Procarbazine Radiolysis and Experiments In Vitro

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Abstract. Steady-state radiolysis of aqueous procarbazine (PC) was studied in air-free, aerated and solutions saturated with N2O. The corresponding G_i(·)-values obtained at pH=7.4 were: 2.85, 5.60 and 3.45, respectively. The investigations in vitro, using E.coli (AB 1157) as a model for living systems, demonstrated that PC acts as a cytostatic in air-free as well as in aerated media. However, it shows radiation protecting ability in the presence of N2O, where OH-radicals are the predominant reactants. Similar results were observed at pH=6.2. The experimental data contribute to a better understanding of the many-sided and frequently contradictory behavior of PC.

Procarbazine (PC, natulan, Ro-4-6467, N-isopropyl-a-(2-methyl-hydrazino)-p-toluamide) is frequently used as a cytostatic drug e.g. for lymphohgranulomatosis (Morbus Hodgkin). However, under certain conditions, it displays carcinogenic properties (1-6).

The present study investigates the relationship between PC-radiolysis under steady-state conditions and presents experiments of the in vitro action of PC using bacteria as a model of living systems.

Materials and Methods

A 60Co-½-source "Gammacell 220"(Nordion Intern. Inc., Canada) assembled with several lead - attenuators for desired reduction of the dose rate was used. A dose rate of 61 Gy/min was applied. The exact substrate radiolysis and product formation was registered by HPLC (Hewlett-Packard, model 1046/1050 in combination with several detectors and computer online). All solutions were prepared with triply-distilled water and analytical grade chemicals. In order to get a better picture of the most reactive radicals towards the substrate, three series of experiments were undertaken: saturated (A) with argon, (B) with N2O and (C) with air. The high purity procarbazine hydrochloride (PC) was provided by Hoffmann-La Roche Corp. (Basel, Switzerland).

The studies in vitro were carried out using Escherichia coli (AB 1157). The handling procedure of the bacteria as well as evaluation of the results have been previously reported (7). The bacteria were added to the buffer and, after a one-hour incubation, they were irradiated with γ-rays. In all other series of experiments the buffer contained a known PC-concentration as indicated in each series at pH=7.4 or 6.2, respectively. The media were saturated with argon, N2O or air and subsequently irradiated with γ-rays at various doses (Gy). The observed survival curves represent the N/N0 - ratio (N0=number of bacteria colonies before and N=after irradiation treatment) as a function of the absorbed radiation dose. The D37 - values, which represent the N/N0 - ratio at given dose (Gy), were taken from the corresponding curves, whereas the AD37 - value (Gy) was calculated by subtracting the D37 - buffer value from the individual D37 - data, e.g. D37 (sample) – D37 (buffer)=AD37(sample). The positive AD37 -values indicate the radiation protecting ability of a given system, whereas the negative values show the corresponding cytostatic efficiency of the media. In all cases the presented data are mean values of 3 to 5 series of experiments. The range of the applied dose was rather high in order to get a better general picture of the radiation-induced processes in the system.

Results and Discussion

Procarbazine radiolysis. As the water content in living systems is more than 70%, the main reactions of water- radiolysis and the G-values* of the primary radiolytic products at pH-range 6 to 8.5 are given in eq. (1):

\[
H_2O \rightarrow e_{aq}^+ + H + OH + H_2O_2
\]

(1)

\[
G_i=(2.7) (0.6) (2.8) (0.45) (0.7)
\]

In the presence of air, H and e_{aq}^+ are converted into peroxyl radicals, whereas in solutions saturated with N2O, the e_{aq}^+ are transferred into OH radicals:

\[
H + O_2 \rightarrow HO_2^* \quad k=2 \times 10^{10} \text{ dm}^3 \text{mol}^{-1} \text{s}^{-1}
\]

(2)

\[
e_{aq}^+ + O_2 \rightarrow O_2^* \quad k=1.9 \times 10^{10} \text{ dm}^3 \text{mol}^{-1} \text{s}^{-1}
\]

(3)

\[
HO_2^* + H^+ + O_2 \quad pK=4.8
\]

(4)

\[
e_{aq}^+ + N_2O \rightarrow \quad \text{OH} + \text{HO}^+ + N_2 \quad k=0.91 \times 10^{10} \text{ dm}^3 \text{mol}^{-1} \text{s}^{-1}
\]

(5)

* G-value = number of species formed or decomposed per 100 eV absorbed energy. G_i=initial G-value: before the start of secondary processes. 1 Gy (Gray)=100 rad=6.24 x 10^{15} eV / g sample

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The decomposition of PC was investigated in media saturated with argon (46% OH, 44% eaq-, 10% H), N2O (90% OH, 10% H) as well as with air (46% OH, 54% O2-) at various absorbed radiation doses. In order to get an overall picture of the specific involvement of the reducing (eaq-, H) and oxidizing radicals (OH, O2-) in the process, relatively high doses of radiation were applied. The course of the PC-radiolysis under these conditions is presented in Figure 1.

Obviously the most efficient degradation of PC is observed by the action of OH-radicals in media saturated with N2O, resulting in GI(-PC)=5.6. (Figure 1, curve B). This is followed by the results obtained in aerated media, GI(-PC)=3.45 (Figure 1, curve C) and finally GI(-PC)=2.85 in air-free solution.

It is interesting to note that the pH-value of the irradiated solutions changes with the absorbed radiation dose, depending on the experimental conditions. This is illustrated for each series of experiments, shown as an insert in Figure 1. In air-free media the pH increases with the absorbed dose. This is a consequence of the preferential eaq- attack on the -CO group (formation of R1 - CO' -R2 radical anion; see 8) as well as on the -NH groups resulting finally in the formation of amines and ammonia (9,10). The R1 - CO' -R2 radical may undergo hydrolysis and cleavage of the chain forming stable products, representing smaller molecules.

The OH-radicals preferably attack the aromatic ring of PC by splitting up the double bonds, forming OH-adducts at various positions and resulting in hydroxylation of the molecule. Hence, the pH of the media in this case is only slightly changed. This process is a matter of theoretical consideration and will be published separately (11). However, in aerated solutions the oxygen adds to the OH-adducts and the resulting peroxyl radicals may undergo hydrolysis, leading to the formation of aldehydes and carboxylic acids, causing a strong pH change. The HPLC-analysis showed the formation of a mixture of various products which have not yet been identified.

Radiation biological studies

Toxicity tests. The toxicity of procarbazine (PC) at concentrations ranging from 5x10^-6 to 10^-3 mol/dm^3 was studied at pH 7.4 and 6.2, using Escherichia coli (AB1157) as a model in air-free (A) and aerated media (B). The obtained data are presented in Figure 2. In all series of experiments with PC doses up to about 10^-4 mol/dm^3, the toxicity was relatively low. However, above this concentration toxicity rapidly increased (decrease of the N/N0-ratio as a function of concentration). This was more pronounced in the presence of air in both pH-ranges.

Experiments in vitro at pH=7.4. As mentioned above, 3 series of experiments in vitro at pH=7.4 were performed: in air-free, aerated and medium saturated with N2O (conversion of eaq- into OH). Figure 3 shows the survival curves: N/N0-ratio of the bacteria as a function of the absorbed radiation dose in air-free medium in buffer (A), in the presence of 10^-4 mol/dm^3 PC (B) and 10^-3 mol/dm^3 PC (C). The calculated ΔD37-values of the corresponding curves are presented as an insert in Figure 3. Obviously, at higher substrate concentration the cytostatic effect of PC is also higher, namely: it increased from ΔD37= -30 at 10^-4 mol/dm^3 PC (curve B) to ΔD37= -65 at 10^-3 mol/dm^3 PC (curve C, Figure 3).
A very similar cytostatic effect of PC was also observed in aerated solution under otherwise identical conditions as illustrated in Figure 4. In this case, however, using $10^{-5}$ mol/dm$^3$ PC, a lower value was found, $\Delta D_{37}=-50$ compared to that in Figure 3.

Finally, in media saturated with $N_2O$, the $\Delta D_{37}$-values were found to be positive, indicating that PC acts as a radiation protecting agent under these conditions (Figure 5). This effect was stronger at lower PC concentration (see insert in Figure 5).

Comparing the $\Delta D_{37}$-values presented in Figures 3 to 5, obtained in different environments it can be stated that:

a) In air-free solution the $\Delta D_{37}$-values demonstrate the cytostatic effect of PC. In this case oxidizing (46% OH) and reducing radicals (44% $e_{aq}^-$ and 10% H) are involved in the process, initiating a rather complicated reaction mechanism. As mentioned before, the pH of the media is increased linearly with the absorbed radiation dose. This is in unison with the formation of ammonia and amines, resulting as a consequence of the $e_{aq}^-$ and H attack on the -NH groups of the PC molecule. On the other hand the mentioned R$_1$ - CO.$^-$ -R$_2$ transients might also be involved in a reaction with DNA in addition to aldehyde formation and so contribute to the observed effect.

As observed by HPLC analysis, the PC-transients produced by OH attack lead to several final products. However, these transients (mainly OH-adducts of PC) seem not to be very much involved in reactions with DNA (compare $\Delta D_{37}$-values in Figure 5).

b) In solutions saturated with air, where 46% OH and 54% $O_2^-$ radicals are present, similar $\Delta D_{37}$-values were obtained. The produced OH-adducts at various positions on the PC molecule can add oxygen, resulting in PC-peroxyl radicals, which might be involved in reactions with DNA or/and undergo
hydrolysis, forming aldehydes and carboxylic acids. As a result of these processes, pH decrease is observed (insert Figure 1). All these radiolytic products are subject to further radiolysis by prolonged irradiation. The resulting secondary radicals can probably attack DNA.

On the other hand, the simultaneously produced \( \text{O}_2^- \) species are slow reacting. However, they can be involved in reactions with DNA by preferably attacking double bonds or / and by electron transfer process on -NH- and -CO groups. In addition to this, \( \text{O}_2^- \) peroxy radicals are also consumed by reactions with products in the bulk of the solution.

c) In the presence of \( \text{N}_2\text{O} \) (90% OH, 10% H) PC apparently acts as an efficient radiation protecting compound. The pH slowly changes at higher radiation doses (Figure 1, insert). As already mentioned the OH - radicals form various types of PC-transients. By means of the pulse radiolysis method, the total rate constant \( k \) (OH + PC) was found to be \( 3.7 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) (11). This rather high \( k \)-value indicates the many-sided induced processes.

Experiments in vitro at pH=6.2. The environment in cancer tissues is slightly acidic (pH=6.2-6.5) in comparison to normal tissues, which have pH=7.4. Hence, a series of comparative experiments were carried out at pH=6.2 under otherwise similar conditions. From the survival curves of buffer and of \( 10^{-3} \text{ mol/dm}^3 \) PC, using the same type of \( \text{Escherichia coli} \) (AB 1157) at pH=6.2 for an absorbed dose up to 650 Gy in media saturated with various gases (argon, air, \( \text{N}_2\text{O} \)), the \( \Delta D_{37} \)-values were obtained. The data are shown in Table I.

The obtained \( \Delta D_{37} \)-values demonstrate that similar effect is observed at pH=6.2 for air-free, aerated and media saturated with \( \text{N}_2\text{O} \), but the values are somewhat lower as reported above for pH=7.4.

## Table I. \( \Delta D_{37} \)-values derived from the corresponding survival curves (\( \text{E. coli, AB1157} \)) expressing the \( N/N_0 \) ratio at pH=6.2 as a function of absorbed radiation dose.

<table>
<thead>
<tr>
<th>System</th>
<th>( \Delta D_{37} )-values in media saturated with:</th>
<th>Argon</th>
<th>Air</th>
<th>( \text{N}_2\text{O} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>( \Delta D_{37} )-values</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>( 10^{-3} \text{ mol/dm}^3 ) PC</td>
<td>-50</td>
<td>-40</td>
<td>+15</td>
<td></td>
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</table>

## References


## Conclusion

Based on the results obtained by steady-state radiolysis and by the experiments in vitro under the influence of \( \gamma \)-rays, it can be postulated that the following factors are responsible for the action of PC as a cytostatic:

i. Availability of a certain type of primary free radicals, e.g. \( \text{e}_{\text{aq}}, \text{H}, \text{OH}, \text{O}_2^- \) etc., as determining factors for the reactivity and formation of secondary transients resulting from PC. This also depends on the dose used.

ii. The oxygen content in the system.

iii. The concentration of PC under certain conditions.

iv. The pH in the medium which can affect the radiation-induced processes.

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