Examination of Homeopathic Preparations in Cell-Free Systems

Günther Harisch, D.V.M., Joachim Dittmann, Ph.D.

Abstract

This pilot study using cell-free systems ascertained the following: that Ubiquinon Injeel® and Ubiquinon Injeel® forte have different effects, that these Injeels® and conventional ubiquinone dilutions have different effects, and that the differences in effects of the single potencies included in the Injeels® do not follow a linear pattern.

The issues raised by this article merit further research.

Resumen

Los que critican la homeopatía requieren una prueba apropiada de la eficacia de las cantidades infinitesimales de sustancias activas. Además, pueden aceptar el fundamento científico de la potenciación sucesiva solamente si se puede verificar que una potencia homeopática y una dilución convencional tienen efectos distintos, aunque contengan cantidades idénticas del ingrediente activo.

In the case of single potencies, such proof is easy to obtain using cell-free test systems. Cell-free systems in this case, buffered enzyme solutions were used as indicators of the effects of the ubiquinone preparations. Every reasonable effort was made to ensure the purity of the enzymes. What can we conclude from cell-free systems? Since such test systems contain only an enzyme and a homeopathic single or mixed remedy, there can be no doubt as to what causes any detected change in the enzyme’s activity. Because cell-free test systems isolate individual functions from the context of the total organism, results obtained from such systems cannot be extrapolated to the organism as a whole. In view of our objectives, however, this limitation is an asset rather than a disadvantage. Critics of our work who are not the same as the above-mentioned critics of homeopathy—often charge us with reductionism, but they are targeting a circumstance that is inherent in the test system and deliberately selected by the investigators.

This article presents a pilot project intended to test the success of similar methods with regard to mixed potencies such as the Injeels. The authors acquired and tested the following preparations: (1) Ubiquinone in all the single potencies included in the Injeels, (2) Ubiquinone Injeel® and Injeel® forte, (3) conventional dilutions corresponding to the single potencies, (4) conventional dilutions corresponding to the Injeels, (5) the solvent used in each preparation, and (6) potentized solvent to compare to each potency.

In addition, we are planning to study preparations successed for different numbers of strokes (10, 50, or 100) at each level of potentization. Our initial work, however, was done with preparations produced exclusively with 10 strokes per potentization level. This preliminary report investigates only Injeels, the corresponding conventional dilutions, and (in some cases) single potencies with regard to their effect on different cell-free systems. A more comprehensive study is planned that will present the data from all of our experiments.

Introduction

Critics of homeopathy demand suitable proof of the efficacy of minute and infinitesimal quantities of active substances and will acknowledge the scientific basis of successive potentization only if it can be proved that a homeopathic potency and a conventionally produced dilution have different effects even though they contain identical amounts of the active ingredient.

In the case of single potencies, such
escogido deliberadamente por los investigadores.

Este artículo presenta un diseño para verificar el éxito de métodos semejantes con respecto a las potencias mezcladas, tal como lo sugieren los Injeel®. Los autores obtuvieron y pusieron a prueba las preparaciones siguientes: (1) Ubiquinon en todas las potencias sencillas incluidas en los Injeel®, (2) Ubiquinon Injeel® e Injeel® forte, (3) Diluciones convencionales que correspondan a las potencias sencillas, (4) Diluciones convencionales que correspondan a los Injeel®, (5) El solvente empleado en cada preparación, (6) El solvente potenciado como comparativo a cada potencia.

Además, vamos a estudiar las preparaciones secundarias (con sucesión) con cantidades distintas de secundarias (10, 50, o 100) a cada nivel de potenciación. Hacemos nuestro nuevo trabajo, sin embargo, con preparaciones producidas exclusivamente con 10 secundarias a cada nivel de potenciación. Este artículo preliminar investiga solamente los Injeel®, las diluciones correspondientes convencionales, y en los casos, unas potencias sencillas con respecto a sus efectos sobre varios sistemas "sin células." Vamos a hacer un estudio más amplio para presentar todos los resultados de nuestros ensayos.

### Methods

#### Active Agents

The active substance we chose was ubiquinon in concentrations of 8X, 12X, 30X, and 200X, all produced using 10 secundaries per potenciacion level. Two mixed potencies or potency chords produced according to the same protocol were also used—Ubiquinon Injeel®, which contains the potencies 12X, 30X, and 200X, and Ubiquinon Injeel® forte, which contains the potencies 8X, 12X, 30X, and 200X. Also tested were mixed dilutions corresponding to Injeel® and Injeel® forte. The dilutions differed from the Injeel® only in that they were not potenciado but produced according to a conventional mixing procedure. All of these preparations and solutions were supplied by Biologische Heilmittel Heel GmbH, Baden-Baden, Germany. Our study used a blinded test format; all preparations were coded prior to use but decoded before the statistical analysis.

#### Enzyme Preparations

The enzymes used in the cell-free test systems were extracted from rat livers processed at 4°C. To extract the cytosolic fraction, the rat livers were first homogenized in 5 mL of buffer A (30 mmol of potassium phosphate buffer, pH 8.3) per gram of liver tissue, using a cell homogenizer, and then centrifuged for 10 minutes at 15000g. The effluent was then centrifuged again at 105,000g for 60 minutes (using the Swing-out Rotor AH-629 manufactured by Sorvall, Bad Homburg, Germany). Aliquot portions (2.5 mL) of the resulting effluent were each applied to a Sephadex G25 column equilibrated with buffer A and then eluted with 3.5 mL of
Figure 2: Catalytic activity of acid phosphatase in the presence of different ubiquinon preparations. The enzyme was incubated with the ubiquinon preparation and the synthetic substrate p-nitrophenyl phosphate at 30°C for 5 minutes. The reaction was stopped and the quantity of enzymatically formed p-nitrophenol was determined. Chart A shows average values (adjusted to the control value) and positive and negative standard deviation. C represents the untreated control. Chart B presents the results of statistical evaluation (n = 72). X denotes significant differences (P < 0.01).

A. The BIO-RAD version (BIO-RAD, Munich, Germany) of Bradford's protein quantitation method was used to determine the quantity of protein. Bovine γ globulin served as the reference substance.

Determining Glutathione S-Transferase Activity

The activity of GSTs in the cytosolic fraction was determined using the method described by Habig et al., which we converted to a microtiter plate format [2]: 100 µL of potassium phosphate buffer (pH 6.5), 100 µL of the test solution (or, in the case of the control, water) and 30 µL of GSH (glutathione, 10 mmol) were pipetted onto a microtiter plate, and 50 µL of CDNB (chloro-2,4-dinitrobenzol, 6 mmol, dissolved in 95% ethanol) were added. Then 20 µL of cytosol extract (protein concentration 0.13 mg/mL) or, in the case of the control, 20 µL of buffer B was added to start the reaction. The course of the reaction was monitored at 32°C for 10 minutes at a wavelength of 340 nm. Readings were taken with a temperature-controlled microtiter plate reader (ATCC 340, SLT Labinstruments, Gräfisch, Germany). A measuring period featuring measurements between minute 3 and minute 7 was used to calculate ΔE/min, which was then multiplied by a factor of 2,333 to yield activity per volume in nmol x min⁻¹ x mL⁻¹ (for 0.02 µL cytosol extract). The molar decrease coefficient for CDNB is 9.6 mmol⁻¹ x cm⁻¹.

Determining the Activity of Xanthine Oxidase and of the Total Xanthine Oxidase/Dehydrogenase System

The activity of xanthine oxidase and of the total xanthine oxidase/dehydrogenase system was ascertained by measuring enzyme-catalyzed urate formation. 100 mL of the cytosolic fraction (final protein concentration, 0.5 mg/mL) was incubated for 30 minutes at 30°C with 300 mL of the ubiquinon preparation (or, in the case of the controls, water) and 600 mL of 0.17 mmol TRIS hydrochloride buffer (pH 8.1) containing 100 mmol of xanthine with or without 0.17 mmol of NAD.

At the end of the incubation period, the reaction was stopped by adding 10 volumes of ice-cold ethanol. After 5 minutes of centrifugation at 4°C and 1,000g, the effluent was incorporated by rotation under vacuum. The resulting precipitate was suspended in 800 mL of 51.2% acetonitrile/0.1% trifluoroacetic acid (pH 2.5) and centrifuged (for 10 minutes at 4,000g). Column chromatography was performed on 100 mL of the effluent using a Knauer high-performance liquid chromatography instrument. A 250.0 x 4.0 mm Spherisorb SODS 2 (particle size
5 mL, Knauer, Berlin, Germany) was used as the column with the appropriate prep column/anterior column. The enzymatically formed urate was eluted from the column with 51.2% acetonitrile/0.1% trifluoroacetic acid (pH 2.5) at a flow rate of 0.5 mL/min. Urate absorption was measured at a wavelength of 292 nm.

The peak area was used to quantitate the enzymatically formed urate according to the reference equation urate (nmol/mL) = 1.705 x peak area - 1.886; correlation coefficient: 0.999. Urate in various concentrations served as the reference substance.

Determining Acid Phosphatase Activity

Acid phosphatase derived from potatoes was supplied by Boehringer-Mannheim. The catalytic activity of this enzyme was determined by measuring the quantity of p-nitrophenyl formed. A microtiter plate format was used for the experiments: 100 mL of the ubiquinone test solution was added by pipette to 100 mL of the synthetic substrate (5.5 mmol of p-nitrophenyl phosphate in 0.1 mol of citrate buffer, pH 5.6), and the reaction was started by adding 20 mL of the enzyme (diluted 1:200 with 10 mmol of acetyl sodium, pH 5.6). After 5 minutes of incubation at 30°C, the reaction was stopped by adding 100 mL of sodium hydroxide (1.0 N), and the quantity of p-nitrophenyl formed was measured at a wavelength of 405 nm using a temperature-controlled microtiter plate reader. The quantity of p-nitrophenyl was determined by applying the reference equation p-nitrophenyl [nmol x mL⁻¹] = 64.82 x OD 405 nm - 3.373; correlation coefficient 0.998. P-nitrophenyl in various concentrations served as the reference substance.

Statistics

The values obtained for each enzyme system (activity per volume in nmol x min⁻¹ x mL⁻¹) were subjected to single-factor variance analysis (balanced design). Subsequently, multiple mean comparisons (Scheffé test) were performed.

Results and Discussion

Presumably, in the cell-free systems described here, homeopathic single and mixed potencies act like the "allosteric effectors" whose effects on cyclic adenosine monophosphate (cAMP) are described in biochemistry texts. As we have shown elsewhere, homeopathic remedies stimulate individual enzymes in situ—that is, in the context of the cell-in very different ways. In vitro, even neighboring enzymes in the same subcellular compartment are influenced differently. Their activity is
controlled-or, to put it in systematic terms, regulated-in different ways. On the basis of this model and of the results already mentioned, we can imagine how the tremendous complex effects expressed in the drug proves on a homeopathic remedy occur. Without earlier studies of intact organisms, it would be impossible to judge the significance of the results we obtained in cell-free systems. On the other hand, in more complex systems than the cell-free model, it would be impossible to prove that homeopathic substances are effective, because direct effects are difficult-if not impossible, given the minute quantities of active agents involved— to prove in the intact organism. Therefore, considerations must precede our discussion so that the significance of the results can be assessed.

Admittedly, it is not easy to explain the effects of the mixed potencies present in Injekt® in terms of the allosteric effect

model, which is based on the interaction of a distinct homeopathic substance with the enzyme bi molecule. Can the three or four homeopathic potencies in an Injekt®, each of which has its own specific effect, as the results of tests with single potencies demonstrate—exert a combined additive effect, or are their individual effects active in different domains of the enzyme molecule? There is a great deal to be said in favor of the latter assumption, which will also be easier to confirm using enzymatic methods and procedures that access protein conformation (the three-dimensional arrangement of the biomolecules in space).

Looking first only at our results with the Injekt® and Injekt® forte, we see that these substances may either depress enzyme activity, as is the case with GSTs (Figure 1) and acid phosphatase (Figure 2), or stimulate it, as is the case with the total xanthine oxidase/dehydrogenase system (Figure 3) in comparison to the untreated control.

Looking at the effects of Injekt® in comparison with those of nonpotentized mixtures (conventional dilutions), we find that the effects of Injekt® and Injekt® forte may work in the same direction as the effects of the conventional xanthine oxidase, but on a quantitatively different level, as is the case with acid phosphatase, GST, and xanthine oxidase (Figure 4), or the effects of Injekt® and dilutions may work in opposite directions, as in the total xanthine oxidase/dehydrogenase system. Since the potentiating process (which in this case involved succession for 10 strokes at each level) constitutes the only difference between Injekt® and conventional dilutions, it can be assumed that potentiization causes the difference in their effects.

If we compare the results obtained using both potentized and nonpotentized forms of Injekt® and Injekt® forte, it is apparent that in all the cell-free systems used here, the effects of Injekt® are more strongly expressed than those of Injekt® forte in terms of reactivating the enzyme system. Since the only difference between Injekt® and Injekt® is that the former includes 8X in addition to 12X/30X/
200X, it is obvious to assume that the effect of Injeel® forte might be fine-tuned by varying the proportion of 8X to 12X/30X/200X. Figure 5 models how this might be played out in the catalytic activity of the detection system—in this case, specific enzymes.

When we consider the effects of the single potencies, it becomes apparent that (in comparison with the untreated control) the effect of 12X can be either less (as in the case of xanthine oxidase and total xanthine-oxidase/dehydrogenase) or greater (as in the GSTs) than that of either 8X or 30X. In the potency series we studied, the 12X potency marks a turning point in the effect. Earlier studies of other homeopathic substances, both in vivo experiments and studies of cell cultures, revealed similar nonlinear effects, which seem to be specific to homeopathy. (It should be noted, however, that the effects of acid phosphatase do not conform to either pattern.)

Fig. 5: Model of possible consequences of successive increases in the amount of 8X in Injeel® forte

References


(10) Dittmann J, Harisch G. Einfluss
BIOMEDICAL THERAPY
INTERNATIONAL JOURNAL OF INTEGRATED MEDICINE

Information for Authors

Biomedical Therapy is a quarterly scientific journal for healthcare practitioners interested in exploring the fields of complementary medicine including homeopathic therapy. Highest priority is given to studies which deal with clinical cases. Most manuscripts fall into one of the following categories:

* Original research: Report involving the collection of data.
* Clinical review: Critical analysis of a medical condition based on a thorough review of the literature.
* Editorial: Opinions on a topic relevant to complementary medicine.
* Letters to the Editor: Brief comments on articles published in BT or other information of interest to readers of BT.

**MANUSCRIPT STYLE**

Type the manuscript double-spaced, including title page, abstract, text, acknowledgements, references, tables, and legends. The manuscript should be on one side on an 8.5x11 inch piece of white bond paper. All sections should have 1-inch margins left and right.

Each manuscript component should begin on a new page, in the following sequence: title page, abstract and key words, text, acknowledgements, references, tables, and legends for illustrations.

Illustrations must be good quality, unmounted glossy prints.

Submit the required number of copies of manuscripts and illustrations in a heavy-paper envelope. The submitted manuscript should be accompanied by a cover letter along with permissions to reproduce previously published material or to use illustrations that may identify human subjects.

**DISKETTES**

If desired, manuscripts may be submitted on diskettes. In such a case, be certain to include a print-out of the document. Put only the latest version of the manuscript on the diskette and label the file clearly. Provide information on the type of format used.

Authors should keep copies of everything submitted as materials will not be returned.

Menaco Publishing Company
P.O. Box 11280
Albuquerque, NM 87192-0280