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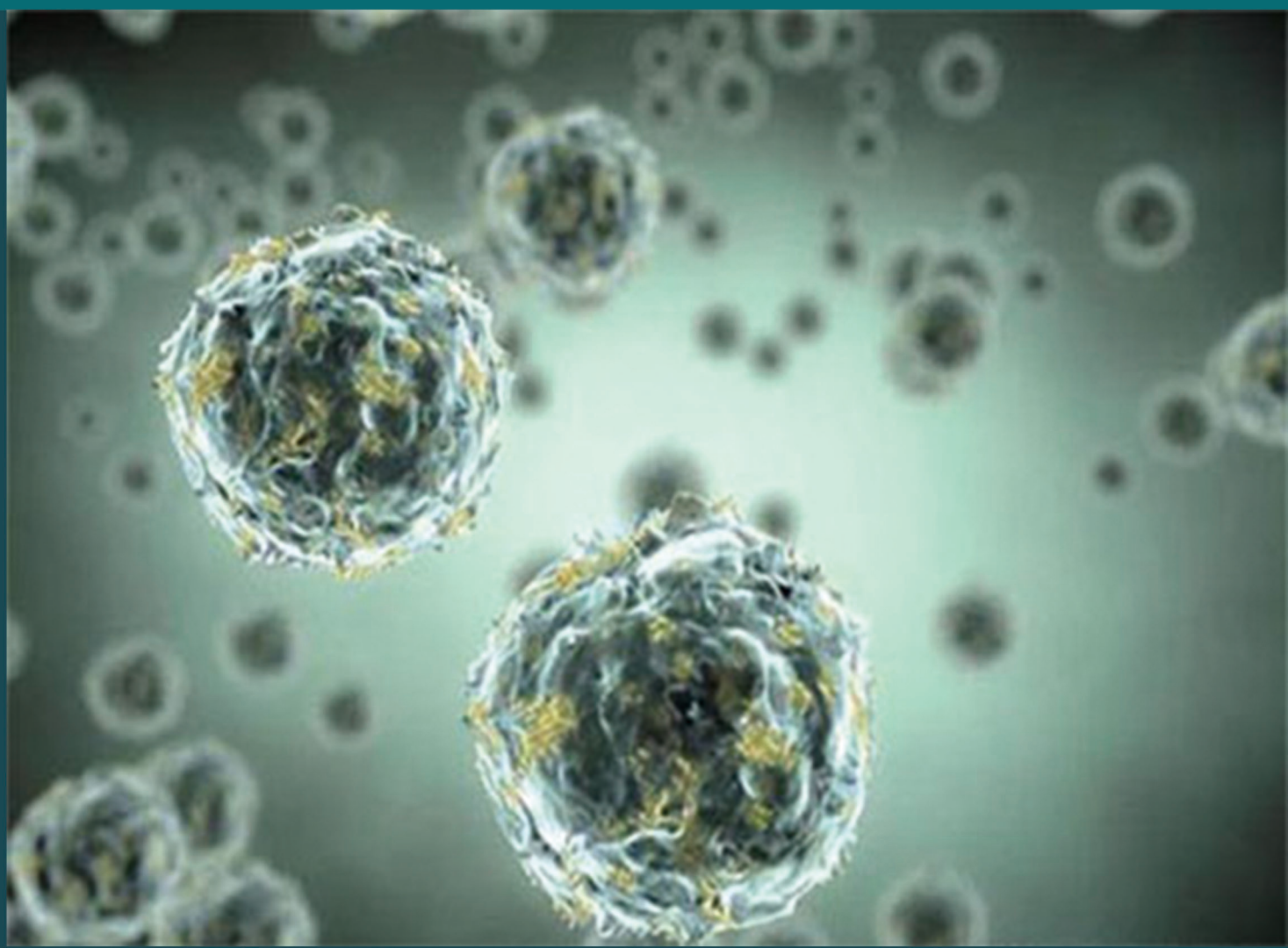

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ARTICLE

Electrified Water as a Regulator of Cell Proliferation

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ABSTRACT

It was previously found that the electric charge of water determines its ability to interact with other substances, including biologically significant ones. It is shown here that the electric charge of water can also determine its ability to penetrate and accumulate in living cells. In particular, it has been shown that the high penetrating ability of positively charged water determines both its active penetration into cells and accumulation in them, which creates favourable conditions for cell proliferation. At the same time, it has been shown that the low penetrating ability of negatively charged water determines its ability to slow down cell proliferation. It also discusses how medics can obtain and use water at different charges.

1. Introduction

The fact that water is the main component of all human biological fluids is beyond doubt. At the same time, the ability of human biological fluids (undoubtedly water in their basis) to change their electric charge (potential) and, therefore, properties, is practically unknown to most. In the process of studying the polymorphism of crystals formed during the drying of tissue fluids of the female body at different stages of the menstrual cycle, I became convinced that these changes do occur (Figure 1) [1].

Figure 1. This is what dried cervical mucus looks like in the first half of the menstrual cycle, i.e. - in the period from the first (left) to the fourteenth (right) day of the cycle [1]

So, studying the nature of this polymorphism (Figure 1), I have found that the shape of the crystals that form when saline solutions dry depends on the electrical charge (potential) of the water on which these solutions were pre-

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pared. In particular, it was found that the drying of saline solutions prepared with positively charged water is accompanied by the formation of cubic or rhombic crystals (Figure 2, left), while the drying of saline solutions prepared with negatively charged water is accompanied by the formation of needle-like or whiskers (Figure 2, right) [1,2]. Since this relationship turned out to be true for sodium chloride [1,2], which is the main salt component of almost all human biological fluids, it was concluded that the observed polymorphism (Figure 1) reflects the variations in the electrical potential of the female body fluids that occur during menstrual cycle.

Figure 2. It is the crystals that formed after the drying of solutions of KH_2PO_4 prepared on the water with potentials of +250 mV (left) and -250 mV (right) [1,2]

Moreover, it was assumed that these variations determine the very existence of the menstrual cycle. One way or another, it was also concluded that the timely use of positively and negatively charged water can normalize the menstrual cycle, and untimely use can cause its disturbances. In addition, it was concluded that the use of positively and negatively charged waters for medical purposes is quite safe, since these waters are normal components of the human body, undoubtedly under its control [1].

It is advisable to add that the established dependence (Figure 2) turned out to be very productive. Thus, the knowledge of this dependence made it possible to purposefully reproduce the conditions under which the arborization of salt crystals occurs. As a consequence, this reproduction made it possible to explain the regenerative effect of pulsed electromagnetic fields on nerve tissues. In particular, this reproduction made it possible to explain why such fields can stimulate the formation of new dendritic outgrowths in neurons, and what importance are the chlorides that are part of nerve tissues (Figure 3) [3].

Figure 3. The crystals formed after drying an aqueous solution of $CuCl_2$, which was previously subjected to the action of EMF, pulsing with a frequency of 10 Hz for 10 minutes; to increase the contrast, the crystals were treated with ammonia vapor [3]

Accordingly, this reproduction (Figure 3) made it possible to quite adequately explain the restorative and stimulating effect of pulsed electromagnetic fields on human nervous tissues, in general [4], and on his brain, in particular [5].

Simultaneously, it was established that positive electrification of water causes its compression, and negative electrification of water causes its decompression. These peculiarities allowed concluding that surface tension of positively charged water is greater significantly than surface tension of negatively charged water. This difference in surface tensions of oppositional charged waters allowed explaining the observed polymorphism of salt crystals (Figure 2). So, it was concluded that more compact crystals are formed due to the rather high surface tension of positively charged water (Figure 2, left), and less compact crystals are formed due to the extremely low surface tension of negatively charged water (Figure 2, right) [2]. Naturally, this dependence made it possible to understand the physical reason for the change in blood pressure in women during the menstrual cycle, in particular, the cause of the increase in blood pressure before menstruation, which manifests itself as premenstrual syndrome [1]. In addition, this dependence suggested that the tone of blood vessels is determined by the electrical potential of the blood, and the tone of the skin is determined by the electrical potentials of both sweat and air. This assumption made it possible to clarify both the sensitivity of a person to the weather and his sensitivity to anomalous zones. Moreover, this made it possible to propose a method for eliminating both of these sensitivities using suitably electrified water. In particular, given the strong negative electrification of the lower layers of the atmosphere both before and during the cyclone, interested people were invited to drink negatively charged water to compensate for the negative effect of such electrification on a person, in particular, to overcome drows-

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iness. The same method of confrontation was proposed to be used by pilots and sailors crossing the Sargasso Sea and the Devil's Sea. At the same time, it was once again noted that electrified water is completely harmless, since it is a normal metabolite of the human body, the activity of which is regulated in a natural way [6, 7]. Thus, the knowledge that the surface tension of water depends on its electrical charge has proven to be very productive in the medical aspect.

Further studies made it possible to establish that the ability of water to hydrate biopolymers, including DNA, also depends on its electrical potential. An inexpensive visualization of this relationship can be done with starch powder. Thus, one can make sure that water with a positive potential hydrates starch noticeably better than water with a negative potential (Figure 4) [1, 2].

Moreover, it can be verified that the electric potential of the water determines its penetrating ability. So, you can see that positively charged water, in contrast to negatively charged, is capable of evaporating even from a closed plastic container (Figure 4, right) [1, 2].

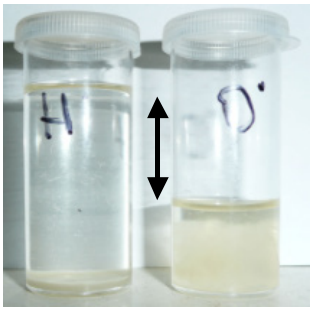

Figure 4. Swelling of starch in oppositely electrified waters. Starch does not swell in water with the potential of -250 (left) and swells in water with the potential of +250 mV (right)

Water with negative potential was obtained by bubbling uncharged water with hydrogen gas (left); water with a positive potential was obtained by bubbling uncharged water with oxygen gas (right).

Water with a positive potential has an abnormally high penetrating ability, which is why it can evaporate even from a closed plastic bottle: the arrow shows how much the level of such water has decreased during the day. In terms of the topic under discussion, it is important that salts dissolved in positively charged water penetrate the plastic along with it.

Both used waters had a temperature of 20-22 ° C [1,2].

Although this phenomenon (Figure 4, right) may be surprising to many, it is a kind of electroosmosis, which is the movement of aqueous solutions in a functioning electrolyzer from the anode to the cathode through a layer of sand or clay (Figure 5), which was first described in 1809 [8,9]. Note that only anolyte, which is a positively charged and oxygenated aqueous solution, passes through layers of sand or clay during electroosmosis (Figure 5), although the same electromotive force acts on the catholyte.

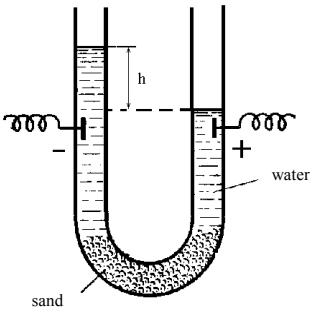

Figure 5. This is an illustration of electroosmosis, which consists in moving water or an aqueous solution from the anode compartment of a functioning electrolyzer to its cathode compartment through a finely porous partition separating both compartments

Thus, the high penetrating ability of water, which acquires a positive charge upon contact with oxygen (Figure 4, right), is no more surprising than the exceptional penetrating ability of anolyte, which determines the very existence of electroosmosis (Figure 5). Additionally, it should be noted that the high penetration of water exposed to ionizing radiation is also explained by the action of active oxygen [10]. Thus, the discussed phenomenon (Figure 4, right) cannot be considered completely unexpected.

Together, it should be noted that the described difference (Figure 4) turned out to be no less productive in medical terms than the previously described addictions. So, this dependence made it possible to better understand the features of water exchange in women at different stages of the menstrual cycle and, in particular, the physical reason for the appearance of edema in them before the onset of menstruation. Accordingly, all of this was seen as evidence that the electrification of the body fluids of women is important for the development of a fertilized egg [1].

It is logical that all this ultimately led to the need to analyze the effect of oppositely electrified waters on metabolism, proliferation and cell viability. Let us consider how the described potential-dependent properties of water help in the analysis of such influence.

2. Discussion

You should immediately accept the fact that water is the main component of the human body. In other words, the human body is primarily a water structure. Thus, Leonardo da Vinci's attractive definition "Life is inspired water" can now be replaced with a more correct definition

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"Life is structured water", which more fully reflects the understanding of the vital importance of structured water. This allows us to assert that it is water (and not peptides, as they say) that is the main building block of all living structures. This also allows us to assert that any growth of living matter is accompanied by both the accumulation of water and its involvement in newly formed structures, in particular, in biomolecules and cells.

Let's consider in this aspect the exceptional properties of positively charged water (Figure 4, right) [1,2]. They suggest that it is positively charged water that is capable of creating hydration shells of newly formed living structures. Moreover, the discovered abilities of negatively charged water (Figure 4, left) [1,2] suggest that it is capable of slowing down the formation of new living structures, in any case, the formation of their hydration shells.

Despite the fact that these explanations seem rather exhaustive and, I hope, convincing, the antagonistic effect of differently charged waters on the growth processes occurring in living matter, including cell proliferation, has both additional justification and limitation. Let's examine them.

2.1 Effect of Electrified Water on Proton Gradients around Cells

It is believed that the presence of proton gradients on the outer sides of the cytoplasmic membranes is characteristic of the cellular life form in general. Moreover, the ability to create these proton gradients is believed to be the exclusive domain of living cells [11]. These statements reflect the importance of such gradients for cell life, in particular, the fact that their energy allows cells to realize two types of secondary transmembrane transport, namely symport and antiport (Figure 6) [11-16].

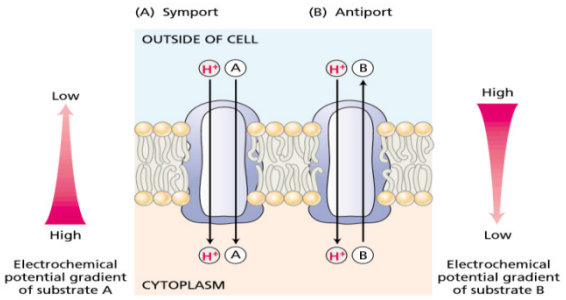

Figure 6. The energy of proton gradients on cytoplasmic membranes allows cells to realize two types of secondary active transport: symport and antiport. At the symptom (A), a proton, penetrating into a cell from the outside, captures one substrate molecule (for example, a glucose molecule). With antiport (B), the energy "scattered" by a proton entering the cell from the outside can be used to remove cations (for example, sodium ions) from the cell [14]

This means that the state of these proton gradients determines both of these types of secondary transmembrane transport (Figure 6) and, therefore, affects the rate of cellular metabolism in general [11-16]. In addition, this suggests that the saturation of the intercellular space with uncompensated protons, in which positively charged water is rich, can increase this proton gradient and, therefore, intensify cellular metabolism.

At the same time, this suggests that the state of these proton gradients can determine the intensity of the penetration of positively charged water, in fact, electroosmotic (Figure 5), into the cells and, accordingly, the intensity of water accumulation inside the cells. In turn, this suggests that the saturation of the intercellular space with positively charged water will also create conditions conducive to cell proliferation. It is obvious that the high penetrating ability of positively charged water, as well as its ability to hydrate biopolymers (Figure 4, right), do not contradict, at least, such assumptions.

At the same time, it cannot be ruled out that cells can burst with an unlimited growth of these proton gradients and, accordingly, an unlimited supply of positively charged water to the cells. Thus, it can be expected that unlimited positive electrification of the internal contents of cells and their external environment can destroy cell membranes in the same way as the surface tension of positively charged water dissipates starch powder (Figure 7, left) [2].

Figure 7. Left: the starch powder applied on the water surface with a potential of +250 mV forms a thin film that completely covers the water surface. Right: On the surface of water with a potential of -200 mV, the powdered starch remains at the application site and sinks [2]

This suggests that the proton guns used by oncologists [17-19] kill cancer cells, probably also by dissolving their membranes, at least cytoplasmic ones, that is, in the same way as positively charged water. The fact that this assumption is not far from reality is confirmed by the fact that a drop of liquid oil forms an invisible layer on the surface of positively charged water, spreading over it in the same way as powdered starch (Figure 7, left), but remains

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unchanged on the surface of negatively charged water like a lump of starch powder (Figure 7, right) [2]. Thus, proton guns initiate apoptosis of the (of the two main) types that arise as a result of irreversible and, therefore, unlimited cell swelling [20].

It is noteworthy that the effect of positively charged water on cells can be compared with the analogous effect of reactive oxygen, low (usually submicromolar) concentrations of which cause cell proliferation, and high concentrations (usually ≥ 10 μM) cause their apoptosis or necrosis [21]. The fact that reactive oxygen provides positive electrification of water [22], which we used earlier (Figure 4, right), makes this analogy more remarkable. In any case, all this correlates well with the opinion of Paracelsus: "Everything is poison, and nothing is devoid of poisonousness; just one dose makes the poison invisible" or, in a more popular interpretation: "Everything is poison, everything is medicine; both determine the dose". Thus, it is quite obvious that it is necessary to control the positive potential of the water used as a therapeutic agent.

Now let's analyze the effect of neutralizing protons in the extracellular space with the help of negatively charged water, or rather, with the help of hydroxyl ions, which this water is rich in: $H^+ + OH^- \rightarrow H_2O$. It is clear that this neutralization will decrease the same proton gradient and, therefore, slow down both types of secondary transmembrane transport (Figure 6) and cellular metabolism in general. Of course, this same neutralization will hinder the growth of living matter, which needs water to form hydration shells of new biopolymers, and, consequently, cell proliferation. Obviously, the low penetrating and hydrating capacity of negatively charged water (Figure 4, left; Figure 7, left) will also prevent it from both penetrating cells from the extracellular space and hydrating intracellular structures, in particular, newly formed ones. All this allows us to consider negatively charged water as a means of preventing cell proliferation.

In addition, all this suggests that the loss of intracellular water by cells upon contact with negatively charged water can also be fatal for them, in accordance with the second pathway of apoptosis, which consists in irreversible drying of cells [20]. This is in complete agreement with the opinion of the authors who assert that the dissipation of proton gradients is invariably accompanied by cell death [11]. Agree, all this also confirms the correctness of the maxim of Paracelsus.

It should be added that the energy of proton gradients formed on the outer sides of bacterial cytoplasmic membranes, which are conjugate, are used by H^+-ATP-synthases integrated into such membranes for ATP synthesis: $ADP + Pi \rightarrow ATP + H_2O$ [12, 15, 23 - 25]. This suggests that neutralization of their cytoplasmic proton gradients is fatal, first of all, for bacteria. Thus, negatively charged water can be positioned as antiseptic, killing bacteria, including pathogenic.

2.2 Effect of Electrified Waters on DNAs

After all that has been said, I hope that you will easily perceive the fact that DNA practically does not dissolve in water, which is sufficiently negatively charged, but dissolves instantly in water, which is sufficiently positively charged [2,26,27]. In turn, this allows us to conclude that positively charged water hydrates DNA better than negatively charged water, and also that the degree of DNA hydration in a positively charged aqueous medium is higher than in a negatively charged one. Let's also take into account that the difference in the degree of hydration of DNA molecules induces their A↔B transitions and, as a consequence, the activity of DNA and RNA polymerases, the intensity of peptide synthesis, cell proliferation, etc. [28-30]. Then there will be every reason to assert that the electrification of the aqueous environment of DNA also affects cell proliferation.

In this aspect, it is useful to compare the UV absorption spectra of lymphocytes from healthy subjects (Figure 8, spectrum 1) and from patients with B-cell chronic lymphocytic leukaemia (B-CCL) (Figure 8, spectrum 2) [31]. You can see that the last spectrum has a noticeable peak at ~ 260 nm, which indicates either a significant positive electrification of the environment of lymphocytic DNA [2,26,32], or their modification with oxygen [33,34] (it is quite possible - and about both at once.).

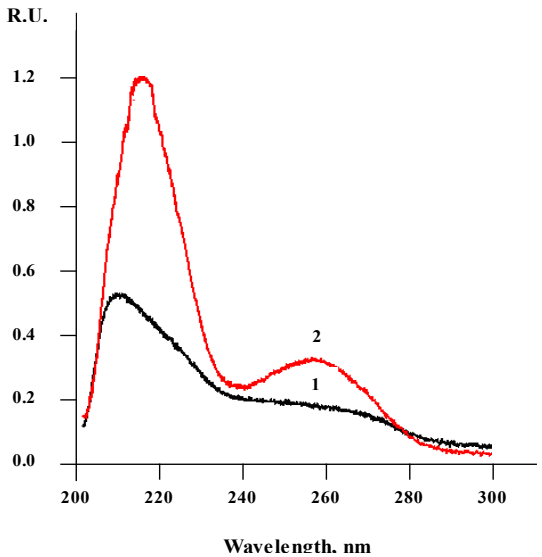

Figure 8. UV absorption spectra of lymphocytes: 1 - lymphocytes of healthy subjects; 2 - lymphocytes of patients with B-CCL [31]

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Negative electrification of lymphocytes from healthy people is confirmed by a peak at 208 nm (Figure 8, spectrum 1), and positive electrification of lymphocytes from patients with B-CCL is confirmed by a peak at 215 nm (Figure 8, spectrum 2) [31,32].

Given that the proliferation of lymphocytes from patients with B-cell chronic lymphocytic leukemia (B-CCL) proceeds more actively than the proliferation of lymphocytes in healthy subjects, we can say that positive electrification of the karyoplasms of lymphocytes contributes to their proliferation, while its negative electrification does not. In addition, all this suggests that neutralizing the positively electrified karyoplasms of lymphocytes with negatively charged water can prevent cancer.

In this aspect, water saturated with hydrogen gas looks very promising, firstly, because such water is negatively charged [22] and, secondly, because hydrogen gas dissolved in such water can restore nuclear DNA, which oxidation usually accompanies (or causes) cancer [35 - 40]. (You should also pay attention to DMSO, which can quickly penetrate into the body through the skin, destroy structured water [22] and remove hydroxyl radicals from DNA [41].)

2.3 Fundamentals of Photodynamic and Thermal Cancer Therapy

Since cancer cells are positively charged, they may be more sensitive to additional positive electrification than healthy cells. Both photodynamic and thermal anticancer therapies (hyperthermia) [42-47] are based, in fact, on these different sensitivities. Let's see this.

Initially, let's take into account that the Pointing vector, which determines, as many people know, the direction of light rays, also determines the direction of movement and positive charges, which few people know about (Figure 9) [48].

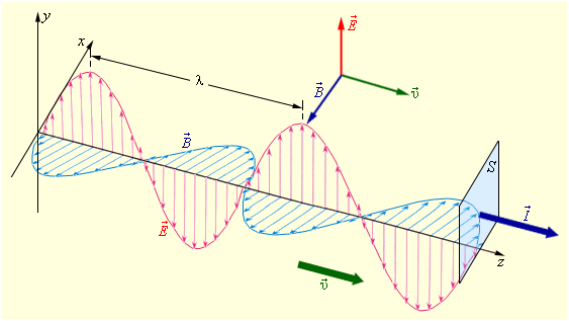

Figure 9. This is a diagram illustrating the structure of the Pointing vector. E and B are the electric and magnetic components of the vector, therefore. v is the speed of positive charges forming a current I in the direction of the Poynting vector [E, B] [48]

This allows us to conclude that focused light itself (that is, without photosensitizes), or rather, the proton flux that it induces, is just as lethal for (positively charged) cancer cells as a proton gun [17 - 19]. Since human tissues are most transparent to red light [43], its use in photodynamic cancer therapy is preferable (Figure 10).

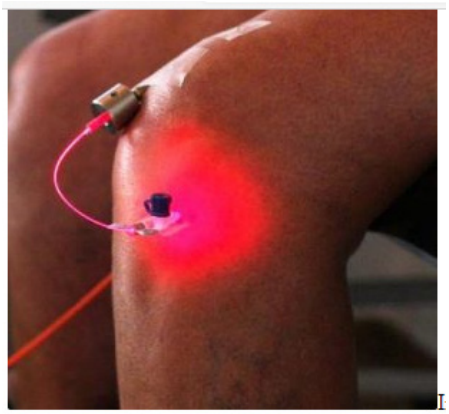

Figure 10. So the tumor is irradiated with a red laser

Before we analyze anticancer thermotherapy (also known as hypothermia), let's remember how starch paste is made. First, a suspension of starch is prepared in cold water, and then this suspension is poured into boiling water in a thin stream. It is during such manipulations that the cold starch suspension extracts protons from boiling water. Remember that starch swells only in water saturated with uncompensated protons (Figure 4). It should also be realized that with such manipulations we form a two-phase system, between the phases of which there is a flux of uncompensated protons.

In this case, the distribution of protons between cold and boiled water follows the Kyon rule: when two phases come into contact, the phase with a higher dielectric constant gets a positive charge, and the phase with a lower dielectric constant gets a negative charge [22]. Since the dielectric constant of water at 20 °C is ~ 81, and the dielectric constant of water at 100 °C is ~ 55 [22], cold water accumulates protons and gains a positive charge, while boiled water loses protons and acquires a negative charge. Thus, anticancer hyperthermia uses the same mechanism of saturation of human tissues with protons, as we do, making starch paste.

It is clear that negative, at least less positive, electrification of healthy cells determines their less sensitivity to both illumination and heating, in fact, to excessive positive electrification.

At the same time, all means aimed at destroying cancer cells with the help of protons simultaneously induce positive electrification of human tissues and, therefore, create favorable conditions for the reproduction of surviving cells. Moreover, the surrounding of the surviving cells is enriched with fragments of killed cancer cells, which the

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surviving cells can use as nutrients. Thus, these types of cancer therapies combine the killing of cancer cells with the simultaneous stimulation of new cancer cells and tumor growth, which is counterproductive. It is also counterproductive to use photosensitizes for additional oxidation of the DNA of cancer cells [42-44], that is, for the oxidation of those DNA that are already oxidized [21,31,36-40].

Based on this, it should be noted that anticancer therapy is more preferable, aimed not at destroying cancer cells, but at their deep chemical recovery [40]. At the same time, negative electrification of cancerous tumors creates conditions for the emergence of new nerve contacts (Figure 3), which means for the restoration of the innervation of damaged tissues, in the absence of which the tissues swell at least uncontrollably. Thus, the use of negatively charged water as an antitumor agent seems to be quite reasonable. In particular, the electron gun, which scientists from the Siberian Branch of the Russian Academy of Sciences are trying to use as an antitumor agent, appears to be a more promising anticancer therapy than a proton gun.

2.4 Obtaining Electrized Water in the Laboratory

If you are interested in all this, it is useful to know how you can get both positive and negative water, in particular - in the laboratory. So, in laboratory conditions, water with a positive electric charge (potential) is convenient to obtain:

(1) by bubbling uncharged water with gaseous oxygen, this is an electron acceptor;

(2) by filtering uncharged water through silica gel, this absorbs hydroxyl ions from the water, OH- [2,22].

When receiving positively charged water, it is advisable to use glassware, since glass absorbs hydroxyl ions, OH- [22,27].

Water with a negative electric charge (potential) is conveniently obtained:

(1) by bubbling uncharged water with gaseous hydrogen, this is an electron donor;

(2) by filtering uncharged water through activated carbon that absorbs hydrogen ions, H+ [22].

When receiving negatively charged water, it is advisable to use polyethylene dishes, since polyethylene absorbs hydrogen ions, H+.

Water with the required value of the electric potential is conveniently obtained:

(1) by varying the thickness of the sorbent layer through which water is filtered;

(2) by varying the time the gas passes through the water and (or) its pressure [2].

As many find this acceptable, both anolyte and catholyte can be used. Moreover, the drying of anolytes is accompanied by the formation of cubic or rhombic crystals (Figure 11, left), and the drying of catholytes is accompanied by the formation of needle-shaped crystals (Figure 11, right). In this, both anolyte and catholyte are clearly similar to positively and negatively charged waters, respectively (Figure 2) [2].

In addition, positively charged water can be obtained by rotating uncharged water counterclockwise with your left hand (Figure 12, left), and negatively charged water by rotating uncharged water clockwise with your right hand (Figure 12, right).

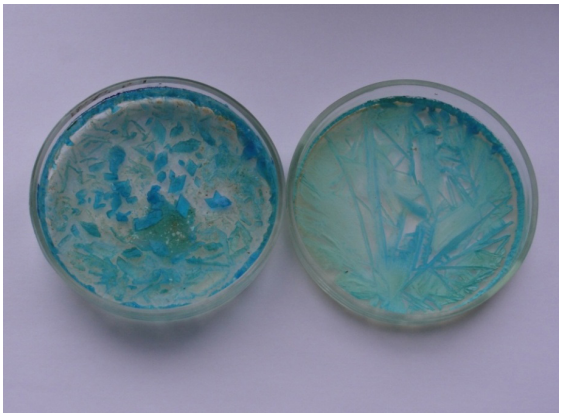

Figure 11. This is how the crystals that formed after drying $CuSO_4$ solutions from anode (left) and cathode (right) departments of functioned electrolyzer look like

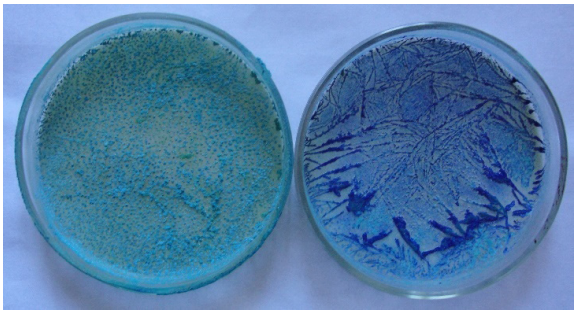

Figure 12. These are crystals formed after drying CuSO4 solutions, which were prepared by turning counterclockwise with left hand (left) and clockwise with right hand (right); both Petri dishes were rotated for 1 min

This method of electrifying water uses the fact that the left hand has a predominantly positive charge, and the right hand predominantly negative [49], as well as the interaction of rotating waters with the electromagnetic field of the Earth [7]. Despite the uncommonness of the latter method of electrifying water, it seems to be the most "clean", since it excludes any pollutants from entering the water.

All described methods should take into account the negative electrification of air at low atmospheric pressure and its positive electrification at high atmospheric pres-

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sure [50]. One should also take into account the fact that positively charged water loses its charge upon evaporation [7], as well as the fact that light and glass enhance the positive electrification of water [27, 48].

2.5 Water Potential Measurement

The electric potential of electrized water, relatively uncharged water, can be measured using a U-shaped tube with a tap at the point of the bend, in both knees of which stainless steel electrodes are soldered. When the valve is closed, one of the elbows of the tube is filled with uncharged water, and the other elbow of the tube is filled with electrized water. The potential difference between the electrodes is recorded after the valve is opened with a voltmeter: this difference is taken as the potential of electrized water [2]. This method of measurement is generally accepted, but it is more convenient to measure the potential of electrized water as shown in the Figure 13.

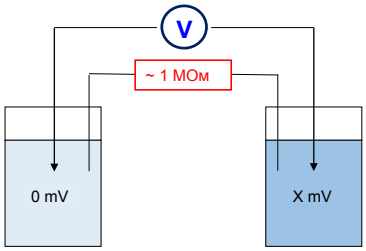

Figure 13. This is the most convenient setup for measuring the electric potential of water: on the left is a vessel with uncharged water (0 mV), on the right is a vessel with water, the potential of which is determined (X mV) from a voltmeter reading (V)

As an additional control, the difference in UV absorption of negatively and positively electrified waters (Figure 14) [2,27,32] can be quite valuable.

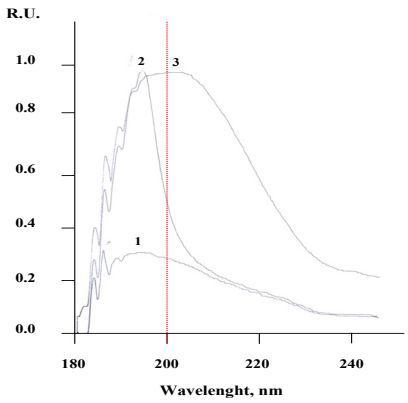

Figure 14. UV absorbance spectra of the water: 1 - fresh distilled water; 2 -water, filtered through activated carbon; 3 - water, filtered through silica gel

The spectra were not processed [2, 27, 32].

3. Conclusion

Since water is both the main component of the human body and the most important nutrient for humans, it should be borne in mind that the properties of water depend on its electrification. It should be especially taken into account that human biological fluids, at least women, can change their own charge (potential) and that these changes are natural. In particular, it should be borne in mind that oppositional charged waters interact differently with biologically significant substances and can affect membrane potentials in different ways, primarily cytoplasmic potentials, affecting both cellular metabolism and cellular activity, including cell proliferation. Without such consideration, many living phenomena will remain incompletely understood. Otherwise, we will have nothing left but powerless to agree with the verdict of Szent-Gyorgyi: “Biology, perhaps, because until now not successful in understanding the most common functions that focused on the matter in the form of particles, keeping away them from two matrixes: water and electromagnetic fields”.

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ARTICLE

Study of the Antitumor Activity of the Drug Dekoglitz on Two Tumors and Some Aspects of Its Mechanism of Action

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ABSTRACT

Aim: Evaluation of the antitumor activity of the new drug Dekoglitz in animals with tumor strains of Sarcoma 45 in comparison with the drug dekocin, from which it was obtained, as well as with 5-fluorouracil and etoposide, and on ovarian tumors (OT) in comparison with the drug dekocin and identification of the effect of Dekoglitz on NA synthesis and internucleosomal DNA degradation. Methods: The study of preparations was carried out on 68 outbred rats with transplanted C-45 and OT tumors. The alkylating effect of the drugs was studied on cells tumor of Sarcoma 180. Results: The antitumor activity of dekoglitz on Sarcoma 45 was high, about 98/96%, with a remission rate of 80%. Its effect was 28-24% higher than that of dekocin. On OT, the effect of decoglitz with intraperitoneal administration reached 89/76% with a remission rate of 40%, with oral administration 96/86% with a remission rate of 60%. Conclusion: The study of the new drug Dekoglitz on animals with a tumor of Sarcoma 45 revealed its higher activity (by 20-27%) in comparison with the original Dekocin, 5-fluorouracil and etoposide with a lower level of side effects. On OT, the effect of Dekoglitz was 35-40% higher, especially after oral administration. Apparently, the great ability to suppress the synthesis of NA and carry out internucleosomal degradation and fragmentation of tumor DNA by the new drugs dekoglitz explains its antitumor efficacy, which is greater than that of Dekocin (K-18) in experiments on tumors.

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1. Introduction

The pronounced general toxic effect of a large number of used cytostatics, rapidly developing resistance, and the lack of sensitivity of a number of tumors to existing drugs dictate the need to create new anticancer drugs.

The use of medicinal preparations based on licorice root has been around for several millennia. The main active ingredient in licorice root is the triterpenoid glycyrrhizic acid (GA). GA as a solubilizer of many water-insoluble organic substances is used to create low-dose, low-toxic drugs [1].

For example, practically insoluble in water, gossypol and its derivatives, hydrocortisone, prednisolone, kracil, nistatine and other drugs in combination with the monoammonium salt of glycyrrhizic acid (MASGA) pass into aqueous solutions [2,3]. All these positive properties of GA and its derivatives are associated with its ability to form supramolecular complexes, which in aqueous solutions have very low critical micelle concentration values. All researchers note the very low toxicity of preparations with GA, MASGK and their derivatives, created on their basis. In addition to the above properties, GA and its derivatives exhibit a pronounced anti-inflammatory, analgesic effect, anti-edema, hypotensive, virus-neutralizing effect, improves tissue regeneration both in the early manifestations of a viral disease and in ulcerative forms.

However, GA was not used for combination with anticancer drugs. We are developing new promising substances based on tropolone alkaloidиs, of which dekocin, a derivative of the alkaloid colchicine, revealed activity in animal studies with 10 tumor strains, which was the highest (above 80%) on Sarcoma S 180, RShM-5 (cervical cancer) and AKATOL [4], which allowed this drug to be proposed for clinical trials. The obtained clinical data of the antitumor drug dekocin indicate a high sensitivity of skin cancer to 3-4% dekocin ointment, which was also effective in combination with radiation [5,6]. However, dekocin is insoluble in water, which complicates both its parenteral administration and bioavailability. In this regard, we used the method of molecular encapsulation of the drug dekocin with glycyrrhizic acid (HA), which has effective solubilizing properties. A new water-soluble supramolecular complex of Dekocin and HA was obtained, which differs in physicochemical parameters from the original Dekocin, as well as a 2.6-fold decrease in toxicity, which is named Dekoglitz.

The aim of this work was to study the antitumor activity of a new colchicine derivative Dekoglitz in animals with tumor strains of Sarcoma 45 and ovarian tumor (OT) in comparison with the effect of decocin, 5-fluorouracil and etoposide, as well as to study the effect of Decoglitz on DNA/RNA synthesis and internucleosomal tumor degradation in comparison with the effect of dekocin (K-18) and etoposide.

2. Methods

2.1 Tumoral Strains

Transplantable tumors, murine sarcoma 180, and two strains of rat sarcoma 45 and ovarian tumors (OT) were used in the work. Strain Sarcoma 180 was purchased from the Tumor Strains Collection Bank (Institute of Carcinogenesis, N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences) Moscow, Russia. The strains of Sarcoma 45 and ovarian tumors (OT) were purchased from the Tumor Strains Collection Bank (Institute of Oncology of Kazakhstan). The tumor strains were passaged to the strain protocol.

2.2 Antitumor Drugs

The following drugs were used in the work: etoposide (Etoposide phosphate, Bristol-Myers Squibb); 5-fluorouracil (Getwell Pharm acutikals, India); the K-18 (Dekocin) and its derivative Dekoglitz (tropolone alkaloids, colchicine derivatives) developed by Prof., Z. M. Enikeeva at the Republican Specialized Scientific Practical Medical Center of Oncology and Radiology of the Ministry of Health of the Republic of Uzbekistan (RSNPMTSO&R MH RUz).

2.3 Animals

In the experiment white outbred, mice weighing 18-20 g (60 individuals) and rats weighing 90-140 g (68 individuals) were used. The animals were kept on a standard diet under natural lighting conditions and had free access to water and food in the vivarium at the RSNPMTSO&R MH RUz.

At the end of the experiment, all rats and mice were euthanized under ether anesthesia, in accordance with the International Rules for the Protection of Vertebrates. All experiments were performed in accordance with the recommendations and requirements of the "World Society for the Protection of Animals (WSPA)" and "European Convention for the Protection of Experimental" (Strasbourg, 1986).

2.3.1 Investigation Antitumor Activity of Drugs

Tumor subinoculation was carried out according to generally accepted methods: tumors of Sarcoma 45 and OT were inoculated subcutaneously with a suspension of tumor cells, 30-60 mg in 0.3-0.5 ml of nutrient medium per rat

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[7]. Treatment of animals began 4 days after tumor implantation, drugs were injected in all groups 10 times, and all experimental groups were injected with drugs in a volume of 0.3 ml per 100 g rat. The animals were slaughtered on the 19-21st day after tumor implantation, the animals were sacrificed using humane methods of working with laboratory animals. Before the introduction and at the end of the experiment, the body weight of the animals was determined.

During the experiment, in order to study the dynamics of tumor growth, the volumes of tumors through the skin of animals were measured in the treated and control groups of mice (in 3 projections) at the beginning of the experiment, every 5 days after the start of treatment, and before slaughter. At the end of the experiment, the efficacy in sacrificed mice was determined by the volume (V) of the extracted tumor tissue, as well as by the tumor mass in the compared groups. Tumor growth inhibition was calculated using the formulas [7]. The tolerability of the treatment was judged by the death of the mice; for an indirect assessment of the possible hematotoxicity in the sacrificed mice, the spleen weight was determined.

2.3.2 Alkylating Action

The effect of drugs on the synthesis of DNA and RNA was studied on Sarcoma 180 tumor cells in vitro. A cell suspension from tumor tissue was obtained according to the method [8]. Cells with a titer of 10,000 were cultured in medium (RPMI-1640 containing 5% fetal bovine serum (FBS), 2mM L-glutamine, 10U/ml penicillin and 100mcg / ml streptomycin), with the absence and presence of therapeutic (TD) investigational drugs , for 24 hours at 370C in an atmosphere of 5% CO_2.

2.3.3 Isolation of DNA/RNA

DNA/RNA preparations from Sarcoma 180 cells were isolated by two methods, a) phenol-chloroform method [9] and according to the protocol of the kit-kit "DNA-sorb-B" (InterLabService), Russia. The DNA/RNA concentration was determined by adsorption at a wavelength of 260 nm on an SF-26 spectrophotometer (Russia).

For the analysis of internucleosomal DNA degradation, total DNA/RNA preparations were treated with the RNase A enzyme according by method [9]. DNA/RNA electrophoresis was analyzed in 1.5% agarose gel for 4 h, 60V according to the method [9].

Statistical processing was performed using Statistica, version 6.0. The level of statistical significance was taken as $p < 0.05$.

3. Results

3.1 Study of Antitumor Activity, in vivo

The study of the antitumor activity of the drugs on the Sarcoma 45 strain began 4 days after tumor subinoculation the drugs were injected 10 times. The slaughter was carried out on day 21. In the control group, there was a mortality of 25%, in the experimental groups with the use of dekoglits and dekocin, there was no death of animals, in the group with 5-fluorouracil all animals died after 10-fold administration, in the group with etoposide, 30% of the animals died.

In group 2, the drug Dekoglitz showed high antitumor activity in 98/96%, 80% of regressed tumors were observed, while the drug caused a slight decrease in body weight (by 5%) and an increase in the spleen by 20% (Table 1).

In group 3, the antitumor effect of the drug dekocin was less high - 70/72%, the drug caused a slight decrease in body weight (by 6%) and spleen (by 20%).

In group 4, the drug 5-fluorouracil at a dose of 15 mg/kg caused the death of all animals on day 15 after inoculation and its antitumor effect could be assessed on day 12 when measuring the volume of tumors in 2 animals, which was in relation to the control for this day is 76%, however, due to the death of animals, it was impossible to assess the effect of the drug on body weight and spleen.

Table 1. Antitumor activity of the drug Dekoglitz in comparison with Dekocin, 5-FU and Etoposide in rats with tumor Sarcoma 45

(Treatment with drugs was carried out on the 4th day after tumor implantation. The slaughter was carried out on the 21st day)

Groups of animals	Number of animals before and after treatment		The mass of animals (gr)		Tumor volume, (cm^3)		
	before	after	before	after	5th day	12th day	after
Control	8	6	131.0±9.3	121.3±9.0	0.3±0.04	2.1±0.5	2.7±0.8
Decoglitz 20 mg/kg	6	6	102.0±5.8	97.0±6.0	0.2±0.1	0.2±0.1	0.04±0.01*
Dekocin 15 mg/kg	6	6	132.0±17	124.0±14.4	0.1±0.02	0.5±0.13	0.8±0.07*
5-FU 15 mg/kg	6	0	109.0±4.4	-	0.1±0.05	0.48±0.08	-
Etoposide 8 mg/kg	6	4	114.0±9.0	106.0±8.0	0.2±0.1	0.7±0.16	0.6±0.16

Note: in the treatment groups n = 6, in the control n = 8; * differences are statistically significant in comparison with control at $P < 0.05$.

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Table 1. Continued.

Groups of animals	Weight tumors (gr)	Weight Spleen (mg)	% inhibition of tumor growth		
			by volume	by mass	% regression
Control	2.5±1.1	0.5±0.03			
Decoglitz 20 mg/kg	0.04±0.01	0.6±0.04	98	96	80
Dekocin 15 mg/kg	0.7±0.07*	0.4±0.03	70	72	0
5-FU 15 mg/kg	-	-	77	-	0
Etoposide 8 mg/kg	0.6±0.06*	0.3±0.02	78	76	0

Table 2. Antitumor activity of the drug Dekoglitz in comparison with Dekocin in rats with ovarian tumor

(Treatment with drugs was carried out on the 4th day after tumor implantation. 10 injections of substances. The slaughter was carried out on the 19th day)

Groups of animals	Weight of animals (gr)		Tumor volume (cm^3)		Weight tumors (gr)	Weight spleen (mg)	% inhibition of tumor growth		
	before treatment	after treatment	for 8^{th} day	for 10^{th} day			by volume	by mass	% regression
Control	160±10.6	162±10.2	1.7±0.5	2.8±0.5	2.5±0,6	0.9±0.07			
Dekocin, 15 g/kg	97±1.3	118±0.02	0.2±0.02	1.3±0.1*	1.4±0.4*	0.8± 0.1	54	44	
Decoglitz 20 mg/kg, (intra-peritoneal)	119±4.6	135±7.0	0.7±0.1	0.3± 0.02*	0.6±0.1*	0.8±0.07	89	76	40
Decoglitz 40 mg/kg, (orally)	110±3.2	139±5.7	0.6±0.1	0.1±0.01*	0.1±0.03*	0.8±0.1	96	86	60

Note: in the treatment groups n = 6, in the control n = 6; * differences are statistically significant in comparison with control at $P < 0.05$.

In the 5th group, the antitumor effect of the drug etoposide was 78/76%, the drug caused a slight decrease in body weight (by 7%) and a more pronounced decrease in the spleen weight (by 40%).

Thus, the new drug Dekoglitz showed the highest activity, both in comparison with dekocin, from which it was obtained, and known cytostatics, moreover, its effect was higher than the comparison drugs by 20-28%, and there was no such side effect as the effect on the spleen. It should be noted that Dekoglitz was studied at a dose that in relation to LD_{50} was significantly lower than that of dekocin, i.e. for GA and MASGK derivatives, it was noted [10] that their activity manifests itself in doses 2-4 times less than the maximum tolerated.

The study of the antitumor activity of the drugs on the Ovarian Tumor (OT) strain began 4 days after tumor transplantation the drugs were injected 10 times. There was no death of animals during the experiment. The slaughter was carried out on the 19th day.

In group 2, the drug dekocin was 54/44% active, while the drug caused a slight decrease in body spleen weight (by 11%) and an increase in body weight by 21% (Table 2).

In group 3, the antitumor effect of the drug Dekoglitz at a dose of 20 mg / kg with intraperitoneal injection was less high - 89/76% than when exposed to Sarcoma 45, but caused tumor regression in 40% of animals. The drug caused a slight decrease in the spleen (by 11%), body weight was 13% more than the initial one.

In the 4th group, the drug Dekoglitz at a dose of 40 mg/kg with oral administration had a higher antitumor effect 96/86%, while it caused tumor regression in 60% of animals, the drug had side effects only in a slight decrease in the spleen (by 11%), body weight was 26% more than the initial one.

3.2 Study of the Mechanism of Action, in vitro

The high antitumor activity of the drug Dekoglitz, as well as its further study as a cytostatic, involves the study of such aspects of its mechanism of action as alkylating, the influence on DNA/RNA synthesis, internucleosomal DNA degradation, and topoisomerase II activity. The effect of K-18 and Decoglitz on DNA and RNA synthesis was investigated in sarcoma 180 cells in vitro in comparison with etoposide, which is a known inhibitor of topoisomerase I/II.

Figure 1 shows the results of DNA/RNA electrophore-

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sis of tumor cells cultured in the absence of preparations (lanes 1,2) and using etoposide, K-18 and Dekoglitz (respectively, lanes 4-6).

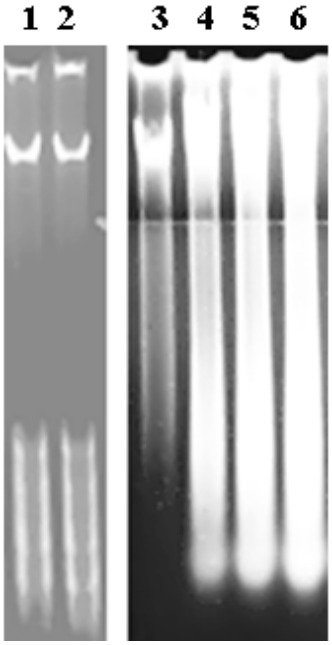

Figure 1. Influence of preparations on native DNA, nucleosoma degradation of DNA and activity topoisomerase II tumor cells of the Sarcoma 180, in vitro

Lanes 1 and 2 native DNA/ RNA of Sarcoma 180 (not treated with RNase A). Lanes 3-4, aliquots of DNA treated with RNase. Lane 3 control, without the using of cytostatics, Lane 4 Etoposide, Lane 5 K-18 (Dekocin), Lane 6 Dekoglitz. Electrophoresis carries out in to 1.5 % TAE agarose gel, 4h, and 60V and visualized by UV transilluminator after staining with ethidium bromide.

In aliquots of DNA/RNA not treated with the enzyme RNase, the electrophoregram shows a high native of nuclear DNA and RNA molecules (Figure 1, lanes 1, 2). In aliquots of DNA/RNA treated with the enzyme RNase, the electrophoregram shows DNA degradation in the form of a plume (Figure 1, lanes 4-6).

The results of the electrophoresis showed: Etoposide, K-18, and Dekoglitz contributed to internucleosomal DNA degradation by: 75.7 ± 3.3, 86.7 ± 3.7, and 94.5 ± 1.7, respectively. Also, according to the pattern of DNA fragmentation, electrophoregram, Etoposide, K-18, and Dekoglitz inhibited topoisomerase II activity by 57.6 ± 2.7, 64.6 ± 2.3, and 79.6 ± 3.0, respectively (Figure 1, Table 3).

Regarding the effect of the three drugs on topoisomerase II (TOPO-II) of Sarcoma 180 tumor cells, the activity of this enzyme was determined visually by the pattern of electrophoresis of fragmented DNA in a gel. Etoposide, K-18, and Dekoglitz inhibited TOPO II activity by 57.6 ± 2.7, 64.6 ± 2.3, and 79.6 ± 3.0, respectively (Figure 1, Table 3).

The results, the effect of the studied drugs on the synthesis of DNA/RNA showed: a) Etoposide, K-18 and Dekoglitz inhibited DNA synthesis by 64.9 ± 2.7, 85.6 ± 2.3, 95.7 ± 3.7, respectively; b) Etoposide, K-18, and Dekoglitz inhibited RNA synthesis by 30.0 ± 3.0, 60.5 ± 1.7, 65.9 ± 2.7, respectively (Table 3).

Table 3. Influence of antineoplastic preparations on synthesis DNA/RNA, TOPO II activity and DNA nucleosoma degradation of cells of the Sarcoma 180 tumor, in vitro

Antitumor preparations (TD)	DNA nucleosoma degradation, in %	Inhibition		
		Activity TOPO-II in %	DNA synthesis in %	RNA synthesis in %
Control	0±0	0±0	0±0	0±0
Etoposide 8 mkg/ml	75.7±3.3	57.6±2.7	64.9±2.7	30.0±3.0
K-18 15 mkg/ml	86.7±3.7	64.6±2.3	85.6±2.3	60.5±1.7
Decoglitz 20 mkg/ml	94.5±1.7	79.6±3.0	95.7±3.7	65.9±2.7

Thus, the results of this experiment showed a high alkylating activity of Decoglitz to targets DNA/RNA and its inhibitory effect on topoisomerase II, resulting in DNA fragmentation, and then cell apoptosis.

4. Conclusion

The study of the new drug Dekoglitz on animals with a tumor of Sarcoma 45 revealed a very high activity with 80% tumor regression, which was 20-27% more than the original Dekocin, 5-fluorouracil and etoposide with a lower level of side effects. Dekoglitz also had a high effect on OT tumor when administered intraperitoneal, which was 30-40% higher than the effect of Dekocin (40% of tumors regressed), however, Dekoglitz showed an even higher activity after oral administration, where 60% of tumors regressed.

This Dekoglitz effect is confirmed by a more intense effect on the synthesis of DNA and RNA of tumor cells. Apparently, the great ability to suppress the synthesis of NA and the activity of topoisomerase II and to carry out internucleosomal degradation of tumor DNA by the new drug Dekoglitz explains its antitumor efficacy, which is greater than that of Dekocin (K-18) in experiments on tumors.

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Conflict of Interest

The authors declare no conflict of interest.

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ARTICLE

Treatment Outcomes of Germ Cell Tumors of Ovary: Single Institutional Study

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ABSTRACT

5% of all ovarian tumours are accounted to germ cell tumours (GCT's). Affecting mostly young women, the highest incidence is seen in second and third decade of life. They are highly malignant but chemosensitive and more curable than their epithelial counterparts. Treating these tumors with effective surgery and combination chemotherapy survival rates have dramatically improved in recent decades. We present our experience of ovarian germ cell tumours in the department of Surgical Oncology, Rajendra Institute of Medical Sciences (RIMS), Ranchi with special emphasis on treatment outcomes. A retrospective review of hospital medical records of patients with ovarian germ cell tumours diagnosed and treated at RIMS from June 2019 to August 2020, was performed. Clinical profile and treatment outcome of patients were recorded. A total of 19 patients met criteria. The median age at diagnosis was 20 years (range 11-42 years) and all had good performance status. All except two patients underwent surgery, 70.6% and 29.4% in upfront and interval debulking surgery (IDS) setting respectively. Fertility preserving surgery was done in 75% patients in the primary surgery group and 60% undergoing IDS. 83.3% patients received BEP as adjuvant chemotherapy whereas 80% as neo-adjuvant chemotherapy.

Majority (31.5%) patients had dysgerminoma as final histology, followed by mixed histology(26.3%), yolk sac tumour (15.7%), immature teratoma (15.7%) and choriocarcinoma (10.5%). 47.3% patients were in Stage I at the time of diagnosis. 78.9% patients were alive without disease, 10.5% recurred, and 10.5% were lost to follow up.

1. Introduction

20 to 25% of all benign and malignant ovarian neoplasms are of germ cell origin. These are uncommon neoplasms arising from primitive germ cells of the embryonic gonad. Affecting mostly young women, the highest incidence is seen in second and third decade of life. Most common histology is teratoma followed by dysgerminoma worldwide.

Before mid 1960's, almost all non-dygerminomatous

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GCT patients died. The patients with dysgerminoma survived owning to its high radiosensitivity but fertility was not spared. The introduction of combination chemotherapy VAC (vincristine, dactinomycin, and cyclophosphamide) achieved 85% cure rate in stage I GCT's whereas metastatic disease had a 50-70% [1-2] mortality. Moreover as BEP therapy does not effect ovarian function much, most GCT patients remain fertile and are able to give birth[3-6]. FIGO stage and elevated tumour markers are seen to be independent poor prognostic indicators [7].

2. Materials and Methods

This is a retrospective study in which data was collected prospectively from hospital medical records. All patients with histologically confirmed GCT and treated at RIMS from June 2019 to August 2020 were included. A total number of 19 patients met criteria.

Clinical profile, stage of presentation, histological classification and treatment received (primary surgery followed by chemotherapy or neo-adjuvant chemotherapy followed by interval debulking surgery) was seen. Treatment outcome and disease free interval were ascertained.

The data was analysed using SPSS software.

3. Results

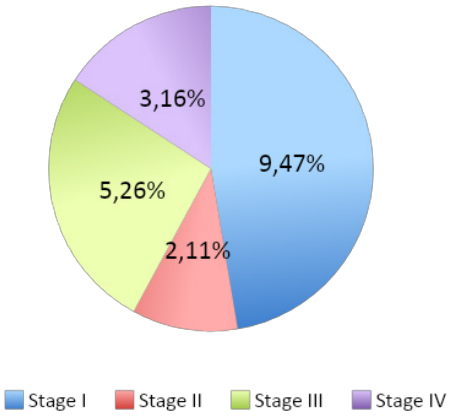
During this 14 month period, a total of 19 patients were admitted and treated in our department. The median age was 20 years (range 11-42yrs) out of which one patient was premenarchal. All patients had a performance status of ECOG 0/1. The median AFP at presentation was 986 ng/mL (range 11-23000 ng/mL), median LDH 843 U/L (range 273-15000 U/L). All except two patients had normal β-hCG (table 1).

Stage wise distribution is depicted in Figure 1. Majority patients(n=9; 47.3%) belonged to stage I followed by stage III (n= 11; 26.3%). Two patients belonged to stage II and three to stage IV.

Table 1. Demographics

Median Age	**20 (11-42years)**
PS	**All except 1 were ECOG 0/1**
Tumour markers at presentation	
AFP	**986 (11-23,000)**
LDH	**843 (273-15000)**
bHCG	**Normal in all except 2**

Figure 1: FIGO Stage Distribution



12 (70.6%) patients underwent upfront surgery out of which 9 (75%) had fertility preservation and 3 (25%) had radical surgery.

In terms of adjuvant chemotherapy, 10 (83.3%) patients received BEP to begin with following which 3 (30%) were switched to EP due to bleomycin induced toxicity, 2 (16.6%) patients received EP, whereas 1 (8.3%) received VIP after disease progression on BEP.

Table 3. Interval debulking surgery details

Surgical details: Upfront surgery		
Type	Details	N=12
Fertility preserved	USO	3
	BSO	1
	USO/omentum	1
	USO/nodes/omentum	4
Fertility not preserved	TAH BSO	1
	TAH BSO/nodes/omentum	2

Table 2. Upfront surgery details

Surgical details: Interval debulking surgery		
Type	**Details**	**N=5**
Fertility preservation	USO/omental biopsy	2
	USO/omentectomy	1
Fertility not preserved	TAH BSO/omentectomy/ LND	2

7 patients received neo-adjuvant chemotherapy (4 BEP followed by VIP in 1 patient, 1 EP and 2 single agent Methotrexate) following which 5 underwent interval debulking surgery (2 patients had choriocarcinoma which

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was cured with chemotherapy). The reasons for NACT were extensive disease in 2 patients out of which 1 patient also had portal vein thrombosis, poor PS in 2 patients, whereas 1 patient had already received NACT before she presented to us. Among the 5 patients undergoing interval debulking surgery 3 had fertility preservation. Complete cytoreduction of macroscopic disease was achieved in all patients who underwent surgery.

Among the 10 patients receiving adjuvant BEP chemotherapy following upfront surgery, majority received 4 cycles (6 patients) out of which 2 patients received subsequent 2 cycles of EP, 3 patients received 3 cycles out of which 1 received subsequent 2 cycles of EP and 1 received additional 3 cycles of VIP. 1 patient was switched to 3 cycles of EP regimen after the first cycle of BEP due to bleomycin toxicity and 2 patients received 4 cycles of EP.

In the interval debulking surgery group, 3 patients received 3 cycles of BEP, 1 received 4 cycles BEP whereas 1 received 1st cycle as single agent carboplatin followed by 4 cycles EP as NACT. The patients with choriocarcinoma received 4 cycles of single agent methotrexate. Postoperatively 2 patients received 2 cycles EP whereas the rest did not need adjuvant treatment.

Table 4. Chemotherapy details

Setting	Regimen	*n* = 19
NACT	BEP	3
	EP	2
	Methotrexate	2
	TOTAL	7
Adjuvant	BEP	10
	EP	2
	TOTAL	12

Table 5. Chemotherapy cycles

Setting	Cycles	n = 40
NACT	Three	3
	Four	4
Chemotherapy (After IDS)	Two	2
	Six	1
Adjuvant Chemotherapy (after upfront surgery)	Three	2
	Four	7
	Five	1
	Six	2

19 (47.5%) patients had yolk sac tumour, 11 patients had mixed histology, 5 presented with dysgerminoma, 4 with immature teratoma whereas 1 patient had choriocarcinoma as depicted in figure 2.

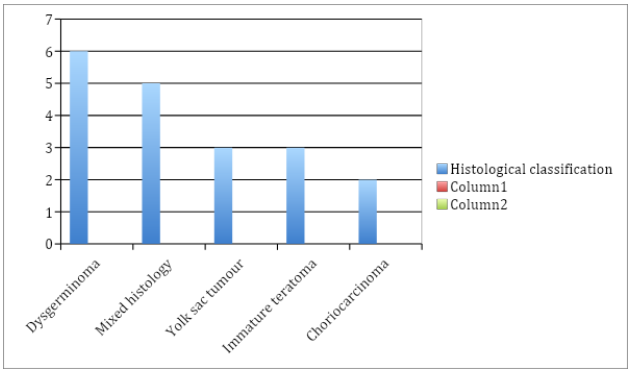

Figure 2.

Follow up data was available for n= 17 patients. This data revealed 15 patients alive without disease and 2 patients alive with disease. 2 patients developed recurrence and 2 were lost to follow up. None of the patients died during this period.

In the recurrence group, one patient had recurrence in both abdomen and pelvis whereas another one in abdomen and thorax. Both received second-line chemotherapy. Two patients were lost to follow up.

4. Discussion

Though accounting for only 5% of all malignant neoplasms, their impact in patient's life is enormous since it mostly affects women in their second or third decade of life [8] . Fearing the sequelae of the disease, many women choose radical surgery but it has been seen that fertility sparing surgery is also an equally effective treatment with possibility of future childbearing [9]. It is now possible to achieve complete cure with fertility preservation however reports on reproductive outcome of GCT survivors are sporadic since it has been only around 30 years since this dramatic improvement due to the implementation of BEP chemotherapy [3-6]. However Zhang et al. in their retrospective study of 32 patients with malignant ovarian germ cell tumour (MOGCT) and sex cord stromal tumours (SCST) showed fertility sparing surgery to be an equally effective alternative to radical surgery with the advantage of menstrual and fertility preservation [10]. In their series all patients underwent fertility sparing surgery (FSS) whereas we could achieve 70.5% FSS rate. This could be due to the fact that all except one patient in their series belonged to either stage I or II whereas 42.1% patients in our series had stage III-IV disease. In addition to a desire to cure, a desire to become pregnant also improves treatment outcomes [25].

Similarly, Turkmen et al. in their series of 69 patients compared survival outcomes of patients undergoing conservative surgery with definitive surgery and established

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surgery type to be insignificant for recurrence [11]. Other studies have also shown that fertility sparing surgery did not affect cancer prognosis in cases of advanced germ cell tumours [6,26,27].

Maheshwari A et al. [12] in their report from India,stated dysgerminoma as the most common histological type which is similar to our series of patients.

Though majority of patients belonged to stage I, a considerable number of patients presented in the advanced stage. This is due to proper staging surgeries performed at our centre along with late referral from other non-oncologic treating institutes. Many patients were operated outside without suspicion of malignancy and presented to us with suspicious final histopathology. One patient with advanced disease even received neo-adjuvant chemotherapy outside before presenting to us. BEP chemotherapy is effective for treating germ cell tumors of the ovary and it has been validated in several trials [13-15].

In the NACT setting, there was complete pathological(or radiological) response in 66.67% patients whereas the rest showed partial response according to the RECIST criteria. S Talukdar et al [16] in their series of 23 patients receiving NACT also showed a complete response of 60.5%. Literature reports 40.7% complete pathological response [17].

10.5% patients recurred and were managed with second line chemotherapy such as VIP (etoposide, ifosfamide, and cisplatin).

Most patients with early disease and healthy looking uterus and ovary underwent fertility sparing surgery. In cases of normal looking contralateral ovary, routine biopsy was not taken however it was thoroughly palpated and any suspicious nodules biopsied. Although occult bilaterality has been reported, biopsy of the contralateral ovary could lead to future infertility related to peritoneal adhesions or ovarian failure [18,19]. Unlike their epithelial ovarian counterparts, GCT's are amenable to fertility preservation. Hence it shoud be the standard of care whenever possible. Many studies document good outcome in this regard [20-24].

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ARTICLE

Research Advance on the Relationship between Wee1 and Tumor Genesis and Progression

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ABSTRACT

In the process of biological genetic information transmission, complete and correct genetic information can make cell mitosis proceed normally. In the development of most tumor cells, G2/M cell cycle checkpoint becomes the key checkpoint in the process of mitosis due to the lack of G1/S cell cycle checkpoint, which mainly depends on the abnormal DNA information blocked by Wee1 protein kinase in G2 phase to enter M phase and prolong the time of G2 phase to complete DNA sequencing So that the normal genetic information can be passed on. Wee1 protein kinase expression is significantly increased in most tumor cells, making it a potential target for tumor therapy.

1. Introduction

Wee1 protein kinase family includes Wee1A, Wee1B and Myt1 members [1]. The human Wee1 gene is located in the P15 region of chromosome 15 [2] (11p15.3-11p15.1), encoding 647 amino acids. Wee1 protein kinase consists of three domains: N-terminal domain, central kinase domain and C-terminal regulatory domain [3]. The N-terminal domain is the activation domain of Wee1 protein kinase, which plays a key role in guiding its destruction, and may inhibit the activity of Wee1 protein kinase [4]. However, the N-terminal domain is also a potential site for inhibiting CyCB/CDK1 dephosphorylation, thus causing cell cycle arrest. The central kinase domain is helpful for Wee1 localization in the nucleus at G2 phase; The C-terminal regulatory domain is the Wee1 protein kinase catalytic domain [5]. Studies have shown that Wee1 is mainly expressed in the nucleus of tumor cells [6]. In recent years, the research of Wee1 protein kinase in DNA repair of cell cycle damage and malignant tumors has become a hot spot. In normal cells, due to the existence of P53, cells can complete the damage repair in G1/S phase when DNA is damaged. However, the mutation of P53 occurs in most malignant tumors, resulting in that the damaged DNA can not be repaired in G1/S phase, and can only be repaired in G2/M phase [7], so that the correct and complete DNA can enter into M phase for mitosis. In this paper, the research progress of the relationship between Wee1 and tumor genesis and development is summarized as follows.

2. Role of Wee1 Protein Kinase in Cell Cycle

Cell cycle is a concept proposed by Howard et al in 1951. It refers to the whole process of a cell from the

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completion of one division to the end of the next division, which is divided into two stages: interphase and division phase. The interphase is divided into G1, S and G2 phases, and the division phase is M phase. In the process of cell proliferation, cell mitosis will encounter a variety of damage factors causing DNA damage and chromosome variation, so someone put forward the concept of cell cycle checkpoint, namely a kind of negative feedback regulation mechanism, which is mainly affected by DNA replication and damage. There are two key checkpoints: G1/S and G2/M phases,which block the heredity of genes with replication errors. In normal cells, DNA damage mainly depends on two pathways mediated by P53(tumor suppressor gene): ATM(Capillary ataxia mutant gene)/ATR-P53-CDK4/CyclinD or ATM/ATR-P53-CDK2/CyclinE inhibit Rb phosphorylation, and make the cell arrest in the G1 phase to complete DNA damage repair. However, studies have shown that most tumor cells lack two pathways in the G1/S phase checkpoint, which makes the DNA damage repair of tumor cells mainly depend on the G2/M phase. Studies have shown that [7] Wee1 and CDC25 play an important role in this checkpoint. ATM/ATR is activated when DNA damage [8], through the phosphorylation of downstream CHK1/2(effect kinase) make its activation [9]. On the one hand, activated CHK1 / 2 can phosphorylate downstream CDC25B/C to inactivate it, inhibit its dephosphorylation of downstream CDK1/CycB, and arrest cell cycle in G2 phase. On the other hand, CHK1/2 can directly activate Wee1 protein kinase. The activated Wee1 protein kinase phosphorylates the thy15 site of CDK1 [10] and inactivates it, which is the key factor for mitosis. The cell cycle is arrested in G2 phase until DNA damage repair is completed. The cell has the opportunity to enter M phase for mitosis. Wee1 protein kinase, as a potential molecular target of tumor cells, has become a focus of current research.

As shown in Figure 1:

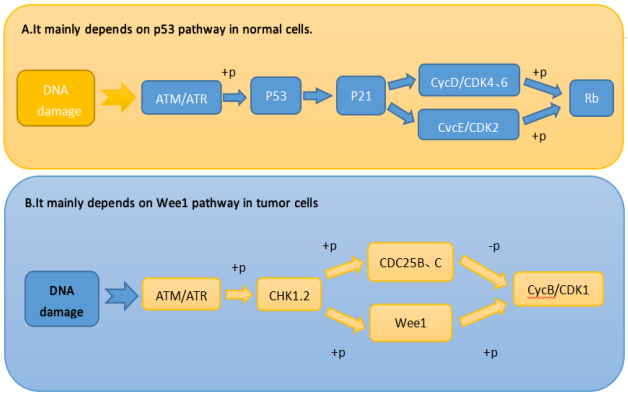

Figure 1. damage and repair process of G2 phase cells

3. Relationship between Wee1 Protein Kinase and Tumor

3.1 Wee1 and Gastric Cancer

Gastric cancer is one of the most common malignant tumors in China. Many chemotherapy drugs can cause DNA damage in gastric cancer tumor cells. Tumor cells lack G1/S phase and most of them rely on G2 phase arrest. Wee1 is a key factor of G2/M checkpoint. Kim et al. [11] first proposed that Wee1 protein kinase might be expressed in gastric cancer. After a series of experiments, it was found that Wee1 was positive in gastric cancer cells, and the positive rate was higher in tumor cells with lymph node metastasis, and the proliferation and invasion ability of tumor cells with Wee1 overexpression was stronger. Zhang et al. [12] also verified that the expression of Wee1 was increased in gastric cancer cells, and further demonstrated that ROP inhibited the proliferation and metastasis of gastric cancer cells by regulating the Wee1 pathway.

3.2 Wee1 and Melanoma

In malignant melanoma, regardless of the status of P53, the high expression of Wee1 can reduce the DNA damage of tumor cells, and is positively correlated with the proliferation, metastasis and poor prognosis of malignant melanoma [13]. Studies have shown that [14], different from most tumors, P53 expression is positive in malignant melanoma. Wee1 is a key signal molecule downstream of BRAF in MAPK signal transduction pathway. Wee1 can inhibit the P53-P21-CDK2/CycE-Rb-E2F pathway in the cell cycle, so that the cell cycle is blocked in the S phase, therefore, the expression of Wee1 protein kinase is still positive in melanoma. Wee1, as the most suitable target, its inhibitor and AKT3 protein kinase inhibitor combined to treat melanoma, so that the treatment effect of AKT3 inhibitor is more effective. In animal experiments, high expression of Wee1 and deletion of MicroRNA-155 (MiR-155) contribute to metastasis of malignant melanoma [15]. However, Bhattacharya et al. [16] showed that compared with primary melanoma, the expression of Wee1 in distant skin metastatic melanoma was down regulated, and the proliferation, migration and invasion ability of Wee1 positive primary tumor cells were decreased.

3.3 Wee1 and Colorectal Cancer

Wee1 can be expressed in both colon cancer tissues and paracancerous normal tissues, but it is highly expressed in colorectal cancer [17]. Experiments showed

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that the expression of Wee1 is mainly positive in the nucleus, but also slightly expressed in the cytoplasm, and the high expression of Wee1 is closely related to distant metastasis of colon cancer, lymph node metastasis and malignant degree of tumor [18]. Yin et al. [19] verified that Wee1 inhibition can reduce the proliferation ability of tumor cells in P53 mutated colorectal cancer, and Wee1 may become a potential target for the treatment of colorectal cancer. Webster et al. [20] also found that the positive expression rate of Wee1 was up-regulated in endothelial cells with liver metastasis from colorectal cancer, and Wee1 may be related to the formation of some branches of blood vessels in liver metastasis from colorectal cancer, which provides a theoretical basis for the research and development of Wee1 protein kinase inhibitors as tumor drugs.

3.4 Wee1 and Breast Cancer

Triple Negative Breast Cancers (TNBCs) are breast cancers that are negative for estrogen receptors, progesterone receptors and human epidermal growth factor receptors. Studies have found [21] that p53 mutations in the vast majority of TNBCs lead to deletion of G1/S stage checkpoints, making Triple Negative Breast Cancer dependent on G2/M stage checkpoints to repair DNA damage. Experimental results showed [22] that Wee1 inhibitor combined with ATR inhibitor can inhibit proliferation and metastasis of TNBCs and induce apoptosis of cancer cells. Ghiasi et al. [23] eliminated G2 phase arrest, accumulated P53, increased G1 phase arrest and significantly reduced the expression of pro-tumor vascular growth factor VEGF by inhibiting Wee1, thus weakening the proliferation ability of cancer cells, indicating the cancer-promoting effect of high expression of Wee1 in breast cancer cells.

3.5 Wee1 and Lung Cancer

Yoshida et al. [24] analyzed 79 patients by immunohistochemistry, including 16 recurrent cases, and found that there was almost no difference in the positive rate of Wee1 between tumor cells and normal cells. Moreover, the recurrence rate and mortality of patients with Non-Small Cell Carcinoma(NSCLC) with negative Wee1 expression were significantly higher than those with positive Wee1 expression.These results suggest that Wee1 expression may act as a protective mechanism against cancer in NSCLC. However, Ku et al. [25] proved that Wee1 protein kinase inhibitor was effective in the treatment of non-small cell lung cancer with KARs gene mutation in TP53 mutated cancer cells, which was similar to the effect of Wee1 inhibitor combined with mTOB inhibitor in the treatment of NSCLC with KARs gene mutation studied by Hai et al. [26]. Jhuraney et al. [27] found that Wee1 and PAXIP1 were commonly expressed in lung cancer,and had no relationship with the status of p53. When both were expressed at the same time, Wee1 inhibitor combined with Cisplatin was effective.Sen et al. [28] a used PCR method to study and found that Wee1 was significantly increased in small cell lung cancer cells compared with normal tissues and non-small cell lung cancer cell lines. Therefore, the mechanism and expression of Wee1 may be different in different types of lung cancer.

3.6 Wee1 and Lymphoma

Lymphoma is a malignant tumor originated from lymphohematopoietic system, which is a systemic disease. At present, the main treatment is chemotherapy, but lymphoma is heterogeneous, and the therapeutic effect is different greatly among different patients. Chemotherapy drugs such as cytarabine can cause DNA damage in B-cell lymphoma. The results showed that [29] in vivo and in vitro, Wee1 inhibitor combined with chemotherapy drugs was only effective in the treatment of B-cell lymphoma with G2 phase arrest. Diffuse large B-cell lymphoma (DLBCL) accounts for about 31% of all non Hodgkin's lymphoma. Although R-CHOP Regimen is more effective, there are still a lot of relapses or deaths.Studies have shown that Wee1 is more significantly expressed in DLBCL [30]. Wee1 inhibitors combined with CDK1 inhibitors may improve the prognosis of patients with DLBCL [31], and Wee1 may become a target for the treatment of Diffuse Large B-cell Lymphoma. De Jong et al. [31] first proposed and verified that Wee1 inhibitor can enhance the anti-apoptotic dependence of DLBCL, and the combination of Wee1 inhibitor and anti-apoptotic inhibitor has better efficacy. Chila et al. [33] demonstrated that CDK1 inhibitors and Wee1 inhibitors were more effective in Mantle Cell Lymphoma (MCL) than solid tumors and other lymphomas, but the high toxic side effects of dual-targeted agents remain to be addressed.

4. Wee1 Protein Kinase Inhibitors

Among tumor therapy drugs, targeted therapy drugs have been widely used in clinic. In recent years, Wee1 protein kinase has attracted more and more attention in tumor cells with G1/S checkpoint deletion, and Wee1 protein kinase plays a key role in G2/M phase, making Wee1 protein kinase become a potential target for clinical treatment of tumors. Wee1 protein kinase inhibitor

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AZD1775, also known as MK1775, is an effective selective inhibitor of Wee1. AZD1775 can inhibit the activity of CDK1 by phosphorylating the Try15 residue of Wee1 protein kinase, so that DNA damage repair can not be carried out smoothly, and cells can not produce substances entering M phase in G2 phase, which leads to apoptosis. Studies have proved that AZD1775 alone is effective in the treatment of tumors. Currently, the treatment methods for patients with ovarian cancer are not perfect. Zhang et al. [34] verified through animal experiments that Wee1 inhibitor MK1775 as a single preparation has an inhibitory effect on tumor cells of ovarian cancer, and Wee1 may become a potential target of ovarian cancer. Bi et al. [35] determined that the expression of Wee1 was increased in esophageal squamous cell carcinoma cells, and thus verified that AZD1775 alone could inhibit the proliferation and metastasis of cancer cells and induce their apoptosis. Jin et al. [36] found that Wee1 inhibitor AZD1775 can block mitosis in S phase in pancreatic cancer, and make the cells in this phase be inhibited and apoptosis, so as to achieve the effect of treating pancreatic cancer.

In recent years, Acute Lymphoblastic Leukemia (ALL) treatment drugs have made patients get very effective treatment effect, but tumor recurrence has become a problem perplexing patients and doctors. It has been proved that [37] AZD1775 combined with CHK1/CHK2 inhibitor can act in S phase to make DNA damage and achieve therapeutic effect on patients with ALL. Junchenghu et al. [38] found that T-ALL was more dependent on G2/M phase in DNA damage due to the lack of G1/S phase, making Wee1 a key therapeutic target. The experimental results showed that Wee1 was closely related to the glycolysis of cells, which verified that Wee1 inhibitor AZD1775 combined with GLS1 inhibitor CB-839 and BPTES had better efficacy in the treatment of T-ALL than using the two drugs alone. According to Cody W. Lewis et al. [39], the presence of Myt1 in the cell cycle can phosphorylate the Thy14 site of CDK1, and also block the mitosis of cells, which can enhance the drug resistance of some tumors such as breast cancer to AZD1775. Therefore, the combined application of Wee1 inhibitor and Myt1 inhibitor in the treatment of some tumors may reduce the resistance of tumor cells to Wee1 inhibitor.

Other studies have demonstrated that Wee1 inhibitors will appear drug toxicity and drug resistance when used alone or in combination with other drugs to treat tumors, while Wee1 inhibitors will have better efficacy and fewer side effects when used continuously with other anti-tumor drugs. Yongfang et al. [40] showed that nausea, weight loss and other symptoms could occur when Wee1 inhibitors were combined with PARP inhibitors, while continuous use of the two drugs alone could not only kill tumor cells, but also improve toxicity in normal cells. There have also been studies [41] showing that sequential therapy with gemcitabine followed by Wee1 inhibitor can increase the number of tumor cell apoptosis more than alternating sequential therapy with gemcitabine followed by Wee1 inhibitor or combination therapy of gemcitabine and Wee1 inhibitor.

5. Summary

In this review, the role of Wee1 protein kinase in the cell cycle and the relationship between Wee1 protein kinase and tumor were summarized, and the development of Wee1 protein kinase inhibitors in tumor therapy was summarized. Wee1 protein kinase inhibitors are potentially targeted tumor therapy drugs. Currently, there are many researches on Wee1 inhibitors, but how to use Wee1 inhibitors to minimize the toxic and side effects of the treatment regimen is still controversial: Some scholars believe that single use is more effective than combined use; some scholars believe that combined use can reduce the side effects caused by Wee1 inhibitors, and combined use can maximize the efficacy; while others believe that neither single use nor combined use can bring the maximum benefit to patients as sequential therapy. As Wee1 protein kinase inhibitors are still in phase II clinical trials [42], how to keep the efficacy of the drug itself, and at the same time control the balance between Wee1 protein kinase inhibitors and other drugs or treatments to bring the greatest benefits to patients is the current research direction.

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CASE REPORT

Rental Mucinous Tubular and Spindle Cell Carcinoma: Case Report

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ABSTRACT

Mucinous tubular and spindle cell carcinoma (MTSCC) of the kidney is an uncommon recently recognized renal cell carcinoma. We reported A 60 year's old man who presented with right flank pain, abdominal swelling and one attack of hematuria. The intraoperative finding was a huge cystic swelling arising from the right kidney occupying almost all the abdominal cavity displacing the bowel to the left side of the abdomen. There was no ascites or evidences of metastasis. Right radical nephrectomy was done. Then the diagnosis of renal MTSCC was established. General condition of the patient was improved and one year prognosis was satisfactory. To our knowledge this is the first reported case of MTSCC in Sudan, and the outcome of treatment was satisfactory.

1. Introduction

MTSCC of the kidney is a rare subtype of renal cell carcinoma (RCC) [1]. It has a female predominance and the mean age of patients is 53 years [2]. The majority of MTSCCs are of low malignant potential, so that it is rarely recur or spread distally [3-6], but exceptionally cases of high grade and/or sarcomatoid features may present with regional nodal or distant metastasis [4-6]. Histologically, this tumour is characterized by a mixture of tubular and spindle cells separated by variable amounts of mucinous stroma. The MTSC has round nuclei and evenly dispersed chromatin. These low-grade nuclear features are the same in both the tubular and the spindle cell elements [6, 7]. Mucin-poor variant of MTSC has little or no extracellular mucin in the stroma [8].

2. Case Report

A 60-year-old man who presented with right flank pain, abdominal swelling and one attack of hematuria. Abdominal CT revealed a grossly hydronephrotic right kidney with septations and peripheral calcification (Figure 1). Radical nephrectomy was done. The intraoperative finding was a huge cystic swelling arising from the right kidney occupying almost all the abdominal cavity (Figure 2), displacing the bowel to the left side of the abdomen. The renal pedicle was approached by mobilization of the ascending colon medially. No ascites or evidences of

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other structures involvement, then radical nephrectomy was done without significant blood loss or intraoperative complication. Postoperative course was unremarkable. Histopathology showed cystic mass; sections showed mucin with columns of poorly formed tubules and clumps of tumour cells; in other areas the tumour was sarcomatous with spindle cells. The diagnosis of renal MTSCC was made. The patient received adjuvant chemotherapy. General condition of the patient was improved on serial follow up, with no evidence of local recurrence or distal metastasis on CT chest and abdomen done six months and one year postoperatively.

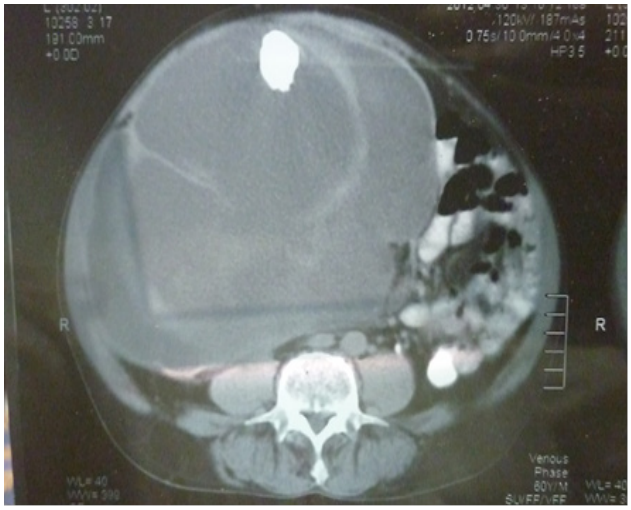

Figure 1. Abdominal CT scan showing grossly hydronephrotic right kidney.

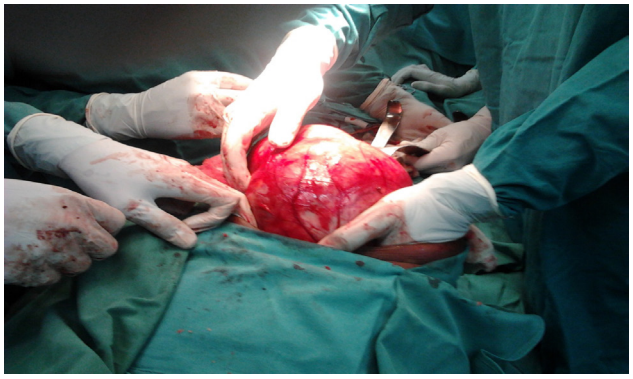

Figure 2. Intraoperative appearance of the tumour.

3. Discussion

MTSCC of the kidney is an uncommon, recently described variant of renal cancers as the first case was reported in 1998, since that time many cases were reported, in 2004 WHO tumour classification recognized it as a distinct entity of renal tumours [10]. The patient presented in this report was a male, although MTSCC is predominant in females. It is described in the literature as a low grade relatively indolent tumour that carry a good prognosis as it is commonly graded as a good differentiation tumour [2, 10, 11]. However, some cases with sarcomatoid features may have more aggressive progression and poor outcome, so adequate sampling of the tumour is required in order not to miss this finding because it may substantially affect the clinical course of the disease [6,5]. MTSCC is mainly diagnosed on histological and morphological grounds, and immunohistochemical studies show mostly specifically positive for RCC marker antigen, vimentin, CK7, and AMACR. [6,5,12].

The clinical presentation and clinical course of our patient was similar to what was mentioned in the literature and reported by many authors [1]. The tumour was generally confined (T1 or T2) in more than 80% of cases [3,13]. The proliferation rate was low, suggesting a low proliferation activity, a finding that may in part explain the low malignancy of this tumour type. The coexistence with other renal abnormalities as a simple renal cyst, a synchronous RCC, a papillary adenoma [13] or angiomyolipoma was reported [8]. Also associated histological findings that may affect the clinical course of the disease were reported, as Jung et al report a case of renal MTSCC with focal neuroendocrine differentiation [9], Simon et al. [6] report a case of MTSCC with extensive sarcomatoid differentiation, multiple metastases, and a rapidly fatal clinical course. Association with tuberculosis was also reported [14].

To our knowledge, this is the first reported case of MTSC in our center and Sudan as well. Management received was satisfactory, the outcome of treatment was satisfactory too and the experience we got from its management was advocated.

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