapidly and no discriminatingly with electrophyle molecules (B) like unsaturated lipids, proteins, nucleic acids, etc. After transferring the energy to oxygen, the photosensitizer can restore its fundamental state (^1S) and the cycle repeats.

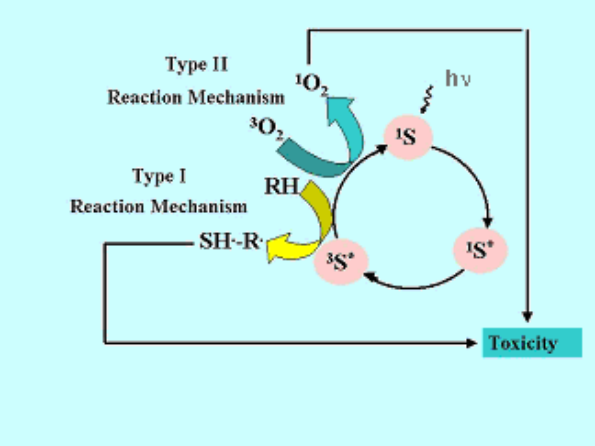

Fig. 1. The mechanisms of photodynamic therapy.

The molecule of the photosensitizer, as a triplet, can react with a target molecule, other than oxygen, by changing hydrogen or electrons. This type of reaction is called type I reaction mechanism:

$$^3S^* + RH \Rightarrow SH^{\cdot} + R^{\cdot}$$
$$^3S^* + RH \Rightarrow S^{\cdot -} + RH^{\cdot +}$$

where:, RH is the target substrate molecule, $SH^{\cdot}$ is the radicalic form of photosensitizer, $R^{\cdot}$ is the radical derived from target substrate molecule, $S^{\cdot -}$ is the radical anion form of photosensitizer, $RH^{\cdot +}$ is the cationic form of target substrate molecule.

Generally speaking, in a given photodynamic system, both types of reactions can appear, their efficiency depending both on the concentration of the photosensitizer that has been accumulated at the level of the tumor and the density of the light radiation energy.

2.2 Effects of photodynamic therapy

They can be direct or indirect.

2.2.1. Direct effects

The direct photodynamic effects are mediated, according to the type II reaction mechanism, by the generation of singlet oxygen. Singlet oxygen has a short lifetime (0.01-0.04 μs) and a limited diffusion distance in a biologic environment (0.01-0.02 μm) due to its reactivity [3], so the cellular deterioration appears in the proximity of the substance in the cell. The location of this molecule is determined by the degree of lipophilicity of the photosensitizing substance.

In general, the anionic lipophilic photosensitizers accumulate at the level of the cellular membrane and its organelles (mitochondria, endoplasmic reticulum, lyssosomes, and nuclear membrane).

Lipid and protein photooxidation in the cellular membrane generates the activation of the phospholypases from the membrane which leads to: changes in membrane

permeability, reduced fluidity and inactivation of enzymatic systems and receptors [4].

Inhibiting the mitochondrial enzymes was considered to be the key of cellular death due to photodynamic therapy [5,6]. Inactivation of transport membrane systems and the depolarization of plasmatic membrane happen before the inactivation of mitochondrial cytosolic and lysosomic enzymes [7,8].

Hydrophilic photosensitizer localize preferentially in lysosomes [9,10]. After the exposure to light radiation, lysosomes disintegrate and the enzymes they contain are spread in the cytoplasm leading to cellular death.

The probability of cell inactivation is higher for the lipophilic photosensitizer than for hydrophilic ones for the same dose of light energy, which means that membranous structures are more vulnerable [11].

Photodynamic therapy can also lead to the deterioration of the DNA. In vitro studies have demonstrated that photodynamic therapy can produce: breaks in the DNA chain [12-14], changes between twin chromatides and chromosomal aberrations [15-17].

Lethal, acute, direct effects on tumoral cells and even normal cells depend on: location of the photosensitizer on/or in the cell, the photodynamic efficiency of the substance in the environment, parameters of irradiation and oxygen transport.

2.2.2. Secondary effects

Due to the direct effect of the photodynamic therapy, most of the tumoral mass is destroyed. In the tumor cells that were not directly affected, secondary effects may appear such as: vascular effects, the initiation of apoptosis, inflammation and generation of an immune response.

- *Vascular effects*

The acute effects of photodynamic therapy most easily observed *in vivo* are on the micro-vasculature which usually lead to stasis [18].

Major determinants for vascular photosensitivity are: the concentration of the circulant photosensitizer and the time from its administration and the tissue irradiation [19].

The demonstrated vascular effects on experimental tumoral systems were: microvascular aggregation followed by transitory vasoconstriction, vasodilatation, blood stasis and hemorrhage [20,21]. When the skin is the subject of photodynamic therapy, edema and erythema are always the first signs. In clinical situations, these vascular events are documented by whitening and hemorrhage of the treated tissue.

- *The initiation of apoptosis*

Apoptosis (programmed cellular death) occupies an important role (besides direct cell death – necrosis) in photodynamic therapy [22]. During apoptosis, the genetic mechanisms of the cell starts a program of cell destruction without involving inflammation. It involves a cytoplasmic condensation with cell destruction in fragments rolled in membrane called apoptotic corpuscles. In the tissue, these corpuscles are destroyed by macrophages.

Cancer cells resistant to apoptosis (trough genetic mutations) are resistant both to chemotherapy and radiotherapy [23].

Studies concerning photodynamic induced apoptosis were conducted both *in vitro* [24-26] or *in vivo* [27] and have demonstrated an association between photodegradation of mitochondria and the apoptotic response of the cell.

- *Inflammation*

Photodynamic therapy usually induces a powerful inflammatory response in the treated tissue. Phototoxic effects on the cell membrane causes the release of lipid metabolites which are important activators of inflammation. It also generates an extended immune response which is very important in long term tumor control.

The complex nature of tissue response to photodynamic therapy is not yet discovered.

3. Photosensitizing substances used in photodynamic therapy

Photosensitizing substances can exist naturally in cells and tissues (flavine, pyridine, bilirubine, etc) or can be administered under control as in photodynamic therapy. Until now many substances have been tested both *in vitro* and *in vivo* (Table 2) none being perfect. Nowadays research is aimed at discovering new substances. A new concept is the biosynthesis of porfirinic photosensitizers after the administration of 5-aminolevulinic acid, a compound from the hem synthesis pathway.

Table 2. Photosensitizers for photodynamic therapy.

Porphyrins	hematoporphyrin derivative
	dihematoporphyrin ether/ester
	porfimer sodium
	tetra-sodium-meso-tetra-phenyl-porphyrin-sulphonate
	metallo-tetra-azoporphyrin
	5,20-Bis(4-sulphophenyl)-10,15-bis(2-metoxi-4-sulphophenyl)-21-tiaporphyrin (2,10tiaporphyrin)
Phthalocyanines	chloroaluminum phthalocyanine tetrasulfonate
	Zn-phthalocyanine
	Si-naphthalocyanine
	cloraluminium phthalocyanine sulphonated
Chlorines	mono-L-aspartil-chlorin e6
	diaspartyl chlorine
	bacteriochlorin a
	benzoporphyrin derivative monoacid ring A
Porphycenes	2,7,12,17-tetraphenylporphycene
	2-hydroxy-ethyl-7,12,17-tris(methoxy-ethyl)porphycene
	21,23-Dithia-3,13-diazaporphycenes
	capronyloxy-tetrakis methyloxyethyl porphycene
Purpurins	metallopurpurin
	tin etiopurpurin
Pheophorbids	pheophorbide a
	bacteriopheophorbide

3.1 Hemoporphyrin (Hp)

Hemoporphyrin is made of several porphyrinic fragments: acetate, biacetate, hydroxy-ethyl, protoporphyrine, vinyl and deuteroporphyrine. Of all these fragments, the active one is biacetate.The results using this fragment are although less good compared with the use of the whole mixture.

The absorption spectrum of hemoporphyrin shows 5 bands in the visibile domain of the electromagnetic spectrum at the following wavelenghts: 400 nm, 520 nm, 540 nm, 590 nm and 630 nm. The only one of interest to photodynamic therapy is 630 nm because in this region the biologic tissues show a low absorption so the effect of the photosensitizer is higher. Hp is better fixed by the macrophages and vascular endothelia.

3.2 Hemoporphyrin derivative (HpD)

HpD is a complex mixture of porphirinic monomers (45%) and oligomers (55%) linked by ether or esther bonds. It can be extracted from haemoporphirine mixed with sulphuric and acetic acids.

The absorption spectrum of HpD presents 5 bands in visibile region. The most important one has its maximum at 400 nm (Soret band). The other absorption bands are less intense and have their maximum at 520 nm, 540 nm, 590 nm, 630 nm.

The methabolism and ways of action of the HpD are not fully understood. Some researchers believe that after the i.v. injection HpD enters all the body cells and is metabolised in the cytoplasm. The concentration of HpD is 2-2.5 times higher in tumoral tissue than normal cells. This great difference permits the application of photodynamic therapy after 48-72 hours without harm on the neighboring normal tissues.

3.3 Porfimer sodium (Photofrin)

Photofrin is a mixture of oligomers formed by linking of porphyrine units trough ether or esther bonds and the tumoral tissues and, under light radiation produces cititoxic effects by generating active oxygen species, which lead to vascular thrombosis and ischemic necrosis of the tumor.

3.4 Phthalocyanines

They are a class of tetra-aza-tetra-benzeneporphyrine [28] which absorb in the red region of the electromagnetic spectrum. They have 2 important absorption bands, one more intense (650-700) nm with a maximum at λ = 670 nm and a less intense one in UV domain with a maximum at λ = 350 nm. The pyrole groups from phthalocyanines are conjugated with benzene rings trough nitrogen bonds. This structure shifts the absorption spectrum to higher wavelenghts and so the absorption band in the visibile spectrum becomes more intense than the one in the near ultraviolet spectrum. For a efficient tissue photosensitization the lifetime of the triplet state of the substance must be long so that the reaction with singlet oxygen can take place. In the case of phthalocyanines, this is done by incorporating a diamagnetic metal ion like Al or Zn.

The most important substance in this class was the cloraluminium phtalocyanine sulphonated (AlPcS). It is a stable compound, very soluble in water and has a powerful absorption at λ = 675 nm [29]. It also has a specific absorbtion in some tumors, a low toxicity in darkness minimal cutaneous photosensitivity and an excellent photodynamic activity at high wavelenght .

Another tested compound from this class was chloroaluminum phthalocyanine tetrasulfonate (AlPcS4). Studies have tested its effect on basal cell carcinoma, Kposi tumor and lung cancer [30].

3.5 Chlorins

Chlorins are reduced porphyrins, synthesized by hydrogenating of a double bond exo-pyrol of the porphyrinic ring [31]. They show intense absorption in the (640-700) nm spectral domain. The compounds studied as photosensitizers are:

- meso-tetra-hydroxyphenyl-chlorin (m-THPC), known commercially as Foscan. The first study was conducted in 1990 in the treatment of human mesotelioma.
- mono-L-aspartil-chlorin e6 (Npe6 or MACE) shows a maximum of absorption at 654 nm. The *in vitro* and *in vivo* experiments with MACE [32] have demonstrated its efficiency in photodynamic therapy due to its great absorption in the tumor and low skin photosensitivity.
- benzoporphyrin derivative monoacid ring A (BPD) shows a maximum of absorption at λ = 650 nm. Clinical trials showed great absorption in the tumors an low skin photosensitivity.
- bacteriochlorins have a powerful absorption at λ = 740 nm and can become good photosensitizing agents, although their stability is under question.

3.6 Porphycene

It is formed through reductive coupling of 5,5'diformil-2,2'bipyrol with titanium in lower covalent form. The UV-VIS spectrum of this substance has 5 bands at wavelengths of 358 nm, 370 nm, 558 nm, 596 nm, and 630 nm.

3.7 Purpurins and verdins

Purpurins have a absorption band at 660 nm and can be synthesized at high purity. Histological studies showed that purpurins and metallopurpurins (octaethylpurpurin and etiopurpurin), exposed to visible light radiation can result in tumor necrosis.

Verdins have a broad absorption band at 700 nm but with much lower intensity compared to the Soret band.

3.8 Pheophorbides

Methyl pheophorbide has an intense absorption band at 650 nm. Studies on biomolecules, human neutrophiles and tumor cells showed that their efficiency is comparable with Hp [33].

Research in this field continue and are directed towards finding new substances with properties closer to ideal.

4. Light sources

For photodynamic therapy of tumors both coherent (lasers) and non-coherent (lamps and LED) light sources are suitable. Both types have their own advantages and disadvantages.

4.1 Lasers

Laser is considered as an ideal light source for photodynamic therapy due to its coherence and monochromaticity. Monochromaticity permits the irradiation with a wavelength near to the maximum absorption of the photosensitizer, and coherence offers the possibility to use optic fibers for the transmission of light to cavities of the body.

Lasers used until present time in photodynamic therapy are: argon laser, copper vapors laser, gold vapor laser, dye lasers, and laser diodes such as gallium-aluminium-arsenide laser.

4.2 Lamps

Lamps have proven useful in photodynamic therapy especially in the cure of skin diseases [34,35]. Their main advantages are linked to the low cost and reduced dimensions. But they can be used only for superficial diseases. Although the light is filtered, the wavelength bands are wide compared with the absorption bands of the photosensitizing substances which leads to heat release which enhances the tumoricid result [36]. Metal halogen lamp and short arc xenon lamp were used in photodynamic therapy.

4.3 LEDs

These are non-coherent light sources that are scarcely used in photodynamic therapy. They generate wavelength bands wider than those from lasers, usually around 25 nm [28].

5. Methods of determining the biologic response to photodynamic therapy

The biologic response to this therapeutic method depends on a number of variables among which are: the light flux and its spatial distribution, the concentration of photosensitizer in the target tissue and the oxygen delivery to that tissue [29].

Determining these parameters represents an important step in experimental studies or clinical trials concerning photodynamic therapy. This field of research is nevertheless less developed and has been limited to: radiolabelling of the photosensitizing substance [30], *in vivo* reflexion spectroscopy [31] and *in vivo* fluorescence spectroscopy in vivo [37].

5.1 Radiolabelling the photosensitizing substance

A possible method of determining the amount of the substance and its concentration is to label it with a radioisotope. This method was employed only by B.C.Wilson [29-31,37] to determine the concentration of HpD and Photofrin II labeled with ^{64}Cu.

The method has a great sensibility and does not depend on the tissue type but it is expensive, difficult to reproduce in different studies on photodynamic therapy.

That is why, there is a growing interesting in developing optic methods to asses *in vivo* the treatment parameters and the biologic response, methods that are easier to apply for a wide range of photosensitizers. Such methods are fluorescence spectroscopy and diffuse reflectance spectroscopy.

5.2 Fluorescence spectroscopy

The tissue fluorescence can be studied in vivo by point-monitoring of fluorescence [32] or recording the fluorescence images [38].

5.2.1. Point-monitoring of fluorescence

Point-monitoring of tissue fluorescence was first employed in 1990 by Profio [33] to determine the concentration of Photofrin II in tumors and normal tissues. The method is based on recording the fluorescence emission spectrum of living tissue for a certain excitation wavelength. The quantification analysis is based on measuring the intensity of the fluorescent bands which is proportional to the concentration and width of the tissue sample through which the incident radiation passes and can lead to real time evaluation of the treatment parameters and helps in choosing the procedure of photodynamic therapy [39,40].

5.2.2. Imaging fluorescence spectroscopy

Imaging fluorescence spectroscopy is based on recording the fluorescent images of the tumoral tissue. The capture, processing and analyzing these images can be accomplished in several ways:

- the incorporation of a device with spectral resolution that measures in points in an image forming instrument and spectroscopic investigation of the regions of points on the image [41];
- sequentially or parallel image forming on several wavelengths of the excitation or emission wavelength (to subtract self-fluorescence) [42-47];
- combining several wavelengths of excitation or emission radiations to compensate for the variations in

optical properties of the tissues and extracting of linear signals depending on the concentration of the exogenous photosensitizer [48,49];

- using a dividing optical device to obtain several images of an object filtered at different wavelengths of the emission wave. These images can be further processed by a computer to obtain an optimal contrast image [47].

Research continues in this field aiming at obtaining better fluorescence images and developing processing software for image analysis.

5.3 Diffuse reflectance spectroscopy

Like fluorescence spectroscopy, diffuse reflectance spectroscopy can be used in photodynamic therapy both for determining the concentration of the photosensitizer in the tumor and for treatment monitoring.

The concentration of the substance is measured by determining the variation of the diffuse reflectance of the tumoral tissue due to the presence of the photosensitizing substance in that tissue. The relation that links the variation of the diffuse reflectance to the absorption coefficient of the tissue (which is directly proportional to the substance concentration $\mu a=\varepsilon C$) can be obtained by solving the radiative transfer equation [50].

The diffuse reflectance can be determined by *steady-state measurements* (using light sources with continuous or pulse emission (with the pulse duration greater than the time of propagation of the photons in the target volume) or detectors with temporal resolution greater than the photon propagation time) or *time-resolved measurements* (using light sources with very short pulses (ps) or detectors with high temporal resolution).

5.3.1. Steady-state measurements

The principle of the method is to send a radiation trough a optic fiber on the sample to be studied and reception of the reflected radiation trough another optic fiber situated at a distance r to the sample.

The variation of the local diffuse reflectance ($R_0(r)/R(r)$) of the tissue induced by the photosensitizing substance is determined by measuring the local diffuse reflectance before the substance is administered ($R_0(r)$) and after its administration ($R(r)$).

Using a calibration curve, $Ro(r)/R(r) = f(c)$, the concentration of the photosensitizing substance can be determined.

Mostly, this method is not used for quantification, but to establish the time when the concentration of the photosensitizing substance is at its maximum (the ideal moment to iradiate the tissue).

5.3.2. Time-resolved measurements

Recently appearance of light sources with very short emission pulses (ps) has lead to the development of time-resolved reflectance spectroscopy. The method is based on the irradiation of the living tissue with a pulsed laser and the measurement of the temporal distribution of photons reflected from the tissue. From the dependence of temporal distribution of the photons on the optical properties of the tissue, it can be determine the aborption and scattering coefficints of tissue.

6. Conclusions

In the last years, photodynamic therapy has had important results and is clinically used in some countries for curing: esofagian cancer (USA, 1996; France, 1997; Japan, 1997) and lung cancer (France, 1997; Germany, 1997; Japan, 1997).

The response to photodynamic therapy is complex in nature and its efficiency depends on the type of photosensitizing substance, ways of administration, light sources and the treatment procedure Although some questions have been answered in the last decade, a complete understanding of the mechanisms involved in tumor distruction through the photochemical effect generated by light radiation is still beyond our reach .

Recently [51], we have drawn attention on the analysis and matching of the refractive indices of the tumoral tissue and photosintizer aiming the improvement of the light delivery in the tissue.

Nowadays research are aimed at answering the questions that have been raised so far and finding new directions for further studies.

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