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ORIGINAL PAPER

The similia principle: Results obtained in a cellular model system

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This paper describes the results of a research program focused on the beneficial effect of low dose stress conditions that were applied according to the similia principle to cells previously disturbed by more severe stress conditions. In first instance, we discuss criteria for research on the similia principle at the cellular level. Then, the *homologous* ('isopathic') approach is reviewed, in which the initial (high dose) stress used to disturb cellular physiology and the subsequent (low dose) stress are identical.

Beneficial effects of low dose stress are described in terms of increased cellular survival capacity and at the molecular level as an increase in the synthesis of heat shock proteins (hsps). Both phenomena reflect a stimulation of the endogenous cellular self-recovery capacity. Low dose stress conditions applied in a homologous approach stimulate the synthesis of hsps and enhance survival in comparison with stressed cells that were incubated in the absence of low dose stress conditions. Thirdly, the specificity of the low dose stress condition is described where the initial (high dose) stress is different in nature from the subsequently applied (low dose) stress; the *heterologous* or 'heteropathic' approach.

The results support the similia principle at the cellular level and add to understanding of how low dose stress conditions influence the regulatory processes underlying self-recovery. In addition, the phenomenon of 'symptom aggravation' which is also observed at the cellular level, is discussed in the context of self-recovery. Finally, the difference in efficiency between the homologous and the heterologous approach is discussed; a perspective is indicated for further research; and the relationship between studies on the similia principle and the recently introduced concept of 'postconditioning hormesis' is emphasized. *Homeopathy* (2010) **99**, 3–14.

Keywords: Similia principle; Self-recovery; Adaptive response; Symptom aggravation; Postconditioning hormesis; Homeopathy

Introduction

The essence of homeopathy is the stimulation of recovery and healing by the application of substances according to the similia principle. This principle indicates that a substance, which in higher dose causes specific symptoms in a healthy organism, may be applied in a curative sense to a diseased organism showing similar symptoms. In mainstream medicine this therapeutic approach is generally viewed as paradoxical.

Our aim has been to develop a research program at the cellular level focused on the stimulation of defense and recovery processes by application of agents according to the similia principle. This research program was initiated almost two decades ago and includes various different aspects. The results have been described in a number of publications.[1–3]

This overview will focus on three crucial aspects: 1) criteria for research on the similia principle in general and for a research program at the cellular level in particular, 2) the homologous approach in which low dose stress conditions

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support self-recovery in cell cultures that were previously exposed to a higher dose of the same stress condition, 3) the specificity of the similia principle as revealed by the heterologous approach in which the low dose stress conditions are different from the high dose stress condition to which cells were initially exposed.

In addition to verifying the validity of the similia principle at the cellular level, we also investigated phenomena like: cellular 'provings', 'symptom aggravation', and the possible therapeutic superiority of the heterologous (or 'heteropathic') rather than the homologous (or 'isopathic') approach.

Finally, in the discussion we will elaborate on perspectives for further research on the beneficial effect of low dose stress within the framework of the similia principle and the link with the recently introduced concept of 'postconditioning hormesis'.[4]

Criteria for experimental studies on the similia principle

A main starting point of any research program focused on the similia principle is that it should accord with clinical (homeopathic) practice. Therefore, the following criteria should be taken into account and need to be operationalized in experimental protocols.

1. Disturbed state: Healthy organisms must be subjected to moderate disorder or damage.
2. Recovery: In case of mild disorder, recovery must be observed. When their homeostasis is disturbed by stress, biological organisms invariably activate their innate recovery and adaptive capacity to restore homeostasis and to protect against future challenges. Stress responses have been interpreted as purposeful homeostatic reactions.[5] Recovery processes usually show large individual variability in speed and efficiency. Organisms that show sub-optimal development of self-recovery are particularly suitable for research to analyze the stimulatory action of agents applied according to the similia principle.
3. Disease picture: The organism must allow classification of characteristic symptoms observed during the diseased state ('disease picture'). This state may result from disturbing physical, chemical, biological and/or emotional factors. Disease represents a disturbance in the body's ability to heal itself and an intensified attempt to restore a balanced state. According to homeopathy, the patient's symptoms represent the totality of the response to a given insult. If disease is seen as a reactive effort, it is reasonable to interpret symptoms as a manifestation of this beneficial reactive phenomenon. Note that detailed analysis of the 'disease picture' is essential for later steps.
4. Matching of 'disease-' to 'remedy-picture': To select an effective remedy, the 'disease picture' is matched to a 'remedy picture', i.e., the symptoms provoked by a remedy or a (high dose of a) stressor applied to healthy organisms. These symptoms must therefore be known in order to select an appropriate remedy for curative actions as indicated by the similia principle. For research purposes, it should be possible to calculate the degree of similarity between the symptoms expressed in the diseased state as well as those provoked by compounds in 'provings' (or 'pathogenetic trials') to obtain the 'remedy pictures'.
5. Accepted outcome parameters: Finally, the effect of an application of the selected treatment on recovery should be evaluated using clear-cut parameters. These parameters should refer not only to the regulatory processes underlying the stimulation of recovery (such as endogenous repair, maintenance and defense processes), they should also be described in terms of mainstream biomedical knowledge.

In order to perform fundamental research on this complex sequence of damage-disease-treatment-effect, in principle any biological system (cell, organ, plant, animal, human) can be used provided that it can be brought in a diseased/disordered state from which it should be able to recover. The process of recovery from an insult can then be modulated by application of compounds according to the similia principle and analyzed using molecular and functional parameters.

A research program at the cellular level

Our research program at the cellular level meets most of the above-mentioned criteria to study the similia principle, since:

Disturbed state: In cells, well-defined states of disturbances can be induced and the subsequent process of self-recovery can also be studied. Highly specific disturbances of cellular organelles and of biochemical pathways can be induced causing particular responses. General disturbances can also be induced at the macromolecular level (such as at the level of DNA, lipids or proteins) leading to more general responses. In our research we have chosen to study '*proteotoxicity*', originally defined by Hightower[6] to indicate the detrimental action of denatured proteins in cells.

Denatured proteins expose hydrophobic parts that cause aggregation of other proteins. These aggregates interfere with normal cell function leading to an array of problems. Proteotoxicity is a phenomenon of increasing interest in biomedical disciplines, since it occurs after a variety of stress conditions including heat shock (HS), ischemia, free radicals (oxidative stress), hypertension, inflammation, pathogens such as viruses and bacteria, as well as after ingestion of environmental pollutants such as heavy metals.

Damaged or denatured proteins are increasingly recognised as crucial factors in the development of various chronic diseases including neurodegenerative, atherosclerotic and diabetic diseases as well as in the process of aging.[7–11] In experimental studies, protein damage can be induced to different degrees using a variety of stress conditions.

In our studies, protein denaturation was induced using both physical (such as a HS) and several chemical stressors. Cells were exposed to different strengths of stress conditions by varying dose and time in order to select both the harmful (high dose) and the curative low dose effects. We use Reuber H35 rat hepatoma cells since many characteristics have been described from this well-differentiated and stable cell line and it has shown to be very useful in cellular stress research.[12,13]

Recovery: An important underlying premise of the similia principle as therapeutic strategy is that a disturbed/diseased biological system activates its innate (endogenous) self-regulatory capacities (often called 'self-recovery') to regain health and increasing the probability of survival. The symptoms expressed during this process are generally thought to reflect active self-recovery processes including the repair of macromolecular damage.[2,11,14]

In response to proteotoxicity, cells react with an up-regulation of heat shock proteins (hsps). These proteins function primarily as molecular chaperones, facilitating the folding of other cellular proteins. In repair situations, they repair structural damages by forcefully disentangling aggregated proteins, unfolding and refolding them into 're-educated and born again' functional proteins, or targeting improperly folded proteins to specific pathways for degradation. The role of chaperones in enhancing stress tolerance and in increasing the survival capacity is well established.[8,15,16] There are different families of these proteins (known as hsp28, hsp60, hsp68, hsp70, hsp84 and hsp100) that are differentially synthesized in response to various stress conditions. We evaluated activation of the molecular stress response and the increased survival capacity using standard procedures.[2,17]

Disease picture: Following exposure to a variety of stress conditions, characteristic alterations have been described at the molecular level as well as in cellular morphology. A short exposure to elevated temperatures causes immediate changes in the stability of the cytoskeleton, decreases the synthesis of proteins in general, enhances the synthesis of hsps in particular, decreases DNA replication and results in enhanced sensitivity to additional stress.

These cellular phenomena reflect both damage and recovery processes. Enhanced synthesis of hsps is generally interpreted as reflecting the beneficial adaptive process required for recovery of cellular homeostasis: increased synthesis of hsp70 has been defined as a positive cellular symptom that can be used as molecular marker of enhanced cellular survival capacity. The relation between stress induced synthesis of hsps and the capacity to survive stress has been the subject of numerous reviews.[15,16,18]

The phenomenon of enhanced sensitivity when homeostatic balance is disturbed merits further attention. Hahnemann noted that patients are more sensitive (hypersensitive) to a similia medication (similar) than healthy people. Administration of a similar remedy may augment the patient's existing symptom pattern, labelled by Hahnemann as an 'aggravation' of the symptoms. The concepts of symptom aggravation and hypersensitivity was rediscovered in 1891 by Robert Koch who noted that tuberculin could be injected in considerable quantities into normal animals, while tuberculous animals reacted violently, even to small doses, some dying within hours. Hypersensitivity was initially associated with infectious disease. Later, the hypersensitive state came to be understood as the body's reaction to any external insult, not just to highly toxic or infectious substances. Selye[5] formulated a phase of enhanced sensitivity following stress and during the initiation of the adaptive response. At the cellular level, sensitization was originally described using the so-called step-down protocol in which cells are exposed to an initial heat treatment immediately followed by a second treatment of lesser degree.

A sensitizing effect has been observed in all *in vitro* and *in vivo* studies.[18] Sensitization is usually followed by development of reduced sensitivity (known as 'tolerance'). The state of enhanced sensitivity, reflecting the disturbed state of the living system, is an essential characteristic of our model. We observed it following exposure to a HS,[19] to arsenite[20] and to cadmium.[21] It offers the opportunity to study the stimulation of self-recovery by low doses of stressor since, due to this enhanced sensitivity, cells react to stimuli to which they normally would not react.

Matching of 'disease picture' with 'remedy picture': Cellular responses induced by different stress conditions can be taken as symptoms aimed at, or involved in, cellular recovery. The stressor-specific induction of the different hsps has been analyzed as molecular symptoms (in order to define the 'remedy picture' at the cellular level). For our research program it was of crucial importance that the various hsps are differentially induced depending on the type of stress condition to which cells are exposed.[17,22]

Based on both the type of induced hsp as well as the degree to which they are induced, an overlap between the 'disease picture' and the 'remedy picture' could be established. This allowed us to determine the degree of similarity between the (high dose) stress conditions used to induce the 'diseased state' and the (low dose) stress conditions used to improve self-recovery processes. Based on the degree of similarity, the efficiency of stimulation of recovery by specific low dose stress conditions could be predicted and experimentally evaluated.

Generally accepted outcome parameters: Parameters to study cellular recovery processes should include both molecular and functional aspects. The presence or enhanced synthesis of stress proteins is of crucial importance, these are involved in both recovery as well as in the building-up of defense (adaptive response), which can be evaluated in terms of cellular survival values under threatening conditions. The method to establish a cell's survival capacity (as functional parameter) is based on its colony-forming ability and has been described before.[2,20]

Steps in research

The protocol to study the beneficial effect of low dose stress applied according to the similia principle includes that cells were first exposed to a harmful (high dose) stress condition. This condition was carefully selected in order to prevent the occurrence of much irreversible damage or compromised resilience. Both survival capacity and the pattern of induction of hsps were identified. This allows a possible increase in hsp synthesis and/or in survival capacity when cells are subsequently exposed to a low dose of the same or similar stress condition to be evaluated. The actual increase in survival capacity was evaluated using a final stress condition. The experimental protocol is shown in Figure 1.

Our research on therapeutic effects has been divided in two main parts; a homologous and a heterologous one.

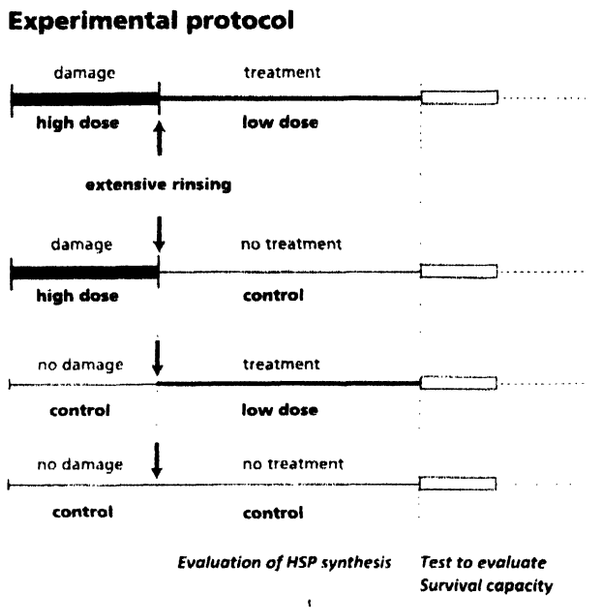

Figure 1 Schematic presentation of the experimental protocol to study the similia principle. Initial high dose damaging stress condition is followed by extensive rinsing and subsequent application of low dose stress condition. Evaluation of hsp synthesis occurs during low dose exposure. Evaluation of survival capacity takes place at the end of the low dose treatment by exposure to an additional high dose stress condition. The various control conditions are indicated.

The homologous strategy (also called 'isopathic' approach) is the most elementary version of the similia principle, since the stress condition used in a low dose to cure is the same as the high dose which disturbs the biological system. We first focused on the effect of a HS followed by a fever-like condition. Later studies have focused on cells initially exposed to 'damaging' doses of arsenite or cadmium, followed by exposure to diluted doses of arsenite or cadmium, respectively. The aim is to determine whether the intrinsic capacity of a cell's defense against harm can be stimulated with a diluted dose of the disturbing agent.

In the heterologous strategy (or 'heteropathic' approach) different substances or stress conditions are used for cure and initial disturbance of cell cultures. The main question refers to the specificity of the stimulation of self-recovery: whether the degree of recovery by low doses is related to the degree of similarity between the conditions used as disturbance and cure.

We used dilutions which were not submitted to a specific potentiating process but were diluted and vortexed according to usual laboratory procedures. Nor did we use dilutions beyond Avogadro's number. The reasons for this choice were that: 1) a large percentage of remedies used in homeopathic practice is in the concentration range of milli- to micro-molarity, 2) remedies used in homeopathic practice are not always potentized in the same way or to the same degree, 3) the use of agents in non-potentized form as low dose stress condition is in agreement with the use of non-potentized remedies in the early years of homeopathy.[2]

Results of homologous studies (isopathic approach)

Schamhart *et al.*[19] exposed cells initially for 30 min at either 42°C or 43.5°C and subsequently incubated them at mild (fever-like) temperatures (38–41°C) for 6 h. The survival capacity was then tested by exposing cells to a test treatment of 43.5°C for 1 h.[19,23] When cells were initially heat shocked at 42°C, a posttreatment of 40°C enhanced the survival capacity compared to cells that were exposed to 42°C and post-treated at 37°C. With this mild postconditioning, heat treatment appeared to be beneficial following the 42°C heat treatment, but it depressed the survival capacity of cells that had been exposed to 43.5°C pretreatment. Interestingly, the exposure of untreated cells to 40°C for 6 h was without effect on survival capacity.[23]

Schamhart *et al.*[19] were the first to study whether the synthesis of specific stress proteins (hsp28, hsp60, hsp68, hsp70, hsp84 and hsp100) induced by a 30 min HS at 42°C was influenced when the heat-shocked cells were subsequently exposed to a lower hyperthermic temperature for a period up to 8 h. In their analysis, they compared the expression of hsps in this postconditioning situation with the expression of the various stress proteins in cells after a HS at 42°C followed by incubation at 37°C. The production of these proteins was found to increase to a larger extent when cells heat shocked at 42°C were subsequently incubated at 41°C. Hsp28 and hsp68 demonstrated a particularly interesting behavior. They were not induced at 41°C and only to a small degree after the HS at 42°C. However, the combination (42°C followed by 41°C) caused a significant induction.[19] Similar observations were reported for mRNA levels of hsp68 and hsp84 when heat-shocked cells were post-exposed to 40°C and 41°C.[23]

These observations were supported by the work of Delpino *et al.*[24] who demonstrated enhanced synthesis of stress proteins and enhanced survival capacity due to step-down heating.

In essence, we made the same observations when cells were damaged with chemical compounds such as arsenite or cadmium and subsequently exposed to low dose conditions of arsenite or cadmium respectively. In case of homologous (isopathic) exposure, both hsp synthesis as well as defense and survival capacity were enhanced. These results are described in Wiegant *et al.*[20,21] and in Ovelgönne *et al.*[25] (Table 1).

The phenomenon of enhanced sensitivity and 'symptom aggravation', in terms of enhanced stress protein production, was observed in all three conditions we examined. The high dose stress induces a sub-optimal level of hsp synthesis. Subsequent incubation of cell cultures in the presence of the low dose stress condition results in an enhanced synthesis of stress proteins. It should be noted that without high dose pretreatment, the low dose stress condition did not induce a detectable synthesis of stress proteins. A representative example is shown in Figure 2.

In summary, using three different stress conditions, this line of research clearly demonstrates the biphasic action of a substance. A small dose can exert a stimulatory effect on

Table 1 Overview of the research program on the similia principle at the cellular level (for which H35 cells were used)

Approach	*High dose (pretreatment)*	*Low dose (posttreatment)*	*Results*	*Reference*
Homologous	Heat shock (HS)	hs ('fever'-like condition)	- Enhanced synthesis of hsps due to posttreatment - Thermo-sensitization due to pretreatment - Delayed recovery of protein synthesis due to posttreatment	Schamhart *et al.*[19]
Homologous	HS (30′ 41.5°, 42.5° or 43.5° C)	hs (40° or 41° C up to 6 h)	- Enhanced synthesis of hsp–mRNA (hsp68, hsp84) - Enhanced development of survival capacity against heat test treatment (60′ 43.5° C) due to posttreatment	Van Wijk *et al.*[23]
Homologous	HS	hs	Delayed recovery of luciferase activity due to posttreatment	Souren *et al.*[27]
Homologous	Arsenite (As) (1 h 100–300 μM)	arsenite (as) (1–10 μM up to 9 h)	Arsenite sensitization due to pretreatment	Wiegant *et al.*[20]
Homologous	As (1 h 100–300 μM)	as (1–10 μM up to 8 h)	- Delayed recovery of total protein synthesis due to posttreatment - Enhanced & prolonged synthesis of hsps due to posttreatment - Enhanced synthesis of hsp mRNA's due to posttreatment - Enhanced development of survival capacity against arsenite test treatment (2 h 300 μM) due to posttreatment	Ovelgönne *et al.*[25]
Homologous	Cadmium (Cd) (1 h 10 or 30 μM)	Cadmium (cd) (0.01–1 μM up to 10 h)	- Cadmium sensitization due to pretreatment - Enhanced & prolonged synthesis of hsps due to posttreatment - Enhanced development of survival capacity (higher resistance) against a test treatment (2 h 50 μM Cd) due to posttreatment	Wiegant *et al.*[21]
'Provings'	HS, As, Ethanol, Cd, Dinitrophenol		Stressor-specific synthesis of hsps, which provides the opportunity to identify 'remedy pictures' at the cellular level	Wiegant *et al.*[17]
'Provings'	HS, As, Cd, Cu, Hg, Pb, Men, DDTC		Stressor-specific synthesis of hsps	Wiegant *et al.*[3]
Heterologous	HS, As, Cd	hs, as, cd	- Enhanced synthesis of hsps in homologous (HS–hs; As–as; Cd–cd) as well as in heterologous conditions (HS–as; HS–cd; As–hs; As–cd; Cd–hs; Cd–as). Homologous appears to be more efficient. - Pattern of enhanced synthesis of hsps resembles that of the 2nd stressor applied as low dose (phenomenon of 'syndrome shift')	Wiegant *et al.*[31]
Heterologous	HS (30′ 42° C)	hs, as, cd, cu, hg, pb, men, ddtc	- Enhanced & prolonged synthesis of hsps due to posttreatment correlates with similarity in hsp induction pattern ('proving') of high and low dose stress condition - Enhanced development of survival capacity against heat test treatment (30′ 43.5° C) due to low dose posttreatment depends on the similarity in stressor-specificity of hsp induction - Pattern of enhanced synthesis of hsps resembles that of the low dose stressor which supports the phenomenon of 'syndrome shift'	Wiegant *et al.*[3]

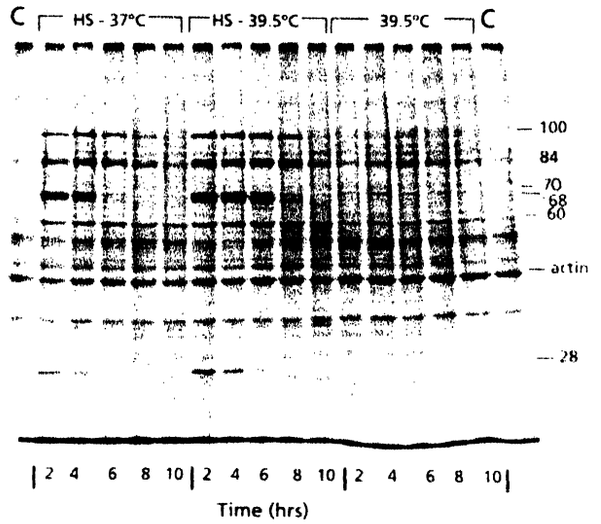

Figure 2 Induction of synthesis of hsps in cell cultures pretreated with a HS of 42.5 C for 30 min, followed by a continuous treatment at the control temperature (HS-37 C) or by a mild hyperthermic temperature (HS-39.5 C) for 10 h. In addition the effect of incubation at 39.5 C without previous HS is shown (39.5 C). At various times following the pretreatments, the cultures were incubated with [^{35}S]-labelled amino acids for 2 h periods (0–2, 2–4, 4–6, 6–8 and 8–10 h). Subsequently samples were prepared for one-dimensional gel-electrophoresis. Also shown are the polypeptide compositions of control cells kept at 37 C. The different hsps are indicated on the right with their molecular weight in kD. Note that different hsps increase in density as cellular 'symptoms' following HS (HS-37 C) and that in case of low dose posttreatment (HS-39.5 C) these hsps are more pronounced and are present for a longer period, representing 'symptom aggravation'. Note that the low dose stress condition itself (39.5 C) hardly induces 'symptoms'.

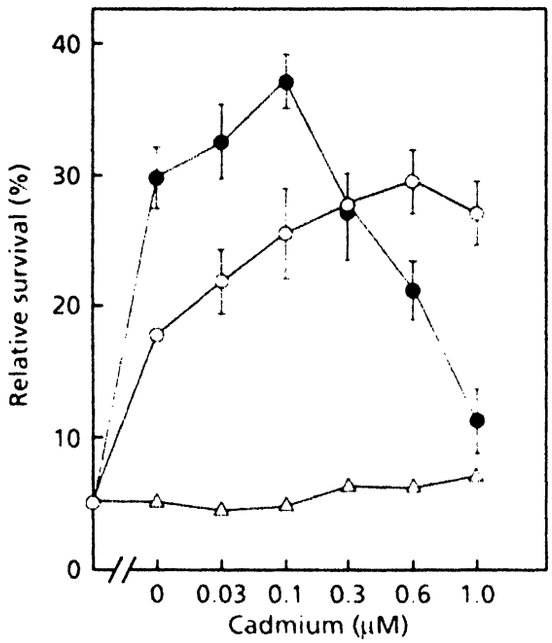

Figure 3 Level of tolerance (survival capacity) induced by step-down cadmium treatment. Survival percentage is shown of cultures which were pretreated with either 0 μM (open triangles), 10 μM (open circles) or 30 μM (closed circles) cadmium chloride for 1 h, followed by a 10 h low dose posttreatment with either 0, 0.03, 0.1, 0.3, 0.6 or 1.0 μM cadmium, and finally exposed to a test treatment with 50 μM cadmium for 2 h. Results were corrected for pretreatment survival values. (Adapted from: Wiegant *et al.*[21]).

the recovery and the development of survival capacity of cells that have been previously disturbed by a high dose of the same substance. It is of interest that this stimulatory effect of low dose stress is dependent on the initial exposure condition. The more severe the initial stress conditions, the smaller the concentration required for stimulating survival and hsp induction. However, small doses can unexpectedly merge into the harmful range, especially when the initial stress condition has been severe. An example of this phenomenon is shown in Figure 3. Generally, the initial exposure has an influence on survival capacity (as measured in terms of tolerance development) which is also stimulated in a dose dependent manner.

These observations in the homologous approach are in agreement with the "Law of Initial Values" formulated by Wilder.[26] This law states that the response of a (cellular) function to any (outside) agent depends to a large degree on the "initial level" of that function at the start of the experiment. According to this law, the higher the initial stimulus, the smaller the response to a 'function-raising' substance and the greater the response to 'function-depressing' agents. Conversely, the lower the 'initial level', the greater the response to function-raising agents and the lesser to function-depressing ones.

Molecular explanation of the sensitized state and the beneficial low dose effect

With respect to the molecular background, the question was raised 1) why 'diseased' cells react to low dose stress to which they would not react in normal, control conditions and 2) how this stimulatory effect on synthesis of hsps and the enhanced survival capacity can be explained.

Sensitization has been explained as a consequence of shortage of available hsps in the cell to deal with all the denatured proteins induced by the high dose stress.[2] The large amount of denatured proteins are detected by cellular quality control mechanisms, and all available hsps recruited to deal with them, leading to a shortage of hsps. When cells are exposed under these conditions to an additional stress challenge, a lack of available hsps is reflected in enhanced sensitivity. Due to signal transduction feedback mechanisms, additional synthesis of hsps is induced, supporting recovery and defense processes.

With respect to an explanation of the stimulatory effects of low dose stress applied according to the homologous approach, the question was asked whether the low dose stress intensifies the trigger for the induction of hsp synthesis. Studying the activation of the heat shock factor (HSF), being the main transcription factor responsible for the expression of HS genes and production of hsp mRNA.

Ovelgönne *et al.*[25] were able to show that the low dose stress applied following the higher dose indeed exerted this stimulatory action. Fever-like hyperthermic conditions

lead to a prolonged presence of an activated form of HSF as well as an enhanced level of hsp mRNA in cells that were previously heat shocked, which explains the prolonged induction and synthesis of hsps shown in Figure 2.[25] The prolonged activation of HSF in the presence of low doses might be explained by the possibility that renaturation of aberrant proteins is hindered by the presence of low doses of stressors during the period of enhanced sensitivity. Souren *et al.* [27] indeed demonstrated that the presence of inactivated proteins in cells can be prolonged by application of a low dose stress when applied following the damage-inducing high dose stress condition, even if this low dose condition did not cause any denaturation of proteins in healthy cells. Furthermore, their studies demonstrated that reactivation of heat denatured proteins play a crucial role in the modulation of induction and synthesis of hsp70.[28] Based on these studies it is suggested that low dose stress may prolong the signal responsible for an ongoing synthesis of hsps.

The enhanced level of hsps may represent an advantage leading to a prolonged and more sustained recovery process at the cellular level and thus be responsible for the observed beneficial effect of low dose stress. In agreement with this is the observation that an overproduction of molecular chaperones following treatments with resveratrol or sodium salicylate is responsible for the reduction of damage induced by reactive oxygen species as well as for a reduction of induced programmed cell death in various damaging contexts.[29]

Results of heterologous studies (heteropathic approach): the specificity of low dose stimulation

To evaluate the specificity of the recovery enhancing effect of low dose stress, the question was asked whether survival capacity and hsp induction can also be stimulated by low doses of (non)-related stress conditions.[2,3,31]

Stressors and their response patterns; 'provings' at the cellular level

In homeopathy, the selection of a remedy is based on the overall symptom pattern of the patient and includes subjective as well as objective symptoms. However, not all symptoms are equally important. A symptom like headache, for example, is too general to be of any value. Likewise in a cell model not all changes in cell structure, metabolism and physiology observed following stress exposure are useful 'symptoms'. A change in cell morphology for instance is, like headache, rather general. In contrast, the pattern of induced stress proteins is more specific and has been selected in our research program as molecular symptoms at the cellular level, to analyze the specificity of the similia principle (see also Table 1). The pattern of induced hsps was considered the sole indication to direct research as to the choice of a low dose agent. The major stress proteins (both hsps and glucose-regulated proteins [grps]) were selected and quantified in order to compare the response patterns and to determine the degree of similarity between the different stressors used.[3] The response in terms of selective induction of specific stress proteins is highly variable, leading to qualitative as well as quantitative differences (Figure 4). Qualitative in the sense that different sets of proteins are induced by different stressors. For instance, lead (Pb) induces the grps (grp78/ grp94), which are not induced by HS, other heavy metals or oxidative stressors. Only arsenite induces grp94 slightly. HS and arsenite induce hsp60, whereas cadmium and ddtc do not. Quantitative in the sense that one 'symptom' (for instance hsp28) is induced to a different degree by various stress conditions (Figure 4).

The determination of the degree of similarity

To this end, one particular stress condition was taken as standard; all the other stress conditions were then compared to this. We selected HS as the standard condition. To determine the degree of similarity in response patterns, the density of each of the bands (hsps and grps) was quantified and compared to the density of the respective band in the standard condition. The ratio was calculated in such a way that a percentage between 0 and 100% was obtained for each of the analyzed proteins. Subsequently, the mean of these

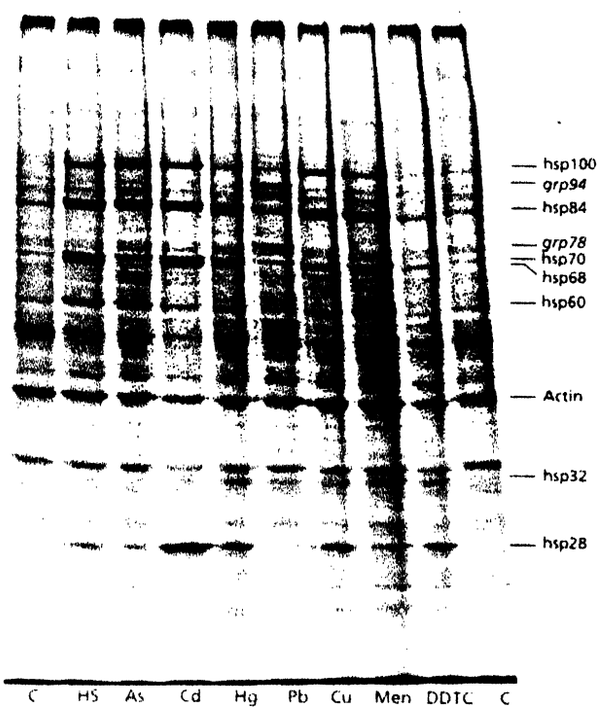

Figure 4 The patterns of synthesized proteins in H35 hepatoma cells in response to different stressors, representing 'provings' or 'symptom pictures' at the cellular level. Stressor conditions were selected that had a similar impact on inhibition of protein synthesis (20–30% over an 8-h period) (HS at 42°C for 30 min; and chemical stressors for 1 h: 100 μM arsenite; 10 μM cadmium; 200 μM mercury; 400 μM lead; 2 mM cuprum; 100 μM menadione and 0.5 mM diethyldithiocarbamate (ddtc)). Exposure to these stressors was followed by monitoring of incorporation of [^{35}S]-labelled amino acids over 8 h. The degree of similarity between stress conditions was calculated using the density of the different bands representing the hsps and the grps which are indicated by their molecular weight in kD. Results are from a representative experiment. (Adapted from: Wiegant *et al.*[3]).

percentages was calculated to obtain the overall percentage of similarity of the stressor pattern with the HS-induced pattern. The mean similarity between the response patterns [based on the major hsps (hsp28, 32, 60, 68, 70, 84 and 100) and on the major grps (grp78 and 94)] ranged from 21.7% (ddtc) to 69.7% (arsenite) with 100% similarity between initial high dose HS and subsequent low dose heat treatment. For a more detailed description see:[2,3]

In conclusion, these data demonstrated that different stress conditions induce a characteristic set of molecular symptoms (qualitatively and quantitatively). Analogous to the symptoms that an agent is able to induce in healthy biological systems, the stressor-specific patterns of induced hsps can be considered 'remedy pictures' at the cellular level (i.e., the set of characteristic (molecular) symptoms an agent is able to induce in healthy cells).[2,3]

The ability to quantify the 'overlap' between disease picture and remedy picture, a crucial prerequisite to study the similia principle, has now been met. Once this degree of similarity was established, the research program was at the stage to study the specificity of low dose stimulation, and could thus focus on the following questions:

1) Is the development of cellular survival capacity after exposure to stress A only stimulated in a homologous way (i.e., by small doses of A) or, is it also stimulated in a heterologous way by small doses of other stress conditions such as B and C?
2) If heterologous stimulation occurs, does the degree in which survival capacity is stimulated (induced tolerance for the stress condition) correlate with the degree of (non)similarity between the symptom pattern manifested by a diseased cell and the remedy picture of the small dose stress applied?

Modulation of HS-induced development of survival capacity

To characterize a possible low dose stimulatory effect due to different stress conditions, the following experimental conditions were required: (a) a high dose stress that initiates a non-optimal self-recovery and defense and allowing for an enhancement of survival capacity by subsequent treatments; (b) low dose stress conditions that are just below the inducing limit of an effect on survival capacity or hsp induction in naïve cell cultures; and, (c) the combination of pre-exposure and subsequent treatment with low dose stressors that allow determination of synergistic effects on survival capacity. Research data regarding these steps is described below as well as in Table 1.

(a) Selection of a HS as high dose pre-exposure condition: As pretreatment condition a HS was selected. For several reasons, HS is an attractive stress condition for experimental research. Unlike chemicals, there are no problems associated with the uptake or compartmentalization of the stressor. The temperature reached in the cell is equal to that in the media surrounding the cells. After lowering the temperature, an immediate normalization of the ambient temperature occurs. In contrast, removal of a chemical compound does not result in an immediate release of the accumulated moiety, but rather a gradual release into the medium.

Pre-exposure HS was thus selected to stimulate sub-optimal development of thermotolerance without substantially affecting actual cell survival. The increased survival capacity under hyperthermic conditions was then determined 8 h after pretreatment by exposing cell cultures to a more severe heat-treatment test (43.5°C for 30 min). Only 8% of non-preheated control cells survived this treatment. Exposure to 40° or 41°C for 30 min applied 8 h prior to test treatment did not significantly enhance survival. Pre-exposure at 42.5°C for 30 min led to a near optimal degree of thermotolerance (evaluated after 8 h). An intermediate, sub-optimal increase in the capacity to survive the test treatment was observed when cells were pre-exposed to a HS at 42°C for 30 min. Therefore, this condition was selected as a pre-exposure condition to further evaluate the stimulatory action of small doses of chemical compounds.[3]

(b) Selection of low dose stress conditions: To test the capacity of a low dose chemical stressor to modulate the HS response, 8 different stress conditions were selected, which included a mild hyperthermia, arsenite, several heavy metal ions (cadmium, mercury, lead and copper) and two different oxidative stress conditions (menadion and ddtc).[3]

The low dose stress conditions should not have any effect on survival capacity, nor on the synthesis of stress proteins during the 8-h time period studied. The selected conditions were: a temperature of 39.5°C (hs), 1 μM arsenite (as), 0.3 μM cadmium (cd), 0.1 μM mercury (hg), 10 μM lead (pb), 10 μM copper (cu), 20 μM menadione (men) and 1 μM diethyldithiocarbamate (ddtc). When these were evaluated, no significant effect was observed after 8 h of exposure to the small dose conditions.[2,3]

(c) HS followed by small dose application of different chemical compounds: Once the required conditions have been selected, the crucial experiments in which HSs were followed by one of the selected low dose stress conditions were conducted.

The question is whether the heat-induced development of survival capacity (measured as development of survival capacity towards a lethal HS) is stimulated by application of low doses of various stressors and if so, whether its degree of stimulation correlates with the degree of similarity. As mentioned above, the selected low dose conditions did not significantly affect the thermotolerance of control cultures. However, when the various low dose stressors were applied after a pre-exposure to HS, the survival capacity of these heat-shocked cultures increased. This scale of increase appeared to depend on the nature of the low dose stressor.

Cells pre-exposed to HS without any subsequent low dose treatment had a survival rate of 34%. When the pre-exposed cultures were subsequently treated for 8 h at 39.5°C (instead of 37°C control), this was enhanced to 66.5%: a 'survival stimulation factor' of '1.95' increase. This factor (the ratio of relative survival with low dose treatment versus HS only) represents the degree of stimulation of survival capacity by low doses of stressors. Low doses of arsenite

enhanced the HS-induced survival capacity to a relatively high degree (1.65 ± .24). In contrast, lead, ddtc and cadmium demonstrated a relatively low increase (1.17 ± .04, 1.11 ± .14 and 1.10 ± .15, respectively). Intermediate values were obtained with small doses of mercury, copper and menadione (1.25 ± .16, 1.38 ± .17 and 1.45 ± .19, respectively).[3]

Correlation between similarity and stimulation of survival capacity

The question was then asked whether the stimulation of additional survival capacity was correlated with the degree of similarity between the symptom patterns of heat-shocked cells ('disease picture') and the 'remedy' picture of the compounds that were applied in low doses? The null-hypothesis was that *"the degree of similarity between the effect of the first and second stressor is not related to the degree of tolerance stimulation"*.

As depicted in Figure 5, the survival stimulation factor (the stimulation of additional tolerance development) plotted against the degree of similarity demonstrates a highly significant correlation ($r = .898$; $p < .001$). In general, the synergistic action observed with the combination HS-hs or HS-as (characterized by high degrees of similarity) was larger than the combination HS-ddtc or HS-pb (characterized by lower degrees of similarity). Cadmium was an

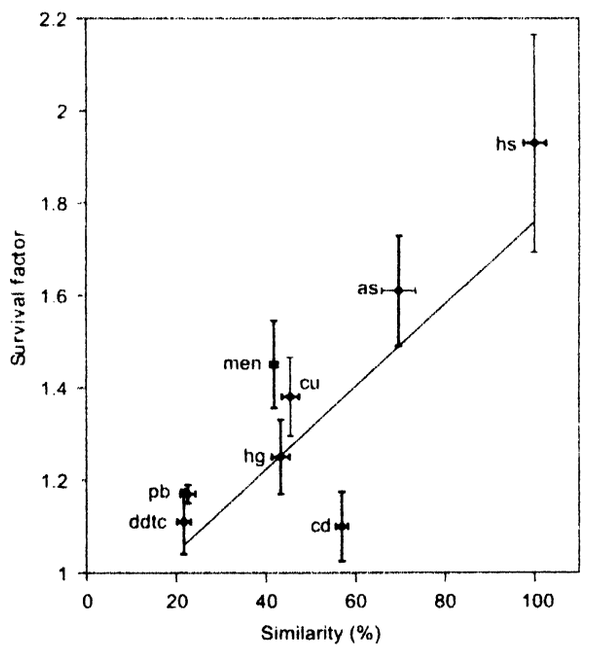

Figure 5 Relationship between the degree of similarity and the survival factor. The percentage of similarity was calculated between the effect of HS as high dose stress condition (30' at 42 C) and the subsequent stress conditions applied as low dose conditioning. Results are the mean of 2 experiments (± standard error of the mean). (Adapted from: Wiegant *et al.*[3]). The effect of an 8 h exposure to the following low dose conditions are indicated: 39.5 C (hs); 1 μM arsenite (as); 0.3 μM cadmium (cd); 0.1 μM mercury (hg); 10 μM lead (pb); 10 μM copper (cu); 20 μM menadione (men) and 1 μM diethyldithiocarbamate (ddtc). The selected low dose conditions were those at the limit of detectable effect on the synthesis of stress proteins with 8 h incubation.

obvious exception to this relationship between similarity and stimulation of tolerance development. Despite its relative high percentage of similarity (56.9%), a survival factor of only 1.10 was observed. Nevertheless, it was concluded that, in general, a higher percentage of similarity predicts a higher stimulation of tolerance development and survival capacity. The null-hypothesis is not supported by our data. To our knowledge this is the first time that unequivocal experimental support has been reported on the similia principle at the cellular level.[3]

To explain the variability of small dose stimulatory action on heat-shocked cells, it was hypothesized that such conditions only induce an increased survival stimulation factor if they are able to stimulate the specific endogenous defense and recuperative mechanisms required by damaged cells. Since stress proteins are viewed as a reflection of the initiation of endogenous defense at the molecular level, the research evaluated whether observed differences in stimulation by small doses was related to the specificity in the overall pattern of hsps. Only those chemical stress conditions that were able to induce similar hsps or a similar pattern of hsps to HS can be considered capable of stimulating heat-induced synthesis of individual stress proteins. Indeed a significant correlation was observed for most hsps between enhanced additional synthesis due to low dose stress ($p < 0.001$) and the degree of similarity between the inducing effect of individual hsps by high and low dose stress.[3]

Is the pattern of enhanced stress proteins determined by the pre-exposure or by the small dose treatment?

We asked whether additional stress protein synthesis is induced with a pattern typical for: a) the first stressor (HS), or b) the pattern characteristic for the secondary low dose stress condition? For this analysis, we used potential hsp patterns that different stressors could induce. To obtain the additional stress protein patterns, the amounts of different synthesized stress proteins were corrected for the values observed in cultures that only received the HS pretreatment. This provided the synergistic effect of the low doses for each stress protein.

No significant correlation was observed between the pattern of low dose enhanced hsp synthesis and the HS-induced pattern ($r = .111$; n.s.). However, a significant correlation was documented with the pattern of additional hsps synthesis induced by the posttreatments ($r = .621$; $p < .001$). Thus, the pattern of additional hsps production is determined by the low dose stressor. These results confirm previous observations in a smaller number of stress conditions.[30,31]

It can be concluded that during the period of enhanced sensitivity, cells react to stressors applied in low dose, to which they would normally not react, and that the stimulation of recovery processes depends on the similarity in effect between high dose and low dose stress condition. Further, the response in terms of enhanced pattern of synthesized hsps is determined by the low dose stressor. The latter observation of a shift in response upon low dose stimulation may have interesting possibilities to study the phenomenon of 'syndrome shift' which is known in

homeopathy as the phenomenon that 'chronic illness may heal after, or being displaced by, acute illness'.

Loss of sensitivity for low doses of homologous and heterologous stress conditions

An initially enhanced sensitivity towards a second application of the same stressor is followed, as part of the recovery process, by a reduced sensitivity (desensitization or tolerance) at a later time. The biphasic change in sensitivity was observed after heat treatments as well as after treatments with arsenite[20] or cadmium.[21] The development of tolerance is usually monitored as cell survival, but it also carries consequences for stressor-induced synthesis of protector proteins like the hsps. During and after the period of heat-induced increased synthesis of hsps, a state of refractoriness (or tolerance) in terms of further induction of the synthesis of hsps by a second HS has been described.[32]

Thus, the capacity to further induce hsps by low doses of the identical substance which was used to disturb the cells initially is a transient phenomenon. These observations led to the conclusion that low doses of homologous substances are effective only during the early period of recovery.

Another question is whether this desensitization during cellular recovery is stressor-specific and to what extent heterologous stimulation of induction remains possible. The specificity of desensitization of hsp induction was tested employing high doses of HS, sodium arsenite and cadmium chloride as primary and secondary inducers of hsps.[30,31]

Normally, the development of tolerance is observed when a fractionated treatment protocol is used. In this protocol, an initial exposure to heat is followed by an incubation at a normal temperature (37 C) and subsequently by-incubations at high temperatures again, in order to determine the degree of developed thermotolerance. More pre-treated cells survive this high temperature than non-pre-treated cells. Examination of the kinetics of induction and decay of thermotolerance showed that an interval of about 4–8 h at normal temperature was required before maximum thermotolerance manifested. The tolerance persisted for at least 24 h.

The same fractionated treatment protocol was applied using the chemical stressors. Stimulation of hsp synthesis following arsenite as the secondary stressor is severely inhibited in arsenite-pre-treated cells, while reinduction following cadmium and HS are specifically inhibited in cadmium- and HS-pre-treated cells respectively.[30]

However, when different stressors were used as first and second stressor, it was observed that reinduction of hsps is still possible. Interestingly, it was observed that the pattern of protector proteins induced by the secondary applied stressor still shows a stressor-specificity related to the second treatment and independent of the pretreatment. Thus, the pattern of re-induced hsps correlated significantly with the pattern of the second stressor when applied singly and not with the pattern induced by the first stressor ($p < .01$).[31]

The possible implication of the results mentioned so far is that after disorder or injury, that is, during the period of homologous tolerance, production of hsps and/or increase in survival capacity may be further stimulated only by application of low doses of heterologous stressor conditions. These observations may have consequences for understanding the relation between the homologous and the heterologous approach as components of the similia principle.

Conclusion

In view of the cytoprotective role of hsps, these data support the use of low level stress conditions following high level stress conditions, with the aim to prolong and increase hsp synthesis and to potentiate cellular recovery defense capabilities. In summary:

1. Brief and moderate HS to the cells used in our research program initiates a) a rapid increase in the synthesis of hsps and b) the development of thermotolerance resulting in an increased ability to survive exposure to otherwise lethal temperatures.
2. Low doses of various chemical stressors (arsenite, cadmium, mercury, lead, copper, menadione and diethyldithiocarbamate (ddtc), at concentrations that do not exert any effect in control cultures (no previous HS), are able to enhance the synthesis of hsps and to stimulate the development of survival capacity when applied to cultures pretreated with a mild HS. This observation may relate to the specificity of 'symptom aggravation' at the cellular level.
3. The degree of stimulation appears to be stressor-specific. This specificity is not only present in the development of the survival capacity, but also in the subsequent enhancement of the HS-induced synthesis of stress proteins.
4. The degree of stimulation of survival capacity by sequential low doses of the mentioned stressors is determined by the degree of hsp pattern similarity between the stress condition used as low dose and initial high dose. This observation unequivocally supports the principle of similarity at the cellular level.
5. The different hsps that demonstrate enhanced induction (additional stress protein synthesis) when heat-shocked cultures were exposed subsequently to various small dose chemical stressors. The hsp pattern induced was that characteristic of the secondary stressor and not the initial HS.
6. The data supported the hypothesis that small doses of toxic compounds may, under certain conditions, have beneficial effects related to stimulation of endogenous cytoprotective mechanisms.

The evidence obtained in our program of experiments supports the validity of the similia principle at the cellular level.

Perspective

The observations as described in the present overview merits further attention. With current techniques in the field of genomics, including DNA arrays, more precise determination of similarity in gene induction patterns is now possible, allowing characterization of different stress conditions

in terms of 'disease-' and 'remedy' picture. In addition, techniques used in the field of proteomics and metabolomics, make feasible more precise evaluation of self-recovery processes to characterize response patterns of cell cultures and/or tissue samples of organism treated according to the similia protocols. It is hoped that the scientific questions raised will attract others to build on the findings described in this paper.

Another interesting development has recently occurred in the field of hormesis. Although a comparison of homeopathy and hormesis has often been made, and not always to mutual benefit, this development is suggested to be of relevance. Hormesis refers to the process in which exposure to a low dose of an agent that is toxic at higher doses induces a beneficial effect on cells or organisms.[4,33–36]

However, in hormesis the effect of low doses is studied in naive cells, or in case of 'pre-conditioning' when the low dose precedes a harmful high dose stress leading to an enhanced resistance. But, Calabrese *et al.*[4] have recently introduced the term 'postconditioning hormesis' to indicate the possibility that small stimuli could also exert a beneficial effect when applied to cells or organisms that previously experienced a severe harmful stress. As an example, a paper by Zhao *et al.*[37] in which a low level of hypoxic stress was applied subsequent to myocardial infarction, which reduced the cellular damage was cited.

Our research program, as described in this paper, is a perfect example of 'postconditioning hormesis' at the cellular level: cells that experienced a harmful (cytotoxic) stress condition show beneficial effects when subsequently exposed to low doses of these stress conditions. The facts that the low doses of chemical stressors used in our research program were not diluted beyond Avogadro's number and were not potentized, being in agreement with the use of remedies during the early years of homeopathy,[2] are additional arguments to suggest that further studies on the similia principle at the cellular level could also take place within the framework of postconditioning hormesis. The preconditioning element of hormesis might primarily pertain to medical prophylactic strategies, whereas postexposure conditioning would then relate primarily to therapeutic strategies.

Calabrese *et al.*[4] believe that postexposure conditioning hormesis might eventually provide important biomedical clinical possibilities, though they only mention one example. We recently reviewed other experimental and clinical examples that do fit the definition of 'postconditoning hormesis'.[38] These examples are also of interest for homeopathy since they emphasize the regulatory principles as well as the physiological processes through which low dose stress conditions, applied within or without the context of the similia principle, exert a beneficial effect in terms of supporting recovery processes.

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