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A Biokinetic Model to Describe Consequences of Inhibition/Stimulation in DNA-Proofreading and -Repair, Part 2. Calibration of the Model

Heinz Haschke*

Scientific Board of *CONSTANTIA*-Group, Opernring 19, A-1010 Vienna, Austria

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Summary. A *biokinetic model* has been developed to describe the mathematical consequences of inhibition, respectively stimulation of proofreading. According to data reported in the literature, a first approximative calibration of the model has been carried out in an attempt to make it both: practically applicable and comparable with experimental data and clinical facts. The model is open for further improvements and adjustable according to results of further researches via the parameters chosen. In a first test of the model it is shown that it does well reflect the results described in the literature upon proof-reading-inhibition and its consequences, *i.e.*, the reduction of replication-fidelity (→ exponential increase of malignant cells with time). As a further result it is shown that the model also does well describe in its kinetic approach opposite effects as, *e.g.*, a reduction of wrong genetic information by classical cancer-therapies like chemotherapy and surgergy.

The system is orientated towards known *biochemical relations* and *chemical similarities* together with a discussion of the potential chance which offer special combinations of chemically identifyable substances (like nucleotides' precursors or effector-molecules contained in low-molecular-human-placenta-extracts as an alternative to umbilical cords'-blood/cells) as stimulators of the enzymatic proof-reading- and -repair-machinery.

Keywords. Aminoimdazolecarboxyamide (AICA); Biokinetic model; Carboxamidoimidazoles (CAIs); Nucleotides' precursors; Proofreading-stimulation; Cancer-therapies.

Introduction and Theory

Zhang and *Mathews* [1], experimenting *in vitro*, detected just recently[1] a significantly lower level of mutant-fractions, if DNA-replication occurred in the presence

* E-mail: Haschke.H@isovolta.com

[1] The first manuscript of the paper presented here with the biochemical model and with the biokinetic program was written during May/June 1995 and was given to attorney's deposit on June 22, 1995; unpublished because of obligations with respect to employer's orders for observing patent-interests

of "*symmetric nucleotide-pools*", *i.e.*, in the presence of equimolar concentrations of all of the four *main-nucleotides* (*i.e.*, at *equimolarity* of the dNTPs).

By further *in vivo* experiments with bacterial or mammalien cells, the authors found that replication was much less accurate if it occurred in the presence of strongly asymmetric nucleotide-pools rather than (the only slightly asymmetric) *biologically* biased dNTP pools. Furthermore, these authors detected an increase in the level of mutant-fractions up to several orders of magnitude if DNA-replication occurred while proofreading was inhibited and they concluded that "maintenance of high (rem.: *replication*-)-*fidelity* is highly dependent upon proofreading of insertion errors that do occur".

Nomenclature used: dNTP is used in the following as an abbreviation for deoxyribose-based nucleoside-triphosphates of the type: ATP, GTP, TTP or CTP; these nucleotides are also named: "*main-nucleotides*". In contrast to them other structural analogous chemical compounds which may replace one of such a main-nucleotide in a DNA- or in a RNA-chain are called: "*pseudo-nucleotides*".

As to the efficacy of the proofreading- and repair-machinery, *Kornberg* [2], demonstrated that *purine*- ("*PU*-") and *pyrimidine*- ("*PY*-")-nucleotides are requested by these cellular mechanisms in *equimolar ratio* and that from molecular biology it has become a well established knowledge that the activity of a typical enzyme in the proofreading- and repair-machinery, (*i.e.*, DNA polymerase-I) is stimulated when all the four main-nucleotides are available for it [3]. This effect has been thus described by *Kornberg* [2]: "*without DNA, there is no template for hydrogen bonding, and without all four triphosphates, synthesis stops early and abruptly for lack of a hydrogen bonding mate for one of the bases in the template*".

These more qualitative findings of *Kornberg*, have been supported by a huge volume of results upon enzymatic activities, enzymatic systems – especially upon polymerases – which are described in later biochemical scientific papers and even by results of carefully executed kinetic measurements and data upon the speeds of such enzymatic activities [4–7].

However, while (a) these kinetic data – again especially those concerning the polymerases – do give a good insight into the kinetic speeds (*c.f.* Table 3) and even to some degree into the mechanisms how such enzymatic systems do work, and while (b) the findings of *Zhang* and *Mathews* [1] give an indication of what could happen if the *nucleotide-pools* were *asymmetric* or the activity of an enzymatic DNA-repair-system were reduced till *inhibited*, plus (c) the exciting data of Japanese researches upon successful therapies with nucleodites-pool-proliferative substances like nucleotides'-precursors as there is AICA (Amino-Imidazole-Carboxy-Amide or: 5-Amino-4-Carboxamido-Imidazole, see Refs. [8–15]), they (a–c) were neither interpreted together nor discussed in the light of kinetics. Moreover, just recently reported [16–19] (d) successes in cancer-therapy with chemically similar structurized (but "cytostatic adjusted") substances used as *angiogenesis-suppressors* and (e) in similarity but also in contrast to the attack by cytostatica, the results in cancer-therapy by repair-/immuno-system stimulation reported by *Ackermann* [20–24] have not led till now to an attempt for a unifying discussion of all these encouraging results (a–e). Such a kinetic approach including the activity of the repair-machineries, especially focused on what would happen, if also the opposite of the findings of Ref. [1] upon repair-system-inhibition – *i.e.* a *repair-system-stimulation* – were possible and including the dramatic consequences which arise therefrom by shifting

the *repair/replication-ratio* (the *R/R-ratio*: see Ref. [33]) to higher repair-rates, seems highly interesting.

This approach is supported by extremely valuable results of kinetic researches as mentioned above together with the updated findings upon molecular mechanisms for DNA-repair (*Sancar* and *Sancar* upon photolysases [25], *Meselson* [7] upon *excision-repair*-mechanisms, *Wagner* and *Meselson* [26] upon *postreplication repair*, *Modrich* [27] upon DNA-mismatch-correction, *Hanawalt et al.* [4] upon DNA-repair in bacteria and mammalian cells, *Hare* and *Taylor* [28] and *Lindahl* [5, 6] upon DNA-repair-enzymes and upon the instability of DNA primary structure by mismatched regions, up to *Cheson* [29] with new prospects in the treatment of indolent lymphomas with purine-analogues).

All these facts fit to a similar large volume of results reported in biochemic and medicinal literature upon cancer-influencing and "soft" cancer-therapies: *Ackermann* ([20–24], HPE-therapy) *via Bursch* ([30]: apoptosis-therapy]), the results reported at the Japanese AICA-conference [8–15] to just recent results like those reported by *Rubinstein* [31], but also by *Vasudevan et al.* [32].

Consequently, a biokinetic model was developed by *Haschke* [33] to explore (at least hypothetically), if these findings could be described kinetically and further, especially what were the consequences if also an opposite effect to *Zhang* and *Mathews*' findings [1] upon a proofreading- and repair-inhibition occurred; *i.e.*, for a proofreading- and repair stimulation.

It is a fact that lesions of DNA-molecules very often do occur: A typical example for the damage to a DNA-chain ("*DNA-lesion*") by external factors is the well-known generation of pyrimidine-dimers by UV-light leading to dim-cytosine- or dim-thymine-links which covalently bind together two DNA-single-strands which may belong to two different DNA-double-helices (*Schrödinger* [34]; *Lindahl* [6]). By this, any further replication past this lesion is immediately stopped ("*stop-effect*").

It is a further fact that such lesions are identified and repaired continuously in a living organism by a suitable *enzymatic* "*proofreading- and repair-machinery*" [35–38].

One of the best studied enzymes of the repair-machinery is *DNA-polymerase-I*, which exemplifies many key-principles of the proofreading and repair-systems, applying to both, procaryotic and eucaryotic systems. *Kornberg* [2] has also shown that mutants are easily avoided by its activity, provided the activity of this enzyme (complex) is not stalled by the lack of one of the main nucleotides.

However, it is also a fact that the chances for a successful repair in reestablishing the original, correct genetic information ("*ocGInfo*") will decrease if a cell has undergone mitosis and by this replicates of the DNA-strand bearing the lesion which has been formed or even propagated via daughter-cells. The reason for this is that in the case of a lesion of the type of a "*complementarity mismatch*" ["*C-mismatch*", *i.e.* the insertion of a "wrong" main-nucleotide (*i.e.* not the right complement opposite to a nucleotide; such mismatches were for example the insertion of a T opposite to a G, or an A opposite to a C) in a DNA-strand]. An enzymatic repair-system will not have any more a chance for reestablishing the ocGInfo as soon as the information is lost, which one is the correct DNA-strand and which one is complementary with the lesion. In another case (which is much more likely for attacks of DNA-molecules by cancerogens), *i.e.* in the case of a "*substantial mismatch*" ["*S-mismatch*", *i.e.* the conversion of a nucleotide in a DNA-strand to a *pseudo-nucleotide* (for example by hydroxylating deamination of cytosine to uracil leading to an UMP instead of a CMP in the DNA-chain)], an enzymatic proofreading and repair-machinery will always be able to identify the pseudo-nucleotide as a "wrong" chemical substance in a DNA-chain. By this, the repair chances for lesions based upon S-mismatches are better (see [33]: conclusion #6).

By the same reasons, the chances for reestablishing the ocGInfo do strongly increase if the Repair/Replication-ratio (the "*R/R-ratio*") is increased, *i.e.*, if the ratio of the number of repair-activities per time-unit to the number of replications

occurring per time-unit is higher; see Haschke [33] about the critical influence of the $k_{cG} \cdot \xi \cdot ACG/k_H \cdot ADPoI$-quotient. [$k_{cG} \cdot \xi$ indicates the suceptibility of cells to the attack of a cancerogen of the activity ACG (cancerogenity) and their kinetic influence, while $k_H \cdot ADPoI$ does indicate the kinetic efficacy of an enzymatic repair-system].

As mentioned above, it is a fact that lesions of the DNA-molecule may cause the development of a cancer, even if this is a multi-step-process:

1. DNA-lesion. (Attack of nucleotides by cancerogenic chemicals, by energy-rich radiation, radioactive or UV), and as a consequence conversion of the nucleotides to pseudo-nucleotides, further by changing to longer sequences in the ocGInfo by viral integration, or just only by forming a C-mismatch during cell-replication). → 2. By this, creation of a wrong genetic information if this is not repaired in time due to a too poor ratio of repair-events to replications per time unit (R/R-ratio: see above and [33]), and → 3. While its replication is neither stopped ("stop-effect"; see [33]) nor it is leading to mutants with "insignificant or even benefitial behaviour", nor leading to an early death or apoptosis of the mutated cells: → 4. Propagating wrong genetic information transferred via m-RNA into wrong complements and via t-RNA into wrong codons. → 5. Expriming "wrong proteins" and "wrong enzymatic activities" and starting with fail-functions of the malignant cells. → 6. Overswitching the standard-regulation-systems for cell-growth and -division and cell-integration into the organisms cell-"community" as it was controlled by the ocGInfo (*i.e.* totally or partially loosing the function for which these cells have been specialized during their "evolution" from stem-cells or by replication of already specialized cells). → 7. Producing and emitting cell-signal-transduction substances and creating wild cell-propagation proliferative conditions and invasion of the organism by malignant genetic information respective malignant cells and formation of locally manifested aggregations of such malignant cells (= a tumor). → 8. Induced by the signal-transductant-substances creation of additional energy-supplys for the tumor (angiogenese). → 9. Breaking away of some malignant cells from the tumor and spreading them over the organism by the blood- and/or lymphs-streams → metastases].

However, if repaired in time, *i.e.* the (*0*) *"optimum-case"* (see above: R/R-ratio and its central role), such a dangerous development can definitely be avoided.

It is obvious that the chances for a successful repair diminish with the length of the damaged sequence, for which reason a cancerous development introduced by DNA-lesions by viral integration will be much more persistent. However, if this first chance of a repair is missed, there are still further biological protective mechanisms: (I) on a cellular level: (*I.1*) an *immediate stop* of the cell's further ability to participate in mitosis (remember: only a single thymidine-dimer in the whole of the human genome can prevent a human cell from moving into division) or, apart from these most favourable cases: (*I.2*) *the lesion* (respectively the mutants replicated past it) *will lead to a serious disturbing* of the further development of the cell bearing it, up to a case which may be again more favorable, *i.e.*, (*I.3*) an *early death of these cells* (which could act similarly positive by avoiding more damage to the organism as does a therapeutically triggered apoptosis). Or, (II) for the whole organism: (*II.1*) *the insertion of the "wrong" amino-acids* into biosynthesized proteins leading to proteins with disturbed structure and consequently disadvantagous behaviour in the organism (for example being less flexible and by this becoming one of the causes to develop the symptoms of ageing or even developing some toxic etc. properties), or (*II.2*) *switching on or off the "wrong" biochemical and/or cellular control mechanisms* of which some at the end may even lead to the development of a cancer. To be comprehensive, two further possibilites should be mentioned, *i.e.* the case: (*I.4*) *the DNA-lesion occurred at a DNA-section which is of no importance* for the further development of the cell or the change in the genetic information only leads to an absolutely insignificant change in the cellular behaviour (case of *indifference*), or (*II.3*) the DNA-lesion undergoes an enzymatic "*pseudo-repair*" (where the pseudo-nucleotide is not replaced by the correct one reestablishing the *ocGInof*, but by any other of the four

main-nucleotides), creating thereby by chance a mutant which opens the way for an *advantagous evolutionary development of the organism* (as it is programmed in biology by the slightly asymmetric nucleotide-pool: see also Ref. [1].

These options for the fate of a DNA-lesion show the importance of: a) an early repair and b) of a high R/R-ratio and by this demonstrating the *chance for a stimulation of the proofreading- and repair-system*.

This introduction should also demonstrate that it might happen rather often in the fate of an organism that this organism is exposed to a *cancerogenic impact* (''*C.I.*'', *i.e.*, to the aggression of a cancerogen[2] or DNA-lesions producing radiation for some time) and, depending on the intensity[3] of this C.I. with respect to the activity of the enzymatic repair-machinery of the organism, the development of a cancer may be avoided *in statu nascendi*.

An organism with a more active enzymatic repair-machinery will be better prepared to withstand such a C.I. This is the chance for a *repair-system-stimulation therapy.*

Additionally – as demonstrated above – such a repair-system-stimulation-therapy is also benefitial to avoid the development of a cancer, even after the first C.I., *i.e.* when already the first cells bearing the cancerous genetic information have divided and the identification of a thus propagated S-mismatch has already started. Even if the repair-machinery has failed in reestablishing the ocGInfo, it has at least exchanged the most dangerous (cancerogenic) genetic information against one which is less fatal (cb. cases I.1–I.4, II.1 and II.3).

Further, even malignant cells in a manifested cancer are identifyable by their altered behaviour compared to the normal somatic cells: they produce and emit signal-substances (*e.g.* Refs. [16, 17]) and their DNA encodes *via* the complementary m-RNA and the codons of the t-RNA (again complementary to the m-RNA) for the insertion of ''wrong'' amino-acids during the biosynthesis of ''their'' proteins, by this signalizing to the organism and its immuno-system that there is something to attack, respectively to repair. This is supported by the successes of *Ackermann* [20–24] against manifested cancers with a therapy by his ''low-molecular-weight human-placenta-extracts'' (*HPE*, to be well distinguished from ''stem-cells-'' (german: *Stammzellen-*) or ''placenta-blood-therapies''). The HPE-therapy seems to be based upon the stimulation of an enzymatic machinery (also stimulable similar to the DNA-polymerase-I-complex, see *Haschke* [33], *i.e.*, by offering symmetric nucleotide-pools via nucleotide-precursors). It is similar to modern attempts with ''xenogenic antigen-therapies'' for stimulation of the immuno-system as a therapy for ovarial-cancer, plus with the findings of *Karmali* and *Pokotilow* [39] (*cf.* at: Experimental evidences for an increased efficacy of AICA-*plus* OA-administrations). There are indications, that stimulation-therapies do also have chances beyond the ''*cancer in statu nascendi*'': Therefore such mechanisms seem worth to be explored by further research, whether – apart from the known proofreading- and repair-machinery for repairing DNA-lesions via the proofreading-process – further repair- and regulation-systems might exist which could help to avoid the definite switch-on of the fatal propagation-chain of malignant cells and/or which could even be activated to eliminate malignant cells respectively their malignant genetic information.

It is true that it is extremely difficult to demonstrate such effects *in vivo*, because of the facts that (a) the actual concentrations of nucleotides and especially of their precursors are extremely difficult to

[2] The activity of a cancerogen (ACG) being measured on a scale between 0 and 1, compared to benzo[a]pyrene = 100% (*i.e.*, $ACG \equiv 1$)

[3] The intensity of a *cancerogenic impact* (*C.I.*) being the mathematical product of the activity of a cancerogen multiplied by the time for which a cell is exposed to it ($CI = ACG \cdot t$)

determine locally where DNA-replication occurs, and because it is true that *in vitro*-results are not directly and easily convertible to *in vivo*-systems. It is therefore one objective of the paper presented here, to check, at least in a model with strongly simplifying assumptions, the consequences which are to be anticipated in case where *Zhang* and *Mathew*'s [1] findings are also significant *in vivo*, and especially in case that also a proof-reading- and -repair-stimulation is possible.

Based upon these praemissae, and keeping in mind the ''*Kornberg-effect*'', *i.e.* that the repair-machinery stops immediately for lack of even only one of the four main-nucleotides, *intermediate cases* seem to be the most likeliest: An appreciable high percentage of repair enzymes may be activable, but they stay inactive due to the lack of at least one of the four main-nucleotides at the site where repair should start. And this reservoir of inactive repair-enzymes can be activated by offering the lacking nucleotides or better by depots of nucleotide-precursors which may easily and in only few biochemical steps be converted to the lacking nucleotides. *I.e.* such an enzymatic repair-system should be stimulable by the administration of such nucleotide-precursors. Starting from the well known *Monod*-equation, a principal dependence of a stimulation-function from the concentration of a stimulator to describe the efficiency of a stimulated repair-system has been formulated by *Haschke* ([33], program CSUBMOD.xls).

In the first part, this lead to a pseudo-*Michaelis-Menten*-shaped function, converging to a horizontal asymptote, *i.e.*, showing the typical saturation-effect as it has to be anticipated till all activable enzymes are supported by enough of the four main-nucleotides. The function derivable from this might be taken as an example for simple cases such as procaryotes. However, if the more complicated auto-stabilizing and nucleotide- etc. -pool-concentration-regulating systems and -cascades are taken into account, it leads to a function with a definite maximum. Below an example to describe very simply such a dependence for an eucaryotic system:

$$\mathrm{ADPoI_{inh.}} = \mathrm{ADPoI_{max}} \cdot \frac{C}{CM2 + C} \cdot \frac{1}{2^{(C/C_i)}} \tag{1}$$

Formula 1 describes the relative activity of a DNA-repair-system in an eucaryotic system ($\mathrm{ADPoI_{inhib}}$) as a function of a stimulator's concentration (C)

CM2 is corresponding to *Monod*'s *K* (see [33] and indicating the accessable Purine-Nucleotides-Precursors' (PUNP) + Pyrimidine-Nucleotides-Precursors' (PYNP)-concentrations (*i.e.*, concentration as far as equimoarity is given) at which (the uninhibited) ADPoI reaches half of its possible maximum value;

C_i is indicating the stimulator's concentration at which the activity of the repair-system is diminished to its half due to auto-regulation- and inhibition-effects.

with

$$1 = \mathrm{ADPoI_{max}} \cdot \frac{C_\mathrm{N}}{CM2 + C_\mathrm{N}} \tag{1a}$$

$$\frac{CM2}{C_\mathrm{N}} = \mathrm{ADPoI_{max}} - 1 \tag{1b}$$

Formulae 1a and 1b describe the relation between $\mathrm{ADPoI_{max}}$ and *CM2*

C_N is corresponding to the concentrations of PUNP + PYNP and their corresponding accessible nucleotides as usually present in the unstimulated case due to normal metabolism.

In the second part of the paper [see: *Haschke* ([33], program CANCER.xls) a kinetic formulation for the influenceability of the timely development of malignant

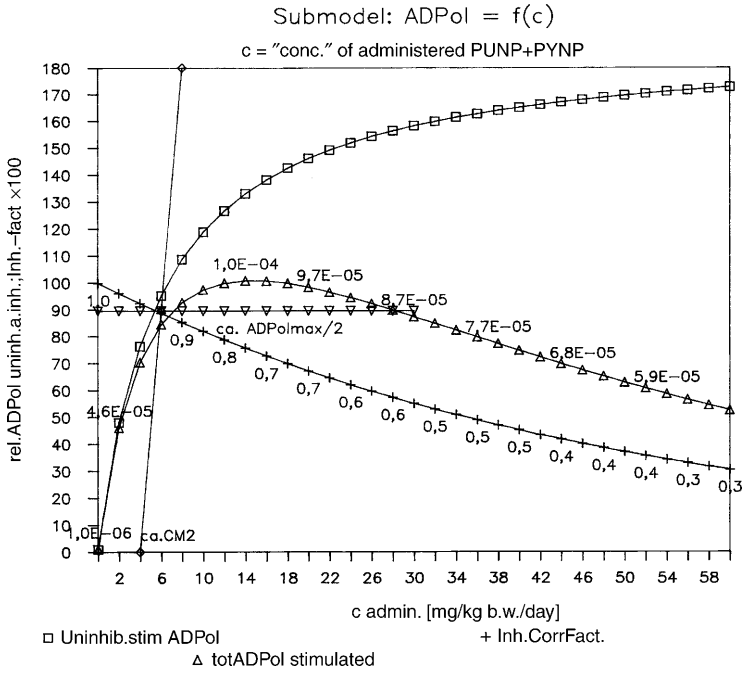

Fig. 1. Model for the dependence of the activity of a DNA-Polymerase-I-similar repair-system from administered doses of stimulating PUNP + PYNP; Submodel: ADPoI = f(C); Abscissa: C [mg accessible PUNP + PYNP/kg b.w./day]; Ordinate: Relative ADPoI; □ uninhib. stimulation; + correction-factor for inhibition; △ total ADPoI (stimulated)

cells in competition to normal somatic cells is given:

$$\mathrm{d}N_\mathrm{H}/\mathrm{d}t = (k_\mathrm{bH} - k_\mathrm{dH}) \cdot N_\mathrm{H} - k_\mathrm{cG} \cdot \xi \cdot \mathrm{ACG} \cdot N_\mathrm{H} \tag{2a}$$

wherein N_H means the number (see also (2b)) of healthy (normal somatic) cells (in the organ under observation); t = time [weeks]; k_bH respectively k_dH are the kinetic constants in the equations describing the formation respectively the death-rate of the cells (dimension of k: [week^{-1}]; ξ is the susceptibility of the cells for being influenced by a cancerogen and ACG representing the activity of a cancerogen (measured on a scale between 0 and 1, compared to 100% = benzo[a]pyrene = ACG ≡ 1. Benzo[a]pyrene as a component of the emissions of *Diesel*-engines; if adsorbed to exhauster-black-particles, might be even more dangerous due to the long-time benzo[a]pyrene-desorption-ability together with the high density and load in benzo[a]pyrene on the rough particles-surface if such particles are desposited on the alveolae (similar to the higher toxic acitvity of vanadium-oxide-loaded-furnace-black compared to pure vanadium-oxide: see *Leuschner et al.* [40], Figs. 5a and 5b).

and

$$+\mathrm{d}N_\mathrm{M}/\mathrm{d}t = k_\mathrm{cG} \cdot \xi \cdot \mathrm{ACG} \cdot N_\mathrm{H} + k_\mathrm{bM} \cdot N_\mathrm{M} + k_\mathrm{E} \cdot {N_\mathrm{M}}^2 - k_\mathrm{dM} \cdot N_\mathrm{M} - k_\mathrm{H} \cdot \mathrm{ADPoI} \cdot N_\mathrm{H} \cdot N_\mathrm{M} \tag{2b}$$

wherein N_M means the number of malignant cells in the organ under observation; k_E is the kinetic constant in the kinetic-velocity-equation for autocatalytic-like effects ("metastasisation constant") and k_H the constant in the kinetic equation describing the velocity of the repair of malignant genetic information.

Note: N_H or N_M, respectively $\mathrm{d}N_\mathrm{H}/\mathrm{d}t$ or $\mathrm{d}N_\mathrm{M}/\mathrm{d}t$ should be understood as an actual quantified measure for a population of cells of such type at definite time t, respectively the changing-rates of the population's size – *i.e.* N_H and N_M do not indicate a number of cell-individuals.

Or abbreviating it *via* the definitions:

$$N_H \equiv x,\ N_M \equiv y;\ a \equiv (k_{bH} - k_{dH}) - k_{cG} \cdot \xi \cdot ACG,\ b \equiv k_{cG} \cdot \xi \cdot ACG,$$
$$c \equiv k_H \cdot ADPoI,\ d \equiv k_{bM} - k_{dM},\ e \equiv k_E,$$

the equations were written (*Haschke* [33]) in easier readable form:

$$\mathbf{d}y/\mathbf{d}t = b \cdot x - c \cdot xy + d \cdot y + e \cdot y^2; \quad \text{while} \quad \mathbf{d}x/\mathbf{d}t = a \cdot x \tag{2c}$$

As a first conclusion it can be derived:

$$\text{Cellular half-life-time is: } t_{1/2} = \frac{\ln 2}{k_d} \tag{2d}$$

Because of the fact that the (half)-life times of cells might differ appreciably from type to type of the cell (for example a neural-cell might have a life-time of the whole life of the organism, *i.e.* for several decades of years, while for a prostata-cell or for an erythrocyte, much shorter $t_{1/2}$ must be anticipated; even substitutional taking over of functions from some cells by others are possible), the choice of a reasonable corresponding k_d from a *virtual* $t_{1/2}$ *via* (2c) (for accordingly reasonable k_{dH^-} and k_{dM^-}, respectively k_{bH^-} and k_{bM}-values) might be a way to adjust the model for significantly different types of cancer.

To calibrate these relations, or in other words, to adjust them to biological frame-conditions, it was necessary to adjust their free parameters to measured facts, *i.e.*, to bring them into relation with biochemical measurements and with clinical data as given in the literature.

Graphical outputs of the mathematical solutions of the *a.m.* equations by numerical integration for several typical cases of the development of malignant genetic information (indicated as the timely development of the number of malignant cells) and of their kinetic propagation-velocities, respectively of the repair-velocities are given in Figs. 3a–4c. Further examples including the predictions of the model for the efficiency of a *DNA-repair-system-stimulation-therapy* together with clinical results of a medical study with a potentially related therapy will be given in the next paper.

Calibration of the Mathematical (Biokinetic) Model According to Facts Reported in the Literature

The method was inspired by reports of successes in cancer-therapy by *Kohn* and *Liotta* [16–19] and the FDA [41] by using 1N-(4″-chlor-2′,6′-dichlor-benzo-phenonyl)-methyl-4-carboxamido-5-amino-triazole (I; also known as L 651 582) and similar substituted imidazoles, *i.e.*, compounds of the group of the so called ''*C*arboxy*A*mino*I*midazoles'' (CAIs, *e.g.* I) to suppress angiogenesis (Fig. 2a and 2b) and the way towards the outstanding role which nucleotide-precursors might play in inhibiting respectively stimulating DNA-related enzymatic systems.

By the proposal of *Liotta* and *Kohn* [16, 17], a potentially significant new break-through in cancer-therapy might be possible: Rather than to attack the malignant cells, respectively the tumor only, *i.e.*, rather than to attack just only the symptom of the disease, this new strategy is aimed to attack steps of the pathways required for cancer, namely proliferation, invasion, and metastasis. Thus, instead of violating healthy cells to an appreciable degree by using the classical methods such as chemotherapy or radiotherapy only to attack malignant cells (''*1st-generation strategy*''), it may be possible by such a ''*2nd-generation-strategy*'' to reduce

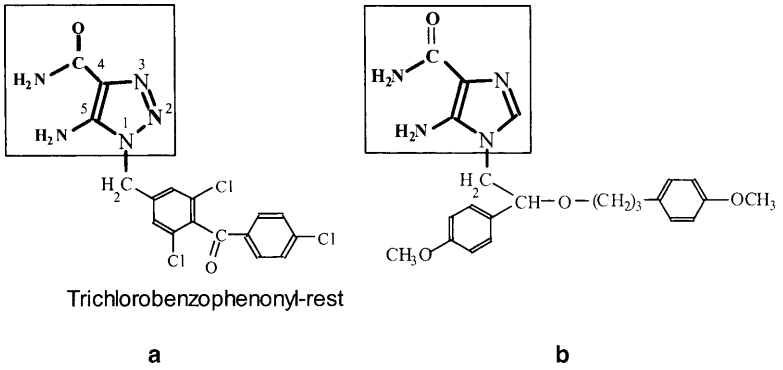

Fig. 2. (a) L 651 582; (b) A real CAI

significantly the undesired side effects of a weakening of the whole organism by classical therapies. By reduction of the energy-supply of malignant cells and suppressing angiogenesis instead of attacking the cells directly might be better.

As to angiogenesis – *i.e.* the formation of new blood-vessels to nourish malignant cells up to tumors – it was found by *Kohn et al.* [19], that such CAIs are inhibitors of non-voltage-gated calcium-channels (including ionophore channels) which are important in calcium-regulated signalizing in the mechanisms for angiogenesis (similar to the mechanisms in the kinetic model presented by *Haschke* ([33], Fig. 4 and Formula 5b).

Relation to a Potential Stimulator I: 4-Carboxamido-5-amino-imidazole (AICA) and Purine-Nucleotides'-Precursors (PUNP)

From the chemical point of view it is surprising how closely related the CAIs are to the well known key-precursor of the natural (in mammals) purine nucleic base precursor AICA or its glycoside the 5′-phosphoribosyl-AICA (also known as AICAR), a key-precursor for the purine-nucleotides. There have been found such surprising successes in using AICA in several therapies that many – predominantly Japanese – scientists focussed their interest especially to this substance dedicating extraordinary work and many conferences to it [8–11]; further publications include "*The Effect of AICA-Orotate in the Treatment of Liver Diseases*" by *Yamada et al.* [12], supported by measurements of the incorporation of ^{14}C-marked AICA by *Seegmiller et al.* [13] and *Miura et al.* [14]), and with respect to AICAR, its nucleotide-analogon by *Wakisaka et al.* [15]. However, as intense the efforts with AICA might have been, it was not concluded that the extraordinary therapeutic successes with this substance could get a still much more interesting dimension if AICA were used in combination with orotic acid (OA), opening the chance for a stimulation-system for enzymatic repair machineries. From this point of view it is again of interest to reconsider this older literature.

According to the established biochemical pathways, AICA is easily converted into inositolmonophosphate (IMP), which is the node-point of the biochemical pathway, because the purine-nucleotides' biosynthesis is splitted there: IMP is easily aminated to adenosine-5′-mono-phosphate, or *via* a metabolic introduction of a second hydroxyl-group to xanthine-5′-mono-phosphate (XMP) giving *via* amination guanosine-5′-mono-phosphate (GMP).

Conclusion: *To create both purine-nucleotides AMP* (*or ATP*) *and GMP* (*or GTP*), *just only one precursor – like AICA – is necessary.*

Experimental Evidences as to the Efficiency of CAIs, AICA, and Purine-Nucleotides'-Precursors (PUNPs)

To find additional to the *in vitro*-results of *Zhang* and *Mathews* [1] a more practically applicable basis for the calibration of the biokinetic model given here, reports in the literature containing practical experiences with AICA-related compounds as therapeutica have been collected:

The efficacy of CAIs like L 651 582 as a therapeuticum against cancer has been proven. However such CAIs only can act as a cytostatic, but not acting as a bio-stimulator. However, the good "first-step-acceptance" of a CAI might open a highly interesting new tool in cancer-therapy by the findings of *Kohn* and *Liotta* [16], and will be an important enrichment in fighting cancer.

Therapeutic successes by similar attack to malignant cell-systems by exploiting also a further step, *i.e.* by the combination of the efficiency of a purine-nucleotide-analogous chemotherapeuticum with an enzyme-inhibitor, aiming definitely towards the interference of fludarabine with DNA-repair-mechanisms by combining it with the topoisomerase-II-inhibitor mitoxantrone, were reported by *Mc Laughlin et al.* [44].

A further proof that AICA itself also enters the pathway of the purine-nucleotides-biosynthesis was given by tests of *Seegmiller et al.* [13] upon the *in vivo* incorporation of radioactive marked AICA. A significant incorporation (*i.e.*, 20–23% of the administered AI-4C^{13}A (at administrations of 14 mg AICA/kg b.w.)) into human uric acid was detected. (Uric acid is an indicator for bio-synthetisized purine-nucleotides because uric acid presents a product of the biodegradation of GMP *via* the biochemical steps: splitting-off the nucleic base guanine, further its deamination to xanthine, followed by hydroxylation to uric acid). These findings get an additional dimension, as it also was found during these investigations that simultanously with the incorporation of AICA, the withholding of glycine-N^{15} was stimulated.

Additionally, a further series of very encouraging reports on therapeutic effects of AICA, have appeared however, mainly concerning the therapy of liver-diseases (acute hepatitis including serum hepatitis, chronic hepatitis, liver-cirrhosis, hepatic or bile-duct cancer [9]). The results were significant even taking into account that the effects on chronic hepatitis and liver-cirrhosis were inferior. Model tests executed by *Miyoshi et al.* [10] with CCl_4-induced acute liver-injuries on rats supported these findings by delivering basic informations on AICA having an inhibitory effect on liver-cell-necrosis, fatty-infiltration, and fibrogenesis and showing an ability to accelerate liver-cell-regeneration.

Relation to a Potential Stimulator II: Orotic Acid (OA) and Pyrimidine-Nucleotides'-Precursors (PYNP)

The therapeutic effects of CAIs, which seem related to the outstanding position of AICA in the purine-nucleotides-biosynthesis, makes it interesting to check if there were any similarities to the second type of nucleotides, *i.e.*, the pyrimidine-nucleotides. The node-point in the PY-nucleotide biosynthesis corresponding to AICA (for the PU-nucleotides) is orotic acid.

In analogy to AICA as the key-precursor for the purine-nucleotides *via* IMP, orotic acid plays a role as the key-precursor for the pyrimidine-nucleotides *via* the node-point UMP, i.e., again for *both*: Cytidine-monophosphate (CMP) and thymidine-monophosphate (TMP).

Conclusion: *To create both pyrimidine-nucleotides TMP* (*or TTP*) *and CMP* (*or CTP*), *just one precursor – like OA – is necessary.*

Experimental Evidences to the Efficacy of OA and Pyrimidine-Nucleotides'-Precursors (PYNPs)

Orotic acid has already been described by *Gordonoff* and *Schneeberger* [45] as an important component of milk, obviously especially focused by evolution as a protective agent for very young children, *i.e.*, where a high reduplication-rate of cells is given. Consequently a high risk of forming erroneous copies of DNA during replication is given. The same criterion, *i.e.*, high mitosis-rates with consequently high risk of erroneous copies and consequently the necessity of corresponding protective agents is given where the embryo is formed, *i.e.* near the placenta. Placenta-preparations in cancer-therapy have been described by *Ackermann* [20–24]).

Based upon tests with N^{15}-marked orotic acid, *Gordonoff* and *Schneeberger* [45] also claimed that OA is incorporated into pyrimidine-nucleotides, while, of course, administration of the nucleic bases cytosine, uracil and thymine – instead of their precursors – does not lead to their incorporation in DNA nor into RNA. Nucleic bases, instead of their precursors are just biodegraded in the liver, while higher concentrations of the mono-phosphates of the nucleosides are even known from the research of *Zhang* and *Mathews* [1] to act as proofreading-inhibitors.

Uridine-5′-phosphate (UMP) is related to PYNP-2 (pyrimidine-nucleotides-precursor minus 2) and orotidine-5′-phosphate/orotidine/OA, the PYNP-1, is consequently also showing positive therapeutic effects as reported by *Gordonoff* and *Schneeberger* [45].

Experimental Evidences for an Increased Efficacy of AICA-plus OA-Administrations (Synergism of Combined Administration of PUNPs plus PYNPs)

Combinations of AICA and of OA are described by *Miura et al.* [14] as being better incorporated *in vivo*, and it is reported by *Kosaka et al.* [11] that such combinations have enhanced therapeutical efficacy, the latter signalizing that there is a real synergism between AICA and OA.

In agreement with these findings are the results of *Katsuki et al.* [8, 46] with C^{14}-marked AICA injected to mice indicating that AICA and orotic acid (OA), when given in combination, are better incorporated into ribonucleic acid (RNA) compared to separate application.

Further results were reported by *Karmali* and *Pokotilow* [39] with PUNP/PYNP-therapy-analoga (from test-series according to the androgen-independent *Dunning* R-3327-AT-1 rat prostate tumor model with Copenhagen rats); these series were focused on the clinical efficacy of AICA-salts, especially on "AICA-HCl-salt": Single PUNP-derivatives and also the comparatively tested single PYNP-derivative" L-651582 orotate" did not show any effect. This result had to be anticipated from the hypothesis given here (a PUNP-hydrochloride or a L-651582-PYNP-salt instead of the equimolar combination of a PUNP + a PYNP must be inactive as a repair-system stimulator). But it has been found a significant lower tumor volume in a group also inoculated with prostatic tumor cells, to which was administrated the PUNP + PYNP-combination ORAZAMID (generic name for an equimolar mixture of AICA plus OA, usually administered as its dihydrate, "ORAZAMID $\cdot 2H_2O$", as registered in Ref. [47]: Drug No.: 6817). It was concluded that: "*Orazamid was ineffective in vitro but effective in vivo in inhibiting the growth of AT-1 prostate cancer cells*". According to the model, such a result was also to be expected (equimolar combination of a PUNP plus a PYNP). It was to be expected too that even with ORAZAMID no effect should be recognised *in vitro*, obviously due to the fact that the PUNP + PYNP-combination therapy does not attack the malignant cells directly, but needs enough repair-enzymes/proteins in a living organism to trigger any effect.

The facts described above, suggest that nucleotide-precursors are well accepted *in vivo*, while the *ready nucleotides are not incorporated into RNA nor into DNA and are rather* rejected *by the organism* (''screened-out'' by biological regulation-systems) *if administered as such*; *they are biodegraded in the* liver [45]. Administered

from external sources and not biosynthesized when there is a local need for them, they might be the source for increasing errors in DNA-synthesis.

This problem of replication-fidelity determinated by nucleotides'-pool-asymmetry in context with base-sequence has been confirmed by *Zhang* and *Mathews* [1].

Conclusions

(i) Strong *excess-concentrations* of single nucleotides (like GMP) might even *act as proof-reading-inhibitors*.

(ii) Following the rules which are given by the biochemical pathways of the nucleotide-biosynthesis, *just only two of them*, *one PUNP plus one PYNP*, *are necessary* to serve as easily accessible raw-material sources (*i.e.* precursors), convertible in only few steps of biosynthesis to all the 4 main-nucleotides and the four corresponding triphosphates as well (AMP, respectively ATP; GMP, respectively GTP; CMP, respectively CTP; TMP, respectively TTP), ribose-based for the biosynthesis of RNA or deoxyribose-based as they are needed for the bio-synthesis of human DNA.

(iii) According to *Kornberg* [2], DNA-Polymerase-I does need all the 4 nucleotides to be active as a repair-enzyme; similarly – as the same physico-chemical laws for the necessity of templates for hydrogen-bonding have to be valid – it might be anticipated that *for the more complicated repair-systems in eucaryotes*, *an* ''*in-time*''*- and* ''*locally where repair should happen*''*- accessibility of all the 4 nucleotides must be given to allow the repair-system's full efficacy* (see also [33]).

(iv) Because full accessibility at any time and at all cellular localities is not very likely, it may be also anticipated that *the repair-systems* (respectively their enzyme-/protein-systems) usually are only partially active and the rest of them (*i.e.*, the rest of the inactive repair-enzymes/proteins) *might be stimulated by making them available the lacking nucleotides*. For this, the best way were from depot-pools, which may be available at much more localities (without disturbing the normal DNA-biosynthesis which might happen, if – instead of the precursors – the ready nucleotides were administered). In such depot-pools the nucleotides should be presented not as such, but presumtive, *i.e.*, as/*via* their precursors (which even might better ''*undertunnel*'' the many nucleotide-concentration-control-mechanisms and by this allowing a real influenceability of the DNA-repair-mechanisms).

(v) It is proven that *asymmetries in nucleotides-concentrations do lead to increased mutant-fractions* during DNA-replication and that the mutant fractions are increased by magnitudes if proofreading-inhibitors are present. Therefore, there are good reasons to anticipate - at least hypothetically - that an opposite effect, i.e., a decrease of the mutant-fraction may be reached not by inhibition, but by stimulation of the DNA-proofreading and the DNA-repair-systems.

Thus it is anticipated that such stimulation is possible by offering an increased accessibility of all the 4 nucleotides to the enzymes and proteins of the DNA-repair-system, especially locally wherever a DNA-repair might be needed. To reach this ''*locally-wherever-needed-availability*'' by creating depots of nucleotides'-precursors: Due to the biochemical pathways mentioned above, only two precursors are necessary as sources for all the 4 nucleotides. Because symmetric

nucleotides'-concentrations do cause a minimum of the mutant-fraction one should try *to administer as DNA-repair-system-stimulators "adaequate combinations of always one PUNP plus one PYNP". I.e.*, to administer such combinations of PUNPs plus PYNPs which – due to their biochemical/biokinetic distance to the nucleotides – do create equimolar concentrations.

Therefore the mass of results upon the extraordinary therapeutic efficacy of AICA as they were described in Japanese literature [8–15], and moreover, the sum of these findings seen in the light of later clinical results upon therapeutica with similar chemical structure described by *Kohn et al.* [16–19], the FDA [41], and even the data recently reported by *Cheson* [43] and *Mc Laughlin et al.* [44] upon the efficacy of so called nucleotides-analogue-cytostatica, together with the results of *Gordonoff* and *Schneeberger* [45], *Katsuki et al.* [48], combined with those of *Karmali* and *Pokotilow* [39], and all these compared to the successes of *Ackermann* [20–24] and the mechanistic options which his therapeutic measures do include, this seems more than a sum of separate results. Seen together and extracting the basic facts which all these findings do have in common, plus the exciting results of *Zhang* and *Mathews* [1], it seems that this does open the opportunity for a *"3rd-generation-strategy" in cancer-therapy* if all those results are interpreted in the light of modern molecular biology and biokinetics. This option is further supported by the reports of *Rubinstein et al.* [31] upon successes in cancer-therapy with placental blood compared to bone marrow.

However, this approach should not be misunderstood as it is undoubtly clear that the classical cancer-therapies like surgery, radio- and chemotherapy are still the essential tools to fight cancer and even the *2nd-generation-strategy* influencing angiogenesis will keep its importance, while a not attacking, but repair-stimulation-therapy only can have chances as a prophylactic, a supporting-postoperative or a ''better-than-nothing'' therapy in cases of inoperable cancers like leukaemia.

It is also undoubtly true that cancer is not cancer: Absolutely different organs might be affected by the generation of malignant cells leading to the growth of a tumor. Also mobile cells and therefore those not generating a distinct tumor (thus inaccessible by surgery) like leukemia, may become malignant. Nevertheless, many types of malignant cells give reason to cancer symptoms if these cells have ceased normal behaviour by being erroneously programmed in their genetic code. The ideal cancer therapy would be one which is able to mend such erroneous genetic information. The main attraction of the model presented here is the possibility which it offers to understand better the timely developments of a cancer, possibly also to optimize the scheduling of therapeutic measures and to have rational interpretations for otherwise unmotivated relapses during a therapy.

Safety-Remarks to a DNA-Proofreading and -Repair-Stimulation by Administration of PUNPs and/or PYNPs

The known activity of orotic acid – independently whether exogenously supplied or endogenously synthesized – if creating inbalances[4] in nucleotide pools and therefrom to act as a promotor for

[4] Based upon an article from *L.A. Liotta* and *E. Kohn* in the popular-scientific journal ''Spectrum of Science'' (April 1992) on carboxyamidoimidazoles, especially on L 651 582, and after having become acquainted with parts of the first manuscript of the paper presented in the Journal of Theoretical Biology [33] as it was given to attorneys deposit on June 22, 1995 (see footnote Nr. 1), *F. Wehrmann* applied on July 21, 1995 for an US-patent claiming ''salts of AICA'', especially ''its inorganic salts as hydrochlorides and/or phosphates, or its organic salts as those with lactic, succinic or maleic acid or the L651 582-salt of orotic acid'' as cancer therapeutica (patented under No. 5,728,707 on March 17, 1998). For safety-reasons it should be mentioned that such measures, which would create imbalances in the nucleotides'-pool (analogously to the cancerogenic activity of imbalances created by administrations of the PYNP orotic acid alone) could very likely also trigger an opposite effect than desired by creating imbalances in the nucleotides'-pool from the PUNP-side. The combination of a pseudo-antimetabolite like L651 582 with orotic acid could even be more dangerous (see below: combination-therapies)

cancerogenic processes, as it must be also expected by the model presented here, is often described in the literature, rather recently again by *Vasudevan et al.* [32]. Nevertheless, as mentioned there, this is a consequence of induced asymmetry in nucleotidic-pools due to an administration of such a single-precursor: Insofar it is not comparable to the effect of a cancerogen, which attacks chemically a nucleic base (for example like nitroso-compounds, hydroxylating deamination of nucleic bases, or their alkylation as does *f.e.* dimethylsulfate), nor comparable to a usual cancer promotor.

However, OA should not be excluded by these effects from being a possible component in formulations for fighting cancer via a stimulation of the proofreading- and DNA-repair-system, if such formulations do contain also a balancing amount of a purine-nucleotide-precursor.

It is a fact, that *just an equimolar mixture of any two precursors* – even if one of them is of the type of a purine-nucleotides'-precursor and the other one of a pyrimidine-nucleotides'-precursor, *i.e.* for example AICA or AICAR are not necessarily the optimum to check the hypothesis (by simplifying the condition of adequate accessibility to equimolar administration), nor does an administration of such a mixture implicate the possible additional necessity to contribute supporting to the body-own reservoir of proofreading and repair enzyme(s) by *offering/administering the triple*: a) PUNP plus b) PYNP plus c1) DNA-repair-system including c2) a repair- and/or blocking-system for established malignant cells (respectively its enzymes, protoenzymes, enzyme-precursors and proteins). In any way and in spite of these facts, a possibility for a first, time-saving, preliminary check of the hypothesis is given.

A concept of a kinetic competition of repair-mechanisms against the reproduction of malignant cells' – as it is given in the hypothesis mentioned – would also explain, why curing effects by the administration of nucleotide-precursors do need longer time (real therapeutic successes are reported in literature not to happen usually earlier than after approx. 2 years of therapy), and also that the delay between start of the therapy and the clinic success is dependent on the amount of malignant genetic information already produced at the start of the therapy. Meaning that an old and manifested cancer will need a much longer time of therapy and will not be stabilized to such an extent. (*F.e.*, in best cases to a tumor without further metastasis, but not to reach a regression, *i.e.*, make tumors shrinking, nor even one, which is completely stabilized, than it could be the case, if the number of malignant cells were still rather small at the start). In such cases of manifested cancers the removal of the tumor by surgery will not only be the method necessary to avoid lethal effects by the tumor itself and its cells with malfunction and/or by metastasis, but it will also be the first step necessary to create a reasonable start-ratio of residual malignant DNA and its propagation-rate to the power of the DNA-polymerase-I-similar repair-system and its kinetic competition.

Clinically Found Synergisms and Indications for the Possibility of a Stimulation-Therapy

An approach as attempted by the model presented here would also explain the clinically found synergisms by administration of AICA or any other PUNP combined with OA or any other PYNP (see *Katsuki et al.* [8, 46], *Miura et al.* [14] and *Karmali* and *Pokotilow* [39]; and the importance of PUNP- to PYNP-equimolarity.

However, up to now such combinations have only been tested clinically in the therapy of liver-diseases, but in any way, by the molecular-biological reasons described above, such therapy seems also to have chances of success if used as a prophylactic, as a post-operative, and in lighter cases even as a supporting (adjuvant) cancer therapy. Nevertheless it must be kept in mind, that the suggested model-strategy for an additional cancer-therapy arising therefrom, is not the suggestion of a new group of cytostatica, nor is it the suggestion of an alternative therapy: It is not a measure to attack and/or to kill malignant cells. Interest should be focused also to the fact, that it is necessary to *avoid as far as possible any new generation of wrong genetic information* and consequently to try to repair its sources by stimulating or accelerating the body-own DNA-repair mechanisms, especially in that cases, where elimination of malignant cells is practically impossible and/or in the usual cases, where life-style

Table 1. Administered doses of (AICA) or ORAZAMID · $2H_2O$ to reach therapeutic effects in clinical tests

Source	Administration (dose)	Corresponds to C (approx.) expressed in mg ORAZAMID · $2H_2O$[a]/kg b.w.
First therapeutic effects to be anticipated following the recommendations for the administration of AICORAT[b] (1 Tbl = 100 mg ORAZAMID · $2H_2O$)	Minimum: 1 Tbl/day Maximum: 3 × 2 Tbl/day	1 mg/kg b.w. 9 mg/kg b.w.
Fujisawa et al. [1962]	Minimum: 300 mg AICA/day Maximum: 600 mg AICA/day	7.5 mg/kg b.w. 23 mg/kg b.w.
Seegmiller et al. [1955]	$AIC^{13}A$: 900 mg AICA/day	35 mg/kg b.w.

[a] By "expressed as ORAZAMID · $2H_2O$" it should be understood in this context the concentration of ORAZAMID-dihydrate administered directly as such or corresponding to the dosage of AICA (**5a**) administered. As the molecular weight of **5a** (AICA) is 126.1 and the molecular weight of OA (**13a**) is 156.1 (meaning that the molecular weight of ORAZAMID-dihydrate were 318.2, *i.e.*, containing 40% of AICA), any administration of AICA only would correspond to the 1/0.4 = 2.5-times higher quantity of ORAZAMID-dihydrate offering the same amount of AICA; [b] AICORAT is the trade-name of a drug produced by the pharmaceutical company Heinrich Mack Nachf. in D-89252 Illertissen, Germany

cannot be changed fundamentally enough so that some exposure to cancerogenous conditions stays still present, in addition – or *rather than* – to kill malignant cells by agents which are so aggressive, that also a too significant number of healthy cells were violated.

To calibrate the model to practical applicability, there was tried to interprete the corresponding data given in the literature (Table 1) as to clinical results found with the administration of AICA and/or with ORAZAMID (see The Merck Index [47].

It may be assumed that *Fujisawa et al.* [9] have found by clinical trial/error methods with a maximum-dosis of administered 600 mg AICA/day (which corresponds approximately to 9.5 mg AICA/kg b.w. or to 9.5/0.4 = approx. 24 mg orazamid-dihydrate/kg b.w.) also a dosage at, or beyond the maximum (*C* at $ADPoI_{max}$) in general efficiency for the stimulation of the repair-system. According to Fig. 1, this would indicate, that *CM2* should be of a magnitude of approximately 6 mg/kg.

Analogously it might be reasonable to assume in the model C_i to be in a magnitude where clinical tests seem to show already a significant depression of therapeutic efficiency at a further increase of the dosage, *i.e.*, somewhere after the maximum efficiency – supported by the relatively high doses chosen by *Seegmiller et al.* [13] for the detection of incorporations with 14 mg AICA/kg b.w. (*i.e.*, calculated on the weight of a human with 60–70 kg, approximately 900 mg AICA/day; corresponding to approx. 35 mg orazamid-dihydrate/kg b.w.) which lead already to a significant excretion, indicating that some overloading of the organism already has been reached. Thus, a reasonable magnitude of C_i therefore might be a value of approx. 35 mg/kg.

The reasonability of the choice of C_i has been confirmed by later findings by *Karmali* and *Pokotilow* [39] in their *in vivo* experiments: Significantly reduced mean tumor volume over the whole period of 35 days observation in that tumor-cells-inoculated group to which were administered 10 mg ORAZAMID/100 g b.w.; while the group to which were administered the higher dose of 20 mg ORAZAMID/100 g b.w. did not show such effect. (*i.e.* the ORAZAMID-effect at this dosage had fallen back to practically zero (Fig. 1).

Using relation (1b) and calibrating $ADPoI_{max}$ in a way that Eq. (1) gives a relative value for ADPoI = 100 for maximum stimulation: $ADPoI_{max}$ becomes approx. 190 at a reasonable (for humans) $C_N = 0.03$ mg/kg b.w. The reasonability of such a C_N might be checked by the well known analytical data (*Gordonoff* and *Schneeberger* [45]) on concentrations of nucleotides' precursors in biological material: approx. 324 mg/l of orotic acid (OA) in sheeps' milk; 80–100 mg/l in cows' milk; 7 mg/l in women's milk – usually strongly dependent from the alimentation – but in any way always significantly higher at the beginning of lactation. After confirmation that N^{15}-marked OA is incorporated *in vivo* into pyrimidine-systems, this phenomenon has been interpreted by the authors that OA is especially important for the very young child.

Assuming that milk has been designed by evolution not only as a food-stuff for very young mammals, but also as a transfer-medium for protective substances (as it is definitely known as to immunizing substances), some content of nucleotides' precursors are to be expected therein, if such nucleotides' precursors play a role in preventing the generation of ''erroneous'' copies of DNA – especially during the extremely high replication-rates due to rapid growth of cells' numbers in very young organisms. As to such a requirement, it is even to be expected, that milk (but also other mother's body-liquids which are supplied to an embryo like placentae- or umbilical-cord's-blood) do contain effector-molecules to stimulate the DNA-repair-machinery, and by this may contain such precursors significantly enriched, while their concentrations in less specialised biological material are much lower. Thus a usual concentration in other biological material of approx. 1/100, *i.e.*, < 0.07 mg/kg b.w. for human organisms, as it is derived for C_N by the other assumptions of the model, is understood as ''reasonable'' in this context.

Based upon these parameters an example for a dependence of the activity of a DNA-polymerase-I-similar repair-system from the concentration C of any administered PUNP + PYNP can be given as expressed by Fig. 1. Figure 1 shows with the plot of the function ''uninhibited'' a graph of a pseudo-*Michaelis-Menten*-like type, which reflects the analogy of the ''ammunition'' of DNA-polymerase-I with the 4 nucleotides to a *Michaelis-Menten*-type enzyme-substrate-complex.

To derive reasonable values for the kinetic constants to be used in these equations, there can be used data of earlier kinetic measurements (Table 3) and those upon average life-times of cells – depending from the type of the cell – and such cells' half-life-times for which there do exist mathematical relations connecting them to kinetic-constants (see for example Haschke [33] Eq. 3c). For the choice of a reasonable corresponding k_d (kinetic constant for the cells' dying-rates) from a *virtual* $t_{1/2}$ and for accordingly reasonable k_b-values (for the kinetic constants for the cells' propagation-rates), to adjust the model for significant different types of cancer *c.b.* Eq. (2c) ff.

Obviously kinetic relations are applicable for healthy cells (in number N_H and with a ''constant'' of decay k_{dH}) and for malignant cells (in number N_M with

Table 2. A model for the dependence of the activity of the DNA-Polymerase-I-analogous DNA-repair-system from stimulating PUNP + PYNP-administrations (some typical values as used below in example-cases of the kinetic model[a])

Administrated dose	Resulting ADPoI according to the model	
[mg orazamid · $2H_2O$/kg b.w.]	Relative	Absolute[b]
No administration (C=C_N)	1	1×10^{-6}
0.15	6	5.6×10^{-6}
1.1	30	3×10^{-5}
2.2	50	5×10^{-5}
4	70	7×10^{-5}
18	approx. 100	1×10^{-4}
100	25	
200	4	
250	1	

[a] A diskette with a program named CSUBMOD.xls or CSUBMOD.wr1 calculating ADPoI in dependence of the doses administrated according to Eq. (2) with respect to the calibrations and the relations (1a) and (1b) is available for academic purposes on request; [b] *cf.* Table 3, footnote f

constant of decay k_{dM}) as well. By similar logics there are also evaluated the other kinetic constants as given as a first approximation (''a first guess'') in Table 3.

Additionally to the kinetic ''constants'' given, there are also to be kept in mind the definitions used by *Haschke* [33]:

ACG, the *relative activity-indicator for a cancerogen* – as different cancerogens do trigger different responses of an organism – is defined on a scale between 0 and 1 (0 meaning no cancerogenity, while 1 meaning ''100%'' cancerogenity, for example comparable to 1,2-benzopyrene. Together with the time-period during which this cancerogen is anticipated to be active, both factors together are exprimming the intensity of the ''*cancerogenig impact*'' *C.I.*

ADPoI for ''*Activity-of-the-extended-DNA-polymerase-I-analogous-system*'', *i.e.*, the activity of a DNA-proofreading and -repair system as derivable from Fig. 1, respectively Eq. (1).

The *Michaelis-Menten*-effect was interpreted as the consequence of the formation of an enzyme-substrate complex first, giving reason to a dependence of biochemical kinetic ''constants'' from substrate-concentrations and leading them to an asymptotic approach to limited maximum-values (saturation-effect). Similar this is correctly reflected by the equation for ADPoI (Eq. (1) and Fig. 1, pseudo-*Michaelis-Menten*-effect). It exemplifies the key-principles for both: for procaryotic and for eucaryotic systems. Thus, with the plot ''inhibited'' (see also: Fig. 1) the model is extended to take into account regulation-systems'-influences, as they must be taken into consideration for the more complicated systems in eucaryotes. According to this, the plot ''inhibited'' shows a typical maximum at dosages of approx. 20 mg [AICA + OA]/kg b.w. (with a maximum value of relative ADPoI of approx. 100) and a fall-back due to inhibition at very high dosages: again reaching such low values like only 4 or 1 (*i.e.* similar to the unstimulated case) at 200–250 mg/kg b.w. (in accordance with the findings of *Karmali* and *Pokotilow* [39]). For some other typical dosages, the model yields the results listed in Table 2.

However, in the case of the kinetic speeds of cell-division which is to be assumed to follow a ''unimolecular'' mechanism (''as long as mitosis does not follow a ''bisexual'' mechanism''), or for the

Table 3. Typical parameters chosen for the kinetic model

$k_{bH} = 0.25$[a] [per week and per 100 cells]
$k_{dH} = 0.26$ [per week and per 100 cells]

simulating (symbolically but not necessary for the model) the ageing effect; the k_{dH}-values chosen above, are adjusted according to (2d) (*i.e.*, via the half-life-time-relation) for an average cell-life-time of 10–11 years (in this connection named as: ''type-1 cancer''); other parameters might be chosen to describe other types of cancer

$k_{bM} = 5$[b] [per week and per 100 cells]
$k_{dM} = 6$ [per week and per 100 cells]

$k_H = 0.025$[c]

together with

ADPoI[d] adjusts the DNA-repair-activity to approximately 10-P-diester-links per second (*i.e.*, the known DNA-polymerase-I's working-speed) at ADPoI = 1, *i.e.*, at standard conditions, respectively to ADPoI = 100 for ''fully stimulated'' conditions reaching approximately a speed comparable to DNA-polymerase-III

$k_{cM} = k_{cG}\,\xi^{e}$, while $k_{cG} = 1, 24$; ξ (expressed in %) should indicate the susceptibility of a cells' aggregation to become malignant by a cancerogenic impact

together with:

ACG a measure for the efficiency of a cancerogenic agent
$k_E = 3 \times 10^{-6}$[f]

[a] At an average life-time of 10–11 years $\rightarrow t_{1/2} = 5.5$ a $\rightarrow \ln 2/t_{1/2}$ (according to Eq. (3c) in Haschke [33]) $= \ln 2/5.5 = k_{bH}$; adjusted to the units [week^{-1} and per 100 cells] $k_{bH} = \ln 2/5.5 \cdot 100/52 =$ approx. 0.25; [b] reflecting the higher propagation-rates of cancer-cells than those of normal cells. (k_{bM} should rely in its magnitude to a ratio to k_{bH} to the known speed ratio of DNA-polymerase-III's activity to DNA-polymerase-I's activity, which ratio is in the order of 1000:10 P-diester-links per s); [c] as a measure of the normal kinetic constant describing the speed of the DNA-repair-system including the fraction of mismatches which are/stay reachable by the system (In this value for k_H there are summarized all activities of DNA-Polymerase-I – analogous systems as far as they are contributing to a repair-effect of malignant DNA (*i.e.*, $k_H = k_H^{max} \times$ %M-rep.accessible/100, expressed in a kinetically suitable reciprocal time measure); [d] by the factor ADPoI the stimulation of the repair-system's activity is reflected: *i.e.*, stimulations of enzyme working at low speed plus activating of inactive (because of being not ''ammunitioned'' with nucleotides, *i.e.*, so called: ''sleeping'') enzymes/proteins; during this process it must be also anticipated that by the repair-system-stimulation by the suggested ''ammunition'' with adequate amounts of PUNPs + PYNPs also the DNA-polymerase-I-analogous enzyme's ability to catalyze ''nick-translations as described by *Voet* and *Voet* [1992] is activated: but, however, such a biochemical reaction would kinetically be reflected just in a lower value of the product of multiplication of ADPoI $\times k_H$ – as some of the enzyme's repair-contributing activity is lost by such a process. But as ADPoI in any way is defined as a relative activity-increase-indicator, such a ''side-reaction'' and even its stimulation (if there were any) will not affect the principal results delivered by the model; [e] kinetic standard-speed-measure for the influence of a cancerogenic substance or radiation to the conversion of a healthy cell to a malignant cell; [f] a measure to take into account also feed-back-effects (therefore also ''metastasisation-constant''); (with respect to the second-order of this term, to be adjusted according to $(+\,dN_M/dt)_{\text{by emitted subst.}} = k_E N_M^2$ to be understood as: $(k_E N)\,N = k\,N$ (with $k_E\,N = k$) meaning that compared with a k for 1st-order-terms at a similar magnitude of contribution of this 2nd order term, $k_E = k/N$ – for an actual average N – should be in a magnitude of $k/10^{-6}$

velocity of cells' death (cells' decay), none of such an effect would affect the corresponding kinetic constants because such kinetics were not dependent from precursing formations of similar association-complexes or the like. Nor were it therefore necessary to introduce a "squared *Michaelis-Menten*-effect" for the case of the "bimolecular"-type interaction of healthy cells delivering the repair-system and malignant cells as their "substrate", especially as the corresponding kinetic velocity-equation does already contain an ADPoI as defined above in its velocity "constant".

Following the above mentioned approach, the set of differential equations describing the number of normal somatic cells, respectively the number of malignant cells in an organism (or in the part under observation of it) at each point of time as the result of a pseudo-stationarity at this very point of time resulting from the propagation-speed of such cells minus their dying-away-rate has been developed in the previous paper of this series [33].

Calibration of the Kinetic Constants Used to Describe the Competition of Propagation Respectively Dying of Malignant Cells Among/in Competition to Normal Somatic Cells

All typical parameters are compiled in Table 3.

Results and Discussion

Just for a first check of the model's outputs in its application to known cases:

There was calculated by the model (by the program CANCER.xls) a "zero-case" first: (*Case 0*: *i.e.*, no C.I. (realized by: ACG = 0) and by this describing an organism with no cancer; Fig. 3a.

This kinetic approach delivers a practically not declining number of the healthy (*i.e.*, the normal somatic) cells. (Except the slight effect by the symbolically chosen

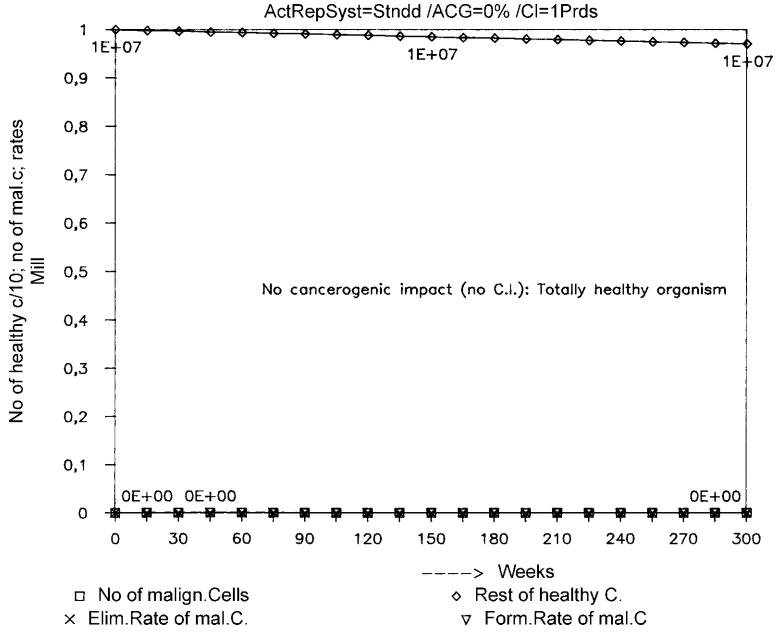

Fig. 3a. *Case 0*: *No Cancerogenic Impact* (*C.I.*) → *No cancer* (healthy-cells: scaled to 1/10)

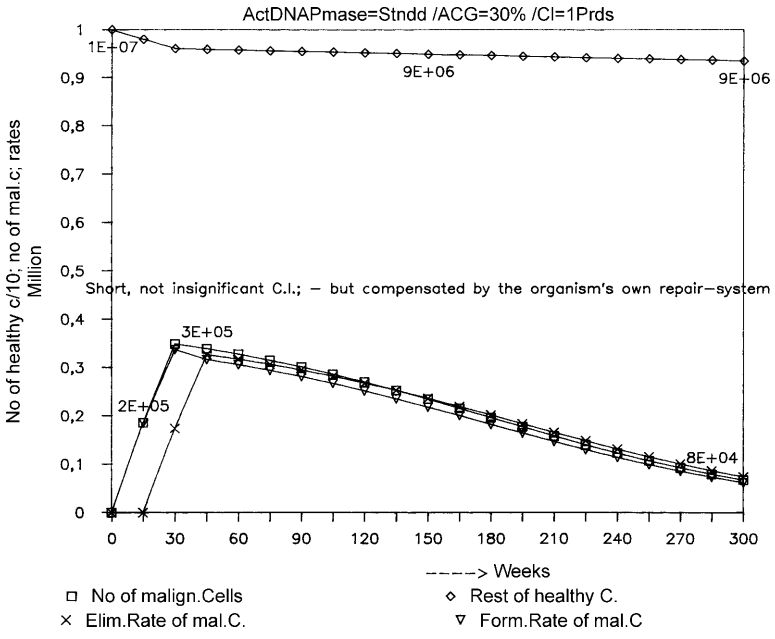

Fig. 3b. *Case 0a*: Significant, but very short C.I. → Endogenically compensated (healthy-cells: scaled to 1/10)

ageing-effect – which should merely be understood as indicating the slow reduction over time of healthy cells with their full functionality).

Further, by the program CANCER.xls there was calculated the case of a not insignificant (ACG = 1/3) but very short (C.I. for only 1 period) cancerogenic impact (which might often occur during the life time of an organism), the C.I. staying undetected, but compensated by the organism's own repair-system ($ADPoI_{abs}$ = standard = 1xE-6: *Case 0a*, Fig. 3b). In this example of a short, but significant cancerogenic impact, the acting of the repair-system is clearly shown, leading at the end to a complete eliminating of any malignant cells which might have been formed intermediately.

For further comparison: the same situation as in case 0a, but with the DNA-proof-reading and -repair-system inhibited ($ADPoI_{abs} = 0$: *Case i*, Fig. 4a (general parameters chosen for all cases mentioned before: time of observation = 300 weeks, i.e., time per period = 15 weeks). This example of *case i* illustrates in analogy to the findings of *Zhang* and *Mathews* [1] a cancer-break-through after approximately 300 weeks (*i.e.*, 6 years) if DNA-proofreading and -repair is inhibited.

The effectiveness of classical cancer-therapies are also well described by the model: Taking as an example *case s*, which describes a typical development of a serious cancer (ACG = 0,3; C.I. = all 20 periods, Fig. 4b) and the effect of a surgical extraction of the tumor formed. The surgical extraction is simulated in the kinetic model by setting N_M to zero in the 8^{th} period, *i.e.*, it is assumed that the tumor is detected and extracted when the number of malignant cells has already reached a significant proportion (in the example $> 10\%$) of the whole organ under observation.

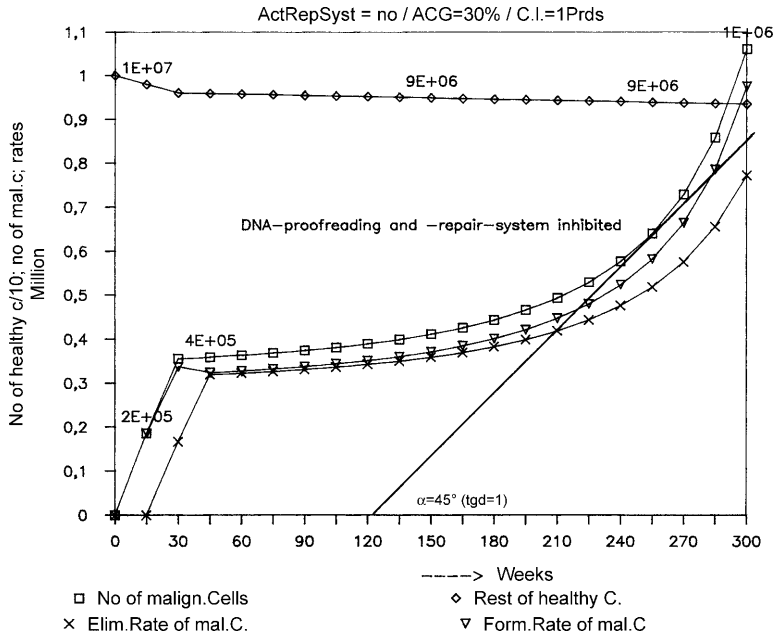

Fig. 4a. *Case i*: C.I. as in *case 0a* (ACG = 0,333; 1 period); *proofreading inhibited* (healthy cells scaled to 1/10)

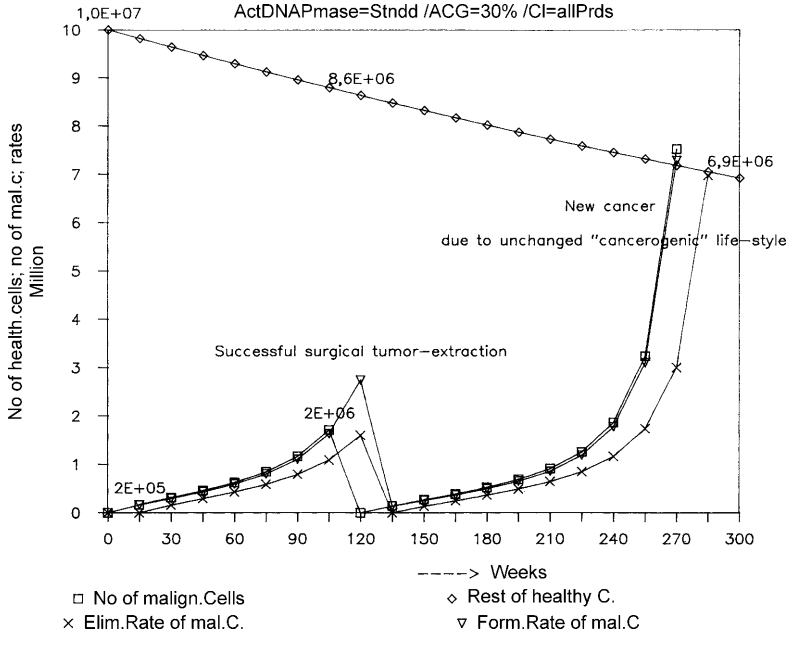

Fig. 4b. *Case s*: ACG = 0,3; all 20 periods (ACG = 0,333; 1 period); *surgical extraction* in 8th period (unchanged life-style) (healthy cells scaled to 1/10)

As a result, the model shows clearly the significant therapeutic effect of the surgical measure; also with a consequent following of the rates of the organism-own formation- and repair-rates for malignant cells going to zero.

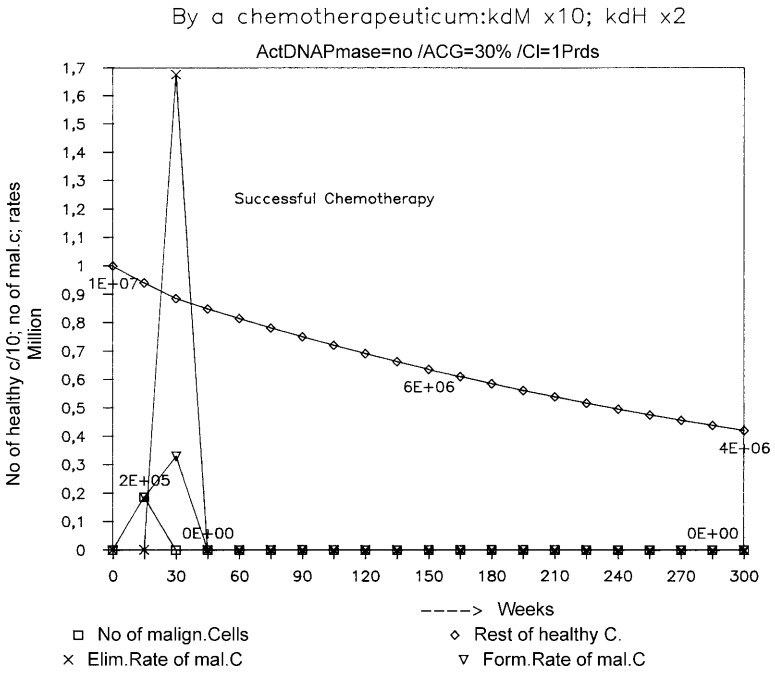

Fig. 4c. *Case c*: C.I. as in *case 0a* (ACG = 0,333; 1 period; proofreading inhibited); *successful chemotherapy* (healthy cells: scaled to 1/10)

However – just for demonstration-purposes – in this special example an extreme case is chosen: Additionally to the description of a normal therapy, it is anticipated that the patient keeps his life-style, which has led to the first cancer-formation, completely unchanged: The model answers with showing the development of another cancer – even after the first one had to be classified as being really healed due to a total success of classical medicinal therapy after approximately 3 years since its development. The other cancer (which is not a rezidive of the first one in this case) is as assumed in this case to be untherapied as a consequence of the patient's belief to be healed for ever – and leading finally to death after approx. 6 years (300 weeks) since the beginning of this case.

Another example is shown in case c, using the same start-values as in *case 0a* (C.I.: ACG = 1/3 for 1 period) and the inhibited situation as in *case i*, both situations together describing *de facto* a typical development of a serious cancer, and inserting strongly increased values for the kinetic constant of the malignant cells' death-rate (k_{dM} increased by a factor 10). This were an example (translated into the physico-chemical/kinetic language of the model) to simulate a chemotherapy, *i.e.*, to simulate the activity of a strong, classical (malignotoxic) chemo-therapeuticum.

Anticipating even that the chemotherapeuticum is highly specific in its maligno-toxicity and therefore its influence against healthy cells leads only to a comparatively moderate increase of the healthy cells' -death-rate (k_{dH} increased by a factor of only 2), the model shows the development for a case of a typical successful cancer-therapy (Fig. 4c).

As a summary, the model does well describe the exponential growth of a population of malignant cells if they are triggered once and if the organism's own proof-reading and repair-systems are inhibited or not efficient enough.

However, it is also shown by the model, that – as it is the typical behaviour of an exponential function – the exponential development of malignant cells (in the case after an induction of mismatches in DNA – by a cancerogenic agent or radiation) leads to a rather long *induction-period*, where no significant number of them and consequently no significant depression of the number of healthy cells are to be observed. By this, a tumor-detection is extremely difficult or even impossible:

The dimension of a (big) cell may be estimated in the magnitude of $10 \times 20 \times 30\,\mu m$, its volume is about $6000\,\mu m^3$; this means that a tumor as soon as it is detectable, *i.e.*, as soon as it has at least approximately pea-size with about 5 mm in diameter or $65\,mm^3$ volume ($=65.10^9\,\mu m^3$), does already contain approximately $6500.10^7/6000 \approx 10$ millions malignant cells, while smaller tumors are practically not detectable by x-ray- or touching-diagnosis nor by mammography.

Now, in the case of an untherapied cancer this induction-period will be followed inevitably by a dramatical increase-phase (the "*explosion-phase*"), as it is also typical for an exponential development of the type $y = e^{n.t}$ at higher values of t (or as it might be defined at a $dN_M/dt = \text{tg}\ \alpha > 1$ of the tangent to the curve; see Fig. 4a), respectively as it happens in this case for a $N_M \sim e^{n.t}$ -function, which finally leads to an overflooding of the organism by malignant cells.

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