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Radiosensitization Mechanisms of Diphenoquinone on Supracellular Level *

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The diphenoquinone by its chemical structure, with two carbonyl grup, is unsaturated, conjugated, resonant, their electron being capable of moving through a large path of the molecule i. e. very good condition for a radiosensitizer.

We have prepared the DQ by KONING's processes and it was purified by recristalization in acetone.

The DQ is administrated to mice orally or intraperitoneally in gum arabic at 1 % or carboxymethyl cellulose suspension.

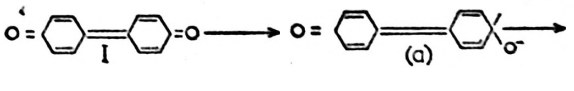
The toxicity is determined by DIXON and MOOD method and the mice irradiation with X-rays at a speed of 26.5 r/min. The potentiation factor as radiosensitizer is determined according to MITCHELL.

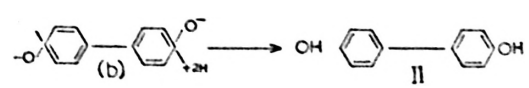
We have done a histopathological study of radiosensitized mice organs by DQ and we study also the action of cystein on this radiosensitizing effects.

These experiments demostrate clearly the radiosensitizing action of the DQ, and as the cystein abolished the effect of DQ, there would exist a mechanisms which acts by means of blockading the SH-groups. We can say that DQ acts in radiosensitization as a Sulfhydryl-Binding Agent.

In previous investigations (4, 10, 11) we have studied the diphenoquinone (DQ) as a radiosensitizing agent. In this one the main objective is to study some mechanisms for which the DQ acts, in addition we try to seek the best condition for the subsequent application of this compound as a radiosensitizer.

The DQ by its chemical structure, is an unsaturated and conjugated system, which can be in resonance, and by its quinonic character is a powerful oxidant (Redox potential $E_0 + 0.954$ volts.). It can produce free radicals of semiquinone type, that increase the electron-affinic properties associated with its chemical structure, such as:





Diphenoquinone Redox System

* Communication IV International Congress Radiation Research: Abstracts, p. 11, n.º 28. Evian (France), 1970.

Materials and Methods

As DQ does not exist as a market product, we prepared it by KONING'S process (7) and it was purified by recristalization in acetone.

The product is not soluble in water and it is administered to Swiss mice by two diferent methods: first orally in a suspension of 1 % of gum arabic in one single dose of 1 mg/g (mouse), one hour before the irradiation and the other at intervals of: 48, 24 and 1.5 hours before irradiation, 1 mg/g at each interval, with a total of 3 mg/g. The applied doses of X-rays are 370 r (LD_{50}) in the first case and 400 r (LD_{75}) in the second one.

With the result of these previous proofs, the potentiation factor (PF) is calculated according to MITCHELL (9),

$$PF = \frac{c}{1/2(a + b)}$$

in which c is the percentage of deaths when the two agents (radiation and product) act together and a and b the corresponding percentage when they act separately.

In these proofs we have clearly seen that the DQ is a good radiosensitizer, though it was observed that the gum arabic acts as a vehicle for radioprotection.

Other experiments were planned and we looked for another vehicle that would not have a protector effect and could also be administrated by the intraperitoneal way.

Experiments of toxicity are being made with carboxymethylcellulose (CMC) and the lethal dose fifty (LD_{50}) is determined by the DIXON and MOOD method (6). It is observed that DQ is a toxic agent when introduced by intraperitoneal method at a dose 0.4 mg/g, while orally at 3 mg/g is a non toxic agent.

To make a comparative study of absorption of the product by these two methods, two conditions are fixed: DQ, 0.2 mg/g in CMC; X-rays, 400 r.

The two agents, radiation and product are combined in the indicated conditions in two ways, orally and intraperitoneally.

Out of the studies we have made with variations the following factors of: 1) Radiation doses: 350 r and 400 r (X-rays); 2) Product doses: 0.2, 1 and 3 mg/g; 3) Administration methods, orally and intraperitoneally; 4) Administration vehicles, gum arabic and CMC.

HISTOPATHOLOGICAL STUDY OF RADIOSENSITIZED MICE ORGANS BY MEANS OF DIPHENOQUINONE.

We have studied the injuries in organs of animals that received jointly 0.2 mg/g DQ and 400 r X-rays and others that received these agents separately.

The organs studied were: lungs, liver, spleen, intestine and kidney. These organs were extracted on the: 1st, 3rd, 5th and 7th days after irradiation and were soaked in paraffin and stained with hematoxylin-eosin according to the VAN-GIESON technique (11).

THE ACTION OF CYSTEIN ON THE RADIOSENSITIZING EFFECT OF DIPHENOQUINONE.

Because of the oxidant character of the DQ, we think that one of its possible action mechanisms as a radiosensitizer could be the reaction with SH groups. To explain this possible mechanisms, we have performed the following experiments: *a*) Action of cystein on the toxicity of the DQ, administered by the intraperitoneal method. *b*) Action of cystein on the radiosensitizing effect of the DQ.

Results

The radiosensitizing action of the DQ in mice, according to what is indicated before is shown in Table I, in which we collect the results obtained in a population of mice (forty by group) expresed in

Table I. *Radiosensitization factors of DQ in mice.*

Vehicle *	X-rays r	DQ mg/g	Deaths %	P.F.**
—	370	—	50	
Gum arabic (P.O.)		—	40	0.8
Gum arabic (P.O.)		1	35	0.875
—	400	—	75	
Gum arabic (P.O.)		—	30	0.4
Gum arabic (P.O.)		3	65	2.16
—	370	—	50	
C.M.C. (P.O.)		—	50	1.00
C.M.C. (P.O.)		1	95	1.9
—	400	—	75	
C.M.C. (P.O.)		—	75	1.00
C.M.C. (P.O.)		0.2	80	1.06
—	370	—	50	
C.M.C. (I.P.)		—	50	1.00
C.M.C. (I.P.)		0.2	90	1.8
—	400	—	75	
C.M.C. (I.P.)		—	75	1.00
C.M.C. (I.P.)		0.2		

* Routes of Administration: (P.O.), oral; (I.P.), intraperitoneal.
** Potentiation factor.

deaths percentage according to the dates review in experimental part.

The potentiation factor, the larger found by us, correspond to 3 mg/g of product and 400 r, obtained according to MITCHELL (9), PF = 2.16, and this is reduced to PF = 0.875, when we apply a single dose of 1 mg/g inmediately before to radiation of 370 r, which would indicate that this product has a latence period to act as radiosensitizer.

To compare the effects of oral and intraperitoneal administration, we can examine the four experiments in Table I, in which a comparative study apears at doses of 370 r (LD_{50}) and 400 r (LD_{75}) applyed to mice of this test. It is found in case that the administration of a single dose of 1 mg/g by oral way, a PF = 1.9, and intraperitoneally give, a PF = 1.8, with 0.2 mg/g.

In both cases LD_{50} of X-rays was applied. Therefore the intraperitoneal way is more effective than the, although an exact study can not be performed because 1 mg/g by this way is lethal for the mice.

When the X-rays doses are increased up to 400 r, practically radiosensitization does not exist with doses of 0.2 mg/g, neither orally nor intraperitoneally.

The comparative results can be seen in the survival curves (figs. 1, 2, 3).

INCREASE OF TOXICITY PRODUCT WITH RADIATION.

In the first experiment DQ was administered orally in a suspension of 1 % of gum arabic in doses of 1 mg/g and 3 mg/g. No increase in deaths was observed during the first three days after irradiation, apply in the first case 370 r, LD_{50} and in the second 400 r LD_{75}. Ho-

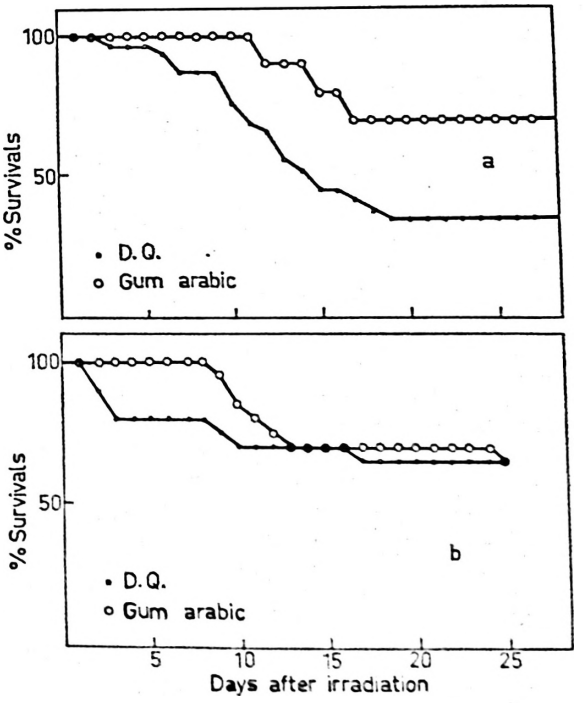


Fig. 1. *Effect of Diphenoquinone (DQ) in gum arabic (1 %) in mice irradiated.*
a) DQ: 3 mg g; X-rays: 400 r; b) DQ: 1 mg g; X-rays: 370 r.

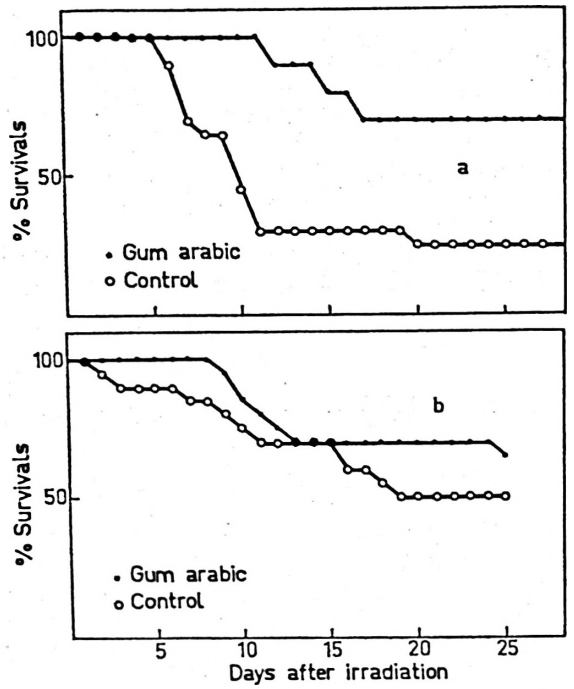


Fig. 2. *Effect of gum arabic in mice irradiated with X rays.*
a) 400 r and b) 370 r.

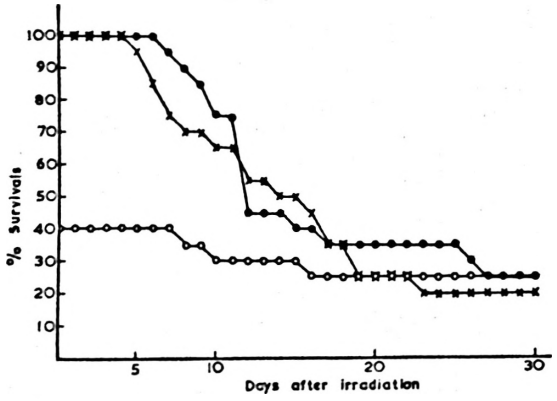


Fig. 3. *Effect of Diphenoquinone 0.2 mg/g in Carboxymethylcellulose in mice irradiated with 400 r.*
● controls; × orally way; ○ intraperitoneal way.

wever, the results were not the same when DQ was administered orally and intraperitoneally in CMC. When administered orally with doses of 1 mg/g and 370 r. under the same conditions as in one of the previous cases with gum arabic the increase in deaths was highly significant. When administered intraperitoneally in doses of 0.2 mg/g, the increase in deaths was even greater.

Thus, gum arabic reveals itself as a radioprotector and CMC does not have a significant action (Table II).

Table II. *Increase of the DQ toxicity with radiation.*

Vehicle *	mg/g DQ amount.	Radiation X rays dose in r	Percentage of deaths Days after irradiation		
			1st	2nd	3rd
Gum arabic (P.O.)	3	400	0	0	0
Gum arabic (P.O.)	1	370 (LD_{50})	0	0	0
C.M.C. (P.O.)	1	370	0	25	25
C.M.C. (P.O.)	0.2	400 (LD_{75})	0	0	0
C.M.C. (I.P.)	0.2	370	45	35	5
C.M.C. (I.P.)	0.2	400	25	35	0

* Routes of Administration: (P.O.), oral; (I.P.), Intraperitoneal.

HISTOPHATOLOGICAL STUDY OF RADIOSENSITIZED MICE ORGANS BY MEANS OF DIPHENIQUINONE.

We have found manifest injuries in: spleen and lung. In the spleen with observed congestive phenomena and polinucleated cells with more intensity and in greater number when the DQ and X-rays were applied together, that when, which DQ and X-rays were applied separately. In both cases most injuries increased up to the 3rd day after radiation.

After the 3rd day the splenic regeneration starts but it is smallest when the two agents act jointly and it is complete on the 7th day when the two agents act separately (Fig. 4).

In the lungs hemorrhagies appear for congestive crossing, but polinucleated cells do not appear. There is no regeneration of injuries as in the case of spleen and

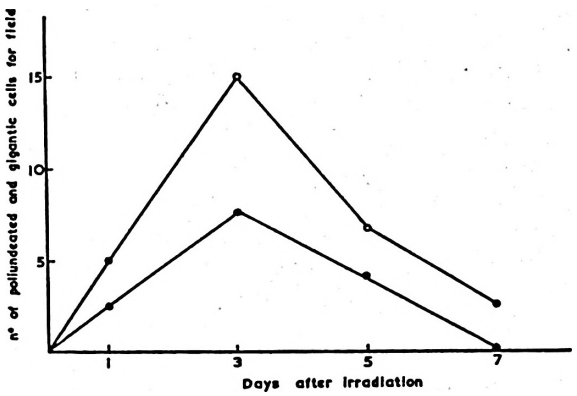


Fig. 4. *The histopathological study of spleen in radiosensitized mice by means of Diphenoquinone.*
● irradiated; O irradiated and with Diphenoquinone.

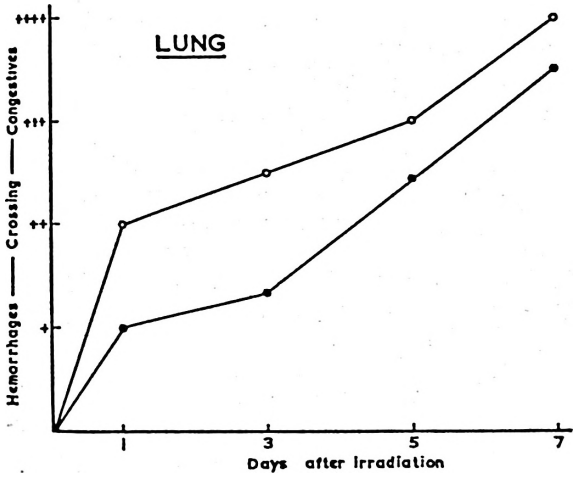


Fig. 5. *The histopathological study of lung In radiosensitized mice by means of Diphenoquinone.*
● irradiated; O irradiated and with Diphenoquinone.

these injuries increase up to the 7th day after irradiation and higher when DQ and X-rays act jointly than when both act separately (Fig. 5).

ACTION OF CYSTEIN ON THE RADIOSENSITIZING EFFECTS OF THE DIPHENOQUINONE.

Toxicity tests of DQ and cystein, administered jointly are being carried out mixing them before injection and as well injecting first the cystein and 30 minutes afterwards the DQ. The dose of DQ used has been 0.55 mg/g, LD_{100}, both injected together with cystein at 0.55 mg/g, as when injected with an interval of 30 minutes. The toxicity of the DQ was completelly nullified by cystein.

To prove the effect of cystein on the radiosensitizing action of DQ, experiments were made in which the animals are treated with 0.60 mg/g of cystein and 30 minutes afterwards, 0.2 mg/g of the DQ are administered and they are irradiated with X-rays at a dose of 400 r. With the results, we have drawn the survival curves that appear collected in Figure 6.

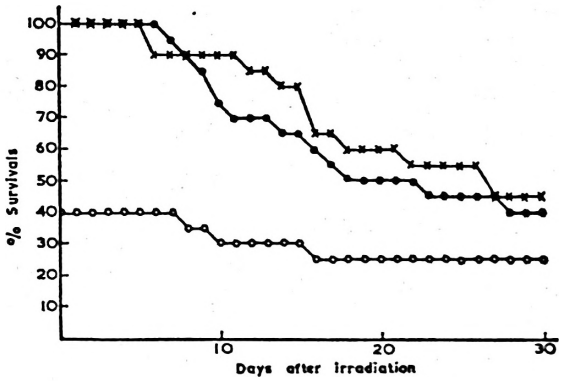


Fig. 6. *The action of Cysteine on the radiosensitizing effec of Diphenoquinone by intraperitoneal way.*
● Cysteine; × Diphenoquinone and Cysteine; O Diphenoquinone.

Discussion

Various hypotheses have been proposed in explanation to the radiosensitization mechanism, based in electron-affinic properties of different compounds, which can be interpreted in terms of redox phenomena. We believe that diphenoquinone is governed by this mechanisms.

By the published until now, ADAMS and DEWEY (2), ADAMS and COOK (1) on the radiosensitizing effects of N-Ethylmaleimide (NEM), we consider that the DQ acts

by a similar process. DQ is a conjugated system, with two carbonyl groups, that according to LOVELOK (8), this molecules type, can have a great radiosensitizing potential, coming from the resonant interaction between the two electron-acceptor groups. In attachment to molecules of this type, the electron can be associated with the orbital extending over the region of conjugation and linking the acceptor groups. This property of electron delocalization is responsible for quasi-stability of the radical-ions and the high electron affinity of the parent molecule.

BRIDGES (5) indicates that NEM itself is too toxic for use with mammals, but other sensitizer agents (quinones and hidroquinones) may sensitize preferentially under anoxia by a similar mechanism to (NEM) N-Ethylmaleimide.

ADAMS and MICHAEL (3) have found that quinones give very long-lived negative radical-ion when irradiated. It they could be introduced into the cell, they might well be efficient sensitizers.

All these considerations have stimulated us to follow this study with DQ as radiosensitizer, either in mammals and in microorganisms.

According to the results obtained up to the present we can take the following conclusions:

That DQ acts as a radiosensitizer due to the electron-affinic properties associated with its chemical structure.

That DQ is toxic administered in small doses intraperitoneally 0.4 mg/g (LD_{50}), while doses 3 mg/g administered orally are not toxic.

There is an increase in the DQ toxicity with irradiation, a high percentage of deaths was observed during the first 48 hours following irradiation.

That increase depends on: *a*) The amount of product administered; *b*) The method of administration; *c*) The vehicle with which the compound is supplied.

According to the results, we assume that the best conditions as well as the more effective ones for the DQ as a radiosensitizer, are by oral administration and the highest doses of radiation and product applied by a suspension of 1 % of gum arabic.

From the histopsthological study of radiosensitized mice organs by means of the DQ, we can say that the injures in the spleen and lungs demostrated clearly the radiosensitizing action of DQ.

That as cystein abolishes the toxic effect of the DQ, it has been shown that there is reaction with SH groups due to a Redox process.

That as cystein abolished the radiosensitizing effect of DQ there would possibly exist a mechanisms which act by means of blockades of the SH groups existing in the organism and which perform the role of radioprotectors.

Resumen

La diphenoquinona por su estructura química, con dos grupos carbonilos, es insaturada, conjugada y resonante, sus electrones son capaces de moverse a través de un gran camino en la molécula, condiciones muy buenas para un radiosensibilizante de esta clase.

Se ha sintetizado por el procedimiento de Koning y se administra a ratones por vía oral e intraperitoneal, en suspensiones de goma arábiga al 1 % y en CMC. Su toxicidad se determina por el método de Dixon y Mood.

La irradiación se realiza con un equipo de rayos X de alta terapia. El factor de potenciación, como radiosensibilizante, se determina según MITCHELL, obteniéndose, en algunos casos, un PF = 2,16.

Se hace un estudio histopatológico de los órganos de animales irradiados y tratados con la DQ y se estudia, experimentalmente, la acción de la cisteína sobre los efectos radiosensibilizantes de la DQ. Estudios que demuestran algunos de los mecanismos por que actúa, especialmente por el bloqueo de los grupos SH, a los que se les asigna un papel radioprotector.

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