THE NEED FOR RATIONALE EXPLANATIONS OF HOMEOPATHY

- As reported by Kleijnen et al. in their seminal review on clinical trials in homeopathy*:
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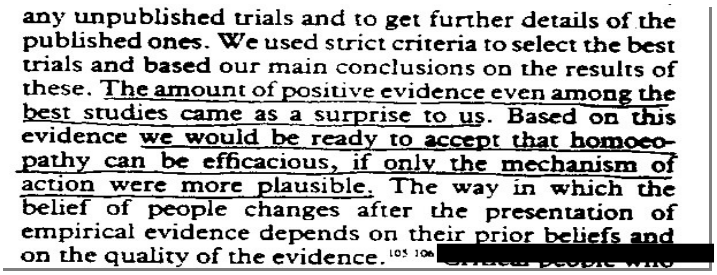
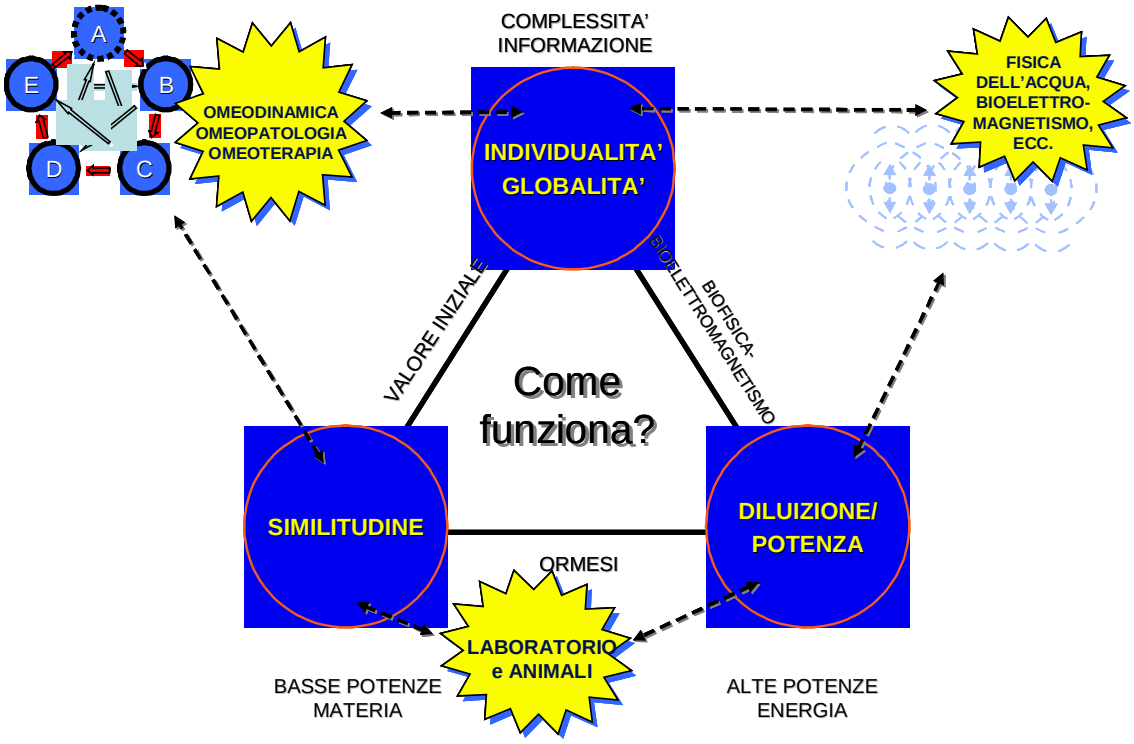


any unpublished trials and to get further details of the published ones. We used strict criteria to select the best trials and based our main conclusions on the results of these. The amount of positive evidence even among the best studies came as a surprise to us. Based on this evidence we would be ready to accept that homoeopathy can be efficacious, if only the mechanism of action were more plausible. The way in which the belief of people changes after the presentation of empirical evidence depends on their prior beliefs and on the quality of the evidence.[105 106]

* Kleijnen, J., Knipschild, P. & Ter Riet, G. 1991. Clinical trials of homoeopathy. *Brit. Med. J.* **302**: 316-323.

- It is only through patient, unrestricted, and methodical research conducted on several planes - clinical, laboratory, epidemiological, and physicochemical - that we shall be able to shed light on the many issues which so far remain unsolved.

LA TRIADE OMEOPATICA E LA RICERCA DI BASE



PPT – 2.1

Effects of homeopathic treatment on animal models of cancerogenesis (part 1)

Animal	Disease Model	Intervention	Results	Ref
Mice	Cytogenetical damage induced by exposures to ultrasonication	Actinomycin D or Arnica 30/placebo	AMD had genotoxic effects of its own. Sonicated mice fed with Arnica 30 showed appreciably reduced genotoxicity	(Chakrabarti, Biswas, and Khuda-Bukhsh 2001) Indian J.Exp.Biol. 39 (12):1235-1242.
Mice	p-dimethylaminoazobenzene (p-DAB)-induced hepatocarcinogenesis	Two potencies of Chelidonium (Ch-30, Ch-200)	Feeding of Chelidonium reduced genotoxic effects to a significant extent ($p < 0.05$ to $p < 0.001$).	(Biswas and Khuda-Bukhsh 2002) BMC.Complement Altern.Med 2:4.
Mice	p-DAB-induced hepatocarcinogenesis	Chelidonium-30 (Ch-30) and Chelidonium-200 (Ch-200),	Both Ch-30 and Ch-200 also modulated favourably some toxicity marker enzymes	(Biswas and Khuda-Bukhsh 2004) Indian J.Exp.Biol. 42 (7):698-714.
Mice	Genotoxic Effects Produced by Mercuric Chloride	Mercurius Solubilis(Merc Sol-30 and Merc Sol-200)	Less chromosome aberrations in the drug-fed series. The amelioration by Merc Sol-200 appeared to be slightly more pronounced.	(Datta, Biswas, and Khuda-Bukhsh 2004) Evid.Based.Complement Alternat.Med 1 (3):291-300.
Mice	p-DAB-induced hepatocarcinogenesis	Carcinosin 200, fed alone and in combination with Chelidonium 200	Carcinosin 200 and Chelidonium 200 when administered alone show considerable ameliorative effect on cytogenetical endpoints and toxicity biomarkers	(Biswas et al. 2005) J Altern.Complement Med 11 (5):839-854.
Mice	Sarcoma 180	Canova, a homeopathic complex medicine*	Delay in the development, increased infiltration by lymphoid cells with active treatment. Increased number of leukocytes and lymphocytes.	(Sato et al. 2005) Homeopathy 94 (1):26-32.

*The formula of Canova is composed of 19x Thuya occidentalis, 18x Bryonia alba, 11x Aconitum napellus, 19x Arsenicum album and 18x Lachesis muta (Viperidae) venom, all extracted and diluted in 70% alcohol, in equal parts.

Effects of homeopathic treatment on animal models of cancerogenesis (part 2)

Animal	Disease Model	Intervention	Results	Ref
Rats	Prostate cancer (rats injected with MAT-LyLu cells)	Thuja occ. 1000c, Conium mac. 1000c, Sabal serr. 200c, Carcinosin 1000c from MAT-LyLu cells	23% reduction in tumor incidence ($P < .0001$), and 38% reduction in tumor volume ($P < .02$). Tumors showed a 19% increase in apoptotic cell death ($P < .05$).	(Jonas et al. 2006) Integr.Cancer Ther. 5 (4):343-349.
Mice	p-DAB-induced hepatocarcinogenesis	Lycopodium 30c and 200C	Protection from chromosome aberrations and toxicity biomarkers	(Pathak et al. 2006 and 2007) Mol.Cell Biochem. 285 (1-2):121-131. Forsch.Komplementarmed. 14 (3):148-156.
Mice	Genotoxicity induced by repeated injections of arsenic trioxide	Arsenicum album 200 alcohol (Alcohol-200)	Drug fed mice showed reduced toxicity at statistically significant levels in respect of all the parameters studied	(Banerjee et al. 2007) J Vet.Med.A Physiol Pathol.Clin.Med. 54 (7):370-376.
Rats	N'-nitrosodiethylamine (NDEA) induced hepatocellular carcinoma and sarcomas	Ruta, Hydrastis, Lycopodium and Thuja (200C), Phosphorus 1M	Homeopathic drugs retarded the tumor growth and reduced the marker enzymes (Ruta 200c of liver tumor, Ruta 200c and phosphorus 1M of sarcomas)	(Kumar et al. 2007) Asian Pac.J Cancer Prev. 8 (1):98-102.
Mice	Human prostate xenografts cancer growth	Sabal serrulata, Thuja occidentalis, and Conium maculatum	Xenograft size was significantly reduced in Sabal serrulata-treated mice compared to untreated controls ($P=.012$). No effect was observed on breast tumor growth.	(MacLaughlin et al. 2006) Integr.Cancer Ther. 5 (4):362-372.
Mice	Hepatocarcinogenesis through P-DAB and Phenobarbital	Natrum Sulphuricum 200 (Nat Sulph-200)	Less number of liver tumors and of chromosome aberrations reeduced toxicity parameters	(Bhattacharjee, Pathak, and Khuda-Bukhsh 2007) eCAM Advance access

Homeopathic medicine and animal (murine)cancer models: SUMMARY

Drug	Effect	Group and reference
Arnica 30CH	Reduces sonication-induced Genotoxicity	{CHAKRABARTI2001} University of Kalyani, Nadia, India
Chelidonium 30CH and 200CH, alone or with Carcinosin 200CH	Reduces p-DAB-induced tumors incidence, genotoxicity and improves Biomarkers	{BISWAS2002} {BISWAS2004} {BISWAS2005} University of Kalyani, Nadia, India
Mercurius Sol 30CH and 200CH	Reduces mercury-induced genotoxicity	{DATTA2004} University of Kalyani, Nadia, India
Lycopodium 30CH and 200CH	Reduces p-DAB-induced tumors incidence, genotoxicity and improves Biomarkers	{PATHAK2006} {PATHAK2007} University of Kalyani, Nadia, India
Natrum sulf. 200CH	Reduces p-DAB-induced tumors incidence, genotoxicity and improves Biomarkers	{BHATTACHARJEE2007} University of Kalyani, Nadia, India
Canova complex (Thuja, Bryonia, Aconitum, Arsenicum, Lachesis)	Inhibit development of sarcomas-180	{SATO2005} Universidade do Vale do Itajai, Brazil
Thuja 1000CH, Conium 1000CH, Sabal S. 200CH Carcinosin 1000CH	Reduces prostate cancer incidence and tumor volume	{JONAS2006A} Samueli Institute, Alexandria, VA, USA
Sabal S. 200CH	Reduces prostate cancer incidence and tumor volume	{MACLAUGHLIN2006} Georgetown University, Washington, DC
Ruta, Hydrastis, Lycopodium, Thuja 200CH, Phosphorus 1000CH	Inhibits liver tumor development (Ruta max effect in one paper and reported as the only remedy in another)	{KUMAR2007} {PREETHI2006} Cancer Research Centre, Kerala State, India

PPT – 3.6

Effects of homeopathic preparations on human prostate cancer growth in cellular and animal models

B. W. MacLaughlin et al. Integr. Cancer Ther., 5 (2006) 362-372.

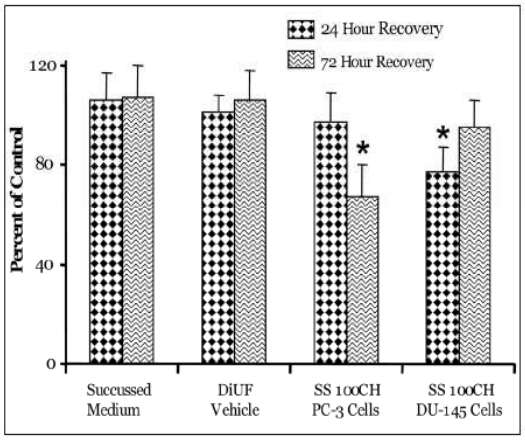


Figure 1 *Antiproliferative effect of 100 CH* Sabal serrulata *(SS 100CH) on both human prostate cancer cell lines PC-3 and DU-145. Cells were treated with 1 dose (10 µL) every 4 hours of* Sabal serrulata *and left to recover for either 24 or 72 hours. DiUF = succussed deionized ultrafiltered water; SS 100CH =* Sabal serrulata *100 CH dilution. Crystal violet technique was used. Error bars represent ± SD. PC-3, 72 hour and DU-145, 24 hour recovery are statistically significant,* P < .01 (*) *as assessed by Student's* t *test.*

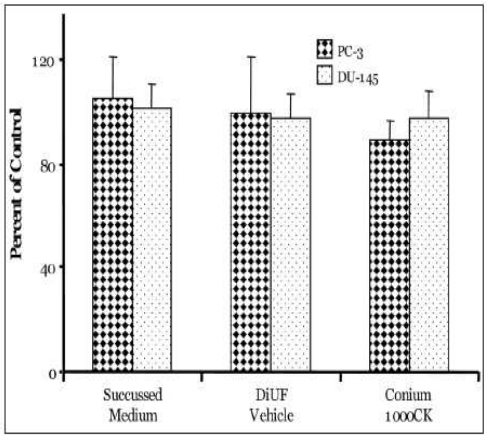


Figure 4 *Effect of 1000 CK* Conium maculatum *on both human prostate cancer cell lines PC-3 and DU-145. They were treated with 1 dose every 4 hours and left to recover for 24 hours. MTT technique was used. DiUF = succussed deionized ultrafiltered water. Error bars represent ± SD. No statistically significant effect was observed,* P > .05.

PPT – 3.5

Effects of homeopathic treatment on cell models of cancerogenesis

Cells	Laboratory test	Intervention	Results	Ref
Normal and leukemic lymphocytes	Cell viability	Pretreatment with either low concentrations (nM-microM) or high potencies (15-20c) of cadmium (Isopatic)	Increased cell viability in primary lymphocytes. Weaker effect in cancerous cells	(Walchli, Baumgartner, and Bastide 2006). J Altern. Complement Med. 12 (5):421-427.
Ehrlich carcinoma	Continuous recording of Na(+), K(+), and Ca(2+) with selective electrodes	Phosphoric acid (PA), at low (10(-14) and high 10(-42)) dilutions	Stimulates ionic transport and Na,K-ATPase	(Nadareishvili 2006) Georgian.Med.News (136):99-101.
Prostate tumor cells	Tumor cell viability and apoptosis gene expression	Thuja occidentalis 1000c, Conium maculatum 1000c, Sabal serrulata 200c	No effects on cell viability or gene expression	(Jonas et al. 2006) Integr.Cancer Ther. 5 (4):343-349.
Prostate and breast cancer cell lines	Tumor cell viability and apoptosis gene expression	Conium maculatum, Sabal serrulata, Thuja occidentalis, Asterias, Phytolacca, and Carcinosin in several potencies	No effects on cell viability or gene expression	(Thangapazham et al. 2006a) Integr.Cancer Ther. 5 (4):356-361.
Copenhagen rat tumor tissues	mRNA levels of the apoptotic genes or the cytokines in prostate tumor	Conium maculatum, Sabal serrulata, Thuja occidentalis, and a MAT LyLu Carcinosin nosode	No significant changes of any treatment	(Thangapazham et al. 2006b) Integr.Cancer Ther. 5 (4):350-355.
Human prostate cancer and breast cancer cell lines.	Cell proliferation	Sabal serrulata (saw palmetto) 100CH	33% decrease of prostate cell proliferation, no effect on breast cells.	(MacLaughlin et al. 2006) Integr.Cancer Ther. 5 (4):362-372.
Lymphoma Ascites and others	Cell growth	Ruta graveolens (MT and 200C)	Cytocidal action of both preparations	(Preethi, Kuttan, and Kuttan 2006) Asian Pacific J Cancer Prev 7:439-443.

EXAMPLES OF HIGH-DILUTION EXPERIMENTS

System	Agent	Dilution	Effect	Ref.
Human basophils	**IgE**	**10^{-60}**	**Stimulation (not confirmed by others)**	Davenas 1988
Human basophils	**Histamine**	**10^{-24} →10^{-32}**	**Inhibition**	Belon 1999-2006 et al (including Verona Group)
Cicken embrio	**Bursin**	**15 CH 10^{-27}g**	**Immunomodulatory and endocrine activity**	Youbicier-Simo 1993-97
Human neutrophils	**Magnesium phos Phosphorus**	**12 x to 30 x**	**Inhibition of superoxide**	Chirumbolo 1993
Rat Hypothalamus	**Sodium chloride**	**10^{-60}**	**Reduces firing rate in rats under high-salt diet**	Sukul 1991-98
Mice nervous system	**Nux vomica**	**30 CH**	**Reduction of alchol-induced sleep time**	Sukul 1999
Rat duodenum	**Atropa belladonna**	**60 CH 200 CH**	**Increases Ach-induced spasm Increases Ach-induced spasm**	Cristea 1991-98
Mouse blood	**Acetylsalicylic acid**	**10^{-30}**	**Pro-thrombotic**	Doutremepuich 1998
Mouse ears	**Silica**	**10^{-60}**	**Speeds up wound healing**	Oberbaum 1998
Wheat germination	**Arsenic Silver nitrate**	**10^{-45} 26 D**	**Protect from toxicity Enhances growth**	Betti 1997/2001 Pongratz 1998
Neurons	**Cycloheximide**	**10^{-27}**	**Increases viability**	Marotta 2002
Saccharomyces	**Axoxystrobin Phosphorus**	**9-30 CH**	**Affect growth kinetics**	Scherr 2006

FARMACOLOGIA DELLE ALTE DILUIZIONI
Studi sulle basi biochimiche e fisiche del medicinale omeopatico

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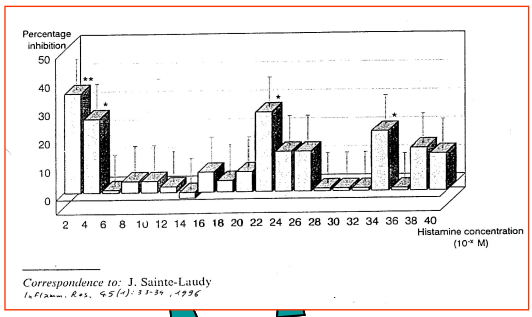
1. **Modi per preparare i rimedi omeopatici e per conservarli.**
2. **Evidenze cliniche ottenute a sostegno delle alte diluizioni nell'uomo, unitamente ad esperimenti di laboratorio**
3. **Caratteristiche fisiche dei medicamenti in diluizioni ultra-alte (risonanza magnetica nucleare e spettri all'infrarosso).**
4. **Possibili meccanismi d'azione delle alte diluizioni sui sistemi viventi.**

Basic research in homeopathy
TYPICAL CELLULAR MODELS FROM THE LITERATURE

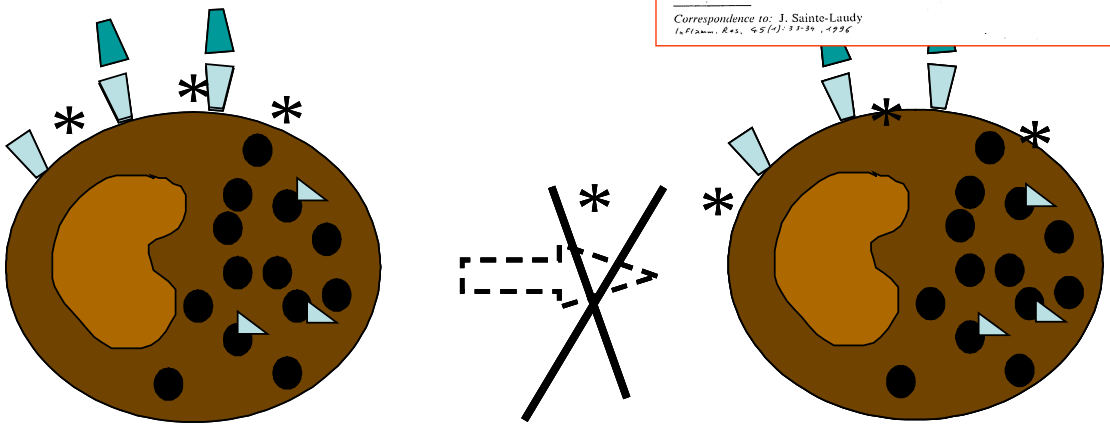
- Lymphocytes (Colas, Bildet, Bastide, Wagner, Chirila)
- Basophils (Poitevin, Sainte Laudy, Belon, Davenas, Ovelgonne, Hirst, Mannaioni, Ennis)
- Granulocytes (Poitevin, Fletcher, Chirumbolo, Bellavite)
- Cell lines: Fibroblasts (Boiron, Mansvelt, Fougeray) Renal cells (Delbancut), Hepatoma (van Wijk, Wiegant), Neurons (Jonas)
- Vegetable cells (Guillemain, Bornoroni, Betti)

CONFIRMATION OF PHENOMENON WITH CD63 EXPRESSION
(S.LAUDY, BELON ET AL., 1996-2006, BROWN AND ENNIS, 2001)



* Ultra-high dilution (10^{-22}, 10^{-34} M) of **Histamine**

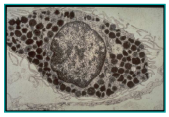
Anti-IgE
(Medium doses)



Resting cell

Inhibition of response to Anti-IgE

PPT – 3.5



HISTAMINE DILUTIONS MODULATE BASOPHIL ACTIVATION

Belon P, Cumps J, Ennis M, Mannaioni PF, Roberfroid M, Sainte-Laudy J, Wiegant FA. - *Inflamm Res.* 2004 May;53(5):181-8.

- Study 1 used a blinded multi-centre approach in 4 centres.
  Study 2, related to the confirmation of the multi-centre study by flow cytometry, was performed independently in 3 laboratories.
  Study 3 examined the histamine release (one laboratory) and the activity of H(2) receptor antagonists and structural analogues (two laboratories).
- RESULTS: High dilutions of histamine (10^{-30}-10^{-38} M) influence the activation of human basophils measured by alcian blue staining. The degree of inhibition depends on the initial level of anti-IgE induced stimulation, with the greatest inhibitory effects seen at lower levels of stimulation. This multicentre study was confirmed in the three laboratories by using flow cytometry and in one laboratory by histamine release.
- Inhibition of basophil activation by high dilutions of histamine was reversed by anti-H2 and was not observed with histidine these results being in favour of the specificity of this effect
- We are however unable to explain our findings and are reporting them to encourage others to investigate this phenomenon.

PPT – 3.5, 3.9

Improvement of flow cytometric analysis of basophil activation inhibition by high histamine dilutions. A novel basophil specific marker: CD 203c.

- Sainte-Laudy J, Belon P.
- Homeopathy. 2006 Jan;95(1):3-8.

We investigated if the use of CD 203c, a basophil specific, earlier marker than CD 63 of the activation cascade, increased the sensitivity of the method, testing two target histamine dilutions, 10(-4) (2C) and 10(-32) M (16C).

- Basophils, obtained from buffy coats, were pre-incubated with the histamine dilutions and activated by two agonists: anti-IgE and fMLP (formyl-methionyl-leucyl-phenylalanine peptide).
- The cells were labelled with anti-IgE, anti-CD 13 and anti-CD 14 for basophil selection, and anti-CD 63 and anti-CD 203c for basophil activation. Results were expressed in up-regulation percentage for CD 63 or mean intensity of fluorescence (MFI) for CD 203c.

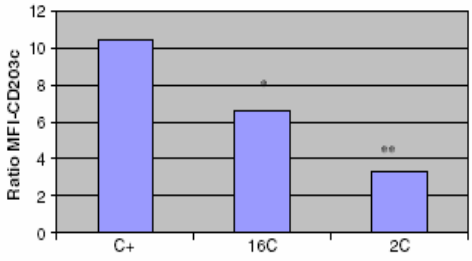
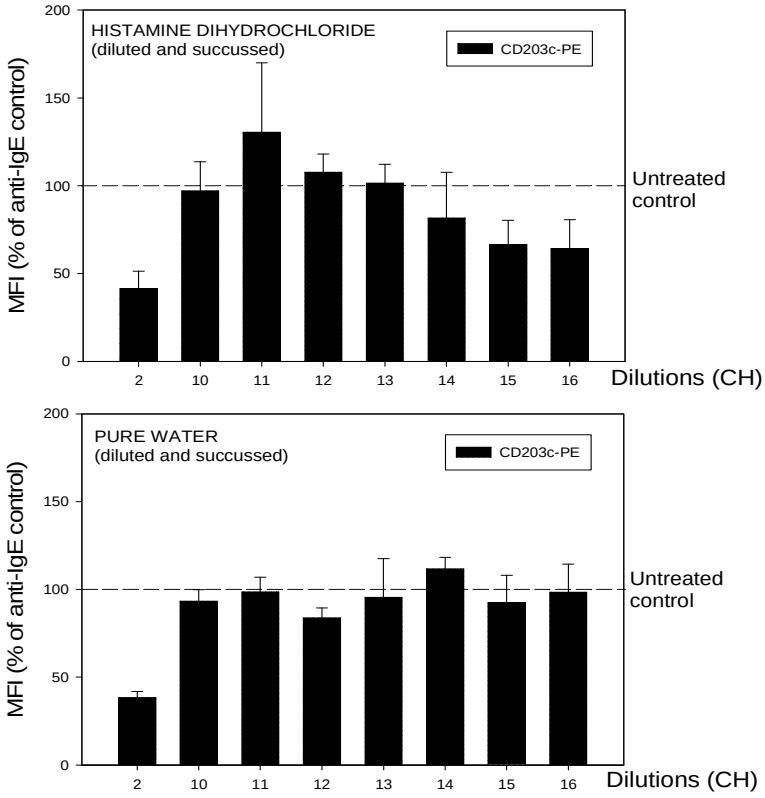


Figure 5. Inhibition of basophil activation by histamine dilutions 2C and 16C analysed by protocol 3 *vs* the fMLP histamine free control (C+) (mean of five experiments). $*P = 0.005$, $**P < 0.001$.

- RESULTS: Histamine 10(-4) M (2C) and histamine 10(-32) M (16C) were capable of inhibiting both IgE-dependent (anti-IgE) and IgE-independent (fMLP) basophil activation. The percentage inhibition depended on the activation marker used. The highest inhibition for histamine dilution 16C was observed with CD 203c (38%, P<0.001), approximately half the inhibition observed with histamine 2C (73%).
- CONCLUSION: The use of CD 203c instead of CD 63 increased the magnitude of the response.

EFFECTS OF HISTAMINE DILUTIONS/DYNAMIZATIONS (POTENCIES)
ON THE HUMAN BASOPHIL ACTIVATION *IN VITRO* (CD203 expression)
RECENT (UNPUBLISHED) EVIDENCE FROM VERONA'S LAB (Bellavite, Chirumbolo, Ortolani, Vella)



This study is supported by a research grant from Boiron s.r.l. to Verona University

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